CANCER EPIDEMIOLOGY IN CHINA: WHAT WE HAVE LEARNT SO FAR?

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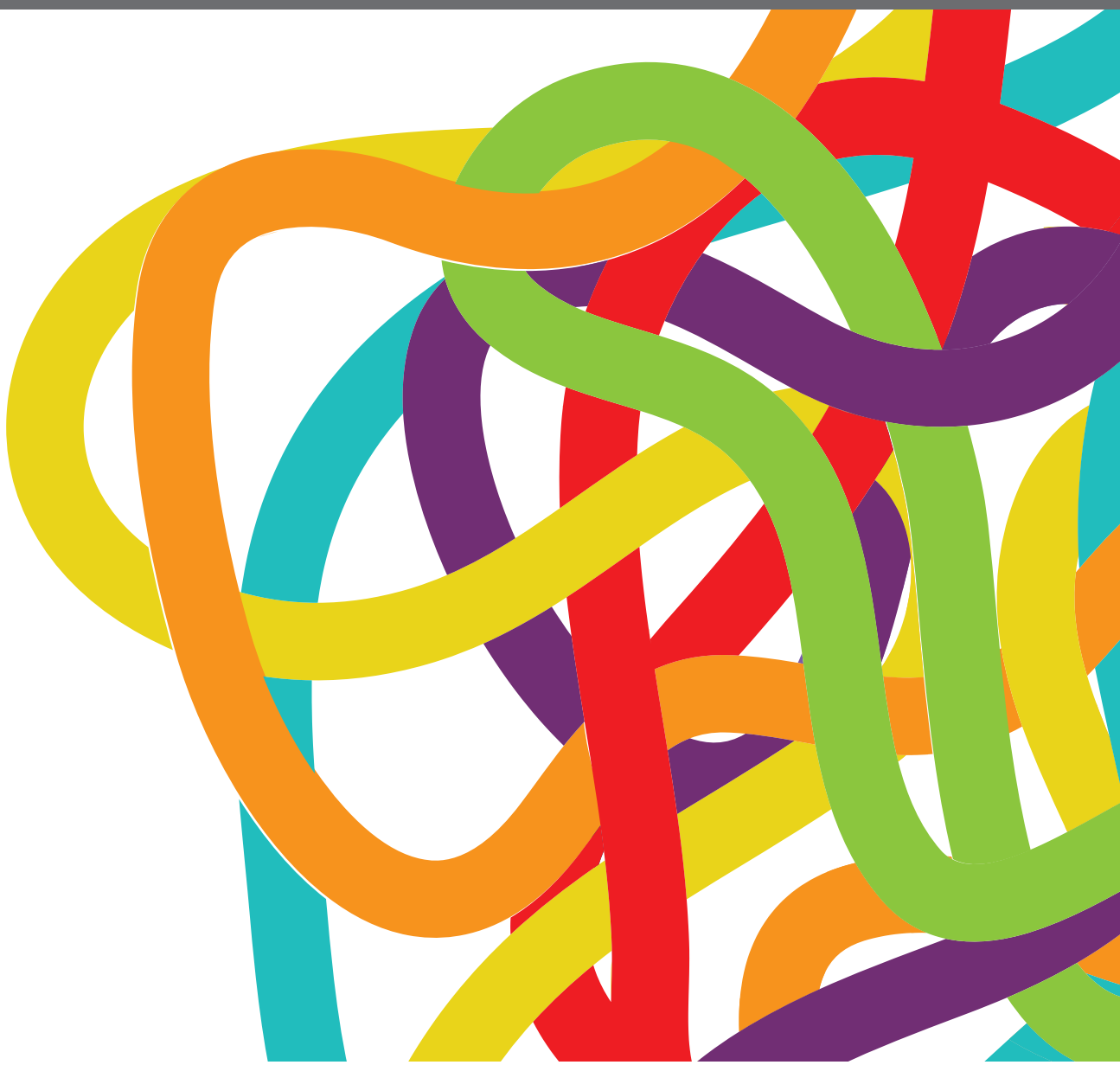
PUBLISHED IN: Frontiers in Oncology



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ISSN 1664-8714
ISBN 978-2-88963-290-9
DOI 10.3389/978-2-88963-290-9

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CANCER EPIDEMIOLOGY IN CHINA: WHAT WE HAVE LEARNT SO FAR?

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After several decades of development, the socialist market economy of China is now the world's second largest economy by nominal GDP. China is also the largest economy by purchasing power parity according to the International Monetary Fund. In tandem with the development of the Chinese economy, China's cancer burden is rising rapidly due to an ageing population and the adoption of unhealthy lifestyle behaviours. According to the data from the National Central Cancer Registry (NCCR) of China, the incidence and mortality of cancer have been increasing rapidly in China. In recent years, cancer has been the leading cause of death among city residents and the second cause of death among rural residents, which has become a stark public health issue in China. According to the NCCR, an estimated 4.29 million new incident cases (12 thousand per day) and 2.81 million death cases (7.5 thousand per day) would occur in 2015 in China. This corresponds to the age-standardized incidence rate (ASIR) of 201.1 per 100,000 and age-standardized mortality rate (ASMR) of 126.9 per 100,000, respectively.

Due to the geographical and ethnical disparities in living habits and healthcare level, the cancer spectrum differs between different regions and ethnical groups in China. According to the estimation from IARC, the incidence of nasopharyngeal carcinoma and liver cancer is the world's highest in specific regions of China. The incidence of some cancer types in Chinese urban areas, such as colorectal, prostate, kidney and bladder cancers, is similar to that in developed countries or regions where the incidence of cancer is highly associated with obesity and westernised lifestyles. Nevertheless, the incidence of some common cancer types in rural areas, including oesophageal, stomach, liver and cervical cancers, shares similarity with less developed countries or regions in the world where cancers are associated with chronic infectious agents due to poverty. In addition, the mortality rate is higher in rural areas, which suggests a poorer cancer prognosis due to late diagnosis and/or unsatisfying clinical treatment. The distinct cancer patterns of different regions and/or ethnic groups indicate a need for precise cancer prevention and control plans tailored for different geographical regions and/or ethnic groups.

The overarching goal of the proposed Frontiers in Oncology Research Topic is to present current perspectives on cancer epidemiology in Chinese characteristics and provide current knowledge of cancer burden as well as cancer mortality to academic investigators, clinicians and stakeholders from the translational, clinical and public health communities.

Citation: Ji, J., Chen, T., Shu, X., Liu, H., eds. (2020). Cancer Epidemiology in China: What We Have Learnt So Far?. Lausanne: Frontiers Media SA.
doi: 10.3389/978-2-88963-290-9

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frontiers in Oncology

EDITORIAL
published: 07 February 2020
doi: 10.3389/fonc.2020.00106

Editorial: Cancer Epidemiology in China: What We Have Learnt So Far?

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Keywords: cancer epidemiology, China, cancer incidence, cancer mortality, cancer survival, cohort

Editorial on the Research Topic

Cancer Epidemiology in China: What We Have Learnt So Far?

Cancer epidemiology has developed greatly during the past decades in China. Overall 36 cancer registers in China were included by the International Agency for Research on Cancer and the International Association of Cancer Registries for their joint publication "Cancer Incidence in Five Continents" (Volume XI) which provides the reference source of data on the incidence of cancer in China. Many scientists have been successfully recruited from comprehensive research institutions, and accelerate the development of cancer epidemiology in China. In this Research Topic, we have received a total of 36 submissions. We selected 16 articles contributed by 216 authors, which have received 24,262 views and nearly 4,000 downloads so far. Our collection covers different types of cancer and various study designs.

Using colorectal cancer (CRC) incidence data from the Cancer Incidence in Five Continents, Volume XI dataset and the age-standardized incidence rate and age-standardized mortality rate of CRC from the 2016 Global Burden of Diseases Study, Dr. Tianjiang Ma's group found a steady increase in the CRC incidence in China over the past three decades and predicted a further increase in the near future (Zhang et al.). Using data from local cancer register in Yangzhou city, the incidence and mortality rates of esophageal and gastric cancers showed a downward trend whereas CRC was on the rise as a whole, suggesting heterogeneous risk factors for these common digestive cancers in China (Shao et al.). Another study leaded by Profs Maode Lai and Yimin Zhu's group found the prediction of CRC prognosis might be improved by using informative differentially expressed gene (DEG) compared to that using the TNM staging system (Pan et al.). As for CRC cancer screening, Wu et al. found that cutoff points of risk score should be optimized and stool-based test should be improved for large-scale usage in Chinese population.

The contribution of infectious microbes on cancer might be more predominant in developing countries such as China. High prevalence of HPV-16 was found to be associated with the incidence of head and neck cancers, as evidenced by a recent meta-analysis using publications on Chinese population during 2006–2018 (Guo et al.). Prof. Guangwen Cao's group found HBV promotes the aggressiveness of primary liver cancer in Chinese population, and the contributions of HBV to intrahepatic cholangiocarcinoma and other etiological factors to HCC might be indirect via arousing non-resolving inflammation (Yang et al.). However, another study found no correlation between Epstein-Barr virus (EBV) and thyroid cancer in a cohort from southern China (Yu et al.).

The articles leaded by Prof. Guangwen Cao investigated urban-rural disparity in cancer burden during 2002–2015 in Shanghai, China. They concluded that female breast cancer and CRC occurred more frequently in urban than in rural populations, while the mortality of female breast cancer significantly declined in urban and rural areas. For all cancers combined, the 5-year survival estimate was higher in urban than in rural areas. These findings provide evidence to optimize the strategy for cancer control and prevention in Shanghai, China (Li et al.).

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Specialty section:
This article was submitted to Cancer Epidemiology and Prevention, a section of the journal Frontiers in Oncology

Received: *26 December 2019*
Accepted: *21 January 2020*
Published: *07 February 2020*

Citation:
Chen T, Shu X, Liu H and Ji J (2020) Editorial: Cancer Epidemiology in China: What We Have Learnt So Far? Front. Oncol. 10:106. doi: 10.3389/fonc.2020.00106

Using the Kailuan men cohort study with overall 104,825 men participating in the health checkup during 2006–2015, Prof. Jie He' group from National Cancer Center found that the U-shaped association between waist circumference and liver cancer risk tended to be strengthened among men with hepatitis B surface antigen (HBsAg) negativity, suggesting waist circumference might be an independent predictor of liver cancer risk in men, especially for those with HBsAg negativity (Wei et al.). Another study from Prof. Jie He' group found the diagnostic yield of ultrasound screening for breast cancer in high-risk population was satisfactory by analyzing 72,250 women with high-risk for breast cancer derived from the Cancer Screening Program in Urban China during 2012–2016 (Wang et al.).

Using a multicenter case-control study, Mai et al. from Hongkong found consumption of milk across life periods was associated with lower risks of nasopharyngeal carcinoma (NPC), which might have important implications for dairy product consumption and prevention of NPC. A systematic review from Prof. Zhijun Dai's group found a strong association between incidence and mortality risk of cancers and concentration of Hexavalent chromium [Cr(VI)] in the air and the exposure time (Deng et al.).

He et al. showed a successful application of electronic health record (e-HR) system in cancer prevention and control in Minhang district of Shanghai, China. Pingping et al. reported the first population-based study investigating epidemiology of sarcomas in Shanghai according to anatomic site and histologic type. Men et al. provided the first geographically representative epidemiological study of postoperative radiotherapy (PORT) in non-small cell lung cancer (NSCLC) patients in China, showing a declined trend of PORT use from 2005 to 2014. Lu et al. found the 7th edition of the TNM classification for esophageal carcinoma is poorly recognized and understood in central and southern China, which might contribute to the relatively low rate of appropriate perioperative procedures applied for esophageal cancer patients.

AUTHOR CONTRIBUTIONS

TC drafted the manuscript. TC, XS, HL, and JJ revised the manuscript and approved the submission. TC and JJ take responsibility as the corresponding authors.

FUNDING

This work was supported by grants from Joint Key Program of Zhejiang Province-Ministry of Health [Grant No.: WKJ-ZJ-1714], Qianjiang Talents Fund of Zhejiang Province [Grant No.: QJD1602026], Key Research-Development Program of Zhejiang Province [Grant No.: 2017C03013], and Startup Funds for Recruited Talents at Zhejiang Cancer Hospital.

ACKNOWLEDGMENTS

We deeply appreciated all submissions and contributing authors and reviewers for this Research Topic.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Oncology

ORIGINAL RESEARCH
published: 03 December 2018
doi: 10.3389/fonc.2018.00579

Urban-Rural Disparity in Cancer Incidence, Mortality, and Survivals in Shanghai, China, During 2002 and 2015

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Specialty section:
This article was submitted to Cancer Epidemiology and Prevention, a section of the journal Frontiers in Oncology

Received: 24 September 2018
Accepted: 19 November 2018
Published: 03 December 2018

Citation:
Li X, Deng Y, Tang W, Sun Q, Chen Y, Yang C, Yan B, Wang Y, Wang J, Wang S, Yang F, Ding Y, Zhao G and Cao G (2018) Urban-Rural Disparity in Cancer Incidence, Mortality, and Survivals in Shanghai, China, During 2002 and 2015. Front. Oncol. 8:579. doi: 10.3389/fonc.2018.00579

Introduction: Disparities in the incidence, mortality, and survival of cancer types between urban and rural areas in China reflect the effects of different risk factor exposure, education, and different medical availability. We aimed to characterize the disparities in the incidence, mortality, and survivals of cancer types between urban and rural areas in Shanghai, China, 2002-2015.

Materials and Methods: The incidence and mortality were standardized by Segi's world standard population. Trends in the incidence and mortality of cancers were compared using annual percent change. The 5-year observed and relative survivals were calculated with life table and Ederer II methods.

Results: Age-standardized incidences and mortalities were 212.55/10^5 and 109.45/10^5 in urban areas and 210.14/10^5 and 103.99/10^5 in rural areas, respectively. Female breast cancer and colorectal cancer occurred more frequently in urban than in rural areas, quite in contrast to liver cancer and cervical cancer. Cancers of lung and bronchus, liver, stomach, and colon and rectum were the leading causes of cancer death in both areas. Age-standardized incidence of female breast cancer and colorectal cancer in urban areas increased while gastric cancer and liver cancer decreased in both areas. Age-standardized mortalities of cancers of breast, esophagus, stomach, colon and rectum, liver, and lung and bronchus decreased in both areas. For all cancers combined, the 5-year observed and relative survivals of cancer patients were higher in urban than in rural areas. The 5-year observed and relative survivals of cancers of liver, pancreas, stomach, brain and central nervous system (CNS), and prostate were higher in urban than in rural areas. The 5-year observed and relative survivals of cervical cancer were higher in rural than in urban areas.

Conclusions: Factors promoting female breast cancer and colorectal cancer in urban areas and liver cancer and cervical cancer in rural areas should be specifically intervened in cancer prophylaxis. Improved medical services can greatly prolong the survival of major cancers in rural areas.

Keywords: cancers, urban-rural disparity, incidence, mortality, survival, China

INTRODUCTION

Cancer has been the leading cause of death in China. Data from 2013 showed that 3.7 million new diagnosed cancer cases and 2.2 million people died of cancer in mainland China (1). Approximately 22% of global new diagnosed cancer cases and close to 27% of global cancer deaths occur in China (2, 3). High levels of cancer incidence and cancer death reflect the aged society, different cancer types, increased pollution, and low level of medical services.

Since the founding of the People's Republic of China in 1949, the government has enforced a household registration system, which is different from other countries in the world. The residents are classified into two types: non-agricultural (urban) and agricultural (rural) residents. During the era of planned economy (1949-1992), urban residents mostly worked in fields of industry and commerce, purchased the necessaries of life including grain, meat, sugar, and cooking oil using their salary. Rural residents mostly lived on agricultural fields. Urban residents had the priority to enjoy some social benefits including the allotment of housing, healthcare, and education. Rural residents were usually self-sufficient and low educated (4). Urban residents experienced more industrial pollutions than rural ones while rural population had a low frequency of having refrigerators for food reservation. Since China entered the market economy era in 1993, the government has gradually eased these regulations, but rural residents still encountered barriers in obtaining basic welfares such as medical service and health education (4). Most health resources were allocated to urban residents while rural residents might not afford expensive medical expenditure, resulting in the inequity of health service utilization among urban and rural residents (5). Cancer is the second most common in rural areas and the first leading cause of death in urban areas in China (6). Data from 5 urban and 5 rural areas in the China Kadoorie Biobank cohort showed that cancer burden was different between urban and rural areas of China (7). Disparities in the incidence, mortality, and survival of cancer between the urban and rural populations may help in identifying cancer-determining socioeconomic factors that can be handled for cancer prevention and control. However, trends in the incidence and mortality of major cancer types in urban and rural areas were not fully elucidated at subnational levels in China. Moreover, there are no data interpreting the difference in cancer survival between urban and rural areas.

Shanghai is the largest metropolis in China, having 16 districts. Pudong new district has a resident population of about 5 million, accounting for 20% of the total population in Shanghai. Pudong new district was founded in 1993, and Nanhui county which represented rural areas was combined into the Pudong new district in 2009. Pudong has become the only district with urban and rural populations in Shanghai. Shanghai has established a cancer registration system since 1973 (8). Since 2002, this system has covered 100% of registered population and become one of cancer registry with the largest population worldwide. The cancer registration data are reliable and their quality has been approved by the World Health Organization. Although not exactly same, original Pudong new district and Nanhui county had equal cancer registration system. We selected Pudong new district as a suitable model to compare the disparities of cancer burden between urban and rural areas in Shanghai, which might provide evidences to optimize cancer control strategy.

MATERIALS AND METHODS

Data Source

The geographic location of Pudong new district, Shanghai, China, is shown in **Supplementary Figure 1**. Due to time needed for data collection, quality control, and analysis, the data in this study have a 3-year time lag. The definitions of urban and rural areas in this study were based on the regulation released by the National Bureau of Statistics of China in 2006. Urban area was referred to the area with a population density of over 1,500 persons per km^2, and rural area was the area with a population density <1,500 persons per km^2 (9). A total of 46 communities (32 in urban and 14 in rural) provided electronic data from 2002 to 2015 by Pudong cancer registry system and were involved in the comparison of the incidence, mortality, and survival of cancers between urban and rural areas. The detailed variables of each cancer patient including age, gender, cancer type, date of diagnosis, pathology, treatment, date and cause of death, TNM stage, and registered residence were collected. All cancers combined and 26 cancer types were identified according to the International Classification of Diseases, 10th edition (ICD-10).

Cancer cases reported to Pudong cancer registry system were followed up to check their survivals via home visits or telephone enquiries every year. The date of diagnosis of primary cancer was set as the starting point of observation, and the date of death caused by primary cancer was determined as the end-point. All information of primary cancer patients who survived from January 1, 2002 to December 31, 2015 were checked. Follow-up was finished on June 20, 2017. Population data by 5-year age group and residence were obtained from the Public Security Bureau of Pudong, Shanghai, China. The study was approved by the ethics committee of the Center for Disease Control and Prevention of the Pudong New Area, Shanghai, China.

Quality Control

The data of all cancers were checked for completeness and validity before constructing database. According to the criteria of International Agency for Research on Cancer/International Association of Cancer Registries, two authors (XL and YD) assessed the data quality independently. Three main measures including the proportion of morphological verification (MV%), percentage of cancer cases identified with death certification only (DCO%), and mortality to incidence ratio (M/I) were calculated to evaluate the data quality.

Statistical Analyses

The crude incidence and mortality rates of cancers in urban and rural areas were calculated and shown as per 100,000 ($/10^5$) person-years. Age-standardized incidence rates (ASIRWs) and mortality rates (ASMRWs) by Segi's world standard population were calculated. The incidence and mortality rates between

urban and rural areas were compared according to the Possion approximation method (10, 11):

$$S_{p_1-p_2} = \sqrt{\frac{X_1+X_2}{n_1+n_2}\left(1-\frac{X_1+X_2}{n_1+n_2}\right)\left(\frac{1}{n_1}+\frac{1}{n_2}\right)} \quad \text{(A)}$$

$$u = \frac{p_1-p_2}{S_{p_1-p_2}} \quad \text{(B)}$$

where the X in the formula A represents the number of incidence or death from a large population, and n is the sample size of this population. p in formula B equals to X/n, and it means the crude incidence or mortality rate in this population.

Cancer trends in ASIRWs and ASMRWs were calculated using Joinpoint Regression Program 4.3.1.0 (downloaded from the website of the National Cancer Institute, MD, USA) and expressed as an annual percent change (APC), and the Z test was employed to assess whether the APC was statistically different from zero. Age-specific incidence and mortality rates were calculated for each 5-year age group, from 0–4 to 85+ years. The 5-year observed survivals (OS) and relative survivals (RS) were calculated with life table and Ederer II methods (12, 13). All statistical analyses were conducted using SPSS 21.0 (SPSS, Inc., Chicago, IL) and R (version 3.4.3). P-value < 0.05 was considered as statistically significant.

RESULTS

Incidence and Mortality Rates of All Cancers

The average MV%, DCO%, and M/I for all cancers was 70.40%, 3.42%, and 0.56, respectively (**Supplementary Table 1**). These indicators suggested that overall quality of data was satisfied.

A total of 149,236 new cancer cases from 37,353,102 person-years were diagnosed during 2002-2015, with 111,139 cases from 26,870,661 person-years in urban areas and 38,097 cases from 10,482,441 person-years in rural areas. The mean ages at diagnosis were 64.73 ± 14.97 years in urban cases and 63.50 ± 15.23 years in rural cases. There were 79,223 cases from 18,667,245 person-years in men, and 70,013 cases from 18,685,857 person-years in women. The mean ages at diagnosis were 65.95 ± 13.91 years in males and 62.68 ± 16.05 years in females (**Table 1**). The crude incidence rates of all cancers were $413.61/10^5$ and $363.44/10^5$ in urban and rural areas, respectively. The crude incidence rate was significantly higher in urban than rural areas ($u = 21.30$, $P < 0.01$). No significant difference in ASIRW of all cancer types was found between urban ($212.55/10^5$) and rural areas ($210.14/10^5$) ($u = 1.27$, $P = 0.89$) (**Table 2**).

A total of 87,668 cancer deaths (53,754 men and 33,914 women) were reported from 2002 to 2015, with 64,764 in urban and 22,904 in rural areas. The mean ages at death were 71.26 ± 13.29 years in urban cases and 69.65 ± 13.53 years in rural cases. The mean age were 70.06 ± 12.91 years for men and 72.07 ± 13.98 years for women (**Table 1**). The crude mortality rate was higher in urban than rural areas ($241.02/10^5$ vs. $218.50/10^5$, $u = 12.58$, $P < 0.01$). The ASMRW was higher in urban than in rural areas ($109.45/10^5$ vs. $103.99/10^5$, $u = -4.79$, $P < 0.01$) (**Table 2**).

The Incidence and Mortality of Top 10 Cancers

Cancers of breast in female, lung and bronchus, colon and rectum, thyroid, stomach, liver, prostate, cervix, brain and central nervous system (CNS), and pancreas were the top 10 cancer types in urban areas, accounting for 72.81% of all new diagnosed cancers. Cancers of lung and bronchus, breast in female, thyroid, liver, stomach, colon and rectum, cervix, prostate, pancreas, and brain and CNS were the top 10 cancers in rural areas, accounting for 74.10% of all new diagnosed cancers (**Table 2**). The ASIRWs of cancers of breast in female ($u = 24.42$, $P < 0.01$), colon and rectum ($u = 20.92$, $P < 0.01$), prostate ($u = 9.57$, $P < 0.01$), and brain and CNS ($u = 3.97$, $P < 0.01$) were higher in urban than in rural areas. In particular, the ASIRWs of female breast cancer and colorectal cancer (CRC) were 62 and 27% higher in urban than in rural areas, respectively. By contrast, the ASIRWs of lung and

TABLE 1 | Incidence and mortality rates of all cancers in Pudong new district, Shanghai, China, 2002-2015.

Area	Gender	Incidence				Mortality			
		N	CIR[a]	ASIRW[b]	Age (years)	*N*	CMR[c]	ASMRW[d]	Age (years)
All	Both	1,49,236	399.53	211.27	64.42 ± 15.04	87,668	234.70	105.44	70.84 ± 13.37
	Male	79,223	424.40	225.68	65.95 ± 13.91	53,754	287.96	138.96	70.06 ± 12.91
	Female	70,013	374.68	201.76	62.68 ± 16.05	33,914	181.50	75.35	72.07 ± 13.98
Urban	Both	1,11,139	413.61	212.55	64.73 ± 14.97	64,764	241.02	109.45	71.26 ± 13.29
	Male	58,670	435.25	223.05	66.23 ± 13.91	39,469	292.81	149.70	70.49 ± 12.91
	Female	52,469	391.82	204.93	63.05 ± 15.90	25,295	188.89	75.52	72.46 ± 13.79
Rural	Both	38,097	363.44	210.14	63.50 ± 15.23	22,904	218.50	103.99	69.65 ± 13.53
	Male	20,553	396.18	233.67	65.16 ± 13.91	14,285	275.36	135.28	68.87 ± 12.89
	Female	17,544	331.35	193.03	61.55 ± 16.44	8,619	162.79	74.79	70.94 ± 14.45

[a] *CIR, crude incidence rate (per 100,000).*
[b] *ASIRW, age-standardized incidence rate by Segi's world standard population (per 100,000).*
[c] *CMR, crude mortality rate (per 100,000).*
[d] *ASMRW, age-standardized mortality rate by Segi's world standard population (per 100,000).*

TABLE 2 | Incidences of major cancer types in urban and rural areas in Pudong new district, Shanghai, China, 2002-2015.

Rank	Type	ASIRW[a]	CIR[b]	Proportion (%)	APC (ASIRW) (95% CI) (%)
URBAN					
1	Breast in female	39.17	69.30	8.34	2.30 (1.61, 3.12)*
2	Lung and bronchus	34.48	74.68	18.02	0.89 (−0.20, 2.05)
3	Colon and rectum	23.65	50.66	12.22	1.42 (1.21, 1.82)*
4	Thyroid	19.23	27.65	6.67	18.93 (17.10, 20.79)*
5	Stomach	19.10	40.59	9.79	−3.42 (−4.24, −2.71)*
6	Liver	13.29	26.63	6.43	−5.03 (−7.01, −3.09)*
7	Prostate	11.60	25.74	3.11	5.22 (2.92, 7.54)*
8	Cervix	10.09	14.94	1.80	9.80 (7.81, 11.92)*
9	Brain and CNS[c]	7.32	12.06	2.91	−1.84 (−3.52, −0.17)*
10	Pancreas	6.43	14.58	3.52	0.65 (−1.02, 2.20)
	All types	212.55	413.61	100.00	1.25 (0.71, 1.68)*
RURAL					
1	Lung and bronchus	38.46	72.49	19.74	−2.40 (−6.08, 1.47)
2	Breast in female	24.16	38.89	5.35	1.92 (−0.61, 4.42)
3	Thyroid	20.73	29.66	8.08	20.22 (12.37, 28.65)*
4	Liver	19.94	35.48	9.66	−7.02 (−11.51, −2.25)*
5	Stomach	18.74	35.08	9.55	−6.26 (−9.01, −3.27)*
6	Colon and rectum	18.64	34.46	9.38	−2.51 (−7.45, 2.62)
7	Cervix	14.77	21.16	2.91	15.50 (3.37, 29.18)*
8	Prostate	9.87	18.26	2.46	6.52 (4.73, 8.41)*
9	Pancreas	7.19	14.34	3.90	−2.30 (−4.48, −0.26)*
10	Esophagus	5.70	11.26	3.07	−6.62 (−9.78, −3.52)*
	All types	210.14	363.44	100.00	−0.92 (−2.91, 1.10)

[a]*ASIRW, age-standardized incidence rate by Segi's world standard population (per 100,000).*
[b]*CIR, crude incidence rate (per 100,000).*
[c]*CNS, central nervous system.*
**APC value is significantly different from zero at alpha = 0.05.*

bronchus cancer ($u = 2.55$, $P < 0.05$), liver cancer ($u = -14.61$, $P < 0.01$), and cervical cancer ($u = -8.43$, $P < 0.01$) were lower in urban than in rural areas, especially with a 50% lower in liver cancer (**Figure 1A**).

The top 10 causes of cancer deaths were cancers of lung and bronchus, stomach, colon and rectum, liver, pancreas, breast in female, prostate, esophagus, gallbladder, and lymphoma in both areas. The proportions of top 10 cancer deaths accounted for 79.16% in urban and 81.86% in rural areas, respectively (**Table 3**). The ASMRWs of cancers of colon and rectum ($u = 15.50$, $P < 0.01$), breast in female ($u = 11.45$, $P < 0.01$), prostate ($u = 6.61$, $P < 0.01$) were higher in urban than in rural areas, while ASMRWs of lung and bronchus cancer ($u = 2.08$, $P < 0.05$) and liver cancer ($u = -14.90$, $P < 0.01$) were lower in urban than in rural areas (**Figure 1B**).

Trends in the Age-Standardized Incidence and Mortality Rates

The ASIRW for all cancers combined increased by 1.25% [95% confidence interval (95% CI): 0.71–1.68%, $P < 0.05$] per year during 2002-2015 in urban areas, but it remained stable in rural areas. Among the top 10 most common cancers in urban areas, the ASIRWs increased in cancers of breast in female, colon and rectum, thyroid, prostate, and cervix and decreased in cancers of stomach, liver, and brain and CNS. In rural areas, the ASIRWs increased in thyroid cancer, cervical cancer, and prostate cancer and decreased in cancers of liver, stomach, pancreas, and esophagus (**Table 2**).

The ASMRW for all cancers combined decreased by 7.44% [(95% CI): −8.91 to −5.95%, $P < 0.05$] per year during 2002-2015 in urban areas and also decreased in rural areas, with an APC of −9.45% [(95% CI): −11.91% to −6.96%, $P < 0.05$] per year. Among the top 10 most common causes of cancer death, the ASMRWs for all cancers combined and cancers of breast in female, esophagus, stomach, colon and rectum, liver, lung and bronchus, and lymphoma decreased in both areas. Decreases in ASMRWs of prostate cancer in urban and pancreatic cancer in rural areas were also evident (**Table 3**).

Age-Specific Incidence and Mortality

The age-specific incidence rates were lower in men than in women aged 30–54 years, while a rapid increase was observed in men. Among those older than 55 years, the incidence rates were consistently higher in men than in women. In rural areas, the incidence rate increased slowly from 30 to 59 years and increased rapidly after 60 years, reaching the peak after 80 years in men, while the incidence increased

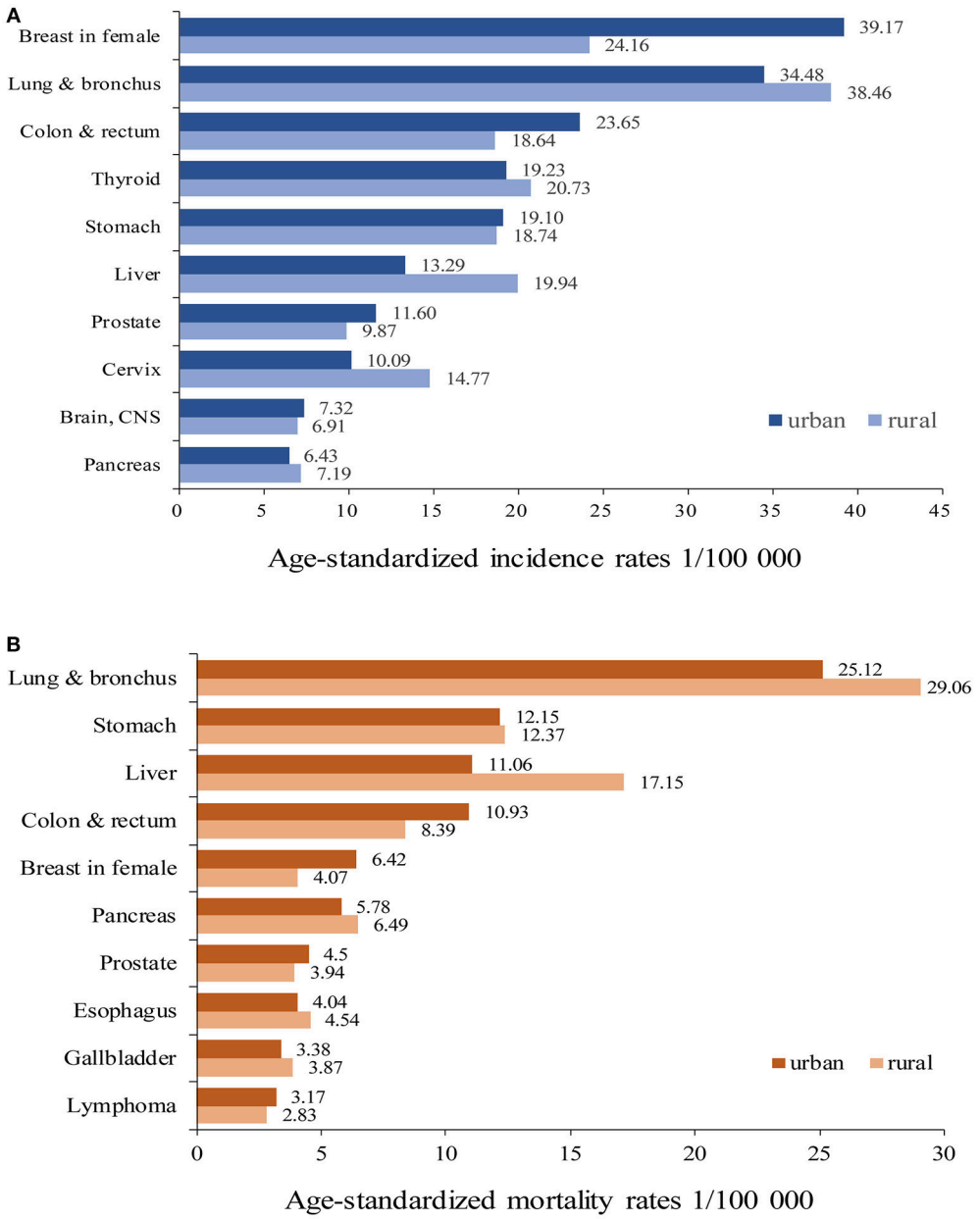

FIGURE 1 | Age-standardized incidence rates **(A)** and mortality rates **(B)** of major cancer types in urban and rural areas of Pudong new district, Shanghai, China.

continuously from 30 years and peaked after 80 years in women. The pattern was similar in rural areas, and a more significant increase was observed in men older than 60 years (**Figure 2A**).

The age-specific mortality rates increased slowly from 30 to 59 years, after which the rates increased rapidly in men. However, the age of switch occurred between 65 and 70 years in women. The mortality rates of all age groups were always higher in rural than in urban areas in men. Compared with urban areas, no significant difference in mortality rates was observed in rural areas in women (**Figure 2B**).

Five-Year OS and RS of Major Cancers in Urban and Rural Areas

For all cancers combined, the 5-year OS of cancer patients was higher in urban than in rural areas (44.05 vs. 41.47%, $P < 0.001$), and the 5-year RS was 4.76% higher in urban areas than in rural areas. Cancers of pancreas, gallbladder, liver, esophagus, and lung and bronchus had the poorest 5-year OS and RS in both areas (**Table 4**).

Cancer types whose 5-year OS were higher in urban than in rural areas were liver cancer (14.37 vs. 11.81%, $P < 0.001$), pancreatic cancer (5.91 vs. 5.03%, $P = 0.004$), gastric cancer

TABLE 3 | Mortalities of major cancer types in urban and rural areas in Pudong new district, Shanghai, China, 2002-2015.

Rank	Type	ASMRW[a]	CMR[b]	Proportion (%)	APC (ASMRW) (95% CI) (%)
URBAN					
1	Lung and bronchus	25.12	57.09	24.90	−5.61 (−7.40, −3.77)*
2	Stomach	12.15	27.31	11.91	−9.12 (−12.64, −5.45)*
3	Liver	11.06	22.58	9.85	−8.54 (−10.63, −6.31)*
4	Colon and rectum	10.93	25.32	11.04	−8.44 (−13.27, −3.40)*
5	Female Breast	6.42	13.11	2.85	−15.98 (−23.43, −7.51)*
6	Pancreas	5.78	13.31	5.81	−1.90 (−4.24, 0.58)
7	Prostate	4.50	10.78	2.36	−10.12 (−16.56, −3.24)*
8	Esophagus	4.04	9.46	4.12	−9.62 (−14.27, −4.81)*
9	Gallbladder	3.38	8.06	3.51	−2.89 (−7.02, 1.65)
10	Lymphoma	3.17	6.44	2.81	−6.52 (−10.73, −2.01)*
	All types	109.45	241.02	100.00	−7.44 (−8.91, −5.95)*
RURAL					
1	Lung and bronchus	29.06	56.53	27.12	−7.84 (−13.25, −2.10)*
2	Liver	17.15	30.84	14.79	−10.03 (−15.34, −4.51)*
3	Stomach	12.37	24.08	11.55	−11.22 (−16.65, −5.43)*
4	Colon and rectum	8.39	16.66	7.99	−10.67 (−14.88, −6.10)*
5	Pancreas	6.49	13.19	6.33	−3.26 (−5.32, −1.15)*
6	Esophagus	4.54	9.20	4.41	−12.20 (−20.17, −3.55)*
7	Breast in female	4.07	7.09	1.72	−14.82 (−18.20, −11.34)*
8	Prostate	3.94	7.45	1.77	−7.11 (−16.27, 3.05)
9	Gallbladder	3.87	7.74	3.71	−2.48 (−5.19, 0.41)
10	Lymphoma	2.83	5.15	2.47	−7.47 (−9.72, −5.10)*
	All types	103.99	218.50	100.00	−9.45 (−11.91, −6.96)*

[a]*ASMRW, age-standardized mortality rate by Segi's world standard population (per 100,000).*
[b]*CMR, crude mortality rate (per 100,000).*
**APC value is significantly different from zero at alpha = 0.05.*

(32.65 vs. 30.52%, $P = 0.037$), brain and CNS cancer (56.10 vs. 54.51%, $P = 0.045$), and prostate cancer (59.20 vs. 53.93%, $P = 0.022$). The 5-year OS of cervical cancer was higher in rural than in urban areas (83.74 vs. 87.22%, $P = 0.002$). Cancers of liver, pancreas, stomach, brain and CNS, and prostate had higher 5-year RS in urban than in rural areas, while the cervical cancer had lower 5-year RS in urban than in rural areas (**Table 4**).

DISCUSSION

In this study, we analyzed urban-rural disparity in cancer burden in Shanghai, China in the past 14 years and confirmed that female breast cancer and CRC occurred more frequently in urban than in rural populations, in contrast to liver and cervical cancers. In 1993, Pudong was set as an Open Economic Zone. Economy has been growing fast. Lifestyle of urban residents has turned to be more westernized, with unhealthy diets and insufficient physical activity. Overweight and obesity, the risk factors for female breast cancer (14), became an increasing public health concern (15, 16). Increasing incidence of female breast cancer is partially attributed to menstrual and reproductive factors such as earlier age at menarche, later ages at menopause and first birth, fewer number of live births, and less duration of breastfeeding (17). The Chinese birth control policy enforced since early 1970s not only limited urban couples to one child and rural couples to two children but also encouraged late marriage and childbearing (18). Compared with rural women, urban women had few number of live births, later ages at birth, earlier ages at menarche, less average duration of breastfeeding, later ages at menopause (19). We showed here that the incidence of female breast cancer increased by 2.3% annually in urban areas during study period. However, some urban women who were affected by this policy have not reached the peak age of disease occurrence (45–55 years) until the end of this study. We speculate that the incidence of female breast cancer will keep increasing. The government has provided free breast cancer screening to rural residents and low-income women since late 2000s, but the coverage rate was only 27.4% in 2010 (20). Our results indicated that the mortality of female breast cancer significantly declined in urban and rural areas, possibly because of the improved diagnosis and treatment, rather than screening (21). Thus, effective and affordable breast cancer prevention and control strategies are urgently needed.

We found that the incidence and mortality of CRC were higher in urban than rural areas. The incidence of CRC increased by 4.2% annually in Shanghai in the past 30 years, which almost reached the incidence level in developed countries (2, 22). The risk factors of CRC are also related to western lifestyles. High red and processed meat intake, overweight and obesity, low

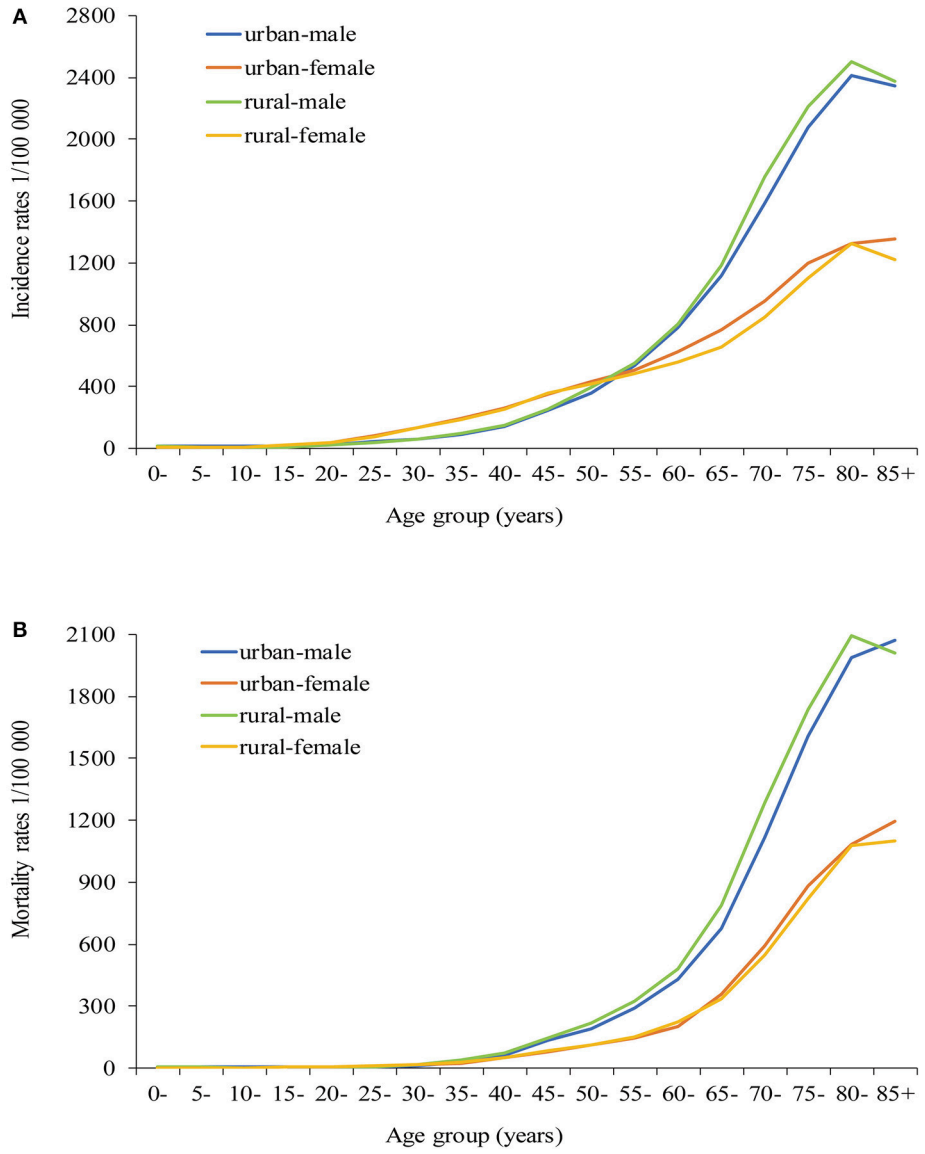

FIGURE 2 | Age-specific incidence rates **(A)** and mortality rates **(B)** of all cancer types in urban and rural areas of Pudong new district, Shanghai, China.

vegetable and fruit intake, tobacco smoking, alcohol drinking, and physical inactivity are major risk factors for CRC (14, 23). Compared with rural residents, urban residents are more exposed to these risk factors (24). Moreover, urban residents are more likely to participate screening of CRC, and improved diagnosis and screening are partially responsible for the higher rates in urban areas. The incidence of CRC increased in urban areas and kept steady in rural areas, but the mortality decreased in urban and rural areas. Shanghai government launched a community-based CRC screening program including 542,430 urban and 267,098 rural residents. A total of 1,630 CRC patients were newly diagnosed, and 51.6% of CRC cases were diagnosed in early stage, which is equal to five times the rate of cancer registry data (22). CRC screening is effective for early diagnosis and treatment, and ultimately reduce the mortality of CRC (25). Thus, the improvement in lifestyle including increased physical activity, health food consumption, and screening are effective in reducing CRC-related death.

The incidence and mortality of liver cancer declined significantly in urban and rural areas. Chronic infection with hepatitis B virus (HBV) and/or hepatitis C virus (HCV), heavy alcohol use, and excessive aflatoxin exposure increase the risk of liver cancer (26–28). After national HBV vaccination in newborns in China from 1992, the prevalence rate of hepatitis B surface antigen (HBsAg) among nationwide population decreased from 9.8% in 1992 to 7.18% in 2006 (29–31). The vaccination rate of HBV in newborn reached over 95% in urban areas and 80% in most rural areas in 2006, thus contributing

TABLE 4 | Five-year observed and relative survivals for cancer patients diagnosed in urban and rural areas in Pudong new district, Shanghai, China, 2002-2015.

Type	Cases		5-year OS[a]			5-year RS[b]		
	Urban	Rural	Urban	Rural	*P*	Urban	Rural	Difference in RSR (Urban-Rural)[c]
Lung and bronchus	19,909	7,503	18.07	16.53	0.613	25.47	22.67	2.80
Liver	7,354	3,834	14.37	11.81	<0.001	18.83	14.98	3.85
Pancreas	3,954	1,498	5.91	5.03	0.004	8.98	7.63	1.35
Gallbladder	2,531	955	11.81	12.86	0.109	18.78	19.64	−0.86
Esophagus	3,168	1,198	17.17	16.08	0.149	25.53	23.52	2.01
Stomach	11,076	3,741	32.65	30.52	0.037	45.62	41.46	4.16
Colon and rectum	13,579	3,586	50.16	52.16	0.284	70.00	70.29	−0.29
Lymphoma	2,831	890	38.77	40.24	0.253	49.95	51.52	−1.57
Brain and CNS[d]	3,318	1,129	56.10	54.51	0.045	69.69	65.82	3.87
Breast in female	9,320	2,048	83.74	83.48	0.534	95.52	92.87	2.65
Cervix	1,965	1,069	83.74	87.22	0.002	90.58	91.84	−1.26
Prostate	3,350	902	59.20	53.93	0.022	96.58	90.02	6.56
Thyroid	6,793	2,768	95.88	96.88	0.209	101.63	101.81	−0.18
All types	1,11,139	38,097	44.05	41.47	<0.001	58.44	53.68	4.76

[a] *OS, observed survival.*
[b] *RS, relative survival.*
[c] *Positive number indicates a higher survival in urban areas.*
[d] *CNS, central nervous system.*

to the decreased rate of liver cancer (32, 33). The incidence and mortality of liver cancer were significantly higher in rural residents than in urban residents in this study, possibly because HBsAg prevalence was higher in rural than in urban areas (29). Currently, there are 94 million chronic HBV carriers in China. How to provide effective and affordable prophylactic options to prevent the occurrence of liver cancer in rural HBV-infected population is a great challenge.

Interestingly, the incidence of thyroid cancer and cervical cancer increased dramatically in both areas. For thyroid cancer, over-diagnosis due to widespread use of screening technologies including ultrasound can explain the dramatic increase (34, 35). The incidence of cervical cancer increased by 9.8 and 15.5% annually in urban and rural areas, respectively. High-risk human papilloma virus (HR-HPV) infection due to multiple sexual partners has been positively associated with the occurrence of cervical cancer (36–38). China has experienced an epidemic of sexual transmitted diseases since reform and opening up in the late 1970s, resulting in an increase in HR-HPV infection. The prevalence of HR-HPV was higher in rural (18.0%) than in urban areas (15.2%), and more rural women had multiple sexual partners (22%) than urban women (16%) (37). Furthermore, ages at the first intercourse and first birth of rural women were 3–4 years earlier than did urban women (36). The Chinese government has provided free cervical cancer screening for 10 million rural women per year since 2009, and HPV vaccine that can reduce the risk of cervical cancer has been available in some big cities in China (37). The increased occurrence of thyroid cancer reflect the profit' seeking medical behaviors-caused over-diagnosis. The increased occurrence of cervical cancer reflect the need of public health education in rural populations.

The socioeconomic factors have important impacts on the urban-rural disparities in cancer survival. Tertiary hospitals, which have ability to provide early detection and effective treatment for cancer, are mostly located in urban areas. In rural areas, poor cancer care and limited access to tertiary hospitals reduces the opportunity, hence the patients are often diagnosed at late stages and receive ineffective treatments in township health centers (4). The urban-rural economic disparity is obvious in China. For example, the annual per capita disposable income of residents was $4,739 and $1,600 in urban and rural households in 2014. The high out-of-pocket expenditure of anticancer drugs may discourage treatments for cancer patients in rural areas. For instance, the average annual medical cost for patients with lung cancer was $11,566 during 2002-2006, which exceeded the financial ability to pay in rural households (39). In order to ensure equal access to the high-quality health care system for all residents, the Chinese government implemented the Urban Employee Basic Medical Insurance (UEBMI) in 1998 and the Urban Resident Basic Medical Insurance (URBMI) in 2007 among urban residents, and the New Rural Cooperative Medical Scheme (NRCMS) in 2003 among rural residents (40). The coverage rates of three main health care systems (UEBMI, URBMI, and NRCMS) have increased to 89% among urban residents and 97% among rural residents by 2012. However, the reimbursement ratios of hospitalized patients covered by the UEBMI (74.64%) and URBMI (59.23%) were significantly higher than patients under the NRCMS (48.04%) (41). More investments are needed to reduce the disparity in health care and develop an equal medical insurance system for urban and rural residents.

Our study have several limitations. First, difference in exposure to risk factors including socioeconomic state, smoking,

alcohol use, dieting habit, and chronic infection with HBV, HCV, and HPV between the urban and rural populations are important to characterize the controllable causes of cancer types. However, these data were unavailable. Second, our study spanned a relative short time period of 14 years (2002-2015), and further researches are needed to assess the long-term disparities in cancer burden between urban and rural areas.

In conclusion, female breast cancer and CRC occurred more frequently in urban than in rural areas, quite in contrast to liver cancer and cervical cancer. Cancers of lung and bronchus, liver, stomach, and colon and rectum were the leading causes of cancer death in both areas. Age-standardized incidence of female breast cancer and CRC in urban areas increased while gastric cancer and liver cancer in both areas decreased. Age-standardized mortalities of cancers of the breast, esophageal, gastric, colon and rectum, liver, and lung and bronchus decreased in both areas. The 5-year survivals of patients with major cancers were higher in urban than in rural areas. Thus, factors promoting female breast cancer and CRC in urban areas and liver cancer and cervical cancer in rural areas should be specifically intervened in cancer prophylaxis. Our results may provide evidence to optimize cancer control strategy.

AUTHOR CONTRIBUTIONS

XL and YD drafted the manuscript, and participated in the collection, analysis and interpretation of data. WT, QS, YC, CY, BY, YW, JW, SW, FY, YD, and GZ contributed to data collection, suggestion for analysis. GC conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

FUNDING

This work was supported by the National Key Basic Research Program of China (973 Program) (2015CB554000), the National Natural Science Foundation of China (81520108021, 91529305, 81673250), the Natural Science Foundation of Shandong Province (ZR2017PH012), the General Project of Scientific Research of Shanghai Municipal Commission of Health and Family Planning (201540407), the Young Medical Talents Training Program of the Pudong Health Bureau of Shanghai (PWRq2017-33), and the Fund of Key Discipline Construction in Pudong New Area Health System (PWZxk2017-28).

ACKNOWLEDGMENTS

We thank all the colleagues who keep maintaining Pudong cancer register system for their great work in data collection and assuring data of high quality.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2018.00579/full#supplementary-material

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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ORIGINAL RESEARCH
published: 12 December 2018
doi: 10.3389/fonc.2018.00607

Waist Circumference Might Be a Predictor of Primary Liver Cancer: A Population-Based Cohort Study

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Background: Waist circumference, as an indicator of central adiposity, has been identified as an important predictor of several specific cancers such as colorectal cancer and gastroesophageal cancer risk, however, a consensus regarding the association between waist circumference and primary liver cancer (PLC) risk has not been reached.

Methods: A total of 104,825 males participating in the health checkup were included in the Kailuan male cohort study (2006–2015). Information on demographic and socioeconomic characteristics, lifestyle, medical records, and anthropometric measures were collected. Restricted cubic spline (RCS) and Cox proportional hazards regression models were used to estimate the hazard ratio (HR) and 95% confidence interval (CI) of association between waist circumference and the risk of PLC in males.

Results: During a median of 8.9 years of follow-up, 346 PLC cases were newly diagnosed in the cohort. The RCS model showed a U-shaped association between waist circumference and PLC risk (*P-overall* = 0.019, *P-non-linear* = 0.017). Overally, males with both high waist circumference ($HR_{Q5vs.Q3}$ = 1.98, 95%CI: 1.39–2.82) and low waist circumference ($HR_{Q1vs.Q3}$ = 1.52, 95%CI: 1.02–2.27) had an increased risk of PLC. Especially, the U-shaped association between waist circumference and PLC risk tended to be strengthened among subjects with hepatitis B surface antigen (HBsAg) negativity ($HR_{Q5vs.Q3}$ = 2.39, 95%*CI*: 1.43–3.98; $HR_{Q1vs.Q3}$ = 2.27, 95%*CI* = 1.29–4.01).

Conclusions: Waist circumference might be an independent predictor of PLC risk in males, especially for subjects with HBsAg negativity. Controlling waist circumference in an appropriate range might be an effective primary prevention to decrease PLC risk.

Keywords: waist circumference, primary liver cancer, cohort studies, Chinese males, restricted cubic spline

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Specialty section:
This article was submitted to Cancer Epidemiology and Prevention, a section of the journal Frontiers in Oncology

Received: *05 September 2018*
Accepted: *28 November 2018*
Published: *12 December 2018*

Citation:
Wei L, Li N, Wang G, Feng X, Lyu Z, Li X, Wen Y, Chen Y, Chen H, Chen S, Wu S, Dai M and He J (2018) Waist Circumference Might Be a Predictor of Primary Liver Cancer: A Population-Based Cohort Study. Front. Oncol. 8:607. doi: 10.3389/fonc.2018.00607

INTRODUCTION

Primary liver cancer (PLC) is one of the most common cancers. According to the estimation of GLOBOCAN 2012 by the International Agency for Research on Cancer (IARC), approximately 83% of all liver cancer occurred in less developed regions, with China accounting for over 50% of the world's burden (1).

It has been established that chronic infection with hepatitis B virus (HBV), causing chronic hepatic inflammation that may lead to fibrosis and cirrhosis, is the leading cause of PLC (2). With the successful introduction of hepatitis B vaccine into the national immunization program in China, the prevalence of hepatitis B surface antigen (HBsAg) among children under 5 years of age has dramatically declined from 9.67% in 1992 to 0.96% in 2006 (3). Hence, HBV, the dominant risk factor of PLC, is unlikely to be the main risk factor of PLC in the future. Thus, it is necessary to explore other important and potentially modifiable risk factors.

Several meta-analyses based on prospective cohort studies have identified increasing body mass index (BMI), the indicator of general adiposity which is often assumed to represent the degree of body fat, was related to higher risks of PLC (4, 5). However, abdominal fat may vary distinctly within a narrow range of BMI (6). In addition, current evidence suggests that visceral adipose is primarily found in the abdominal cavity, which had been confirmed more metabolically active than subcutaneous adipocytes (7–9). Previous study have suggested that waist circumference was a better predictor of abdominal fat compared with BMI in males (10). Hence, waist circumference, as the index considering both the amount and distribution of adipose, could be an appropriate measurement of abdominal obesity compared with BMI (11).

The recent study reported that the abdominal obesity (waist circumference $\geq$90 cm for male and $\geq$80 cm for female) prevalence was approximately quadrupled from 9.53% in 1993 to 36.7% in 2011 among Chinese males (12). Although waist circumference has been identified as an important predictor of several specific cancers such as colorectal cancer (13) and gastroesophageal cancer risk (14) in general, the association between high waist circumference and PLC risk in males has not reached a consensus (15–19). In addition, the effect of low waist circumference has rarely been investigated, leaving evidences to be further strengthened. Therefore, we conducted a large prospective cohort study based on the Kailuan Group to investigate the association between waist circumference and risk of PLC incidence in Chinese males, which might be helpful for identifying a potentially preventable risk factor of PLC.

METHODS

Study Design and Population

The Kailuan male study, a large and dynamic prospective cohort study, was initiated in May 2006 and based on Kailuan Group in Tangshan city, Hebei province, northern China. The Kailuan Group is a functional community managing coal industry, machine manufacturing, coking, chemical engineering, transportation, new building materials, and health care institutions (including 11 affiliated hospitals) (20).

Participants were enrolled in the present study if they met the following criteria: (1) males with age>18, (2) providing informed consent, (3) completing the questionnaire interview. Participants without baseline waist circumference (n = 3,786), with waist circumference lower than 1st percentile (<68 cm, n = 991), and with waist circumference higher than 99th percentile (>112 cm, n = 1,010) were excluded. Ultimately, a total of 104,825 male subjects were enrolled in the present study. This study was carried out in accordance with the recommendations of the ethical review committee of the Kailuan General Hospital. All subjects gave written informed consent in accordance with the Declaration of Helsinki.

Exposure Assessment

Standardized questionnaire and health examination for all participants were conducted by trained doctors and nurses at baseline entry. Information on demographic and socioeconomic characteristics, lifestyle, medical records, and anthropometric measures were collected. Smoking was defined as someone has tobacco smoking at least one cigarette per week for more than 6 consecutive months and was categorize as "non-smoker," "ex-smoker," or "current smoker" according to questionnaire information. Alcohol drinking was defined as drinking at least once per month for more than 6 consecutive months and was classified into "non-drinker," "ex-drinker," "<1 time per day" or "$\geq$1 time per day" using self-reported information. The subjects' weights and heights were measured using standardized stadiometers and scales while wearing light clothes, and the BMI was calculated based on the formula that BMI = weight (kg)/height^2 (m^2). Waist circumference was measured at the midpoint between the lower border of the rib and the supra margin of iliac crest plane. Diabetes history was categorized as "yes" or "no" on the basis of fasting blood glucose (FBG) level according to diabetes diagnostic criteria recommended by International Diabetes Federation (FBG $\geq$ 7.0 mmol/L) (21) and history for antidiabetic medication use. Measurement of FBG was performed using the Hexokinase method (BioSino Bio-Technology & Science Inc., China.). The HBsAg was detected quantitatively by the enzyme-linked immunosorbent assay for HBsAg (SHANGHAI KEHUA BIO-ENGINEERING, KHB, Shanghai, China) with standard operating procedure.

Outcome Assessment

The follow-up of each participant terminated at diagnosis of cancer, death, or administrative censoring (December 31, 2015), whichever occurred first. During the study period, new cases were obtained through self-report when they took part in routine questionnaires and health examinations every 2 years until 31 December 2015. In addition, incident PLC cases were checked yearly by the diagnosis and medical records linkage with the Tangshan medical insurance system and Kailuan social security system. Moreover, discharge lists from the 11 affiliated hospitals and death certificates from state vital statistics offices were also tracked yearly to ascertain the outcome information (22).

The diagnosis of incident PLC cases was confirmed by medical records review by clinical experts. Information on pathological diagnosis, imaging diagnoses (including ultrasonography, computerized tomography, and magnetic resonance imaging), blood biochemical and alpha fetoprotein test was collected to

Abbreviations: PLC, primary liver cancer; RCS, restricted cubic spline; HR, hazard ratio; CI, confidence interval; IARC, International Agency for Research on Cancer; HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; BMI, body mass index; HCV, hepatitis C virus; IGF, insulin-like growth factor.

assess the incident PLC cases (22). All PLC events were coded as C22 according to the International Classification of Diseases, Tenth Revision (ICD−10). Other details relating to Kailuan Cohort has been described previously (22–24).

Statistical Analyses

Subjects were grouped into quintiles according to the baseline waist circumference (<80.0, 80.0–84.9, 85.0–89.9, 90.0–94.9, or ≥95.0 cm), and the third quintile of waist circumference (85.0–89.9 cm) served as the reference. Proportions and chi-square tests were used to describe the categorical variables. A restricted cubic spline (RCS) analysis was conducted to explore the potential non-linear relationship between continuous waist circumference and the risk of PLC in the study (25).

Furthermore, Cox's proportional hazards regression models were constructed to estimate the hazard ratio (HR) and 95% confidence interval (CI) of PLC risk according to waist circumference quintiles. In model 1, only waist circumference was included in this univariate model. In model 2, age (continuous) was added as the underlying time metric. In model 3, multiple factors including education level (illiterate/primary school, junior high school, senior high school, or college and above), dust exposure (no or yes), frequency of alcohol drinking (non-drinker, ex-drinker, <1 time per day, or ≥1 time per day), and smoking status (non-smoker, ex-smoker, or current smoker) were further adjusted. In model 4, disease history including diabetes (yes or no), and HBV infection status (HBsAg negative or positive) served as additional adjustments. In model 5, the main model, BMI (continuous) was added in this multivariate model for exploring whether waist circumference is independent of BMI for PLC prediction.

Subgroup analyses were performed by alcohol drinking status (non-drinker vs. drinker), smoking status (non-smoker vs. smoker), and HBsAg status (negative vs. positive). And the multiplicative models were applied to test for the interaction between waist circumference and these variables.

Sensitivity analyses were conducted to examine the consistency of our findings. Firstly, the PLC cases occurred in the initial 3 years of follow-up were excluded from the analyses to evaluate whether potential preexisting disease influenced the association between waist circumference and PLC risk. Secondly, main models were repeated with exclusion of subjects with BMI < 18.5 kg/m^2 in consideration of the effect of preclinical cancers that may cause weight loss and waist circumference decrement and thus result in overestimation of the association between lower waist circumference and PLC risk.

The data management and all analyses were conducted using SAS 9.4 (SAS Institute Inc., Cary, NC, USA). All statistical test presented were two-side, and $P < 0.05$ was considered statistically significant.

RESULTS

Baseline Participant Characteristics

A total of 104,825 males were included in this study with a mean age of 51.4 years, for a total of 827,352.43 person-years. During a median follow-up time of 8.9 years, 346 members of the cohort were diagnosed with PLC. We compared the characteristics at baseline according to waist circumference quintiles of all subjects. As shown in **Table 1**, compared with subjects with low waist circumference, those with higher waist circumference were more likely to be older and have lower education level. Males in the higher waist circumference categories were more likely to be non-smokers and ex-drinkers. Negative HBsAg and diabetes were more common among males with higher waist circumference (**Table 1**).

The Association Between Waist Circumference and PLC Risk

The RCS model showed a significantly U-shaped association of waist circumference with the risk of PLC among the participants (*P-overall* = 0.019, *P-non-linear* = 0.017) (**Figure 1**). As the 40th quintile of waist circumference (85.0 cm) was chosen to be the reference, the HRs of PLC related to waist circumference rise obviously when waist circumference was over 95.0 cm or lower than 75.0 cm.

Furthermore, subjects were grouped into quintiles according to the baseline waist circumference, the crude PLC incidence rates for males according to waist circumference quintiles were $44.93/10^5$, $35.26/10^5$, $32.25/10^5$, $35.94/10^5$, and $59.75/10^5$, respectively. Compared with the third quintile waist circumference (85.0–89.9 cm), the HRs were 1.98 (95% CI: 1.39–2.82) for highest quintile waist circumference (≥95.0 cm) and 1.52 (95% CI: 1.02–2.27) for lowest quintile waist circumference (<80.0 cm), respectively, after adjusting for age, education, dust exposure, status of tobacco smoking and alcohol drinking, diabetes history, HBsAg status and BMI (**Table 2**).

Population attributable fractions (PAFs) for categorical exposure variables were calculated to reveal the common risk factors' contribution to PLC incidence. As shown in the **Supplementary Table S1**, in addition to HBsAg status (45.64%), the waist circumference (23.01%) also account the main attributable proportions of PLC incidence (**Supplementary Table S1** in Supplementary Material).

Subgroup Analyses Between the Waist Circumference and PLC Risk

Subgroup analyses showed that the statistically significant U-shaped association between waist circumference and PLC risk tended to be strengthened among subjects with hepatitis B surface antigen (HBsAg) negativity ($HR_{Q5vs.Q3}$ =2.39, 95%*CI*: 1.43–3.98; $HR_{Q1vs.Q3}$ = 2.27, 95%*CI* = 1.29–4.01). In addition, high waist circumference (≥95.0 cm) among non-drinkers (HR = 2.14, 95%CI = 1.34–3.41) and non-smokers (HR = 1.80, 95%CI = 1.13–2.86) also indicated a positive association with PLC risk in present study. Interaction analyses were conducted between the waist circumference and these confounders. However, there was no evidence of interaction effect (all $P >$ 0.05) between waist circumference and alcohol drinking, tobacco smoking and HBsAg status (**Table 3**).

Sensitivity Analysis

As shown in **Table 4**, after excluding PLC cases (case No.= 127) having occurred during the first 3 years of follow-up,

TABLE 1 | Baseline characteristics of males stratified by waist circumference, Kailuan male cohort, 2006–2015.

Characteristics	Total No. (%)	Waist circumference (cm)					χ^2	*P*-value
		<80.0	80.0–84.9	85.0–89.9	90.0–94.9	≥95.0		
No. study participants	104,825 (100.00)	16,269 (15.52)	20,898 (19.94)	23,452 (22.37)	20,061 (19.14)	24,145 (23.03)		
Age (year)							2,230.86	<0.001
<40	18,849 (17.98)	4,711 (28.96)	4,132 (19.77)	4,138 (17.64)	2,915 (14.53)	2,953 (12.23)		
40∼	23,099 (22.04)	3,353 (20.61)	4,879 (23.35)	5,578 (23.78)	4,453 (22.2)	4,836 (20.03)		
50∼	36,792 (35.10)	4,868 (29.92)	7,315 (35.00)	8,363 (35.66)	7,447 (37.12)	8,799 (36.44)		
60∼	26,085 (24.88)	3,337 (20.51)	4,572 (21.88)	5,373 (22.91)	5,246 (26.15)	7,557 (31.30)		
Education							256.86	<0.001
Illiteracy/primary school	12,011 (11.51)	1,833 (10.64)	2,216 (11.30)	2,498 (10.69)	2,358 (11.83)	3,106 (12.96)		
Junior high school	69,474 (66.60)	9,913 (67.92)	14,143 (61.13)	16,000 (68.45)	13,307 (66.75)	16,111 (67.21)		
Senior high school	14,388 (13.79)	2,871 (13.45)	2,800 (17.70)	3,110 (13.31)	2,660 (13.34)	2,947 (12.29)		
College or above	8,447 (8.10)	1,599 (7.99)	1,664 (9.86)	1,766 (7.56)	1,612 (8.09)	1,806 (7.53)		
Dust exposure								
No	41959 (40.25)	6045 (37.32)	8442 (40.59)	9468 (40.53)	7797 (39.13)	10207 (42.62)	66.69	<0.001
Yes	62275 (59.75)	10153 (62.68)	12356 (59.41)	13892 (59.47)	12130 (60.87)	13744 (57.38)		
Drinking							191.47	<0.001
Non-drinker	52,360 (50.05)	7,914 (48.70)	10,439 (50.02)	11,953 (51.06)	9,585 (47.90)	12,469 (51.77)		
Ex-drinker	4,528 (4.33)	567 (3.49)	780 (3.74)	926 (3.96)	984 (4.92)	1,271 (5.28)		
<1 time per day	25,873 (24.73)	4,532 (27.89)	5,154 (24.7)	5,669 (24.22)	5,084 (25.41)	5,434 (22.56)		
≥1 time per day	21,862 (20.90)	3,236 (19.92)	4,495 (21.54)	4,862 (20.77)	4,357 (21.77)	4,912 (20.39)		
Smoking							314.72	<0.001
Non-smoker	54,665 (56.59)	8,037 (56.29)	10,941 (52.79)	12,599 (57.86)	10,292 (56.05)	12,796 (58.69)		
Ex-smoker	3,995 (4.14)	505 (3.19)	621 (3.32)	856 (3.93)	847 (4.61)	1,166 (5.35)		
Current smoker	37,943 (39.28)	6,682 (40.52)	7,876 (43.89)	8,321 (38.21)	7,223 (39.34)	7,841 (35.96)		
BMI(kg/m^2)							26,171.84	<0.001
<18.5	2,282 (2.18)	1,265 (7.78)	442 (2.12)	263 (1.12)	133 (0.66)	179 (0.74)		
18.5∼	61,675 (58.87)	14,094 (86.66)	16,796 (80.42)	14,997 (63.99)	9,153 (45.66)	6,635 (27.50)		
25.0∼	36,399 (34.75)	850 (5.23)	3,546 (16.98)	7,885 (33.64)	10,281 (51.29)	13,837 (57.35)		
30.0∼	4,404 (4.20)	55 (0.34)	102 (0.49)	293 (1.25)	477 (2.38)	3,477 (14.41)		
HBsAg status							20.59	<0.001
Negative	96,750 (96.65)	15,046 (96.31)	19,234 (96.29)	21,760 (96.76)	18,456 (96.8)	22,254 (96.98)		
Positive	3,353 (3.35)	577 (3.69)	742 (3.71)	729 (3.24)	611 (3.2)	694 (3.02)		
Diabetes history							1212.49	<0.001
No	92052 (90.37)	15241 (95.13)	19026 (92.85)	20835 (90.96)	17153 (88.74)	19797 (85.64)		
Yes	3319 (9.63)	780 (4.87)	1466 (7.15)	2071 (9.04)	2176 (11.26)	3319 (14.36)		

BMI, body mass index; HBsAg, hepatitis B surface antigen.

there was still a positive association of the risk of PLC related to high waist circumference (HR = 1.78, 95% CI: 1.18–2.69). When excluding individuals without or with BMI <18.5 kg/m^2 (n = 2,347, case No.= 11), the results did not change substantially ($HR_{Q5vs.Q3}$ = 1.90, 95%CI: 1.32–2.72; $HR_{Q1vs.Q3}$ = 1.51, 95%CI = 1.00–2.28).

DISCUSSION

In this large prospective cohort study among Chinese males, we found a significant U-shaped association between waist circumference and PLC risk. The association was robust even after including BMI in the statistical models, supporting the hypothesis that waist circumference is an independent predictor for PLC. In addition, the subgroup analyses showed that the association between waist circumference and risk of PLC differed across categories of alcohol drinking, tobacco smoking, and status of HBV infection, as there were discrepancies among subgroups. To our knowledge, this is the first prospective cohort study to report on the association of both high and low waist circumference with PLC risk in mainland Chinese males which could be a strong evidence suggesting waist circumference is an independent predictor of PLC.

Waist circumference was one of the earliest means of quantifying body fat distribution, as an approximation of central adiposity (26). Results from several prospective cohort studies have examined the association between high waist circumference and risk of PLC (15–19). European Prospective Investigation into Cancer and Nutrition study identified 177 liver cancer cases and

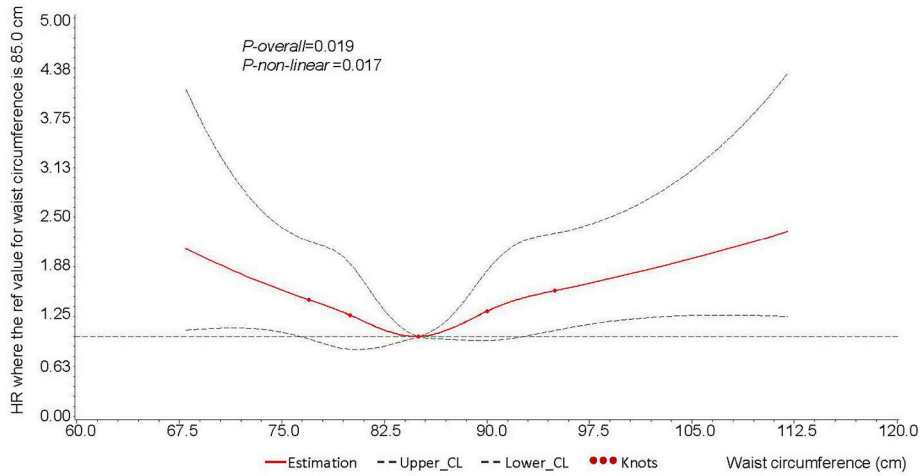

FIGURE 1 | Cubic spline graph of the adjusted HR (represented by solid line) and 95%*CI* (represented by the dotted lines) for the association between waist circumference and risk of male liver cancer in Kailuan male cohort, 2006–2015.
Knots: 77.0, 80.0, 85.0, 90.0, 95.0 of the distribution of waist circumference (cm).
Referent: 85.0 cm, 40th of the distribution of waist circumference.
Adjusted for age (continuous), education level (illiteracy/primary school, junior high school, senior high school, or college and above), dust exposure (no or yes), smoking (non-smoker, ex-smoker, or current smoker), drinking (non-drinker, ex-drinker, <1 time per day, or ≥1 time per day), diabetes (no or yes), HBsAg (negative or positive), and BMI (continuous). HR, hazard ratio; HBsAg, hepatitis B surface antigen; BMI, body mass index.

TABLE 2 | The association between waist circumference and primary liver cancer in males, Kailuan male cohort, 2006–2015.

Model	Waist circumference(cm)				
	<80.0	**80.0–84.9**	**85.0–89.9**	**90.0–94.9**	**≥95.0**
Person-years (Case No.)	129,096.88 (58)	164,484.92 (58)	186,046.82 (60)	158,615.61 (57)	189,108.2 (113)
Incidence($1/10^5$)	44.93	35.26	32.25	35.94	59.75
Model 1 [HR (95% CI)]	1.39 (0.97–2.00)	1.10 (0.76–1.57)	Ref	1.12 (0.78–1.60)	1.86 (1.36–2.54)
Model 2 [HR (95% CI)]	1.50 (1.04–2.15)	1.12 (0.78–1.60)	Ref	1.07 (0.74–1.54)	1.69 (1.24–2.32)
Model 3 [HR (95% CI)]	1.60 (1.10–2.33)	1.23 (0.84–1.78)	Ref	1.12 (0.77–1.65)	1.90 (1.37–2.65)
Model 4 [HR (95% CI)]	1.54 (1.05–2.27)	1.11 (0.75–1.63)	Ref	1.16 (0.79–1.71)	1.96 (1.40–2.74)
Model 5 [HR (95% CI)]	1.52 (1.02–2.27)	1.10 (0.75–1.62)	Ref	1.16 (0.79–1.72)	1.98 (1.39–2.82)

HR, hazard ratio; CI, confidence intervals; HBsAg, hepatitis B surface antigen; BMI, body mass index.
Model 1: Univariate model including waist circumference (<80.0, 80.0–84.9, 85.0–89.9, 90.0–94.9, or ≥95.0 cm).
Model 2: Model 1+age (continuous).
Model 3: Model 2+education level (illiteracy/primary school, junior high school, senior high school, or college and above)+ dust exposure (no or yes)+smoking(non-smoker, ex-smoker, or current smoker)+alcohol drinking(non-drinker, ex-drinker, <1 time per day, or ≥ 1 time per day).
Model 4: Model 3+ diabetes (no or yes) + HBsAg (negative or positive).
Model 5: Model 4+BMI (continuous).

reported that high waist circumference was related to higher risk of liver cancer (highest tertile VS. lowest tertile, HR = 2.60, 95% CI: 1.66–4.07) (17). Similarly, the Liver Cancer Pooling Project also found waist circumference to be an independent risk factor for liver cancer risk in males (waist circumference ≥110 cm VS. <90 cm, HR = 1.88, 95% CI: 1.42–2.49) (15). A study from Taiwan reported that the association between central obesity (waist circumference >90 cm for men and <80 cm for women) and PLC was only restricted in subjects with HBsAg negative and antibody to hepatitis C virus (HCV) positive (HR = 2.16, 95% CI: 1.19–3.92) (18). Our results on waist circumference were in line with the previous findings, whereby we consistently observed a significant association between high waist circumference and high liver cancer risk, even after further adjustment for BMI.

However, few studies have explored the association between low waist circumference and risk of PLC. Our study added a new perspective that the statistically U-shaped association between waist circumference and PLC risk, in which that low waist circumference might also play a potential role in PLC incidence. The RCS model showed that PLC risk increased obviously when waist circumference was lower than 75.0 cm. In addition, the present study also found a significant relationship when the first quintile (<80.0 cm) compared to third quintile (85.0–89.9 cm) in subjects with HBsAg negativity, which support an association between low waist circumference and PLC risk. Perhaps males with low waist circumference were prone to accompany with preclinical disease that can cause weight loss and also

TABLE 3 | Stratified analysis of the association between waist circumference and risk of primary liver cancer in Kailuan male cohort, 2006–2015.

	Waist circumference (cm)					$P_{interaction}$
	<80.0	80.0–84.9	85.0–89.9	90.0–94.9	≥95.0	
Drinking						0.195
Non-drinker						
Person-years (Case No.)	61,374.38 (31)	80,863.64 (23)	93,712.25 (31)	74,794.84 (26)	96,473.2 (66)	
HR (95% CI)[a]	1.68 (1.00–2.82)	0.77 (0.43–1.37)	Ref	1.00 (0.57–1.75)	2.05 (1.31–3.22)	
HR (95% CI)[b]	1.59 (0.93–2.74)	0.75 (0.42–1.35)	Ref	1.02 (0.58–1.78)	2.14 (1.34–3.41)	
Drinker[c]						
Person-years (Case No.)	67,612.11 (27)	83,435.57 (35)	92,094.97 (28)	83,510.19 (30)	92,265.82 (46)	
HR (95% CI)[a]	1.47 (0.83–2.59)	1.48 (0.87–2.52)	Ref	1.34 (0.78–2.33)	1.83 (1.10–3.04)	
HR (95% CI)[b]	1.56 (0.86–2.83)	1.52 (0.89–2.60)	Ref	1.30 (0.75–2.27)	1.70 (0.98–2.95)	
Smoking						0.809
Non-smoker						
Person-years (Case No.)	63,241.66 (28)	85,686.21 (27)	99,570.37 (32)	81,159.41 (27)	100,186.22 (56)	
HR (95% CI)[a]	1.49 (0.89–2.52)	0.89 (0.52–1.54)	Ref	1.09 (0.64–1.84)	1.71 (1.09–2.68)	
HR (95% CI)[b]	1.40 (0.82–2.41)	0.87 (0.50–1.50)	Ref	1.11 (0.65–1.88)	1.80 (1.13–2.86)	
Smoker[d]						
Person-years (Case No.)	57,893.73 (28)	67,854.07 (30)	73,581.92 (22)	64,563.19 (25)	70,931.96 (52)	
HR (95% CI)[a]	1.67 (0.94–2.95)	1.38 (0.79–2.43)	Ref	1.27 (0.71–2.27)	2.24 (1.34–3.74)	
HR (95% CI)[b]	1.76 (0.97–3.21)	1.41 (0.80–2.49)	Ref	1.23 (0.69–2.22)	2.09 (1.20–3.64)	
HBsAg status						0.192
Negative						
Person-years (Case No.)	121,737.26 (33)	155,114.28 (23)	176,440.05 (25)	149,666.76 (34)	178,641.71 (65)	
HR (95% CI)[a]	2.18 (1.26–3.76)	1.10 (0.60–2.00)	Ref	1.61 (0.93–2.78)	2.49 (1.52–4.06)	
HR (95% CI)[b]	2.27 (1.29–4.01)	1.12 (0.61–2.04)	Ref	1.58 (0.91–2.74)	2.39 (1.43–3.98)	
Positive						
Person-years (Case No.)	4,653.08 (24)	5,921.55 (34)	5,900.87 (35)	4,904.05 (22)	5,420.1 (46)	
HR (95% CI)[a]	1.07 (0.62–1.86)	1.09 (0.65–1.81)	Ref	0.83 (0.47–1.46)	1.56 (0.97–2.50)	
HR (95% CI)[b]	0.99 (0.56–1.76)	1.05 (0.63–1.75)	Ref	0.85 (0.48–1.52)	1.68 (1.02–2.77)	

HR, hazard ratio; CI, confidence intervals; HBsAg, hepatitis B surface antigen; BMI, body mass index.

[a] *Adjust for age (continuous), education level (illiteracy/primary school, junior high school, senior high school, or college and above), dust exposure (no or yes), smoking (non-smoker, ex-smoker, or current smoker), alcohol drinking (non-drinker, ex-drinker, <1 time per day, or ≥1 time per day), diabetes (no or yes), HBsAg (negative or positive).*

[b] *Further adjust for BMI (continuous).*

[c] *Including ex-drinkers and current drinkers.*

[d] *Including ex-smokers and current smokers.*

increase risk of PLC, which may confuse the association. However, the association between low waist (<80.0 cm) circumference and PLC risk remained robust with exclusion of participants with BMI<18.5 kg/m^2 (HR = 1.51, 95% CI: 1.00–2.28). In addition, for subjects with HBsAg negativity, the association was stronger (HR = 2.29, 95% CI: 1.28–4.08, data was not shown) when excluded the underweight participants (BMI<18.5 kg/m^2). Therefore, the robust findings indicated that low waist circumference might be a risk factor of PLC.

The previous study suggested that waist circumference was a better predictor of abdominal adiposity in males when compared with body weight or BMI (11). In our study, waist circumference conveyed statistically significant association with PLC risk, even after adjusting BMI in the statistical models, supporting the hypothesis that waist circumference is an independent predictor for PLC. Subjects with high waist circumference tend to be diagnosed with PLC maybe due to the following possible mechanisms. Increased release from metabolically active abdominal fat of substantial adipokines, such as tumor-necrosis factor-α, free fatty acids, leptin and inflammatory markers, and reduced release of adiponectin, contribute to development of insulin resistance, compensatory and chronic hyperinsulinaemia (8, 9, 26–28). Increased insulin levels, in turn, lead to reduced insulin-like growth factor (IGF) binding protein 1 synthesis in liver and other tissues, additionally, generally accompany with reduced levels of IGF binding protein 2 in the blood. Both the reduced IGF binding protein 1 and IGF binding protein 2 give rise to facilitate the biological activity of IGF1. Ultimately, insulin and IGF1 signal through the insulin receptors and IGF1 receptor, respectively, to promote cellular proliferation, inhibit apoptosis, and then contribute to tumorigenesis (8, 17). The mechanisms for subjects with low waist circumference also

TABLE 4 | Sensitivity analysis of the association between waist circumference and primary liver cancer risk in Kailuan male cohort, 2006–2015.

	Waist circumference (cm)				
	<80.0	80.0–84.9	85.0–89.9	90.0–94.9	≥95.0
Exclude cases occurred in the first 3 years of follow-up					
Person-years (Case No.)	129,075.93 (39)	164,437.11 (30)	186,017.32 (45)	158,579.5 (32)	189,053.03 (73)
HR (95% CI)[a]	1.29 (0.82–2.03)	0.75 (0.46–1.21)	Ref	0.84 (0.52–1.34)	1.61 (1.09–2.38)
HR (95% CI)[b]	1.16 (0.72–1.85)	0.72 (0.44–1.16)	Ref	0.87 (0.54–1.41)	1.78 (1.18–2.69)
Exclude BMI<18.5 kg/m^2					
Person-years (Case No.)	119,619.69 (52)	161,043.56 (57)	183,970.46 (59)	157,445.81 (57)	187,580.26 (110)
HR (95% CI)[a]	1.49 (1.00–2.21)	1.12 (0.76–1.66)	Ref	1.17 (0.79–1.73)	1.93 (1.37–2.71)
HR (95% CI)[b]	1.51 (1.00–2.28)	1.13 (0.76–1.67)	Ref	1.17 (0.79–1.73)	1.90 (1.32–2.72)

BMI, body mass index; HR, hazard ratio; CI, confidence intervals; HBsAg, hepatitis B surface antigen.

[a] *Adjust for age (continuous), education level (illiteracy/primary school, junior high school, senior high school, or college and above), dust exposure (no or yes), smoking (non-smoker, ex-smoker, or current smoker), alcohol drinking (non-drinker, ex-drinker, <1 time per day, or ≥ 1 time per day), diabetes (no or yes), HBsAg (negative or positive).*

[b] *Further adjust for BMI (continuous).*

related to high PLC risk is still inconclusive, hence further research to better understand the underlying mechanisms are needed.

In the present study, the association between high waist circumference and risk of PLC differed by status of drinking and smoking. The association was statistically significant in non-drinkers or non-smokers but negative in drinkers or smokers. It is possible owing to the competing risks of tobacco smoking and alcohol drinking. Previous studies have suggested that alcohol drinking may increase 179% (*95%CI*: 2.00-3.87) risk of liver cancer incidence (29) via the induction of cytochrome P-450 2E1, which potentially leads to activation of procarcinogen (30) and inhibition of phase II enzymes (31), thus affecting the clearance of carcinogens (32). And tobacco smoking (HR = 1.51, 95% CI: 1.37–1.67) was also found to be an independent risk factor for liver cancer (33). Therefore, in the presence of a competing risk, the association may be attenuated among drinkers and smokers. However, for non-drinkers, or non-smokers, high waist circumference showed a significant effect on PLC development which could have key scientific and clinical importance for preventing PLC.

The prevalence of HBsAg was 3.30% in the present study, which was similar to the previous study on HBsAg prevalence (<4%) among northern Chinese population (34). As it was estimated that hepatitis B viral infections accounts for more than 60% of liver cancer cases in Asia area (2), our study also proved that HBsAg positivity increased 30.74 (*95%CI*: 24.51–38.55) fold higher risk of PLC when compared with HBsAg negative. Therefore, the effect of waist circumference may be weakened, which explain the finding that increased PLC risk related to higher and lower waist circumference was restricted in subjects with HBsAg negativity. Although the HBV infection currently plays a leading role in the development of PLC (35), it is unlikely to be the main risk factor in the future, as the prevalence of HBsAg among the children under 5 years of age decreasing from 9.67% in 1992 to 0.96% in 2006 (3) with the successful massive hepatitis B vaccination implementation. Whereas, the abdominal obesity prevalence was approximately quadrupled from 9.53% in 1993 to 36.7% in 2011 among Chinese males (13). Thus, the findings of abdominal obesity, related to PLC risk differently depending on HBsAg status may shed some light on preventing PLC in the condition of hepatitis B vaccination application.

One of the main strengths of our study is its prospective design and inclusion of a large population, which gave us high power to detect quite modest associations as well as minimize the potential bias caused by preclinical disease. Furthermore, in our study, anthropometric factors (e.g., waist circumference, weight, and height) at baseline were measured by trained personnel rather than relied on self-reported, which avoid misclassification in analyses. However, there are several limitations that should be discussed when interpreting the results. Firstly, the lack of information on HCV infection is a major limitation of the current study. However, the HCV prevalence rate was only 0.43% in Chinese general population according to a national survey carried in 2006 (36), which attribute little to PLC incidence in China. In subsequent questionnaire interview and health examination, we will complement the HCV infection information to provide more comprehensive results in the future. Secondly, the follow-up time (Median, 8.9 years) was relatively short, which precluded stratified analyses by subtypes of PLC, such as hepatocellular carcinoma and intrahepatic cholangiocarcinoma, owing to limited number of cases. In addition, the subjects focused on male employees from Kailuan Group, it may be difficult in extrapolating to females or general population. So for other population, more studies are warranted to confirm these findings.

In conclusion, our analyses provided convincing evidence that waist circumference might be one of the scientific and important predictor of PLC based on the large-scale prospective study. The findings indicated that both high and low waist circumference could increase the risk of PLC in males, especially for subjects with HBsAg negative. Therefore, controlling waist circumference in an appropriate range might be an effective primary prevention to decrease PLC risk.

DATA AVAILABILITY

The datasets for this manuscript are not publicly available because all our data are under regulation of both the National Cancer Center of China and Kailuan Group. Requests to access the datasets should be directed to Jie He, hejie@cicams.ac.cn and Shouling Wu, drwusl@163.com.

AUTHOR CONTRIBUTIONS

NL, MD, SW, and JH did the study concept and design. GW, XF, YC, HC, and SC carried out the acquisition and quality control of data. LW, ZL, XL, and YW performed statistical analysis, or interpretation of data. LW performed the writing and drafting of the manuscript. NL and MD did the critical revision of the manuscript for important intellectual content. All authors agreed to be accountable for the content of the work.

FUNDING

This study was funded by National Key R&D Program of China (No. 2016YFC1302500 and 2016YFC1302503), Training Programme Foundation for the Talents in Beijing City (No. 2017000021223TD05), National Natural Science Foundation of China (No. 81673265), State Key Projects Specialized on Infectious Diseases (No. 2017ZX10201201-008-002).

ACKNOWLEDGMENTS

The authors thank all participants, the principal investigators and their institutions for their contributions to this study.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2018.00607/full#supplementary-material

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Oncology

SYSTEMATIC REVIEW
published: 12 December 2018
doi: 10.3389/fonc.2018.00619

Prevalence of Human Papillomavirus Type-16 in Head and Neck Cancer Among the Chinese Population: A Meta-Analysis

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OPEN ACCESS

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Reviewed by:
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Specialty section:
This article was submitted to Cancer Epidemiology and Prevention, a section of the journal Frontiers in Oncology

Received: *12 September 2018*
Accepted: *29 November 2018*
Published: *12 December 2018*

Citation:
Guo L, Yang F, Yin Y, Liu S, Li P, Zhang X, Chen D, Liu Y, Wang J, Wang K, Zhu Y, Lv Q, Wang X and Sun X (2018) Prevalence of Human Papillomavirus Type-16 in Head and Neck Cancer Among the Chinese Population: A Meta-Analysis. Front. Oncol. 8:619. doi: 10.3389/fonc.2018.00619

Background: The burden of head and neck cancer in China is heavier, and studies have shown that it may be associated with HPV infection, especially high-risk HPV.

Objectives: We aimed to conduct a meta-analysis to estimate the high-risk HPV-16 prevalence of head and neck cancer in the Chinese population.

Methods: The reports on HPV and head and neck cancer in a Chinese population published between Jan 1, 2006 and Oct 23, 2018 were retrieved via WANFANG/CNKI/MEDLINE/EMBASE databases. The pooled prevalence and corresponding 95% confidence intervals was calculated by a random-effect model.

Results: The meta-analysis included a total of 2,896 head and neck cancer cases from 28 studies. Overall, the pooled HPV-16 prevalence among head and neck cancer cases was 24.7% (20.2–29.3%) in China, 31.6% (21.7–41.5%) in oropharyngeal cancer, 28.5% (18.2–38.7%) in laryngeal cancer and 14.9% (10.1–19.7%) in oral cancer, 25.3% (14.8–35.8%) in fresh or frozen biopsies and 25.0% (19.5–30.5%) in paraffin-embedded fixed biopsies, 36.5% (17.9–55.1%) by E6/E7 region and 14.3% (6.4–22.1%) by L1 region of HPV gene. The highest HPV-16 prevalence was found in Central China.

Conclusions: High prevalence of HPV-16 was found in the samples of Chinese head and neck cancers. Preventive HPV-vaccination may reduce the burden of HPV-related head and neck cancer in China.

Keywords: head and neck cancer, human papillomavirus, prevalence, meta-analysis, China

INTRODUCTION

As a member of the papillomavirus family of viruses, human papillomavirus (HPV) can infect humans by attacking the squamous cell of skin and mucous membranes, including those of the cervix, anogenital region and head and neck (1–3). As we all know, nasopharyngeal carcinoma is related to EB virus infection. Except that, more than 11% of the remaining squamous cell

carcinomas of head and neck are caused by high-risk human papillomavirus infection (4), and the incidence of such subtypes is increasing year by year (5). However, the frequency of HPV infection in head and neck cancers varies between 3 and 84% in different studies (6). Based on the different nucleotide sequences, HPV can be divided into more than 200 genotypes by DNA sequencing, of which HPV16 and HPV18 are more closely related to malignant tumors as the main high-risk types (7, 8). However, unlike cervical and oral carcinogenesis, the role of HPV-16/18 in the development of other head and neck cancers has not been clearly defined.

Even in the same country, the HPV prevalence in head and neck cancers range widely (9). Demographic and racial factors, sample condition, cancer location and the viral detection method have been proposed to identify possible causes of differences in the results. However, as one of the most common subtype of HR-HPV, the HPV-16 prevalence in China has not been estimated so far.

The aim of the meta-analysis is to estimate the prevalence of HPV-16 detected in head and neck cancer cases and the different cancer sites, influence of regions, specimen types, and detection methods in China from all published studies of English and Chinese language literature.

MATERIALS AND METHODS

Literature Search Strategies

This meta-analysis was reported following the guideline of Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (10). The key words "human papillomavirus," "papillomavirus infections," "HPV," "head and neck cancer," "laryngeal cancer," "oropharyngeal cancer," "oral cancer," "neck cancer," "head cancer," and corresponding carcinoma in English language or in Chinese language were used in combination to search. The retrieved databases included MEDLINE (via PubMed), Excerpta Medica database (EMBASE), Wanfang Data Knowledge Service Platform and Chinese National Knowledge Infrastructure (CNKI). Date of the literature was assigned between Jan 1, 2006 and Oct 23, 2018. Firstly, we excluded the duplicates. Secondly, we screened each title and abstract to evaluate its possible relevance. Thirdly, we downloaded full text for detailed evaluation if titles and abstracts weren't enough to make decision. All papers were independently reviewed by two authors (YFN and LSZ). Uncertainties and discrepancies were resolved by consensus after discussing with a senior researcher (SXB). If the data we needed were not explicitly reported or could not be derived from the papers, we also emailed authors to obtain related data. Additional studies were also identified using cross-referencing.

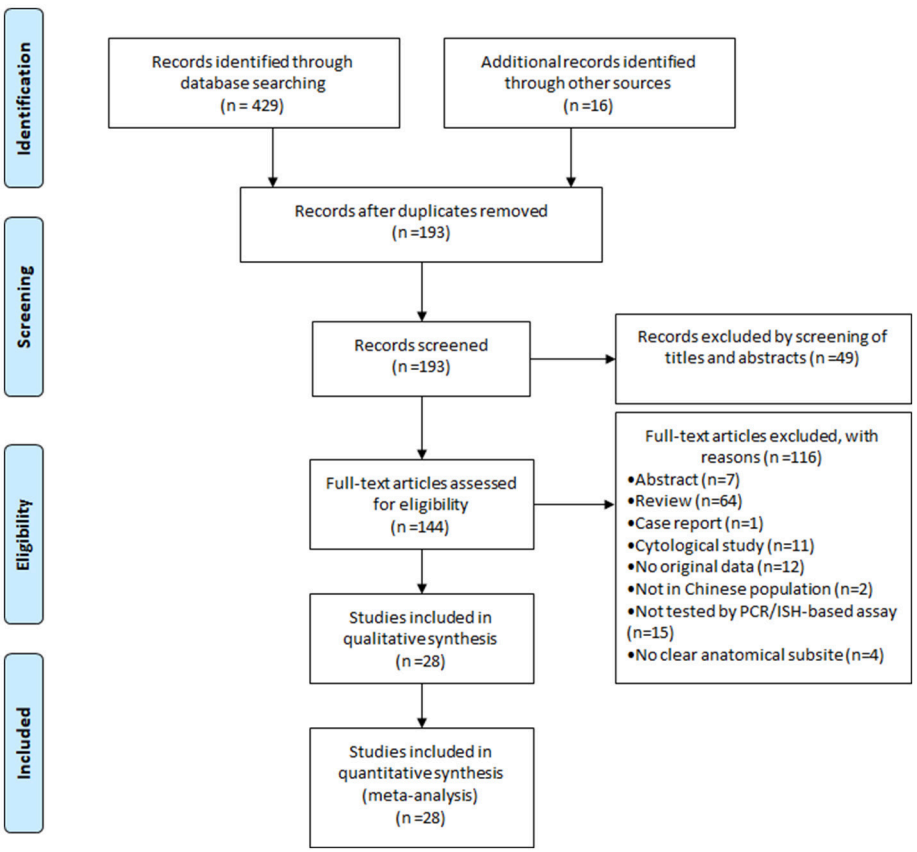

FIGURE 1 | Flow diagram of systematic literature search on HPV-16 infection in head and neck cancer.

Inclusion and Exclusion Criteria

According to the PRISMA statement, the literatures contained in this study must meets the following criteria: (a) to inform at least 10 cases of head and neck cancer confirmed by biopsy or histopathology, (b) to use polymerase chain reaction (PCR)-based methods (including type-specific PCR primers, broad-spectrum PCR primers, or a combination of both kinds of primers) or *in situ* hybridization (ISH) to amplify HPV DNA, (c) to report the prevalence of HPV-16 in head and neck cancer tissue samples with clear anatomical subsite.

The literatures excluded in this study were mainly due to the following reasons: were cellular or animal studies; were not conducted in Chinese; unable to extract or calculate the necessary data directly from the original article; without clear definition of anatomical subsite; reviews.

Data Extraction

All studies included in the final meta-analysis extracted the following data: first author's name, publication year, geographical areas, cancer site, clinical stage, numbers of cases and HPV positive cases, HPV test method, specimen types (formalin-fixed paraffin-embedded biopsies [FFPE], fresh, or frozen biopsies [FF]).

Statistical Analysis

Overall pooled point estimate and 95% confidence interval (95% *CI*) for HPV-16 prevalence were calculated through the method of DerSimonian and Laird (11) using the assumptions of a random-effects model. For a variety of HPV infections (including HPV-16), the multiple HPV types were classified into different types and the HPV-16 type-specific prevalence represents types for cases with either single HPV-16 infection and multiple HPV-16 infection.

Cochrane Q test ($P < 0.10$ indicated a high level of statistical heterogeneity) and I^2 (values of 25, 50, and 75% corresponding to low, moderate and high degrees of heterogeneity, respectively) was used to assess the heterogeneity between eligible studies (12). Subgroup analyses for HPV-16 prevalence were subsequently

TABLE 1 | Studies included in the meta-analysis and their characteristics.

References	Period of recruitment	Province	Region of China	Site	Stage	Method	Cases, *n*	HPV-16 + ve, *n* (%)	Detection method	Specimen
Wei and Qian (13)	NA	Guangxi	West	OC	NA	PCR	37	14 (37.8)	NA	FF
He (14)	NA	Hubei	Central	OC	NA	PCR	16	5 (31.3)	NA	FF
Peng et al. (15)	1998–2008	Chongqing	West	LC	I–III	PCR	123	67 (54.5)	E6/7	FFPE
Zhao et al. (16)	1999–2001	Hubei	Central	OC	I–IV	PCR	52	13 (25.0)	NA	FFPE
Liu et al. (17)	2000–2008	Beijing	East	LC	I–IV	PCR	84	23 (27.4)	L1	FFPE
Yao and Liu (18)	2005–2007	Shanxi	Central	LC	I–IV	PCR	30	22 (73.3)	E6	FFPE
Wang et al. (19)	2000–2008	Beijing	East	LC	I–IV	PCR	84	29 (34.5)	E6/7	FFPE
Cheng et al. (20)	2006–2009	Taiwan	East	OPC	III–IV	PCR	60	12 (20.0)	L1	FFPE
Huang et al. (21)	1999–2009	Beijing	East	OPC	I–IV	PCR	66	8 (12.1)	NA	FFPE
Lee et al. (22)	2004–2006	Taiwan	East	OC	III–IV	PCR	333	26 (7.8)	L1	FFPE
Lu et al. (23)	2011–2012	Shandong	East	LC	I–IV	PCR	57	2 (3.5)	NA	FF
Wu and Zhou (24)	2008–2011	Shanghai	East	LC	I–IV	PCR	46	2 (4.3)	E6/7	FFPE
Xue and Liu (25)	NA	Hubei	Central	OC	I–IV	PCR	30	8 (26.7)	NA	FFPE
Zhang et al. (26)	2004–2009	Beijing	East	OC, OPC, LC	NA	ISH	78	37 (47.4)	NA	FFPE
Gan et al. (27)	2009–2013	Hubei	Central	OC	I–IV	PCR	200	39 (19.5)	L1	FF
He et al. (28)	NA	Fujian	East	OC	I–IV	PCR	75	1 (1.3)	NA	FF
Wang et al. (29)	1999–2009	Guangdong	East	LC	I–II	PCR	163	3 (1.8)	L1	FFPE
Cui et al. (30)	2002–2011	Hunan	Central	OPC	I–IV	ISH	60	29 (48.3)	NA	FFPE
Guan et al. (31)	2009–2013	Shanghai	East	LC	NA	PCR	31	6 (19.4)	NA	FFPE
Chen et al. (32)	2012–2015	Fujian	East	OC	NA	PCR	178	6 (3.4)	NA	FFPE
Chor et al. (33)	2012–2014	Hongkong	East	OC, OPC, LC	NA	PCR	202	14 (6.9)	NA	FFPE
Fei et al. (34)	1995–2010	Yunnan	West	OPC	I–IV	PCR	60	20 (33.3)	E6	FFPE
Lam et al. (35)	2005–2009	Hongkong	East	OPC	I–IV	PCR	207	43 (20.8)	NA	FFPE
Lu et al. (36)	2010–2012	Guangdong	East	LC	I–IV	PCR	82	2 (2.4)	E2/6	FFPE
Ma et al. (37)	2012–2015	Sichuan	West	OC, OPC	I–IV	PCR	180	39 (21.7)	NA	FF
Wang et al. (38)	2014	Hainan	East	LC	NA	PCR	50	29 (58.0)	E6/7	FF
Zhang et al. (39)	2011–2016	Ningxia	West	LC	NA	PCR	101	9 (8.9)	NA	FFPE
Tong et al. (40)	NA	Heilongjiang	Northeast	LC	NA	PCR	211	132 (62.6)	NA	FFPE

OC, oral cancer; LC, laryngeal cancer; OPC, oropharyngeal cancer; HPV-16 + ve, human papillomavirus 16 positive; PCR, polymerase chain reaction; ISH, in situ hybridization; FFPE, formalin-fixed paraffin-embedded biopsies; FF, fresh or frozen biopsies; NA, not available.

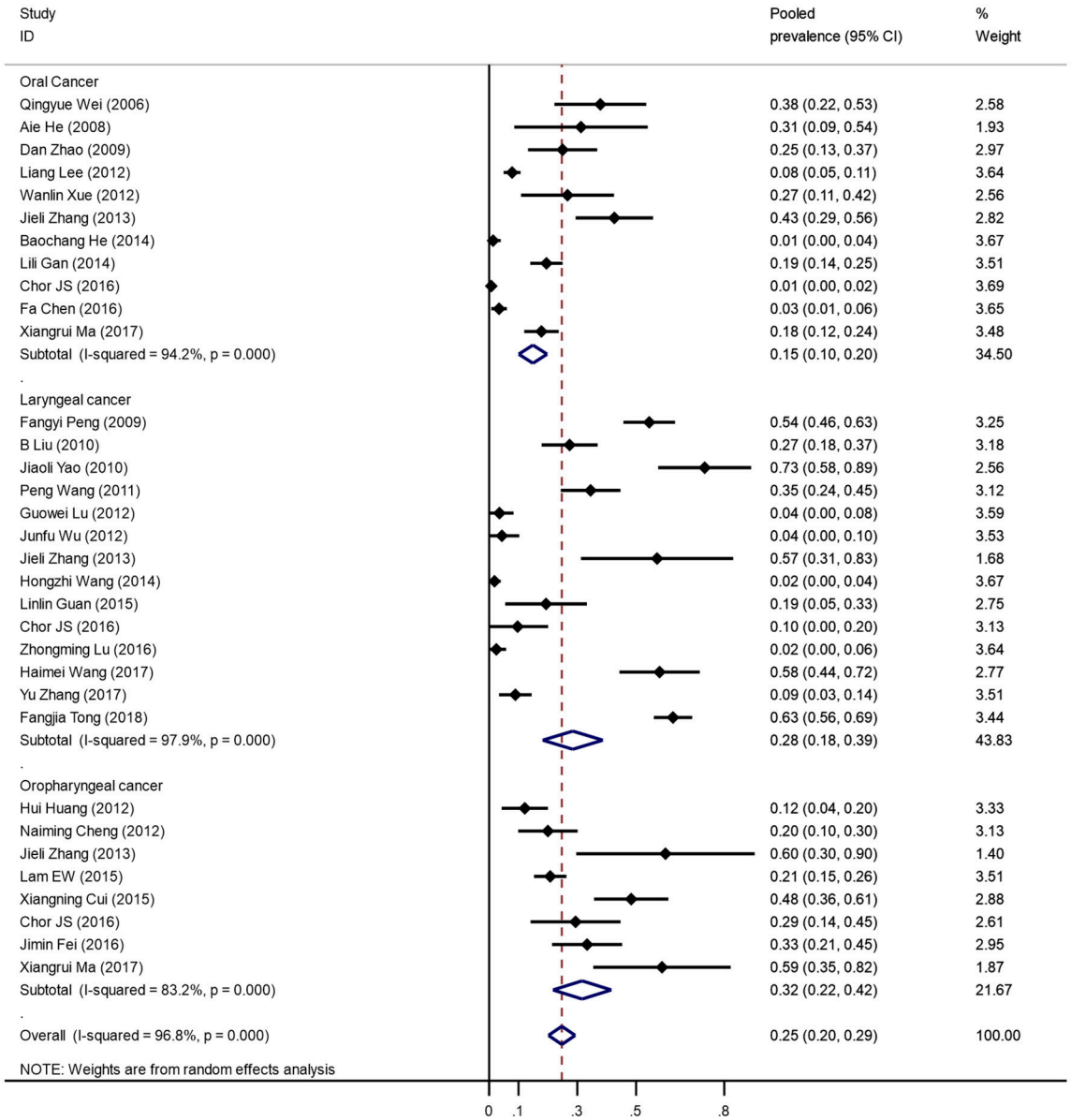

FIGURE 2 | Forest plots of meta-analysis on HPV-16 prevalence in head and neck cancer tissue.

carried out according to the geographical areas of the study origin, cancer site, year of publication, number of patients, HPV detection method and types of specimen. In the eligible studies, three studies, which contained different cancer sites, were treated as the separate studies. Meta-regression analyses were used to examine the association of the geographical areas of the study origin, cancer site, year of publication, number of patients, HPV detection method and types of specimen with the prevalence of HPV-16. Sensitivity analysis was also performed to assess the impact of each individual study on the strength and stability of the meta-analytic results. Each time, one study in the meta-analysis was excluded to show that study's impact on the overall impact size. Funnel plot and Begg adjusted rank correlation test for funnel plot asymmetry were performed to test any existing publication bias.

Statistical analysis was performed using STATA SE version 15.1 (StataCorp LP, College Station, TX, USA) for Windows. $P < 0.05$ with two-tailed was considered statistically significant.

RESULTS

Systematic Review and Study Characteristics

Figure 1 showed the flow diagram of systematic literature search. Generally speaking, the search strategy generated 445 citations, of which 193 were considered of potential value after screening

TABLE 2 | Results of subgroup analyses for HPV-16 prevalence in head and neck cancer lesion.

Variables	Studies, *n*	Cases, *n*	Prevalence (95% CI)	Heterogeneity test	
				p for *Q* test	I^2 (%)
Overall	33	2,896	24.7% (20.2–29.3%)	<0.001	96.8
Region					
East	20	1,796	14.5% (10.9–18.1%)	<0.001	93.6
Central	6	388	37.0% (21.0–52.9%)	<0.001	90.2
West	6	501	33.9% (17.8–50.0%)	<0.001	94.6
Site					
OC	11	1,275	14.9% (10.1–19.7%)	<0.001	94.2
OPC	8	514	31.6% (21.7–41.5%)	<0.001	83.2
LC	14	1,107	28.5% (18.2–38.7%)	<0.001	97.9
Year					
2005–2010	6	342	41.6% (26.7–56.4%)	<0.001	87.8
2011–2015	16	1,490	18.9% (13.7–24.1%)	<0.001	93.8
2016–2017	11	1,064	23.8% (14.9–32.8%)	<0.001	97.9
Number of patients					
<100	23	1,080	28.6% (21.9–35.3%)	<0.001	94.5
≥100	10	1,816	19.1% (11.5–26.8%)	<0.001	98.4
Detection method					
PCR	29	2,758	22.1% (17.6–26.7%)	0.012	96.8
ISH	4	138	47.8% (39.6–56.1%)	0.631	0.0
Specimen					
FF	8	615	25.3% (14.8–35.8%)	<0.001	95.6
FFPE	25	2,281	25.0% (19.5–30.5%)	<0.001	97.1
Detection gene					
L1	5	840	14.3% (6.4–22.1%)	<0.001	94.2
E6/E7	7	475	36.5% (17.9–55.1%)	<0.001	97.8

OC, oral cancer; LC, laryngeal cancer; OPC, oropharyngeal cancer; PCR, polymerase chain reaction; ISH, in situ hybridization; FFPE, formalin-fixed paraffin-embedded biopsies; FF, fresh or frozen biopsies; NA, not available.

of titles and abstracts and the full text was retrieved for detailed evaluation. 116 articles were subsequently excluded from the meta-analysis for various reasons, including 64 were reviews, 15 were not tested by PCR/ISH-based assay, 12 that did not provide *HR*s or *CI*s, 11 were cytological study, 7 were abstract, 4 had not clear anatomical subsite, 2 were not in Chinese population and 1 was case report. So, 28 studies (11 in English and 17 in Chinese) were eligible and included in this meta-analysis (13–40). Individual characteristics of the included 28 studies were summarized in **Table 1**. The size of the study samples ranged from 10 to 333 cases of head and neck cancer (median = 60). Summing up the studies, a total of 2,896 cases of head and neck cancer were identified. As shown in **Table 1**, more than half of the studies were conducted in Eastern China ($n = 16$, 57.14%), and the remaining studies distributed in three other regions of China as follows: 6 (21.43%) studies in Central China, 5 (17.86%) studies in Western China and 1 (3.57%) study in Northeastern China. 14 (50.00%) studies conducted in laryngeal cancer cases, 11 (39.29) conducted in oral cancer cases, and 8 (28.57%) conducted in oropharyngeal cancer cases. For HPV detection methods, 26 (92.86%) studies used PCR, and 2 (3.57%) studies used ISH. 21 (75.00%) studies used formalin-fixed paraffin-embedded biopsies (FFPE), and 7 (25.00%) studies used fresh or frozen biopsies. Besides, 7 (25.00%) studies used the gene detected from HPV E6/E7 region, and 5 (17.86%) used the gene detected from HPV L1 region.

Meta-Analysis of HPV-16 Prevalence in Head and Neck Cancer Cases

Figure 2 was the forest plot illustrated the individual and pooled prevalence estimates derived from a random effect model analysis. In this study, the prevalence of HPV-16 ranged from 1.3 to 58.0%. The pooled prevalence for HPV-16 was 24.7% (95% *CI*, 20.2–29.3%) in head and neck cancer cases in the Chinese population. Overall, high heterogeneity was observed in the studies included (*Q*-test $P_{\text{heterogeneity}} < 0.001$, $I^2 = 96.8\%$).

Subgroup Analysis and Meta-Regression

Table 2 presents detailed results of subgroup analyses. As shown in **Table 2**, the highest pooled HPV-16 prevalence was observed in Central China (37.0%; 95% *CI*, 21.0–52.9%), followed by that in Western China (33.9%; 95% *CI*, 17.8–50.0%), and in Eastern China (14.5%; 95% *CI*, 10.9–18.1%). Oropharyngeal cancer had the highest infection rate of HPV-16 (31.6%; 95% *CI*,

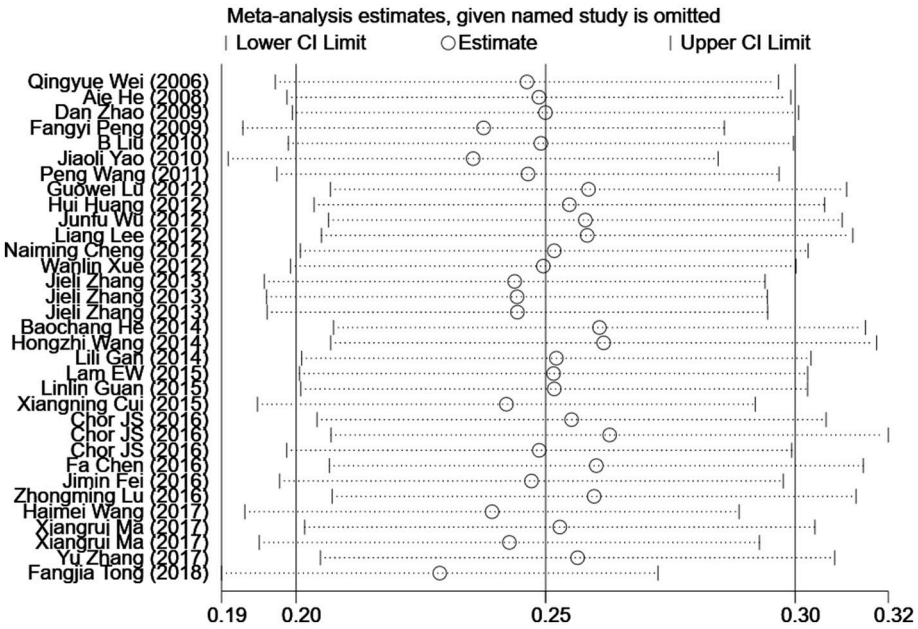

FIGURE 3 | Influence analyses for individual studies on the summary effect.

21.7–41.5%), followed by laryngeal cancer (28.5%; 95% *CI*, 18.2–38.7%) and oral cancer (14.9%; 95% *CI*, 10.1–19.7%). Stratified analysis by published year showed that head and neck cancer before the year 2011 had the highest HPV-16 prevalence (41.6%; 95% *CI*, 26.7–56.4%) as compared with the period from 2011 to 2015 (18.9%; 95% *CI*, 13.7–24.1%) and the period from 2016 to 2017 (23.8%; 95% *CI*, 14.9–32.8%). Stratified analysis by number of patients showed that head and neck cancer patients <100 has the higher HPV-16 prevalence (28.6%; 95% *CI*, 21.9–35.3%) as compared with those more than 100 (19.1%; 95% *CI*, 11.5–26.8%). HPV-16 prevalence with PCR method (22.1%; 95% *CI*, 17.6–26.7%) was lower than with ISH method (47.8%; 95% *CI*, 39.6–56.1%). Hierarchical analysis by specimen types showed that head and neck cancer in FF tissue (25.3%; 95% *CI*, 14.8–35.8%) had almost the same prevalence of HPV-16 compared with the FFPE tissue (25.0%; 95% *CI*, 19.5–30.5%). Regarding the HPV-16 test methods, the prevalence of head and neck cancer in the E6/E7 region of HPV gene (36.5%; 95% *CI*, 17.9–55.1%) was significantly higher than that in the L1 region (14.3%; 95% *CI*, 6.4–22.1%). In short, the estimated heterogeneity for studies included decreased to some extent but not disappeared. Meta-regression analyses found significant association of HPV-16 prevalence with HPV detection method (slope = 2.78, $P = 0.027$).

Influence Analysis of Individual Studies

To address the potential bias due to the quality of the included studies, we performed the sensitivity analysis by calculating pooled HPV-16 prevalence again when omitting one study at a time. **Figure 3** showed the results of sensitivity analysis. The HPV-16 prevalence ranged from 23.0% (95% *CI*, 18.7–27.3%) to 26.4% (95% *CI*, 20.9–31.9%). Point estimates for all results of influence analysis were within 95% *CI*s of the pooled prevalence. The meta-analysis of pooled HPV-16 prevalence in head and neck cancer cases was not significantly affected by any of the 33 individual studies that was not analyzed, indicating that each study did not affect the stability of overall HPV-16 prevalence estimate.

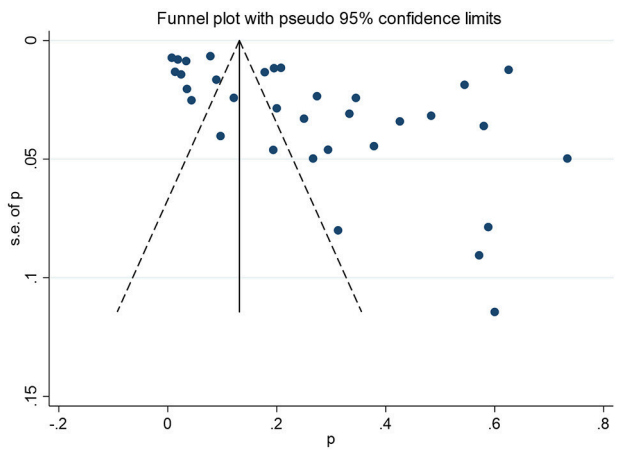

FIGURE 4 | Funnel plots for publication bias.

Publication Bias

The non-significant *P*-values of Begg's test (0.09), Eegg's test (0.07), and the near-symmetric funnel plot (**Figure 4**) demonstrated that there was no evidence of publication bias.

DISCUSSION

China has the highest burden of head and neck cancer around the world, which was rational for studying the prevalence of certain types of HPV (type-16) in China for the highly lethal cancer. As we know, this is the first meta-analysis to exploring the prevalence of HPV-16 in Chinese head and neck cancer tissues. The results of the meta-analysis showed that about 25% cases of head and neck cancer harbored HPV-16, indicating a high level of HPV 16 infection in head and neck cancer cases of China. Our results were little higher than the global prevalence (41).

Characterizing the HPV-16 prevalence in head and neck cancer is an important preliminary step in assessing the relationship between HPV-16 and head and neck cancer. Estimates of the HPV prevalence in various cancer sites of head and neck cancer vary considerably. Our meta-analysis showed that oropharyngeal cancer had the highest HPV-16 prevalence (31.6%), followed by that in laryngeal cancer (28.5%), and in oral cancer (14.9%). Interesting, the prevalence of HPV-16 in oropharyngeal cancer and oral cancer were much lower than Asian average, but much higher in laryngeal cancer (41). More cases involved and areas covered could be helpful in estimating the prevalence of HPV-16 in different head and neck cancer sites in the future.

The detection rate of HPV-16 DNA in FF tissue was found somewhat higher than that in FFPE tissue, given significant DNA degradation could be observed in FFPE tissue (42). The HPV infection status was determined on FFPE tissue in the majority of included studies. It is known that the low detection rate of HPV DNA occur with the fabric of FFPE, especially when a long DNA fragment was amplified.

Some possible reasons for variation in HPV prevalence among studies include small study sizes, different HPV testing, inter-laboratory variability, and manipulation of specimens leading to contamination (43–45). In our meta-analysis, PCR and ISH were just used as HPV detection methods, which focused on the HPV DNA detection method. Besides, for better PCR and ISH sensitivity and specificity, the literature time was limited from January 1, 2006 to Oct 23, 2018. HPV, a double-stranded circular DNA virus, encodes early proteins (E1, E2, E5, E6, and E7) and late proteins (L1, L2, and E4) with about 8,000 bp genome size (46, 47). When stratifying the L1 and E6/E7 gene fragments, we found that, in E6/E7 gene fragment (35.8%), the detection rate of HPV-16 DNA was much higher than in L1 gene fragment (14.3%). This is mainly due to the disruption of L1 region when HPV is integrated into the host genome (48), which may be an important event in promoting and triggering head and neck cancer.

The highlights of this meta-analysis includes a large sample size, both English and Chinese published studies are included with a strict inclusion criteria. By including English and Chinese studies, the selection bias caused by the publication language was avoided. Finally, by astricting studied published after 2006, limiting PCR and ISH detection methods and excluding studies without specific subsites, we tried to minimize the HPV prevalence variation as much as possible.

However, the meta-analysis has several limitations. First, the studies included in the meta-analysis are heterogeneous, which could be explained by changes in the population, the cancer site, the year of publication, the number of patients, the HPV detection method, the sample collection method, and the sensitivity of HPV primer PCR different protocols. To solve this issue, the random-effects model was used in the meta-analysis to combine data if significant heterogeneity was found. We directly tested heterogeneity by describing the HPV-16 prevalence in head and neck cancer cases by study area, cancer site, year of publication, number of patients, method of HPV detection and sample types. Of course, we have not been fully able to explain the heterogeneity. Even in stratified outcomes (for example, studies in different parts of China), the prevalence estimates are still uneven. Second, the study estimates may be biased because the accuracy of these estimates depends on the test method used and the type of HPV evaluated. That is, some studies use multiple probes or wide primers to detect multiple types of HPV, while other studies only detect HPV-16 type. Finally, the possibility of confounder cannot be ruled out. Limited studies have implicated the effect of age and smoking on the prevalence of HPV. We could not determine whether the variation of HPV prevalence was due to differences in environmental factors: age, sexual habits, smoking, alcohol consumption and other ethnic and cultural differences, as little or no information on these potential confounders in the studies involved. Therefore, studies with good designs to explore HPV infection by major confounding factors are likely to be required in future studies.

In short, the current meta-analysis provides a quantitative estimate of HPV-16 prevalence in head and neck cancer lesions in China. Although this review is a preliminary step in assessing the relationship between HPV-16 and head and neck cancer in China, it may be useful to evaluate the effect of HPV-16/18 prophylactic vaccines against carcinogenesis in the future. Considering that HPV infection plays an important role in the tumorigenesis of head and neck, other scientific studies deserve to be done in the future.

AUTHOR CONTRIBUTIONS

All authors have made substantial contributions to the conception and design of the study. LG and FY led protocol design, search, data extraction, statistical analysis, and manuscript drafting. YY and SL contributed to search, data extraction, and manuscript modifications. PL, XZ, DC, YL, JW, KW, YZ, QL, and XW contributed to quality assessment and revision of the manuscript. XS contributed to data interpretation and revision of the manuscript. All authors have reviewed and approved the final version.

FUNDING

This study was supported by the National Natural Science Foundation of China (No. 81703298).

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Oncology

ORIGINAL RESEARCH
published: 20 December 2018
doi: 10.3389/fonc.2018.00638

Time Trends of Gastrointestinal Cancers Incidence and Mortality in Yangzhong From 1991 to 2015: An Updated Age-Period-Cohort Analysis

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Background: Gastrointestinal (GI) cancers are the common cause of morbidity and mortality in China which seriously threaten people's health and lives. The aim of this study was to describe the temporal trend in the epidemiology of GI cancers from 1991 to 2015, with an emphasis on the effects of age, period and cohort in Yangzhong City, Jiangsu province, a high-risk area of GI cancers in China.

Methods: Our study extracted cases of gastric cancer, esophageal cancer and colorectal cancer diagnosed from 1991 to 2015 from Yangzhong Cancer Registry. Age-standardized rates (ASRs) were calculated and joinpoint regression was used to compute the estimated annual percent changes. Age-period-cohort (APC) model was performed to investigate the independent effects of age, calendar period, and birth cohort.

Results: Between 1991 and 2015, 18,006 new cases and 10,262 deaths were registered with GI cancers in Yangzhong. The age-standardized incidence rates (ASIRs) of gastric cancer decreased in both sexes during the study period. And the incidence rates of esophageal cancer stabilized at first then continued to decline, the turning point was in 2005 for men and 2001 for women. Changes in the mortality rates of gastric cancer and esophageal cancer showed significant declined trends around 2000–2010 in both genders. The incidence rates of colorectal cancer increased steadily during the entire study period, and the increase was more pronounced in the mortality rates of men. The results of APC analysis suggest that general decreases in incidence and mortality of esophageal cancer and gastric cancer might be caused by the downward trend of the period and cohort effects, while the increases in colorectal cancer might be caused by the uptrend of the period effects.

Conclusions: The incidence and mortality rates of esophageal and gastric cancers showed a downward trend and colorectal cancer was on the rise as a whole in Yangzhong City. The different burden of gastrointestinal cancer indicating heterogeneous risk factors exist and may have contributed to these temporal variations.

Keywords: gastrointestinal cancers, incidence, mortality, age-period-cohort analysis, trends

OPEN ACCESS

Edited by:
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Specialty section:
This article was submitted to Cancer Epidemiology and Prevention, a section of the journal Frontiers in Oncology

Received: *28 September 2018*
Accepted: *06 December 2018*
Published: *20 December 2018*

Citation:
Shao Y, Hua Z, Zhao L, Shen Y, Guo X, Niu C, Wei W and Liu F (2018) Time Trends of Gastrointestinal Cancers Incidence and Mortality in Yangzhong From 1991 to 2015: An Updated Age-Period-Cohort Analysis. Front. Oncol. 8:638. doi: 10.3389/fonc.2018.00638

INTRODUCTION

Gastric cancer, esophageal cancer, and colorectal cancer are three most common types of gastrointestinal (GI) cancer, which is one of the most commonly diagnosed malignancies and one of the leading causes of cancer-related death worldwide, particularly in China (1, 2). It was estimated that 4,292,000 new cancer cases and 2,814,000 cancer-related deaths occurred in China in 2015, among which 35.72% (1,533,300 of 4,292,000) new cases and 37.81% (1,064,000 of 2,814,000) deaths were attributed to GI cancers (2). According to the National Central Cancer Registry of China (NCCRC) cancer statistics data in 2014, gastric cancer ranked the third highest incidence and the third most common cause of death, with estimated age-standardized incidence rate (ASIR) was 19.51/100,000 and an estimated age-standardized mortality rate (ASMR) was 13.3/100,000. Esophageal cancer is the sixth most common cancer and the fourth in cancer mortality, with ASIR and ASMR reaching as high as 12.17 and 8.75 per 1,00,000, respectively. Colorectal cancer had the fifth incidences and fifth mortality, the estimated ASIR and ASMR were 17.52 and 7.91 per 1,00,000, respectively (3).

In the year 2004, the Ministry of Health of China initiated a population-based endoscopy screening program in the high-risk areas of China (4). As a pilot rural area, massive population-based esophageal and gastric cancer screenings of asymptomatic patients have been performed in Yangzhong City (5). During the last several decades, with socioeconomic development and lifestyle changes simultaneously, declining incidence and mortality rates were observed in gastric cancer and esophageal cancer; however, colorectal cancer has increasing incidence and mortality in the Chinese population (6–8). The overall trends in GI cancer incidence and prevalence can only help us in understanding the changes in the epidemiology of GI cancer, but it won't be able to uncover the potential etiology clue. The longitudinal trends in the epidemiology of GI cancer can be influenced by a variety of risk or protecting factors, such as changes in dietary composition, living styles, and implementation of screening programs (9). The age-period-cohort (APC) analysis plays an important role in understanding time-varying elements in epidemiology. It usually identifies patterns in cancer incidence or mortality rates from population-based count (numerator) and population (denominator) data, which are often retrieved from cancer registry databases such as SEER in the form of a table showing the numbers of cancer cases or cancer deaths (counts) and corresponding person-years at risk (population) for particular age groups and calendar time periods. This analysis can help us separate the independent effects of age, period and cohort patterns, and further explores factors affecting the incidence and mortality of GI cancers from a macro perspective, such as social conditions, economic changes, environmental development (10, 11). By analyzing the overall trends of GI cancer epidemiology with the APC model, we can effectively identify potential risk factors and further make recommendations for prevention and control strategies of GI cancers.

Therefore, in order to identify the trends in GI cancers incidence and mortality rates and to reveal new information about the potential etiology clue of GI cancers, we chose a relatively fixed population from Yangzhong City, Jiangsu Province, which is a high-risk area of GI cancers in China (12, 13) to analyzed the secular trends in the incidence and mortality rates of gastric cancer, esophageal cancer, and colorectal cancer from 1991 to 2015 with the APC model.

MATERIALS AND METHODS

Source of Data

Sex- and age-specific incidence and mortality data of GI cancers were obtained from Yangzhong Cancer Registry, 1991–2015. A detailed description of the cancer registration and reporting system in Yangzhong City can be found in a previous publication (14). Briefly, registered cancer cases were coded using the International Classification of Diseases, 9th Revision (ICD-9) before 2001, and using the 10th Revision (ICD-10) since 2002. Esophageal cancer (ICD-9:150; ICD-10: C15), gastric cancer (ICD-9:151; ICD-10: C16), and colorectal cancer (ICD-9:153-154; ICD-10: C18-21) were included in the APC analysis. The ICD revisions have no effect on the temporal trends for the incidence and mortality of GI cancer in our study. In this study, the demographic data (age composition by sex) from 1991 to 2015 were collected from the Yangzhong Statistics Department. Since our study did not involve interaction with human subjects or personal identifying information, ethical approval, and informed consent were not required.

Quality Control

Quality of registration data was assessed based on the criteria of "Guideline for Chinese Cancer Registration (15)" and "Cancer Incidence in Five Continents Volume IX" by International Agency for Research on Cancer/International Association of Cancer Registries (IARC/IACR) (16). Briefly, the proportion of morphological verification (MV%), the percentage of cancer cases identified with death certification only (DCO%), mortality to incidence ratio (MI), and the percentage of the diagnosis of unknown basis (UB) (%) were used to evaluate the completeness, validity, reliability, and comparability of the data. Data included in the final analysis should meet the following criteria: MV% was not lower than 66%, DCO% was lower than 15%, M/I was between 0.6 and 0.8, and the percentage of the diagnosis of unknown basis (UB) (%) was $<5.0\%$. In the present study, the data have high quality and completeness as the overall MV%, DCO%, and MI ratio were 71.23, 2.98, and 0.65%, UB% was 0.32%, respectively.

Statistical Analysis

Age-standardized incidence and mortality rates of GI cancers were calculated using Segi's World Standard population as the standard population (17).

To evaluate the trends in the epidemiology of GI cancers, we used the Joinpoint Regression Program (18). The log-linear model (based on Poisson distribution) of the joinpoint regression analysis program was employed to estimate trends in the age-standardized incidences and mortality rates of GI cancers, and the results were expressed as the estimated annual percent

changes (EAPC) and their 95% confidence intervals (CIs) for each period. Significant joinpoints were identified by the Monte Carlo permutation test and $p < 0.05$ represents a statistically significant difference at the junction (19).

For the APC analysis, the cancer incidence and mortality data in this study were collected in five successive 5-year periods from 1991–1995 to 2011–2015, and ten 5-year age groups, ranging from 35–39 years to 80–84 years. The goal of APC analysis was to distinguish and statistically estimate the unique effects associated with age, calendar period, and a birth cohort from cross-sectional data respecting historical changes in the risk of morbidity and mortality. Age effects reflect the factors that associated with different age groups. Period effects reflect the factors that affect all age groups simultaneously, while cohort effects reflect the long-lasting effects of factors that influence all age groups simultaneously. Moreover, conventional APC models fall into the class of generalized linear models (GLM) that can take various alternative forms. When it refers to the estimation of cancer data which follow Poisson distributions, the model can be written as a log-linear regression model:

$$ln\left(E\left(M_{ijk}\right)\right) = \ln\left(\frac{D_{ijk}}{P_{ijk}}\right) = \mu + \alpha_i + \beta_j + \gamma_k + \varepsilon_{ijk}$$

where α_i, β_j, and γ_k represented age, period, and cohort effect, respectively. In the full 3-factor model, one inherent identification problem associated with the APC analysis by the exact linear dependency between age, period, and cohort: period = age + cohort (20). A number of methods have been proposed to solve this problem and we utilized the Intrinsic Estimator (IE) algorithm to address the identification problem and to provide parameter estimates because of its superior estimation ability, non-biased approach, validity, and asymptotic features. The IE method based on the estimable functions and the singular value decomposition of matrices, which does not need to have reference categories for the age, period, and cohort coefficients (21). The initial results are expressed as logarithmic coefficients, which can be seen as the natural logarithm of rate ratio (22, 23). To compare the rate ratio across ages, periods, and birth cohorts, we calculated the exponential value of the estimated coefficients (rate ratio = exp(coef.) = $e^{coef.}$) (24). The goodness of fit for age-period-cohort sub-models were estimated using the Akaike information criterion (AIC). In this study, the calculation process was implemented in Stata version 14.0 (StataCorp, College Station, TX, USA).

RESULTS

Incidence

A total of 9,400 gastric cancer cases, 7,107 esophageal cancer cases, and 1,499 colorectal cancer patients were registered during the period of 1991–2015 (**Table 1**). As shown in **Figures 1A,B**, ASIRs were highest in gastric cancer in men, followed by esophageal cancer and colorectal cancer in men. In women, the ASIRs of gastric cancer and esophageal cancer were almost at the same level, higher than that of colorectal cancer (**Table 1**; **Figures 1A,B**).

Joinpoint regression analysis showed that ASIR of gastric cancer significantly decreased and without changes in the joinpoint regression by an average of 3.0% per year in men and 4.5% per year in women during 1991–2015. Regarding esophageal cancer, the ASIR in men has significantly decreased from 2005 to 2015 by an average of 5.4% per year; in women, the ASIR decreased even more rapidly by 7.7% per year from 2001 to 2015. On the contrary, the ASIR of colorectal cancer has increased steadily in both men (3.7%) and women (1.5%) from 1991 to 2015, again without change points during the entire observation period (**Table 2**).

We then carried out the full APC models based on the goodness of the fit (**Table 3**). **Supplementary Table 1** shows the results from the estimation of the full APC models using the IE method. The model-derived age, period and cohort effects were expressed in terms of rate ratio and shown in **Figures 2A–B,E–F, I–J**, respectively. After controlling the period and cohort effects, the age effects for both genders showed that the incidence rates of these three GI cancers had consistently increasing trends in incidence with age for people aged 35–74 year (**Figures 2A–B**).

The period effects for the incidence of both gastric and esophageal cancer showed a steady decline and the decreased more rapidly in women than in men; while colorectal cancer showed an upward trend in both sexes during the period of 1991–2015 (**Figures 2E–F**).

With regard to birth cohort, we observed the effects of increased first and then decreased for GI cancers incidences after we adjusted the age and period effects. For gastric cancer and colorectal cancer, the cohort effects peaked around the year 1927 in both genders (**Table 4** and **Figures 2I,J**). And the highest risk of esophageal cancer incidence was delayed by 5–10 years compared with gastric cancer and colorectal cancer, the apparent peak appearing around 1942 for male, but the highest value was observed in the 1932 cohort for female (**Table 4** and **Figures 2I,J**).

Mortality

Between 1991 and 2015, 5,507 gastric cancer deaths, 4,113 esophageal cancer deaths, and 642 colorectal cancer deaths were reported in Yangzhong City (**Table 1**). The ASMRs of gastric cancer and esophageal cancer increased firstly and then declined, thereafter kept steady; while colorectal cancer remained stable in both sexes in 1991–2015 (**Figures 1C,D** and **Table 1**).

For gastric cancer, the ASMR in men increased by 54.4% on average per year in the period of 1991–1993, followed by a stable rate from 1993 to 2001, and then decreased by 11.9% on average per year from 2001 to 2010, and again an increase of 9.5% per year in the period of 2010–2015 was observed (**Table 2** and **Figures 1C,D**). In women, the ASMR has been significantly decreased from 1999 to 2011 by 13% per year on average (**Table 2** and **Figures 1C,D**). For esophageal cancer, the ASMR in men has been significantly decreased by 9.1% per year on average during 2000–2010. In women, the ASMR was increased during 1991–1993 (66.3% per year) and then reached a plateau from 1993 to 2000, followed by a decrease by 13.9% per year in the period of

TABLE 1 | Crude and age-standardized incidence and mortality rates (ASRs) of gastrointestinal cancers per 1,00,000 person-years in Yangzhong and calendar periods during 1991–2015.

Calendar Periods	Incidence						Mortality					
	Males			Females			Males			Females		
	N	Crude rate	ASR	*N*	Crude rate	ASR	*N*	Crude rate	ASR	*N*	Crude rate	ASR
GASTRIC CANCER												
1991–1995	1,301	189.28	156.76	797	114.88	90.90	702	102.13	86.79	469	67.60	51.47
1996–2000	1,133	164.85	138.39	706	101.82	79.74	856	124.55	106.71	538	77.59	57.35
2001–2005	1,238	180.24	128.81	682	97.65	64.60	818	119.09	86.18	460	65.86	42.18
2006–2010	1,210	177.28	106.76	647	92.47	51.93	496	72.67	43.65	244	34.87	18.59
2011–2015	1,122	162.33	82.81	564	78.71	37.05	650	94.04	46.56	274	38.24	16.27
ESOPHAGEAL CANCER												
1991–1995	696	101.26	81.89	746	107.53	85.84	399	58.05	47.41	430	61.98	47.79
1996–2000	741	107.82	87.76	735	106.00	83.40	515	74.93	63.23	526	75.86	57.18
2001–2005	809	117.78	83.90	700	100.23	65.09	509	74.11	53.42	467	66.87	40.82
2006–2010	820	120.14	72.42	617	88.18	49.36	331	48.50	29.54	239	34.16	17.75
2011–2015	752	108.80	55.05	491	68.52	30.27	422	61.05	30.19	275	38.38	15.55
COLORECTAL CANCER												
1991–1995	88	12.80	10.60	91	13.12	9.93	41	5.96	4.88	44	6.34	4.25
1996–2000	115	16.73	13.55	104	15.00	11.72	56	8.15	7.18	57	8.22	6.03
2001–2005	142	20.67	14.83	120	17.18	11.21	78	11.36	8.25	63	9.02	5.66
2006–2010	206	30.18	17.98	150	21.44	12.44	75	10.99	6.54	46	6.57	3.33
2011–2015	289	41.81	22.18	194	27.07	13.59	108	15.63	7.96	74	10.33	4.38
1991–1995	88	12.80	10.60	91	13.12	9.93	41	5.96	4.88	44	6.34	4.25

ASR, age-standardized rates.

2000–2010 (**Table 2** and **Figures 1C,D**). Over the whole 25-year study period, there has been a significantly increasing trend of ASMR of colorectal cancer in male (2.3% per year), while female mortality trends will not present considerable variations (**Table 2** and **Figures 1C,D**).

The full APC models of GI cancers mortalities had the lowest AIC value and were selected as the model with the best fit based on the goodness of the fit test results (**Table 3**). The age effects for both genders showed that the mortalities of gastric, esophageal and colorectal cancers had consistent increasing trends with age (**Figures 2C,D**).

In male, fluctuating period trends were presented in gastric cancer and esophageal cancer, which showed a rising trend first during the period of 1991–1995 to 1996–2000; then the period effects declined from the second 5-year period, i.e., from 1996–2000 to the period of 2006–2010; and then they increased thereafter up to the last period of 2011–2015. However, colorectal cancer as a whole showed an upward change in men. In the female, there were exhibited a fluctuating periodic pattern in the mortalities of gastric cancer and colorectal cancer, but a continuous decreasing period effect was observed in esophageal cancer (**Figures 2G,H**).

The cohort effect patterns of GI cancers in mortality were similar to those of incidence, showing an Inverted-U shape (**Figures 2K,L**). The cohort effect of gastric cancer peaked at around the year 1927 in both genders. The risk of colorectal cancer-related death in male was highest for those born in 1927, but in the female it was highest in 1932 cohort. And the risk of esophageal cancer-related death in male and female was highest for those born in 1937 and 1932, respectively (**Table 4** and **Figures 2K,L**).

DISCUSSION

In this updated analysis of data from the 25-year period between 1991 and 2015, we observed divergent trends in the incidence and mortality of GI cancers in Yangzhong City. As a whole, the incidence and mortality rates of esophageal and gastric cancers showed downward trends while colorectal cancer was on the rise. Our APC analyses suggested that the incidence and mortality of GI cancers were increased exponentially with age. The fluctuated trends of GI cancers' incidence and mortality are primarily due to the changes of the period and cohort effects, which may indicate the impact of the early diagnosis and treatment of cancer, lifestyle and environmental changes on the risk of GI cancers in Yangzhong, a high-risk area of China.

Age Effect

Similar age patterns were observed in the incidence and mortality trends of GI cancers in both sexes, from 35 to 74 years of age, indicating that the risk gradually increased with age. With modern medical technology and economic development, the proportion of elderly in the general population has increased

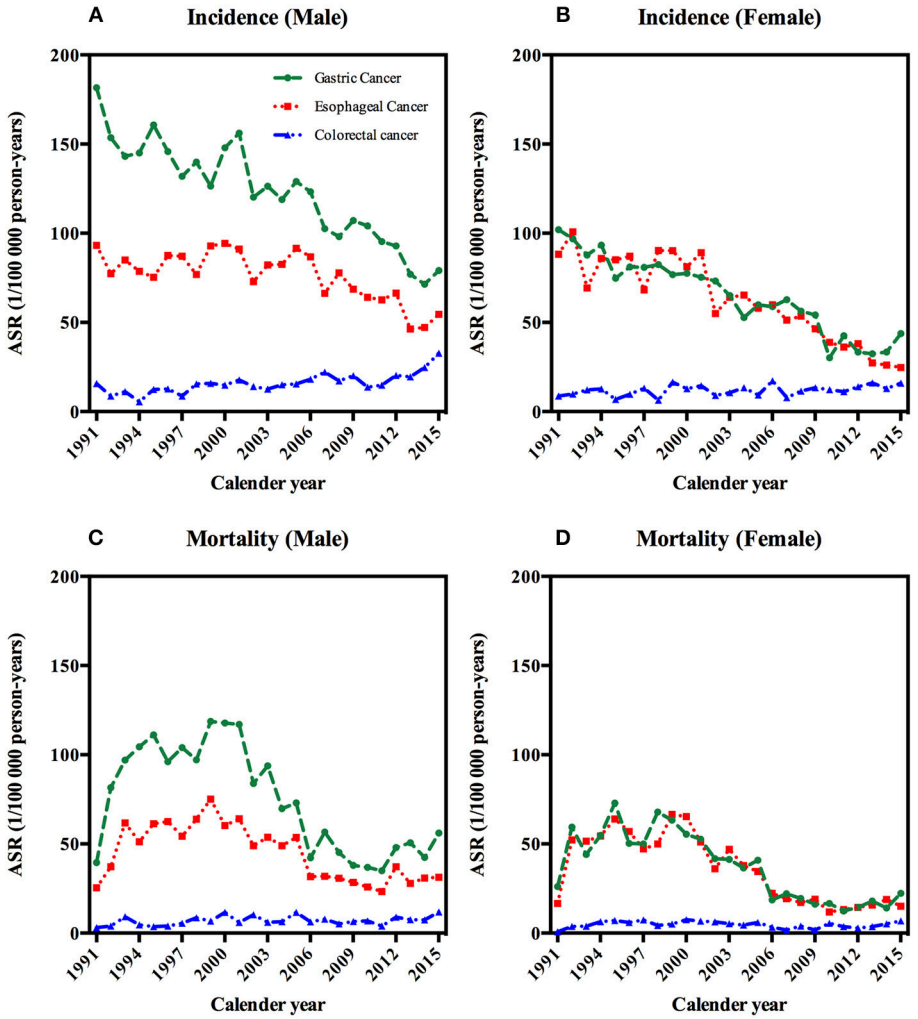

FIGURE 1 | Age-standardized incidence and mortality rates of gastrointestinal cancers by sex in Yangzhong, 1991–2015. **(A)** Age-standardized incidence rates of gastrointestinal cancers in males. **(B)** Age-standardized incidence rates of gastrointestinal cancers in females. **(C)** Age-standardized mortaity rates of gastrointestinal cancers in males. **(D)** Age-standardized mortaity rates of gastrointestinal cancers in females.

dramatically; the burden caused by GI cancers would be a great challenge for China in the future. The average life expectancy was raised from 46 years in 1950 to 75 years in 2010 in China, and that of Jiangsu province is 1.6 years longer than the national level (76.6 years) (25). Elderly people are at a higher risk of developing GI cancers, which may be related to prolonged exposure to carcinogens. The rate ratio of GI cancers incidence and mortality was significantly increased from 40 years old and generally peaked in the age group of 75–79 years. In addition, women had the same level of the rate ratio of developing GI cancers compared to men. This indicated that cancer screening should be focused on those aged 40–74 years of both genders.

Period Effect

The results of APC analyses showed that the trends of period effects in GI cancers were consistent with the age-standardized incidence and mortality rates trends, which indicated that the period effects might be an important factor affecting GI cancers morbidity and mortality. Variations in incidence and mortality over long periods often reflect the impacts of the changes in diet and lifestyle, updated diagnostic techniques, and improved medical interventions.

Since the late 1980s, the Yangzhong City government has paid attention to the prevention and treatment of cancers, especially esophageal cancer and gastric cancer. Under the leadership of the Institute of Cancer Research, a city-town-village tertiary cancer prevention and treatment network was established. And a comprehensive cancer prevention plan for education, environment, and medical care was established in 1998. Therefore, with the popularization of health education, some risk factors of upper GI cancers such as hot-temperature food, long-term stored rice, pickled vegetables, and drinking contaminated water have been greatly reduced (26, 27); meanwhile, the living and nutritional conditions of the local population also have been significantly improved. The incidence

TABLE 2 | Joinpoint analysis of gastrointestinal cancers in Yangzhong, 1991–2015.

Cancer types	Gender	Incidence			Mortality		
		Period	EAPC (95% CI)	*P*	Period	EAPC (95% CI)	*P*
Gastric cancer	Male	1991–2015	−3.0 (−3.6, −2.5)	<0.01	1991–1993	54.4 (3.1, 131.2)	<0.01
					1993–2001	0.6 (−4.7, 6.2)	0.8
					2001–2010	−11.9 (−15.7, −8.0)	<0.01
					2010–2015	9.5 (0.1,19.9)	<0.01
	Female	1991–2015	−4.5 (−5.2, −3.7)	<0.01	1991–1999	7.0 (−0.3,14.7)	0.1
					1999–2011	−13.0 (−16.7,−9.1)	<0.01
					2011–2015	11.3 (−9.1,36.3)	0.3
Esophageal cancer	Male	1991–2005	0.2 (−1.1, 1.6)	0.7	1991–1993	49.0 (−1.9, 126.3)	0.1
					1993–2000	2.6 (−4.4, 10.1)	0.4
		2005–2015	−5.4 (−7.6, −3.3)	<0.01	2000–2010	−9.1 (−12.5, −5.6)	<0.01
					2010–2015	4.7 (−4.6,15.6)	0.3
	Female	1991–2001	−0.7 (−3.5, 2.1)	0.6	1991–1993	66.3 (5.3,162.7)	<0.01
					1993–2000	0.9 (−6.6,9.0)	0.8
		2001–2015	−7.7 (−9.3, −6.1)	<0.01	2000–2010	−13.9 (−17.4,−10.2)	<0.01
					2010–2015	4.5 (−5.7,15.7)	0.4
Colorectal cancer	Male	1991–2015	3.7 (2.2, 5.2)	<0.01	1991–2015	2.3 (0.3, 4.4)	<0.01
	Female	1991–2015	1.5 (0.1, 3.0)	<0.01	1991–1993	156.1 (−18.6, 705.9)	0.1
					1993–2015	−2.6 (−5.2, 0.1)	0.1

EAPC, estimated annual percent changes; CI, confidence interval.

TABLE 3 | Akaike information criterion (AIC) of age-period-cohort sub-models for gastrointestinal cancers incidence and mortality, Yangzhong, 1991–2015.

Sub-models	Gastric cancer				Esophageal cancer				Colorectal cancer			
	Incidence		Mortality		Incidence		Mortality		Incidence		Mortality	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Age-period-cohort	7.98	7.24	7.57	6.71	7.35	7.15	6.71	6.18	5.95	5.75	4.96	4.61
Age-drift	769.17	714.42	887.73	813.44	631.20	993.02	606.14	762.33	334.17	276.28	232.42	227.31
Age-period	489.28	430.97	453.00	368.98	522.95	609.57	402.82	431.81	287.29	275.44	230.83	223.79
Age-cohort	397.51	357.08	477.74	405.07	375.58	363.76	397.99	346.10	291.91	282.57	244.66	233.77

and mortality of esophageal and gastric cancer thereafter have changed significantly.

Furthermore, several screening methods have been developed and tested in the high-risk area of China. From 1986 to 1990, approximately 31,000 people in Yangzhong (age range, 35–70 years) received occult blood detection (28, 29). The latest study using endoscopic examination with Lugol's iodine staining and biopsy conducted in Yangzhong in the year 2004. With special funds from the Ministry of Health, local residents aged 40–69 years were eligible for a free endoscopic screening. Until 2012, 38,917 participants were covered from 26 villages in Yangzhong, which included 21,404 people age 40–69 years. In the target population, 13,888 participants were endoscopic examinations, and screening compliance was 64.89% (13,888 of 21,404 people). Among detected upper digestive tract cancers patients, 98.11% with esophageal cancer and 100% with gastric cancer were defined as at the early stage (ie, carcinoma *in situ* and intramucosal or submucosal [T1N0M0] carcinoma). Results from these studies showed that endoscopic screening and intervention significantly reduced mortality caused by esophageal or gastric cancer (5). This massive endoscopic screening program could largely explain the sharp downward trend of gastric and esophageal cancer mortality from the period of 2001–2005 to 2006–2010. Slight increases were observed in gastric and esophageal cancer mortality from 1991–1995 to 1996–2000, which might be mainly associated with the high prevalence of gastric cancer and esophageal cancer in the early 1990s and the underdevelopment of medical care during that period of time. In addition, a sudden tiny increase was captured in mortality of gastric and esophageal cancer, especially in male sex during the last period of 2011–2015. Reasons for this period effect fluctuation are not completely clear but may be related to aging, the high prevalence of H. pylori infection, and the frequent severe air pollution in China (30, 31). Besides, alcohol production and availability have increased over the past few years in China, and Chinese men have more social interactions to drink than women,

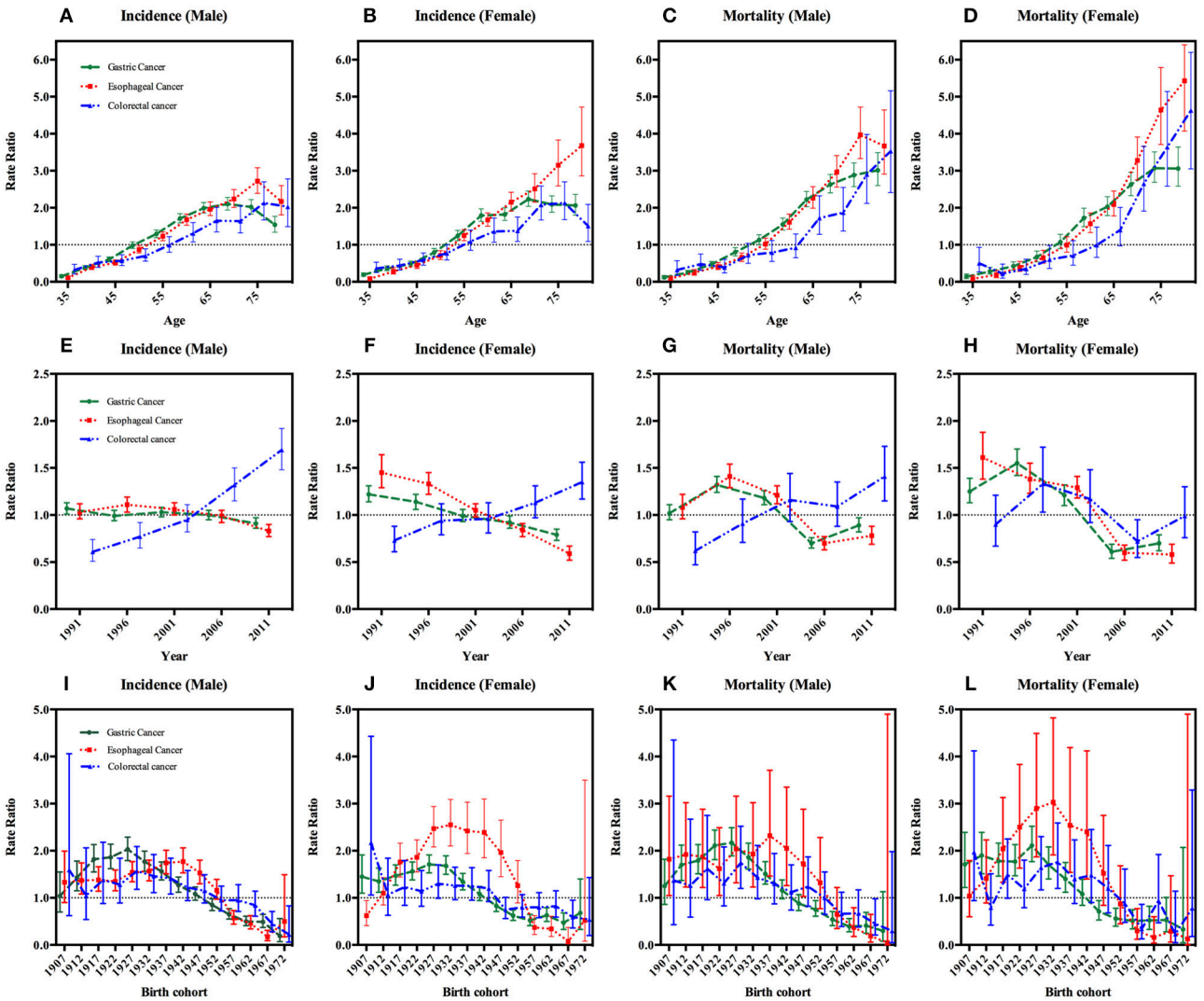

FIGURE 2 | Age, period, and cohort effects on gastrointestinal cancers incidence and mortality with 95% confidence interval, stratified by sex. Footnotes: The age, period and cohort effects are estimated by a log-linear model using the intrinsic estimator (IE) method and expressed as rate ratios. **(A–D)** In the first row represent age effects after adjusting for period and cohort effects. **(E–H)** In the second row are the estimated period effects after adjusting for age and cohort effects. In the year 2004, a population-based endoscopy screening program in high-risk areas was initiated. **(I–L)** In the last row are the cohort effects after adjusting for age and period effects.

which may also contribute to the burden of gastrointestinal cancer (32). There also might exit some potential risk factors which we still could not recognize and its harms outweigh the benefits of screening programs, further studies are needed to explore and primary prevention measures are still needed to reduce the exposure.

For colorectal cancer, the period effect was followed by a generally increasing trend in the incidence of both sexes and showed an upward trend in the mortality of the male. Previous studies based on extensive epidemiologic and experimental investigations have suggested that specific diet pattern, sedentary lifestyles, and obesity are associated with the increased risk of colorectal cancer (33, 34). With the accelerated economic development in Yangzhong, some westernized lifestyle such as excessive intake of high-protein and high-fat foods and less physical activity may contribute to the persistently rising incidence rate of colorectal cancer (35). In addition, some studies have shown higher zinc intake was significantly associated with reduced risk of colorectal cancer (36). According to a national representative cross-sectional study on nutrition and health in China in 2002, dietary zinc intake does not meet the Chinese Dietary Reference Intakes (DRIs) in Jiangsu Province (37), which might be one of the reasons for the rising incidence of colorectal cancer.

The gender difference in colorectal cancer mortality probably related to different exposures to risk factors in male and female. Some colorectal cancer risk factors such as smoking, diet, and obesity have been shown to have disparate effects on sex which

TABLE 4 | Highest cohort effects (Rate ratio) on GI cancers incidence and mortality rates and the corresponding birth cohorts.

	Gastric cancer		Esophageal cancer		Colorectal cancer	
	Birth cohort	Rate ratio (95%CI)	Birth cohort	Rate ratio (95%CI)	Birth cohort	Rate ratio (95%CI)
Incidence male	1927	2.03 (1.79, 2.29)	1942	1.77 (1.53, 2.06)	1927	1.57 (1.18, 2.09)
Incidence female	1927	1.71 (1.53, 1.91)	1932	2.55 (2.10, 3.09)	1927	1.30 (1.00, 1.70)
Mortality male	1927	2.17 (1.89, 2.49)	1937	2.32 (1.46, 3.71)	1927	1.74 (1.20, 2.52)
Mortality female	1927	2.11 (1.77, 2.52)	1932	3.03 (1.91, 4.82)	1932	1.76 (1.20, 2.59)

CI, confidence interval.

may be related to interactions between estrogen exposure, body fat distribution, and the biologic underpinnings of the tumors (38). In addition, significant differences in colorectal cancer survival between men and women were demonstrated in some researches, indicating that higher survival rates persisted in women, which may partly explain the diversity in gender (39). Some studies have speculated that differences in immunological and genomic backgrounds could contribute to the gender-related differences in survival (40, 41). However, the underlying etiology clues driving gender differences regarding colorectal cancer mortality remains to be investigated.

Cohort Effect

The birth cohort effect usually reflects the exposure of early life to specific risk factors that do not exist in other periods (42). Common risk factors such as tobacco smoking and diet may account for the long-term trend according to the similarity of the cohort patterns (43, 44). In our study, a noteworthy phenomenon is that the rate ratio for GI cancers incidence and mortality exhibited highest among those born in the late 1920s to the early 1940s. A possible explanation is that natural disasters have occurred during that period, followed by severe famine and deaths. At the beginning of the Twentieth century, social conditions and public health conditions in China were poor. Low socioeconomic status during childhood may partly explain the result. Individuals with poor socioeconomic status during childhood are characterized by a short length of life, high mortality rates, and independent of subsequent socioeconomic conditions (45, 46). A Study has suggested a possible link between exposure to the Chinese famine during childhood and an increased risk of GI cancers (47). So the newborns who survived those famines may have suffered from malnutrition and have a greater risk of developing digestive diseases. Esophageal cancer and gastric cancer have a relatively higher rate ratio than colorectal cancer during this period, meanwhile, the rate ratio of esophageal cancer was lower in men than in women. So, it can be inferred that esophageal cancer and gastric cancer have a stronger biological susceptibility to malnutrition, especially in women.

Since the early 1930s, the cohort effects on the incidence and mortality rates of gastric cancer and colorectal cancer has been declining, and esophageal cancer has declined since the early 1940s. Subsequently, the cohort effects of GI cancers all have dropped to low levels around the 1950s, which can be explained by improvements in socioeconomic status after World War II and the founding of new China (48). Furthermore, a relevant decrease in the prevalence of tobacco and alcohol consumption among the younger generation could be another possible reason. A study on the prevalence of smoking among different generations in China shows that the rate of smoking has decreased gradually from the birth population in the 1950s to the birth population in the 1980s (49). In addition, the decrease in the number of frequent alcoholics in rural China from those born in the 1940s may partially affect the decline in cohort effect (50).

Strengths and Limitations

The present study has some strengths and limitations. Based on a high-quality population-based of cancer registration database, we conducted a comprehensive analysis of GI cancers incidence and mortality over a time period of 25 years in Yangzhong City, a high-risk area of GI cancers in China. Furthermore, APC analysis can effectively disentangle the separate effects on secular trends compared to traditional statistical methods. Our study also has several limitations. First, due to sample size limitations, the findings of this study may not be representative of other Chinese populations. Second, like other APC model analyses of cancer (51, 52), we analyzed the incidence and mortality of GI cancers without distinguishing histological types. However, the histological types of GI cancers in China are relatively simple. For example, more than 90% of esophageal cancers in China are mainly esophageal squamous cell carcinomas (53), which partly reduce the influence of histological type on this study. Last, the changes in the age, period and cohort effects cannot be specific to which pathogenic factors are caused. Therefore, the APC model of estimated parameters can only provide clues for etiology studies. The relevant hypotheses proposed in this study still need further confirmation in future studies.

CONCLUSIONS

The incidence and mortality rates of esophageal and gastric cancers showed a downward trend and colorectal cancer was on the rise as a whole, indicating heterogeneous etiologies between upper gastrointestinal cancers and colorectal cancer. Significant changes in the period effects of esophageal and gastric cancer suggest that early detection and treatment programs are effective in reducing mortality, while the increasing trend of colorectal cancer probably attributed to the increased risk factors associated with westernized lifestyle. The similarity of cohort

effects supports the influences of early childhood nutritional deficiencies on the development of GI cancers. All results from the APC analysis in this study still need further confirmation with more relevant studies.

AUTHOR CONTRIBUTIONS

YSha, WW, and FL contributed conception and design of the study. ZH collected and organized the database. YSha, YShe, XG, and CN performed the statistical analysis. YSha and LZ draft the manuscript. FL and WW Revised and made the decision to submit for publication. All authors contributed to manuscript revision, read and approved the submitted version.

FUNDING

This work was supported by grants from the National Natural Science Foundation of China (81874277, 81473056, and 81573224), Beijing Natural Science Foundation (7172025) and Scientific Research Project of Beijing Educational Committee (KM201710025006 and KM201510025001).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2018.00638/full#supplementary-material

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Oncology

SYSTEMATIC REVIEW
published: 04 February 2019
doi: 10.3389/fonc.2019.00024

The Effect of Hexavalent Chromium on the Incidence and Mortality of Human Cancers: A Meta-Analysis Based on Published Epidemiological Cohort Studies

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OPEN ACCESS

Edited by:
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Reviewed by:
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Specialty section:
This article was submitted to Cancer Epidemiology and Prevention, a section of the journal Frontiers in Oncology

Received: 09 November 2018
Accepted: 08 January 2019
Published: 04 February 2019

Citation:
Deng Y, Wang M, Tian T, Lin S, Xu P, Zhou L, Dai C, Hao Q, Wu Y, Zhai Z, Zhu Y, Zhuang G and Dai Z (2019) The Effect of Hexavalent Chromium on the Incidence and Mortality of Human Cancers: A Meta-Analysis Based on Published Epidemiological Cohort Studies. Front. Oncol. 9:24. doi: 10.3389/fonc.2019.00024

Background: Hexavalent chromium [Cr(VI)] is an occupational carcinogen that can cause lung and nasal cancers, but its association with mortality and incidence in many other cancers is unclear.

Objectives: In this meta-analysis, we aimed to evaluate the relationship between exposure to Cr(VI) and the mortality and incidence of human cancers.

Methods: We performed a search of the literature and extracted the standardized mortality ratios (SMRs), standardized incidence ratios (SIRs), and their corresponding 95% confidence intervals (CIs), to estimate risk values. Subgroup analyses were conducted by sex, occupation, and types of cancer to identify groups that were at high-risk or predisposed to certain cancers.

Results: A total of 47 cohort studies covering the period 1985–2016 were included (37 studies reporting SMRs and 16 studies reporting SIRs). The summary SMR for all studies combined was 1.07 (95% CI: 1.01–1.15). Summary SMRs were higher among chromate production workers, chrome platers, and masons, and especially male workers. In the subgroup analysis, Cr(VI) exposure was related to a higher risk of death owing to lung, larynx, bladder, kidney, testicular, bone, and thyroid cancer. The meta-SIR of all studies combined was 1.06 (95% CI: 1.04–1.09). Summary SIRs were elevated among cement industry workers and tanners. Cr(VI) exposure was related to an elevated risk of respiratory system, buccal cavity, pharynx, prostate, and stomach cancers.

Conclusions: Cr(VI) might cause cancers of the respiratory system, buccal cavity and pharynx, prostate, and stomach in humans, and it is related to increased risk of overall mortality owing to lung, larynx, bladder, kidney, testicular, bone, and thyroid cancer. In addition, there was a strong association between incidence and mortality risk of cancers and concentration of Cr(VI) in the air and the exposure time.

Keywords: hexavalent chromium, cancer, mortality, incidence, meta-analysis

INTRODUCTION

Rationale

Occupation-related cancers are an important public health issue with serious socioeconomic effects. It is essential to recognize and identify occupational carcinogens for risk prevention, surveillance, and compensation of exposed workers. According to data from the International Agency for Research on Cancer (IARC), hexavalent chromium [Cr(VI)] has been classified as a Group I occupational carcinogen. In addition, there is sufficient evidence that Cr(VI) is related to cancers of the lung, nasal cavity, and paranasal sinuses (1). The European Commission conducted a socioeconomic, health, and environmental impact assessment and found that strong factors related to attributable cancer deaths include hardwood dust, Cr(VI), and respirable crystalline silica (2). Cr(VI) exposure exists in many industries, including chromate production, stainless steel production, welding, chrome pigment production, chrome plating, tanning, cement production, and aircraft manufacturing. Workers are often exposed to Cr(VI) by inhalation and dermal contact (3). A meta-analysis performed by Welling et al. suggested that Cr(VI) is a stomach carcinogen in humans (4). Hara et al. found that Cr(VI) exposure may increase the risk of brain cancer and malignant lymphoma (5). Iaia et al. confirmed that exposure to Cr(VI) increased lung, bladder, and pancreatic cancer mortality among tanners and found that Cr(VI) increased mortality due to myeloid leukemia and tumors of the endocrine glands (6).

Great progress has been made in identifying the correlation between exposure to Cr(VI) and some respiratory cancers. Nevertheless, there is an ongoing need for research on the relationship between Cr(VI) and other cancers, which has not been evaluated owing to inadequate epidemiological evidence and a paucity of quantitative exposure data.

Research Question

Does Cr(VI) pose a risk of increased cancer incidence and mortality in populations with high exposure to Cr(VI)?

MATERIALS AND METHODS

This study was conducted following the 2015 Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) and the Meta-Analysis of Observational Studies in Epidemiology (MOOSE) guidelines (7, 8), which was shown in **Supplemental Table 4**.

Search Strategy

Databases (PubMed, EMBASE, Web of Science, the Cochrane Library, Google Scholar, CNKI, WANFANG, and VIP) were searched by two authors independently to obtain all related epidemiological data up to May 28, 2018. The data covered the period 1985–2016. The search strategies used combinations of the following keywords and phrases: hexavalent chromium, chromium(VI), Cr(VI), chromate, chrome, chromate production, stainless steel, welding, chrome pigment production, chrome plating, ferrochrome production, leather, tanning, tanners, cement, concrete, metal plating, cancer, neoplasia, neoplasm, tumors, malignant neoplasms, malignancy, cohort study, cancer mortality, cancer incidence, cancer morbidity, standardized mortality ratio (SMR), standardized incidence ratio (SIR), with "AND" and "OR" used to narrow the range of articles identified. In addition, we searched all studies in the reference lists of published reviews and all relevant meta-analyses.

Selection Criteria

All contents of the candidate articles and data were read, and the data were extracted by two authors (DYJ and WM) independently. Publications included in this meta-analysis met the following criteria: (1) the exposed population and study region was stated; (2) the exposure factor was clear and exposure was to Cr(VI); (3) the cancer mortality or incidence data were included; (4) the study was a cohort study; (5) the exposure time and dose were declared; (6) a follow-up period was included; (7) the SMR or SIR with confidence intervals (CIs) were listed; (8) for studies with different latency periods, the SMR/SIR for the longest period was selected; (9) for studies with different exposure levels, the result of the highest level was selected.

The exclusion criteria were as follows: (1) unavailable data (no CIs of SMR or SIR, or only data of relative risk, odds ratio, proportional mortality ratio, or hazard ratio); (2) duplicated data; for overlapping populations, only the largest number of cases or most recent data were selected; (3) meta-analysis study, case report, review, or letter; (4) occupational exposure to materials other than Cr(VI), such as asbestos or nickel;(5) unpublished data, including government reports; (6) professions such as shoemaking (non-leather) or general building work; (7) exposure to Cr(VI) in drinking water.

Quality Assessment

Each study was assigned a quality score based on the Newcastle-Ottawa assessment scale (NOS) (9), by two authors independently, to ensure the research quality. Any disagreements were discussed among the group members until consensus was reached. NOS scores range from 0 to 9; the NOS score of the studies included in this meta-analysis ranged from 6 to 8.

Data Extraction

The following data were extracted by two authors independently: first author' name, publication year, country in which the study was conducted, sex of the study population, exposure time, follow-up period, cancer incidence rate, cancer types, occupations, number of cancer-specific deaths, study design, outcome indicator, and standardized mortality ratios (SMRs) or standardized incidence ratios (SIRs) with their 95% CIs.

Statistical Analysis

The meta-SMRs and SIRs with their 95% CIs were calculated using either a fixed or random-effects model to evaluate the mortality and incidence of human cancers. The Cochran Q test and I^2 statistic were used to assess heterogeneity. Heterogeneity was considered significant with $P < 0.10$ or $I^2 > 50\%$. The

Abbreviations: SMR, standardized mortality ratio; SIR, standardized incidence ratio; CI, confidence interval; LL, lower limit; UL, upper limit; NOS, The Newcastle-Ottawa scale; Cr(VI), hexavalent chromium.

random-effects model was used for values of I^2 >50%, and the fixed-effects model was applied otherwise. Subgroup analyses were carried out according to geographical region, sex, cancer type, and profession, to screen susceptible populations and high-risk diseases. Begg's funnel plot and Egger's test were used to estimate publication bias (10, 11). Asymmetry in the funnel plot exists with a value of $P < 0.05$ in the Egger's test, indicating significant publication bias. A sensitivity analysis including more than 10 studies was conducted, to assess the individual effect of each study on the results. All statistical tests were two-sided, with $P < 0.05$ indicating statistical significance. Stata version.14.0 (Stata Corp LL, College Station, TX, USA) was used to analyze the data.

RESULTS

Study Selection and Characteristics

Of nearly 700 entries initially identified in the database search, only 512 articles were included after duplicates were removed and full texts were read carefully. The specific screening procedures are listed in the flow chart (**Figure 1**). We ultimately included 37 cohorts with SMRs reported and 16 with SIRs reported (retrospective or historical prospective) from 47 separate studies. The total number of cases was 1,141,094, ranging from 198 to 892,591 cases per study. Included studies covered 14 countries including the United States, Unite Kingdom, Finland, France, Korea, Japan, Germany, Italy, Lithuania, Sweden, Switzerland, Iceland, Denmark, and Norway. The study populations included both male and female workers who were occupationally exposed to Cr(VI), such as chromate production workers, cement industry workers, stainless steel welders, chrome platers, aircraft manufacturing workers, tanners, painters, and masons. In most studies, the exposure time to Cr(VI) was more than 1 year. Key characteristics of the included studies are summarized in **Tables 1**, **2**. All the results of subgroup analysis were shown in **Supplementary Tables 1**, **2**. Studies excluded from this meta-analysis and the reasons for exclusion are shown in **Supplementary Table 3**.

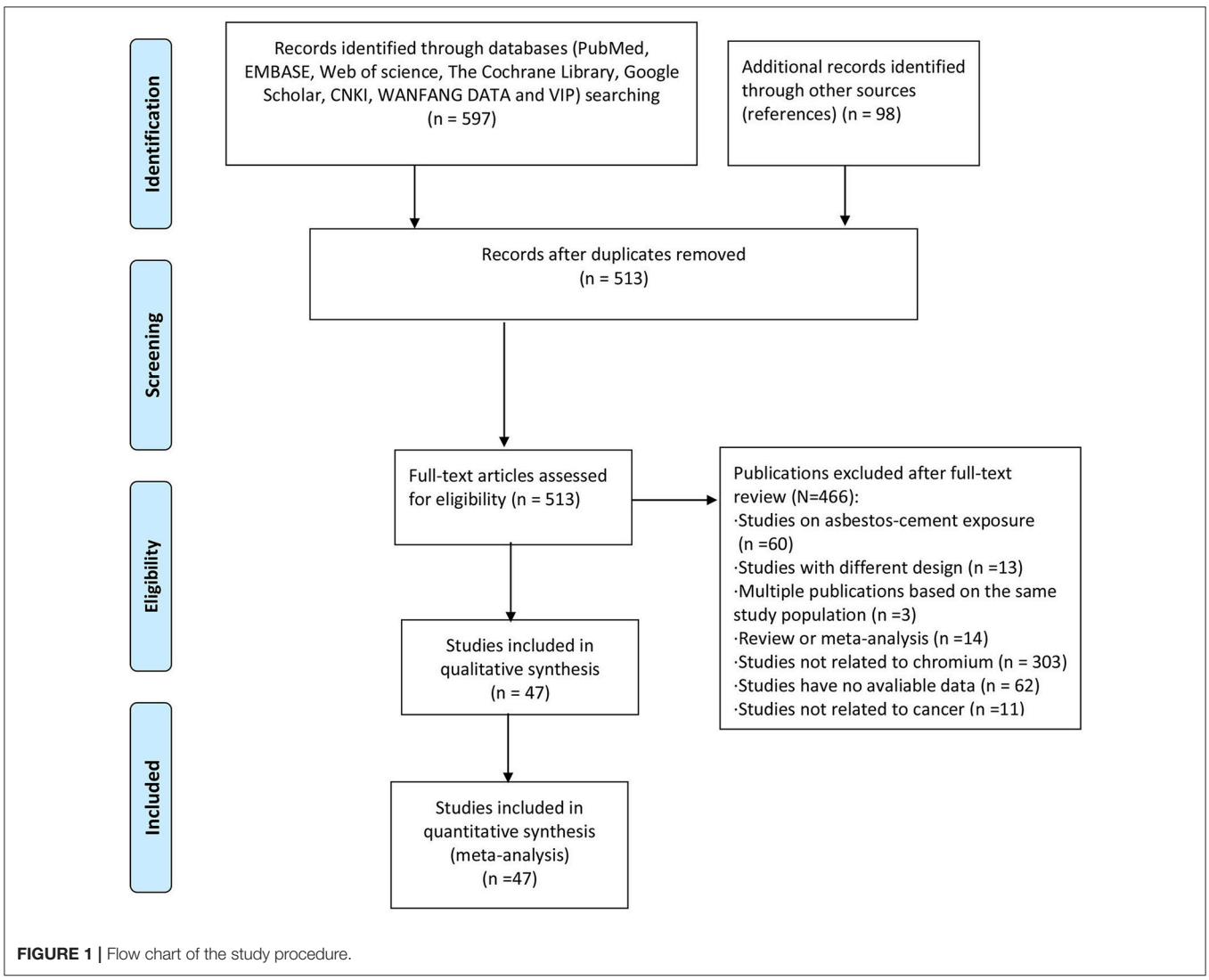

FIGURE 1 | Flow chart of the study procedure.

Cancer Mortality

There were a total of 43 SMR studies comprising 167,397 cases and 8,277 cancer deaths. The aggregate data covered all 14 countries listed above and 8 types of occupation. All studies had follow-up periods of more than 5 years. The specific follow-up information was as follows: 5- to 10-year, 11- to 20-year, 21- to 30-year, 30- to 40-year, 40- to 50-year, and more than 50-year follow-up studies accounted for 11.60%, 16.30, 14.00, 23.30, 18.60, and 16.30% of studies, respectively.

We calculated the overall cancer-specific SMR for all studies and found that significant heterogeneity existed among studies (I^2 = 85.1%, P = 0.0001, **Figure 2**). Therefore, the random-effects model was used and the meta-SMR was found to be 1.07 (95% CI: 1.01–1.15). In subgroup analysis by sex, we found that the SMR was higher for male workers than female ones (SMR = 1.14; 95% CI: 1.06–1.23). When subgroup analysis was conducted by geographical location, we found that Cr(VI) exposure was related to a higher risk of death owing to cancer in North America than in other regions (SMR = 1.19; 95% CI: 1.04–1.35). Among the various occupations, the meta-SMR was higher for chromate production workers (SMR = 1.24; 95% CI: 1.07–1.43) and chromium platers (SMR = 1.22; 95% CI: 1.10–1.34) than for other workers. However, the results differed for workers in Europe and Asia, cement production workers, aircraft manufacturing workers, tanners, welders, and chromium workers.

Respiratory System Cancers

In total, 44 cohort studies were included, with 94,089 cases; a total of 2,938 patients died of respiratory system cancers. We found that Cr(VI) exposure was correlated with a high-risk of respiratory system cancer mortality (SMR = 1.33; 95% CI: 1.19–1.48). In subgroup analysis, the meta-SMR was higher in male workers than in female workers, and higher in North America and Europe than in Asia. The meta-SMR was elevated for chromate production workers, chromium platers, and welders, but not for cement production workers, aircraft manufacturing workers or tanners.

For lung cancer, the random-effects model was used for the 44 included studies (2,805 deaths), and the meta-SMR was 1.31 (95% CI: 1.17–1.47). In subgroup analysis, the meta-SMR was significantly higher among male workers, in North America, and in Europe than in female workers and in Asia. The meta-SMRs were higher in chromate production workers, chromium platers, and welders than in cement production workers, aircraft manufacturing workers, and tanners.

For larynx cancer, the fixed-effects model was applied for 18 SMR studies (100 deaths) and the meta-SMR was 1.22 (95% CI: 0.98–1.51). The results were robust when we stratified by geographical location; however, in subgroup analysis by occupation, the meta-SMR was higher in chromate production workers than in other workers. In addition, we found that the summary SMR was increased in male workers.

Digestive System Cancers

The fixed-effects model was used (I^2 = 14.8%, P = 0.115) for 99 studies (154,688 cases, 1,833 deaths). There were no significant association between Cr(VI) and death risk of digestive system cancers, and the meta-SMR was 0.97(95% CI: 0.92–1.01). In subgroup analysis, the results were robust for esophageal cancer, stomach cancer, pancreatic cancer, hepatobiliary system cancer, intestinal cancer, colon cancer, and rectal cancer, except for rectal cancer in Europe (**Supplementary Table 1**).

Urinary System Cancers

The fixed-effects model was used (I^2 = 35%, P = 0.022) for 36 studies (92,532 cases, 170 deaths), and the meta-SMR was 1.20 (95% CI: 1.07–1.35). The meta-SMR was higher in male workers and higher in European workers than in North American workers. The meta-SMR was elevated for welders, but not for chromate production workers, cement production workers, aircraft manufacturing workers or tanners.

For bladder cancer, the fixed-effects model (I^2 = 35.9%, P = 0.076) was used for 16 studies (71,950 cases, 157 deaths), and the meta-SMR was 1.24 (95% CI: 1.05–1.47). The meta-SMR was higher in male workers, and higher in European workers than in North American workers, and higher in welders than in tanners.

For kidney cancer, the fixed-effects model (I^2 = 6.10%, P = 0.386) was used for 12 studies (62,568 cases, 87 deaths) and the meta-SMR was 1.15 (95% CI: 0.91–1.45). The results were robust when we stratified these studies by geographical location or profession (**Supplementary Table 1**). The meta-SMR was more significant in male workers than in female ones.

Lymphatic and Hematopoietic Cancers

The fixed-effects model (I^2 = 10.20%, P = 0.276) was used for 47 studies (149,026 cases and 274 deaths), and the meta-SMR was 1.03 (95% CI: 0.93–1.13). The results were robust when we stratified studies by occupation or sex (**Supplementary Table 1**). In subgroup analysis, exposed workers in Asia had highest cancer mortality. For multiple myeloma (7 studies, 34,112 cases, 46 deaths), the meta-SMR was 1.10 (95% CI: 0.80–1.50). For leukemia (16 studies, 108,957 cases, 186 deaths), the meta-SMR was 1.00 (95% CI: 0.86–1.16). The results were robust in subgroup analysis (**Supplementary Table 1**). With respect to lymphoma (19 studies, 65,956 cases, 129 deaths), meta-SMR was 1.15 (95% CI: 0.96–1.39). In addition, the meta-SMR was 1.40 (95% CI: 0.87–2.25) for Hodgkin lymphoma and was 1.07 (95% CI: 0.84–1.37) for non-Hodgkin lymphoma.

Genitourinary System Cancers

We analyzed a total of 27 studies (65,784 cases, 315 deaths) of genitourinary system cancers, and the meta-SMR was 1.04 (95% CI: 0.93–1.17). The meta-SMR for 14 studies (61,178 cases, 254 deaths) of prostate cancer was 0.99 (95% CI: 0.87–1.12). These results were robust in subgroup analysis (**Supplementary Table 1**). For breast cancer (4 studies, 20,424 cases, 31 deaths), the meta-SMR was 1.12 (95% CI: 0.76–1.65). The fixed-effects model was used (I^2 =16.90%, P = 0.307) for testicular cancer (4 studies, 23,446 cases, 13 deaths), and the meta-SMR was 2.55 (95% CI: 1.38–4.71).

TABLE 1 | Characteristics and results of SMR studies included in the meta-analysis.

Study	Country	District	Sex	No of cases	Cancer deaths (Observed)	Exposure time(year)	Follow-up time	Cancer type	Occupation	Effect measure	SMR	95% CI		NOS score
												LL	UL	
Proctor et al. (12)	USA	North America	male	714	167	>1	1940–2011, 34.4 (0.1–69.9) years, 24,535 person-years	Lung cancer	Chromate production workers	SMR	1.86	1.45	2.28	8
Proctor et al. (12)	USA	North America	male	198	37	1	1940–2011, 6,549 person-years	mixed	Chromate production workers	SMR	1.28	0.87	1.69	7
Gibb et al. (13)	USA	North America	male	2,354	460	6.4-57.2	1950–2011, 38.9 (+/−14.2) years, 91,186 person-years	mixed	Chromate production workers	SMR	1.16	1.06	1.27	8
Huvinen et al. (14)	Finland	Europe	mix	8,088	133	24.7	1971–2012, 199,760 person-years	mixed	Chromate production workers	SMR	0.88	0.73	1.03	8
Dab et al. (15)	France	Europe	mix	9,118	207	>1	1990–2005, 13.4 years, 122,124 person-years	mixed	Cement production workers	SMR	0.8	0.69	0.92	8
Koh et al. (16)	Korea	Asia	male	5,146	103	35.3(9.6)	1992–2007, 74,123 person-years	mixed	Cement production workers	SMR	0.83	0.68	1.01	7
Lipworth et al. (17)	USA	North America	mix	7,458	980	>1	1960–2008, 226,885 person-years, 31.8 years	mixed	Aircraft Manufacturing Workers	SMR	0.99	0.92	1.05	8
Hara et al. (5)	Japan	Asia	mix	1,193	97	>0.5	1976–2003, 27 years, 26,000 person-years	mixed	Chromium platers	SMR	1.05	0.86	1.27	8
Birk et al. (18)	Germany	Europe	mix	901	47	>1	1958–1998, 14,700 person-years	mixed	Chromate production workers	SMR	0.98	0.72	1.3	7
Iaia et al. (6)	Italy	Europe	mix	972	21	>1	1970–1998, 14,402 person-years	mixed	Tanners	SMR	0.66	0.44	0.95	7
Park et al. (19)	Korea	Asia	male	44,974	801	>1	1992–2001, 414,259 person-years,10 years	mixed	Welders	SMR	0.79	0.7	0.9	8
Park et al. (20)	USA	North America	male	2,357	122	3.1	1950–1992	Lung cancer	Chromium workers	SMR	1.78	1.5	2.11	6
Smailyte et al. (21)	Lithuania	Europe	male	2,498	121	>1	1978–2000, 43,490 person-years	mixed	Cement production workers	SMR	1.3	1	1.5	7
Stern et al. (22)	USA	North America	mix	9,352	548	>1	1940–1993, 265,499 person-years	mixed	Tanners	SMR	0.9	0.83	0.98	7
Luippold et al. (23)	USA	North America	mix	482	90	>1	1940–1997, 14,048 person-years	mixed	Chromate production workers	SMR	1.55	1.25	1.91	7
Steenland et al. (24)	USA	North America	male	4,459	258	>2 (8.5)	1988–1998	mixed	Welders	SMR	1.25	1.09	1.41	6
Moulin et al. (25)	France	Europe	mix	4,897	216	>1 (16.7)	1968–1992, 18.1 years, 87,247 person-years	mixed	Welders	SMR	0.97	0.85	1.11	8
Sorahan et al. (26)	UK	Europe	mix	1,087	131	>0.25	1972–1997	mixed	Chromium platers	SMR	1.35	1.12	1.62	6

(Continued)

TABLE 1 | Continued

Study	Country	District	Sex	No of cases	Cancer deaths (Observed)	Exposure time(year)	Follow-up time	Cancer type	Occupation	Effect measure	SMR	95% CI		NOS score
												LL	UL	
Boice et al. (27)	USA	North America	mix	3,634	251	>1	1960–1996, 88,224 person-years (mean 24.2 years)	mixed	Aircraft Manufacturing Workers	SMR	0.93	0.82	1.06	8
Sorahan et al. (28)	UK	Europe	male	1,762	65	>0.5	1946–1995, 48135.1 person-years	Lung cancer	Chromium platers	SMR	1.25	0.93	1.66	7
Montanaro et al. (29)	Italy	Europe	mix	1,244	123	>0.5	1955–1994,36 414 person-years	mixed	Tanners	SMR	1.12	0.94	1.34	7
Jakobsson et al. (30)	Sweden	Europe	male	727	61	>1	1952–1993,15 years	mixed	Welders	SMR	0.8	0.6	1.1	7
Fu et al. (31)	UK	Europe	mix	4,215	646	>1	1939–1991, 10,3726 person-years	mixed	Tanners	SMR	0.75	0.69	0.81	7
Fu et al. (31)	Italy	Europe	mix	2,008	127	>1	1950–1990, 54,395 person-years	mixed	Tanners	SMR	1.16	0.96	1.38	7
Mikoczy, et al. (32)	Sweden	Europe	mix	2,060	119	>1	1952–1989	mixed	Tanners	SMR	1.09	0.91	1.31	6
Sorahan, et al. (33)	UK	Europe	male	10,438	1.129	>1 (9.3)	1946–1990, 29.2 years	mixed	Welders	SMR	1.19	1.12	1.26	7
Korallus, et al. 1(34)	Germany	Europe	male	1,417	62	>1	1982–1988, 12114.5 person-years	mixed	Chromate production workers	SMR	1.18	0.91	1.51	7
Korallus et al 2(34)	Germany	Europe	male	1,417	81	>1	1982–1988, 13868.2 person-years	mixed	Chromate production workers	SMR	1.58	1.27	1.94	7
Moulin et al. (35)	France	Europe	male	2,721	60	>1 (19.5)	1975–1988, 34,131 person-years	mixed	Welders	SMR	0.93	0.71	1.2	7
Jakobsson et al. (36)	Sweden	Europe	male	2,391	97	>1	1952–1986, 52,177 person-years	mixed	Cement production workers	SMR	0.83	0.68	1.02	7
Becker et al. (37)	Germany	Europe	mix	1,213	48	>0.5	1983–1988, 31,122 person-years	mixed	Welders	SMR	1.09	0.82	1.44	7
Steenland et al. (38)	USA	North America	male	4,459	105	>2	1974–1987,8.5years	mixed	Welders	SMR	1.02	0.83	1.24	7
Simonato et al. (39)	Europe	Europe	male	11,092	303	>1	1964–1984, 164,077 person-years	mixed	Welders	SMR	1.13	1	1.26	7
Moulin et al. (40)	France	Europe	male	2,269	27	>1	1952–1982	mixed	Welders	SMR	1.01	0.67	1.48	6
Horiguchi et al. (41)	Japan	Asia	male	265	7	>1	1965–1979, 3912.1 person-years	mixed	Chromium platers	SMR	1.13	0.45	2.32	7
Costantini et al. (42)	Italy	Europe	male	2,926	85	>0.5(12.3)	1950–1983	mixed	Tanners	SMR	0.97	0.77	1.19	6
Guberan et al. (43)	Switzerland	Europe	male	1,916	96	>1	1971–1984	mixed	Chromium platers	SMR	1.27	1.07	1.51	6
Svensson et al. (44)	Sweden	Europe	male	1,164	51	>0.25	1951–1983,24,624 person-years	mixed	Welders	SMR	0.98	0.74	1.3	7
Hayes et al. (45)	UK	Europe	male	1,879	101	>1	1940–1982, 50,724 person-years	mixed	Chromium workers	SMR	0.93	0.76	1.13	7
Rafnsson et.al. 1(46)	Iceland	Europe	male	449	26	20-30	1927–1982	mixed	Masons	SMR	1.45	0.95	2.13	6
Rafnsson et al. 2(46)	Iceland	Europe	male	389	21	20	1951–1982	mixed	Masons	SMR	1.64	1.02	2.51	6
Rafnsson et al. 3(46)	Iceland	Europe	male	251	17	30	1951–1982	mixed	Masons	SMR	2.23	1.3	3.57	6
Pippard et al. (47)	UK	Europe	male	260	30	>1	1939–1982	mixed	Tanners	SMR	1	0.68	1.43	6

TABLE 2 | Characteristics and results of SIR studies included in the meta-analysis.

Study	Country	District	Sex	No of cases	Cancer incidence (Observed)	Exposure time (year)	Follow-up time	Cancer type	Occupation	Effect measure	SIR	95% CI		NOS score
												LL	UL	
Huvinen et al. (14)	Finland	Europe	Mix	8,146	408	5	1967–2011, 196,484 person-years	mixed	Welders	SIR	1.01	0.93	1.13	7
Koh et al. (16)	Korea	Asia	Male	5,596	174	33.1(9)	1992–2007, 47,233 person-years	mixed	Cement Industry Workers	SIR	1.01	0.87	1.18	6
Sorensen et al. (48)	Denmark	Europe	Mix	4,539	421	>1	1968–2003, 35 years, 125,762 person-years	mixed	Welders	SIR	1.02	0.93	1.13	8
Mikoczy et al. (49)	Sweden	Europe	Mix	2,027	351	>1	1958–1999, 56,022 person-years	mixed	Tanners	SIR	1.16	1.04	1.29	7
Smailyte et al. 1(21)	USA	North America	Mix	1,727	141	>1	1978–2000, 43,490 person-years	mixed	Cement Industry Workers	SIR	1.20	1.00	1.40	7
Smailyte et al. 2(21)	USA	North America	Mix	771	41	>1	1978–2000, 43,490 person-years	mixed	Cement Industry Workers	SIR	0.80	0.60	1.10	7
Knutsson et al. (50)	Sweden	Europe	Female	33,503	3572	>1	1971–1992,19.4 years, 582,225 person-years	mixed	Cement Industry Workers	SIR	1.07	1.03	1.10	8
Vasama-Neuvonen et al. (51)	Finland	Europe	Male	892,591	5072	>1	1971–1995, 15,481,680 person-years	Overian cancer	Chromium workers	SIR	0.70	0.40	1.20	7
Danielsen et al. (52)	Europe	Europe	Male	426	32	>10	1976–1992, 6,632 person-years	mixed	Welders	SIR	0.77	0.53	1.09	7
Rafnsson et al. (53)	Iceland	Europe	Male	1,172	148	>1	1955–1993	mixed	Cement Industry Workers	SIR	1.13	0.96	1.33	6
Jakobsson et al. (30)	Sweden	Europe	Male	719	112	>1	1958–1992	mixed	Welders	SIR	0.90	0.70	1.20	6
Danielsen et al. (54)	Norway	Europe	Mix	606	41	>1	1953–1992	mixed	Welders	SIR	1.00	0.71	1.35	6
Hansen et al. (55)	Denmark	Europe	Male	10,059	190	>1	1964–1986	mixed	Welders	SIR	0.94	0.81	1.08	6
Jakobsson et al. (36)	Sweden	Europe	Male	2,358	162	>1	1958–1986, 46,133 person-years	mixed	Cement Industry Workers	SIR	1.01	0.86	1.18	7
Simonato et al. (39)	Europe	Europe	Male	7510	363	>5	1964–1984, 98,376 person-years	mixed	Welders	SIR	1.20	1.08	1.33	7
Melkild et al. (56)	Norway	Europe	Male	783	252	>0.25	1946–1977	mixed	Welders	SIR	1.03	0.90	1.16	6
Svensson et al. (44)	Sweden	Europe	Male	1,164	84	>0.25	1958–1983, 20,936 person-years	mixed	Welders	SIR	1.03	0.82	1.27	7

Mix, Male and Female; SIR, standardized incidence ratio; CI, confidence interval; LL, lower limit; UL, upper limit; NOS, The Newcastle-Ottawa quality assessment scale; mixed: Various cancer types; Europe (in country): Denmark, England, Finland, France, Germany, Italy, Norway, Scotland, Sweden.

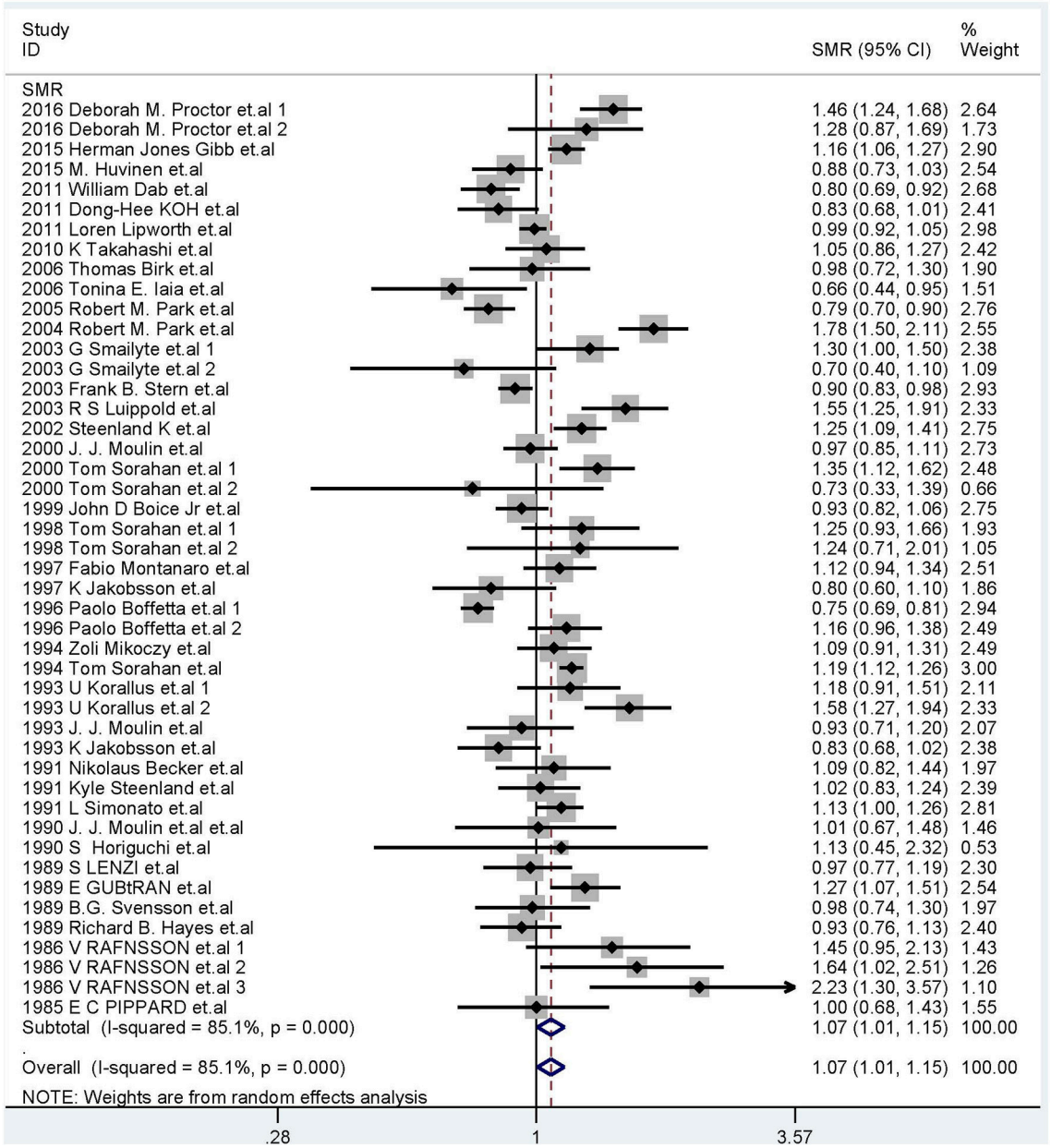

FIGURE 2 | Forest plot of studies included in this meta-analysis of hexavalent chromium with standardized mortality ratios (SMRs) of cancer.

Other Types of Cancers

For cancers of the buccal cavity and pharynx (17 studies, 58,204 cases, and 126 deaths), the meta-SMR was 0.91 (95% CI: 0.75–1.10). The results were robust in subgroup analysis (**Supplementary Table 1**). The combined SMR for skin cancer (6 studies, 30,243 cases, 29 deaths) was 0.99 (95% CI: 0.66–1.48). For melanoma, the meta-SMR was 0.90 (95% CI: 0.52–1.54). For connective and soft tissue cancer (3 studies including 20,444 cases and 11 deaths), the combined SMR was 1.22 (95% CI: 0.62–2.41). The meta-SMR for cancers of other sites (23 studies, 53,010 cases, 427 death) was 1.22 (95% CI: 0.98–1.51).

For cancers of the central nervous system, 9 studies (33,718 cases and 50 deaths) were analyzed and the meta-SMR was 1.22 (95% CI: 0.67–2.23). In subgroup analysis, the meta-SMR was highest among Asian workers. For brain cancer, the meta-SMR was 1.67 (95% CI: 0.62–4.46).

For bone cancer (5 studies, 24,568 cases, 12 deaths), the fixed-effects model was used ($I^2 = 0\%$, $P = 0.422$) and the meta-SMR was 2.06 (95% CI: 1.12–3.81). For thyroid cancer (3 studies, 19,109 cases, 8 deaths), the fixed-effects model was used ($I^2 = 31.20\%$, $P = 0.234$) and the combined SMR was 2.41 (95% CI: 1.19–4.87).

Cancer Incidence

In all, 973,697 workers were involved in 17 SIR studies, and 11,564 of these had cancer. In these studies, the aggregate data covered seven countries and four kinds of occupations. All studies had follow-up periods of more than 15 years; and the detailed data are: follow-up of 15–25 years (9 studies, 52.90%), 26–35 years (4 studies, 23.50%), and 36–45 years (4 studies, 23.50%).

The fixed-effects model was used ($I^2 = 39.4\%$, $P = 0.049$, **Figure 3**) and the combined SIR was 1.06 (95% CI: 1.04–1.09). In subgroup analysis, the summary SIR was elevated for cement industry workers, and tanners, but not for welders or chromium workers. In addition, workers exposed to Cr(VI) had high cancer incidence in Europe. For workers in North America and Asia, the summary SIRs were above 1.00, but the 95% CIs included 1.00.

Respiratory System Cancers

The fixed-effects model ($I^2 = 5.8\%$, $P = 0.375$) was used for 30 studies (22,623 cases, 1,001 cancer patients), and the meta-SIR was 1.27 (95% CI: 1.19–1.36). The meta-SIR was higher in male workers and European workers. The meta-SIR was also elevated for welders and cement production workers.

For larynx cancer (10 studies, 61,940 cases, 73 incidents), the meta-SIR was 1.14 (95% CI: 0.89–1.45). A total of 10 studies (69,883 cases, 894 incidents) of lung cancer were analyzed, and the meta-SIR was 1.28 (95% CI: 1.20–1.37) using the fixed-effects model ($I^2 = 35.2\%$, $P = 0.093$). The results were robust in subgroup analysis (**Supplementary Table 2**). For nasal cancer (3 studies, 10,956 cases, 6 incidents), the meta-SIR was 2.14 (95% CI: 0.79–5.80). The fixed-effects model was used ($I^2 = 0\%$, $P = 0.764$) for 3 studies (34,892 cases, 21 incidents) of pleural mesothelioma, and the meta-SIR was 1.73 (95% CI: 1.08–2.77).

Digestive System Cancers

The fixed-effects model was used ($I^2 = 19.3\%$, $P = 0.12$) for 51 studies (65,737 cases, 1,407 incidents) of digestive system cancer, and the meta-SIR was 1.05 (95% CI: 1.00–1.11). In subgroup analysis, there were no association between Cr(VI) and esophageal cancer (55,927 cases, 58 incidents), pancreatic cancer (8 studies, 55,248 cases, 160 incidents), colon cancer (19,899 cases, 114 incidents), rectum cancer (66,508 cases, 291 incidents), and hepatobiliary system cancer (55,391 cases, 115 incidents). All these results were robust in subgroup analysis (**Supplementary Table 2**).

The fixed-effects model was used ($I^2 = 43.30\%$, $P = 0.042$) for 14 studies (66,508 cases, 420 incidents) of stomach cancer, and the meta-SIR was 1.20 (95% CI: 1.08–1.32). In subgroup analysis, meta-SIR was elevated in male workers, European workers, and cement production workers, but not in welders. For the 14 studies (66,508 cases, 668 incidents) of bowel cancer (intestine, colon, and rectum), the meta-SIR was 1.03 (95% CI: 0.96–1.12), with the SIR being especially high in welders.

Urinary System Cancers

The fixed-effects model was used ($I^2 = 0\%$, $P = 0.6$) for 23 studies (62,986 cases, 562 incidents) of urinary system cancer, and the meta-SIR was 1.03 (95% CI: 0.95–1.13). In 9 studies of bladder cancer (60,489 cases, 360 incidents), the meta-SIR was 1.09 (95% CI: 0.98–1.22). In the 10 studies (54,757 cases, 175 incidents) of kidney cancer analyzed, the meta-SIR was 0.93 (95% CI: 0.79–1.08). These results were robust in subgroup analysis (**Supplementary Table 2**).

Genitourinary System Cancers

For the 13 studies (61,564 cases, 1,083 incidents) of male genital cancer and 13 studies (61,564 cases, 1,047 incidents) of prostate cancer, the combined SIR was 1.14 (95% CI: 1.07–1.21) and 1.15 (95% CI: 1.08–1.22), respectively, with the SIR being especially high among European workers. These results were robust when we stratified these studies by occupation (**Supplementary Table 2**).

For 8 studies (903,535 cases, 5,181 incidents) of female genital cancer, the meta-SIR was 1.02 (95% CI: 0.85–1.23). The results were robust in subgroup analysis (**Supplementary Table 2**). For breast cancer, the meta-SIR was 1.08 (95% CI: 0.84–1.38).

Lymphatic and Hematopoietic Cancer

The fixed-effects model was used ($I^2 = 0\%$, $P = 0.998$) for 24 studies (633,367 cases, 432 incidents) of lymphatic and hematopoietic cancers, and the meta-SIR was 1.10 (95% CI: 1.00–1.22). The results were robust in subgroup analysis (**Supplementary Table 2**). For multiple myeloma (5 studies, 50,444 cases, 72 incidents), the meta-SIR was 1.13 (95% CI: 0.89–1.44). In the 7 studies of leukemia (53,390 cases, 148 incidents) analyzed, the meta-SIR was 1.11 (95% CI: 0.93–1.31). For lymphoma, the combined SIR of 5 studies (88,894 cases, 173 incidents) was 1.12 (95% CI: 0.96–1.30). In addition, the meta-SIR was 0.99 (95% CI: 0.65–1.52) for Hodgkin lymphoma, and 1.14 (95% CI: 0.97–1.34) for non-Hodgkin lymphoma.

Oral Cancers

The fixed-effects model was used ($I^2 = 4.3\%$, $P = 0.404$) for 16 studies (59,537 cases, 163 incidents) of oral cancer (buccal cavity and pharynx), and the meta-SIR was 1.30 (95% CI: 1.11–1.54), with the SIR being especially high in male workers, Europeans and welders (**Supplementary Table 2**).

Cancer of Other Sites

The meta-SIR for 4 studies (48,417 cases, 29 incidents) of thyroid cancer was 0.81 (95% CI: 0.54–1.21). Three studies of brain cancer (42,821 cases, 134 incidents) were analyzed, and the meta-SIR was 1.04 (95% CI: 0.87–1.24). The combined SIR for 13 studies (55,880 cases, 432 incidents) of skin cancer was 1.02 (95% CI: 0.95–1.10). For melanoma, 3 studies (43,802 cases, 117 incidents) were analyzed, and the meta-SIR was 0.92 (95% CI: 0.77–1.11). The meta-SIR for soft tissue cancer (3 studies, 43,676 cases, 35 incidents) was 1.20 (95% CI: 0.84–1.71). The meta-SIR of other cancers (5 studies, 43,511 cases, 180 incidents) was 1.09 (95% CI: 0.94–1.26).

Exposure Level of Cr(VI) With Incidence and Mortality Risk of Cancers

As shown in **Table 3**, in the subgroup analysis of cumulative hexavalent exposure (mg Cr(VI)/m^3-years), the higher the

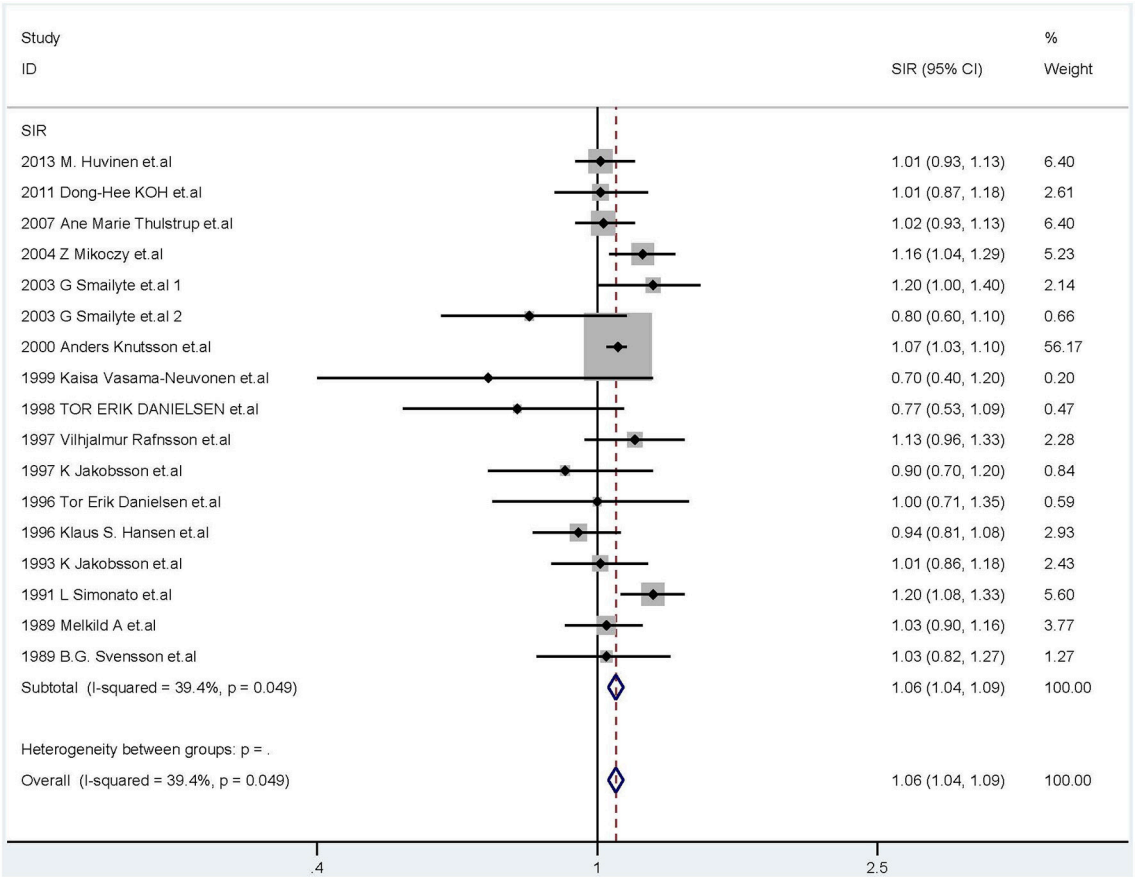

FIGURE 3 | Forest plot of studies included in this meta-analysis of hexavalent chromium with standardized incidence ratios (SIRs) of cancer.

concentration of Cr(VI) in the air, the higher the risk of cancer death for workers. When the concentration is more than 1 mg $Cr(VI)/m^3$, there is a significant increase of risk of cancer death. As for time since first exposure to Cr(VI), an elevated death risk of cancer was observed, especially for lung cancer.

AN interesting finding is that a slight increase of death risk of cancer was observed in workers less 10 years, when the subgroup analysis of duration of employment about Cr(VI) was conducted. Further analysis revealed that long duration of exposure to Cr(VI) was associated with elevated death risk for lung cancer, but not for colorectal cancer. In SIR studies, the duration of employment was related to increased cancer risk, especially when workers were employed more than 15 years.

Publication Bias

Begg's funnel plot and Egger's test were used to investigate publication bias. We found no evidence of asymmetry in the funnel plot of all studies combined (**Figure 4**), or in the funnel plots for each subgroup analysis (**Supplementary Table 2**). The results of the Egger's (SMR: $P = 0.36$; SIR: $P = 0.09$) and Begg's tests (SMR: $P = 0.78$; SIR: $P = 0.14$) also showed no significant evidence of publication bias.

Sensitivity Analysis

Each study was individually eliminated to assess the effect of individual studies on the results. The sensitive analysis (**Figure 5**) showed that the results were significantly influenced by two studies (31, 33). Therefore, the meta-SMR was calculated after excluding these two studies, resulting in a slightly higher meta-SMR (1.08 vs. 1.07). As shown in **Figure 6**, there was no significant change in the merged SIRs, which indicated the stability of results.

DISCUSSION

Summary of the Main Findings

In this meta-analysis, we identified 47 studies specifically investigating cancer mortality or incidence in relation to Cr(VI) exposure among workers. Overall, our meta-analysis provided evidence that Cr(VI) might cause cancers of the respiratory system, buccal cavity and pharynx, prostate, and stomach in humans. This findings differ from the conclusions of Donato et al. (57), who suggested that Cr(VI) was related to increased risk of cancer incidence of the lung, larynx, bladder, kidney, testis, thyroid, and bone. In addition, the incidence and death risk of

TABLE 3 | The results of the association between different exposure level of Cr(VI) and incidence and mortality of human cancers.

Exposure level assessment	Number of study	Cancer type	Effect model	Heterogeneity		Outcome	Effect value	LL	UL
				I^2 (%)	*P*-Value				
CUMULATIVE HEXAVALENT EXPOSURE (MG CR(VI)/m^3-YEARS)									
<0.5	3	All	Fixed	0.00	0.41	SMR	1.35	0.81	2.25
0.5–1	2	All	Fixed	0.00	0.33	SMR	1.50	0.91	2.48
>1	2	All	Fixed	0.00	0.51	SMR	4.17	2.95	5.90
Total	7	All	Random	71.60	0.01	SMR	2.48	1.93	3.18
TIME SINCE FIRST EXPOSURE (YEARS)									
< 20	17	All	Random	50.10	0.01	SMR	1.42	1.18	1.71
≥ 20	19	All	Fixed	10.30	0.33	SMR	1.55	1.27	1.89
Total	36	All	Fixed	36.20	0.02	SMR	1.53	1.38	1.71
< 20	14	Lung cancer	Fixed	0.00	0.55	SMR	1.52	1.24	1.85
≥ 20	15	Lung cancer	Fixed	49.80	0.02	SMR	1.57	1.36	1.81
Total	29	Lung cancer	Fixed	29.50	0.07	SMR	1.55	1.38	1.74
DURATION OF EMPLOYMENT (YEARS)									
1–10	20	All	Fixed	47.70	0.02	SMR	1.11	1.05	1.18
11–20	5	All	Fixed	20.10	0.29	SMR	0.98	0.92	1.05
≥21	12	All	Random	90.30	0.01	SMR	0.92	0.87	0.96
Total	37	All	Random	80.3	0.01	SMR	0.99	0.96	1.02
1–10	18	Lung cancer	Fixed	0.01	0.48	SMR	1.32	1.21	1.43
11–20	4	Lung cancer	Fixed	0.01	0.58	SMR	1.09	0.97	1.23
≥21	10	Lung cancer	Random	91.80	0.01	SMR	1.43	1.01	2.02
Total	32	Lung cancer	Random	80.50	0.01	SMR	1.40	1.21	1.63
1–10	2	Colorectal cancer	Fixed	0.01	0.91	SMR	1.08	0.87	1.34
11–20	2	Colorectal cancer	Fixed	49.70	0.16	SMR	1.00	0.79	1.27
≥21	3	Colorectal cancer	Random	56.10	0.10	SMR	1.03	0.89	1.19
Total	7	Colorectal cancer	Fixed	11.70	0.34	SMR	1.04	0.93	1.16
1–14	6	All	Fixed	0.00	0.88	SIR	1.13	0.90	1.40
≥15	4	All	Fixed	0.00	0.30	SIR	1.34	1.13	1.60
Total	10	All	Fixed	0.00	0.64	SIR	1.25	1.09	1.44

cancer was associated significantly with concentration of Cr(VI) in the air and the exposure time.

The overall cancer mortality risk of 1.07 indicated that workers exposed to Cr(VI) had a higher risk of cancer death compared with their age-matched and sex-matched counterparts in the general population. For lung cancer, a significant relationship existed between exposure to Cr(VI) and cancer death. This result was consistent with a previous meta-analysis conducted by Cole et al. that included 49 SMR epidemiological studies and showed that Cr(VI) was a weak cause of lung cancer (58). Moreover, we found that Cr(VI) was related to a higher risk of larynx cancer mortality, especially among chromate production workers. The meta-analysis did not find evidence for increased risk of death due to digestive system cancers, which was accordant with a formal meta-analysis performed by Proctor et al. Those authors analyzed 32 SMR studies and found that workers exposed to Cr(VI) were not at a higher risk of death owing to gastrointestinal tract cancer (59). However, our analysis of 14 studies showed that the meta-SMR was high for rectal cancer in Europe; these results must be confirmed in further studies.

A major finding here was that for workers exposed to Cr(VI), the mortality risk of urinary system cancer and bladder cancer was high. Among male workers, the meta-SMR of kidney cancer was high. In addition, the present meta-analysis provided additional evidence for elevated risk of death owing to testicular, bone, and thyroid cancers among workers exposed to Cr(VI), however, additional epidemiological evidence is needed to verify the accuracy of these results. However, the associations between exposure to Cr(VI) and the high mortality of some cancers were not significant, like those of breast, lymphatic, and hematopoietic, buccal cavity and pharynx, central nervous system, skin, and connective and soft tissue cancers.

The summary SIR of 1.06 (95% CI: 1.04–1.09) provided evidence for increased risk of cancer among workers exposed to Cr(VI), as compared with the general population. The narrow CI, which excludes 1.0, and the low I^2 value indicate that this result was not due to chance, to a large extent. We also performed subgroup analysis by sex, occupation, cancer type, and geographical location to explore more specific relationships. The results were robust for cement industry workers and tanners,

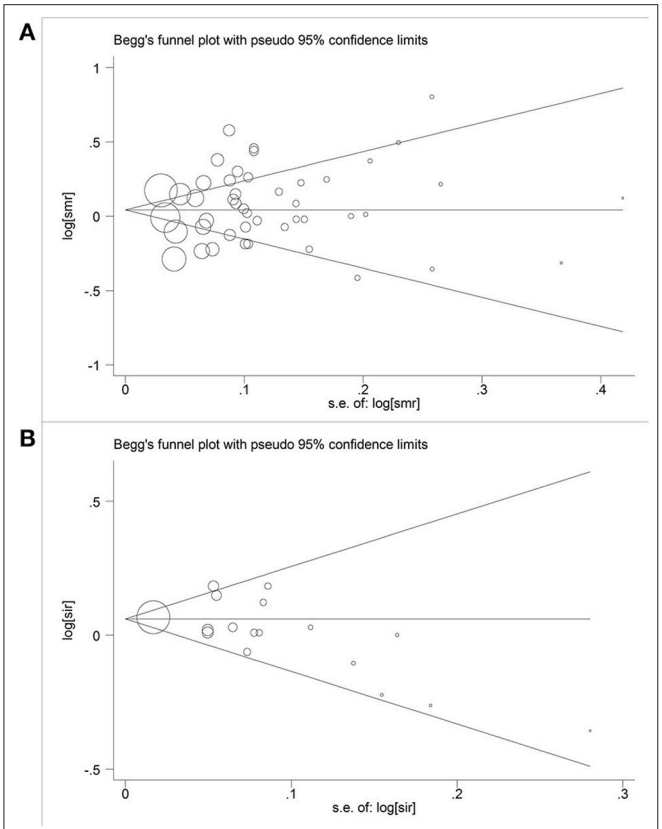

FIGURE 4 | Begger's funnel plot of studies included in this meta-analysis of hexavalent chromium with cancer mortality and morbidity. **(A)** Begger's funnel plot of hexavalent chromium and SMR; **(B)** Begger's funnel plot of hexavalent chromium and SIR.

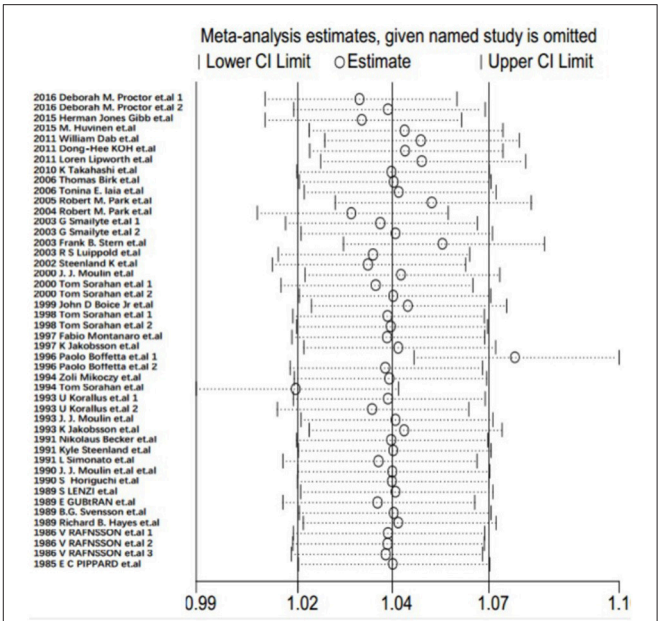

FIGURE 5 | Results of sensitivity analysis for SMRs.

especially in Europe. For studies in North America and Asia, the summary SIR was above 1.00, but the 95% CI included 1.00. For respiratory system cancers, the meta-SIR was elevated, especially in male workers, European workers and welders. The associations were strong and robust for lung cancer and pleural mesothelioma, but not for larynx cancer and nasal cancers. Kim et al. suggested that there was no strong evidence of an association of Cr(VI) with nasal and paranasal cancers (60). However, in 2015, Binazzi et al. conducted a meta-analysis of risk ratios in 28 studies (11 cohort, 17 case-control) and suggested that sinonasal cancer was associated with exposure to nickel and chromium compounds (61). Therefore, more studies of these cancer types are urgently needed.

A novel finding was that workers exposed to Cr(VI) were at risk of oral cancer. Yuan et al. suggested that nickel and chromium play a role in oral cancer (62). The present meta-analysis showed that the risk for genital cancers, such as prostate cancer, was elevated in male workers. With respect to the digestive system, the summary SIR was elevated among exposed workers only for stomach cancer and, not for esophageal, pancreatic, intestinal, colon, rectal, or hepatobiliary system cancers. This finding is in agreement with the results of the meta-analysis performed by Welling et al. (4), as previously described. These results were also consistent with the conclusion of a previous meta-analysis of pancreatic cancer by Ojajarvi et al. Those authors analyzed 92 studies on occupational exposures and found that the meta-risk ratio for chromium was 1.40 (95% CI: 0.90–2.30) (63); the established causal relationship between exposure to Cr(VI) and lymphatic and hematopoietic cancer was found to be weak.

The cohort studies included here were carried out during different time periods, which may account in part for the heterogeneity, even though all follow up periods of the included studies were more than 5 years. However, the distribution of the six follow-up periods in the included SMR studies was well-proportioned, so the heterogeneity may be alleviated to a certain extent. The proportion of occupation types included in the analysis varied: chromate production workers, welders, and tanners comprised nearly 20% each, whereas, other occupations comprised 5–10%. The varying exposure times may be another source of heterogeneity, even though the exposure times of the included cohorts were mostly over 1 year. To some extent, the study populations included in the meta-analysis, which were from different geographical regions, may cause bias and heterogeneity. The SMR is a reflection of relative ratio and depends on the adjustment of confounders in the study and reference populations. Most studies in this meta-analysis included adjustment for confounding factors, such as age and sex.

There is some evidence that clarifies the carcinogenesis of Cr(VI) exposure. In 2014, Ovesen declared that long-term exposure to low-concentrations of Cr(VI) could induce DNA damage (64). Wang et al. found that chronic Cr(VI) exposure is associated with epigenetic dysregulation via an increase in the related histone-lysing methyltransferases expression which

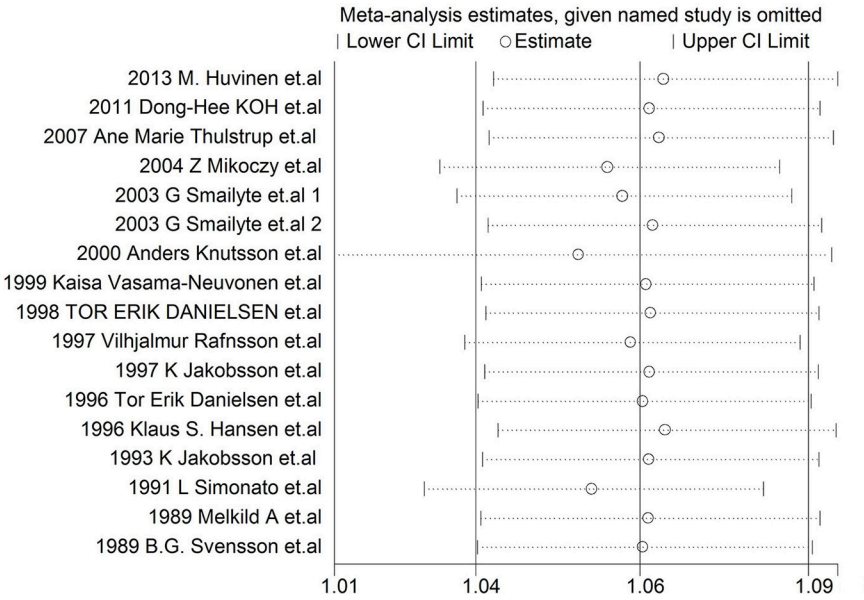

FIGURE 6 | Results of sensitivity analysis for SIRs.

plays an essential role in Cr(VI)-induced cancer stem cell-like property and cell transformation (65). Another study confirmed that Cr(VI) can form protein-Cr-DNA adducts and silence tumor suppressor genes, as well as disrupt CTCF binding and nucleosome spacing (66). In addition, Clementino stated that Cr(VI)is related to oxidative stress and metabolic reprogramming, which contribute to tumorigenesis by participating in enhancement of the anti-apoptosis ability and rapid proliferation of cells (67). Therefore, additional high-qualify studies are needed to further explore the carcinogenesis of Cr(VI) exposure.

Limitations

There are some limitations in the present meta-analysis. Although we used the Begg's funnel plot and Egger's test and found no publication bias, slight publication bias is unavoidable. Owing to the occupational specificity and sex limitations, the study population comprised mostly male workers and specific sex-related SMRs were lacking. Therefore, the finding that high cancer deaths existed among male workers is acceptable. In addition, in a cohort study from 1971 to 1986 among 33,503 concrete workers, Knutsson et al. found that risk of cancer was high in female concrete workers (SIR = 1.17; 95% CI: 1.03–1.10) (50); further evidence is needed to verify this relationship. It should be noted that all studies included in this analysis comprised adults rather than children as the study population, as well as workers exposed to Cr(VI) rather than the general population; therefore, the results should not be generalized or applied to populations other than those who are exposed to Cr(VI). An additional limitation was that the included cohorts were from Europe, North America and Asia, with no available reports from other geographical areas available. For SMR studies, 70% were from Europe, 22% from North America, and 8% from Asia. For SIR studies, 82% were from Europe, 12% from North America, and 6% from Asia. Therefore, the estimates are largely dominated by the European cohorts. Nevertheless, these cohorts may provide more accurate and consistent baseline data compared with others owing to their large sample size and similar geographical conditions.

CONCLUSIONS

In summary, our meta-analysis provides evidence to support the association between exposure to Cr(VI) and increased mortality and incidence of some cancers. Based on our results, Cr(VI) exposure is related to a high-risk of death owing to lung, larynx, bladder, kidney, testicular, bone, and thyroid cancer. In addition, Cr(VI) exposed workers are at elevated risk of cancers of the respiratory system, buccal cavity, pharynx, prostate, and stomach. As with all meta-analyses, publication bias, and heterogeneity cannot be entirely eliminated. These findings require the support of well-designed cohort studies which are capable of addressing the problem of accurate measurement of exposure dose and time and potential confounders in the relationship between exposure to Cr(VI) and cancer.

AUTHOR CONTRIBUTIONS

All authors read, critically reviewed, and approved the final manuscript. YD and MW conducted the database searches, screened titles, abstracts and full-texts for eligibility, performed study quality assessments. ZD and GZ planned and designed the research. CD and PX provided methodological support, advice.

SL tested the feasibility of the study. QH, ZZ, YZ, YW, and QH extract data. LZ performed the statistical analysis. YD and TT wrote the manuscript.

FUNDING

This study was supported by National Natural Science Foundation of China (No. 81471670); the International Cooperative Project of Shaanxi Province, China (No. 2016KW-008); and the Key Research and Development Plan, Shaanxi Province, China (2017ZDXM-SF-066).

ACKNOWLEDGMENTS

We thank all members of our study team for their whole-hearted cooperation and the original authors of the included studies for their wonderful results.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2019.00024/full#supplementary-material

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Oncology

ORIGINAL RESEARCH
published: 21 February 2019
doi: 10.3389/fonc.2019.00098

Trends in and Predictions of Colorectal Cancer Incidence and Mortality in China From 1990 to 2025

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Colorectal cancer (CRC) has emerged as a major public health concern in China during the last decade. In this study, we investigated the disease burden posed by CRC and analyzed temporal trends in CRC incidence and mortality rates in this country. We collected CRC incidence data from the Cancer Incidence in Five Continents, Volume XI dataset and the age-standardized incidence rate (ASIR) and age-standardized mortality rate (ASMR) of CRC by sex and age, from the 2016 Global Burden of Diseases Study. We used the average annual percentage change (AAPC) to quantify temporal trends in CRC incidence and mortality from 1990 to 2016 and found the ASIR of CRC increased from 14.25 per 100,000 in 1990 to 25.27 per 100,000 in 2016 (AAPC = 2.34, 95% confidence interval [CI] 2.29, 2.39). Cancer cases increased from 104.3 thousand to 392.8 thousand during the same period. The ASIR increased by 2.76% (95% CI 2.66%, 2.85%) and 1.70% (95% CI 1.64%, 1.76%) per year in males and females, respectively. The highest AAPC was found in people aged 15–49 years (2.76, 95% CI 2.59, 2.94). Cancer deaths increased from 81.1 thousand in 1990 to 167.1 thousand in 2016, while the ASMR remained stable (−0.04, 95% CI −0.13, 0.05), A mild increase (AAPC = 0.42, 95% CI 0.34, 0.51) was found among males and a significant decrease (AAPC = −0.75, 95% CI −0.90, −0.60) was found among females. Between 2016 and 2025, cancer cases and deaths are expected to increase from 392.8 and 167.1 thousand in 2016 to 642.3 (95% CI 498.4, 732.1) and 221.1 thousand (95% CI 122.5, 314.8) in 2025, respectively. Our study showed a steady increase in the CRC incidence in China over the past three decades and predicted a further increase in the near future. To combat this health concern, the prevention and management of known risk factors should be promoted through national polices. Greater priority should be given to CRC prevention in younger adults, and CRC screening should be widely adopted for this population.

Keywords: colorectal cancer, incidence, mortality, prediction, China

OPEN ACCESS

Edited by:
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Reviewed by:
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Specialty section:
This article was submitted to Cancer Epidemiology and Prevention, a section of the journal Frontiers in Oncology

Received: *07 November 2018*
Accepted: *04 February 2019*
Published: *21 February 2019*

Citation:
Zhang L, Cao F, Zhang G, Shi L, Chen S, Zhang Z, Zhi W and Ma T (2019) Trends in and Predictions of Colorectal Cancer Incidence and Mortality in China From 1990 to 2025. Front. Oncol. 9:98. doi: 10.3389/fonc.2019.00098

INTRODUCTION

Colorectal cancer (CRC) is a common diagnosed malignant neoplasm, which ranks third among all cancers in terms of incidence and second in terms of mortality (1). In 2018, nearly 2.0 million newly diagnosed CRC cases and more than 0.8 million related deaths are expected to occur worldwide (1). CRC incidence rates vary substantially across the world, with the highest rates observed in parts

of Europe (e.g., Slovakia, the Netherlands, Norway, and Hungary), in which the age-standardized incidence rates (ASIRs) have been as high as 60 per 100,000 for males and 35 per 100,000 for females (2), but CRC ASIRs in Asia have been two to three-fold lower than that in Europe (2, 3). The striking disparity in the global incidence of CRC reflects the strong impact of lifestyle factors on the occurrence of this cancer (4). Moreover, variations in genetic background between different populations, such as single nucleotide polymorphisms in certain genes, have been hypothesized to be associated with CRC genesis (5–7). CRC incidence and mortality rates have been stabilizing or decreasing in highly developed countries through the enormous efforts over the last decades (8). However, a rapid upward trend has been seen in many low-income and middle-income countries (4).

China has been the world's fastest-growing developing country for the past four decades. Urbanization, aging population, and shift to sedentary lifestyle and Westernized diet has led to a shift in the disease burden from infectious to non-communicable diseases (9). One of the major public health issues is the rapid rise in CRC incidence and the accompanying increase in disease burden (10, 11). According to cancer statistics for 2010 (12), ∼274.8 thousand new CRC cases and 132.1 thousand CRC related deaths were estimated to occur in China, accounting for nearly one tenth of the global CRC burden. Knowing the epidemiological features of CRC, including its temporal trends and geographical patterns in China, therefore, is critical for both the prevention and management of cancer in this country.

This study used the CRC incidence and mortality data of China from the Global Burden of Diseases (GBD) 2016 Study and the data from the Cancer Incidence in Five Continents Volume XI of the International Agency for Research on Cancer. Both databases provide us with a unique opportunity to understand the CRC landscape in China. We described the contemporary geographical distribution of CRC incidence and assessed the temporal trends in its incidence and mortality from 1990 to 2016. Moreover, we conducted a forecasted CRC incidence and mortality up to 2025. The results yielded by our study should be useful for the assessment of current prevention strategies, to enhance our understanding of and planning to manage the disease burden, and to promote the evolution of China's Health System to respond to future challenges.

MATERIALS AND METHODS

Study Data

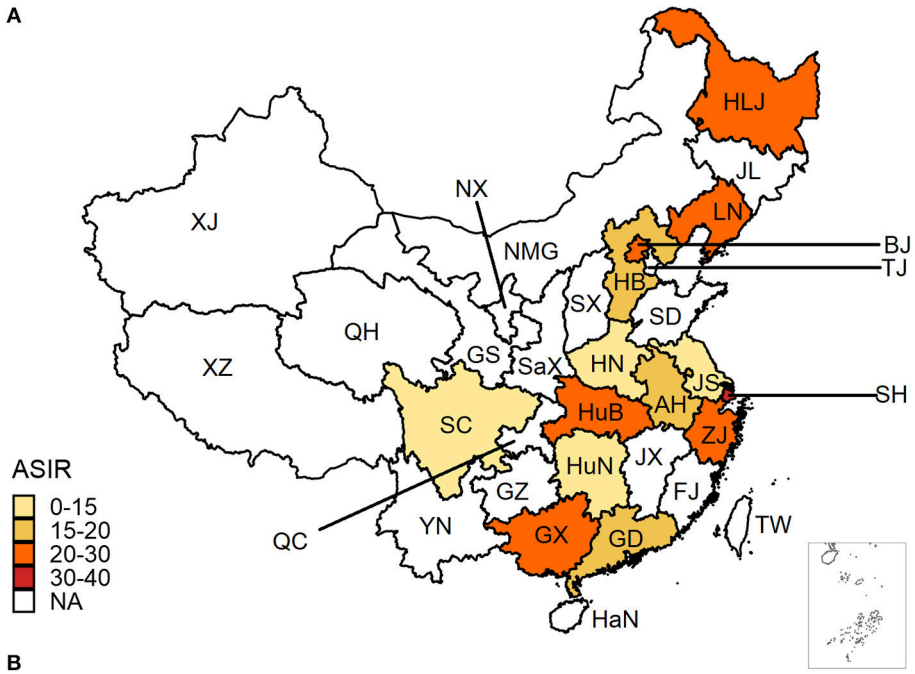
We collected CRC incidence data from the Cancer Incidence in Five Continents Volume XI (CI5XI) dataset (13), which contains the average annual incidence of cancer (2008–2012) by sex, site, and age-group per 100,000 population from 36 cancer registries in China (**Figure 1B**, the table embedded in the **Figure 1**). The codes from 10th revision of the International Statistical Classification of Diseases and Related Health Problems (2010) corresponding to CRC (colon cancer: C18; rectal cancer: C19-20) were used to identify cancer cases. We combined colon cancer and rectal cancer to estimate the overall ASIR of CRC in different regions in China. We merged the data and provided a summary estimate for the provinces with several cancer registries. The ASIRs of CRC were plotted on a national map. We retrieved annual incident cases, ASIRs, mortality, age-standardized mortality rates (ASMRs), and Disability-Adjusted Life Years (DALYs), Years Lived with Disability (YLDs), and Years with Life Lost (YLLs) of CRC from 1990 to 2016, by sex and age group from the Global Health Data Exchange, a comprehensive online catalog of the GBD data (http://ghdx.healthdata.org/gbd-results-tool) (14). The cancer incidence was sought from individual cancer registries or aggregated databases of cancer registries, including Cancer Incidence in Five Continents (CI5) (15) and SEER (Surveillance, Epidemiology, and End Results) (16). Like data in CI5XI database, all cancer data stored in GBD were stratified by cancer site, country or region, age group, sex, and time. The general methods used in the 2016 GBD Study and the methods for estimating the disease burden of CRC have been detailed in previous studies (17). We matched all neoplasms as defined in the International Statistical Classification of Diseases and Related Health Problems to one of the 29 GBD cancer groups (18, 19). In the GBD Study, 95% uncertainty intervals (95% UI) were calculated to provide information on the variability of estimates resulting from errors related to the sampling process, and to non-sampling errors related to adjustments to data sources and modeling (18).

Statistical Analysis

The Standard World Population 2000 was used to estimate ASIRs of CRC per 100,000 person years for all regions. We used the average annual percentage change (AAPC) to quantify temporal trends in CRC incidence and mortality from 1990 to 2016 (20). A regression line was fitted to the natural logarithm of the rates, i.e., $y = \alpha + \beta x + \varepsilon$, where y = ln(ASIR or ASMR), and x = calendar year. The AAPC was calculated as $100 \times (exp(\beta)-1)$. The 95% confidence interval (CI) of the AAPC was also calculated in the regression model. The ASIR (ASMR) showed an upward trend when the AAPC estimation and the lower boundary of its 95% CI were both > 0. In contrast, the ASIR (ASMR) showed a downward trend when the AAPC estimation and the upper boundary of its 95% CI were both < 0. Otherwise, the ASIR (ASMR) was deemed to be stable over time (21). For a clearer depiction of the temporal trend of the ASIR, we divided the study period into two discrete timeframes (1990–2010 and 2011–2016) and assessed the ASIR trend for each timeframe. The study period for the ASMR was also separated into two discrete periods (1990–2002 and 2003–2016), and the AAPC for each timeframe was also assessed separately. Furthermore, we predicted the numbers of cases and deaths from CRC between 2017 and 2025 by conducting a Bayesian age-period-cohort analysis using integrated nested Laplace approximations, which has been well-documented and validated in other studies (22, 23). All statistical analyses were performed using the R program (Version 3.4.1, R core team). The Bayesian age-period-cohort

Abbreviations: CRC, colorectal cancer; ASIR, age-standardized incidence rate; ASMR, age-standardized mortality rate; AAPC, average annual percentage change; GBD, global burden of diseases.

A



B

Province	Total	Registry
Anhui	3	Hefei, Maanshan, Tongling
Beijing	1	Beijing
Guangdong	4	Guangzhou, Jiangmen, Zhongshan, Zhuhai
Guangxi	1	Liuzhou
Hebei	2	Cixian, Shexian
Henan	3	Linzhou, Xiping, Yanshi
Heilongjiang	1	Nangang District (Haerbin City)
Hubei	1	Wuhan
Hunan	2	Hengdong, Yueyanglou District
Jiangsu	8	Guanyun, Haimen, Huaiyin, Jianhu, Lianyungang, Qidong, Sheyang, Wuxi
Liaoning	3	Anshan, Benxi, Shenyang
Shanghai	1	Shanghai
Sichuan	1	Yanting
Hong Kong	1	Hong Kong
Zhejiang	4	Hangzhou, Jiashan, Jiaxing, Xianju

FIGURE 1 | (A) The geographical distribution of the age-standardized incidence rate (ASIR) per 100,000 colorectal cancer cases in China. The deeper the color, the higher the rate. The white areas indicate no data were mapped. **(B)** The cancer registries included in this analysis. (BJ, Beijing; TJ, Tianjin; HB, Hebei; NMG, Inner Mongolia; SX, Shanxi; HLJ, Heilongjiang; JL, Jilin; LN, Liaoning; SD, Shandong; JS, Jiangsu; SH, Shanghai; ZJ, Zhejiang; AH, Anhui; FJ, Fujian; JX, Jiangxi; GD, Guangdong; GX, Guangxi; HaN, Hainan; YN, Yunnan; GZ, Guizhou; XZ, Tibet; SC, Sichuan; QC, Chongqing; HuN, Hunan; HuB, Hubei; HN, Henan; SaX, Shaanxi; GS, Gansu; NX, Ningxia; QH, Qinghai; XJ, Xinjiang).

model was analyzed using the BAPC (Bayesian age-period-cohort) and INLA (integrated nested Laplace approximation) packages in R. A $P < 0.05$ was considered statistically significant.

RESULTS

The CRC incidence data retrieved from the 36 cancer registries in 15 provinces were included in the study (**Figure 1**). The combined CRC ASIRs from 2008 to 2012 showed significant variation across the country, with the highest ASIR observed in Hong Kong (39.97 per 100,000), followed by Shanghai (32.30 per 100,000), Liaoning (29.62 per 100,000), and Zhejiang (24.30 per 100,000). At the national level, the ASIR of CRC increased from 14.25 (95% UI 13.75, 14.93) per 100,000 in 1990 to 25.27 (95% UI 24.02, 26.47) per 100,000 in 2016, with an overall AAPC of 2.34 (95% CI 2.29, 2.39). Cancer cases increased nearly three-fold from 104.3 thousand (95% UI 100.6, 109.4) to 392.8 thousand (95% UI 373.0, 412.4) during the same period (**Table 1**). CRC cases and ASIRs were significantly higher in males than females. Among males, the ASIR increased by 2.76% (95% CI 2.66, 2.85) per year from 1990 to 2016, and the cancer cases increased more than three times compared to 1990. Among females, the ASIR of CRC increased from 12.43 per 100,000 to 18.83 per 100,000, with an AAPC of 1.70 (95% CI 1.64, 1.76). Cancer cases among females increased from 46.9 thousand to 148.9 thousand during the study period (**Table 1**). The specific timeframes of the AAPCs

in the ASIRs are presented in **Figure 2A**. In general, the CRC ASIRs of both males and females rapidly increased from 1990 to 2010; thereafter, the rising trends slowed. We also examined the CRC ASIRs among the three age groups. The highest ASIR was observed among individuals aged 50–69 years in terms of number of cancer cases and in those aged 70+ years in terms of the ASIR. However, the highest AAPC was found in people aged 15–49 years (2.76, 95% CI 2.59, 2.94) (**Table 1**).

Table 2 presents the ASMR and cancer deaths from CRC in 1990 and 2016. Overall, cancer deaths increased from 81.1 thousand in 1990 to 167.1 thousand in 2016, whereas the ASMR remained stable (AAPC = −0.04, 95% CI −0.13, 0.05) during the same period. For males, a mild increase (AAPC = 0.42, 95% CI 0.34, 0.51) was observed in the ASMR during 1990-2016. In contrast, a significant decrease (AAPC = −0.75, 95% CI −0.90, −0.60) was found in females. The AAPC in the CRC ASMR was time dependent. As shown in **Figure 2B**, an increase in the overall ASMR was observed from 1990 to 2002, whereas the ASMR decreased thereafter (AAPC = −0.43, 95% CI −0.56, −0.31). The ASMR increased among males by 0.46 per year from 1990 to 2002, and the rising trend slowed from 2003 to 2016. Among females, a stable trend in the ASMR was found between 1990 and 2002. However, the ASMR showed a downward trend after 2002. The significant increase was detected only among people aged 70+ years (AAPC = 0.51, 95% CI 0.38, 0.63) (**Table 2**). We also retrieved the data on risk factors (including tobacco use, metabolic risks, high body-mass index [BMI], diet high in red meat, and diet high in processed meat) for CRC mortality during the study period. The temporal trends in the risk factors contribution for CRC mortality were estimated (**Figure 3**). Among the five well-established risk factors, the most significant contribution was tobacco use, followed by metabolic

TABLE 1 | The cancer cases and age standardized incidence rate of colorectal cancer in China in 1990 and 2016.

	1990		2016		1990–2016	
	Cancer cases No. × 10^3 (95% UI)	ASIR (/1e5) No. × 10^3 (95% UI)	Cancer cases No. × 10^3 (95% UI)	ASIR (/1e5) No. × 10^3 (95% UI)	Change in cancer cases (%)	AAPC in ASIR No. (95% CI)
Overall	104.3 (100.6, 109.4)	14.25 (13.75, 14.93)	392.8 (373.0, 412.4)	25.27 (24.02, 26.47)	276.6 (257.4, 289.3)	2.34 (2.29, 2.39)
SEX						
Male	57.5 (55.3, 59.7)	16.44 (15.81, 17.07)	243.9 (234.4, 253.1)	32.16 (31.00, 33.33)	324.2 (300.8, 350.1)	2.76 (2.66, 2.85)
Female	46.9 (44.4, 50.7)	12.43 (11.78, 13.42)	148.9 (135.8, 162.7)	18.83 (17.16, 20.52)	217.5 (203.2, 234.3)	1.70 (1.64, 1.76)
AGE (YEARS)						
5–14	0	0	0	0	–	–
15–49	21.6 (20.6, 22.7)	3.35 (3.19, 3.53)	54.6 (51.0, 59.3)	7.32 (6.84, 7.95)	152.3 (129.4, 171.4)	2.76 (2.59, 2.94)
50–69	49.5 (47.5, 52.2)	34.33 (32.98, 36.18)	201.2 (190.7, 212.5)	62.71 (59.42, 66.22)	306.5 (278.6, 332.4)	2.34 (2.21, 2.47)
70+	33.2 (31.9, 34.9)	87.84 (84.30, 92.32)	137.0 (130.2, 143.3)	163.3 (155.3, 170.9)	312.7 (297.4, 340.8)	2.57 (2.50, 2.64)

UI, uncertainty interval; ASIR, age standardized incidence rate; AAPC, average annual percentage change; CI, confidence interval.

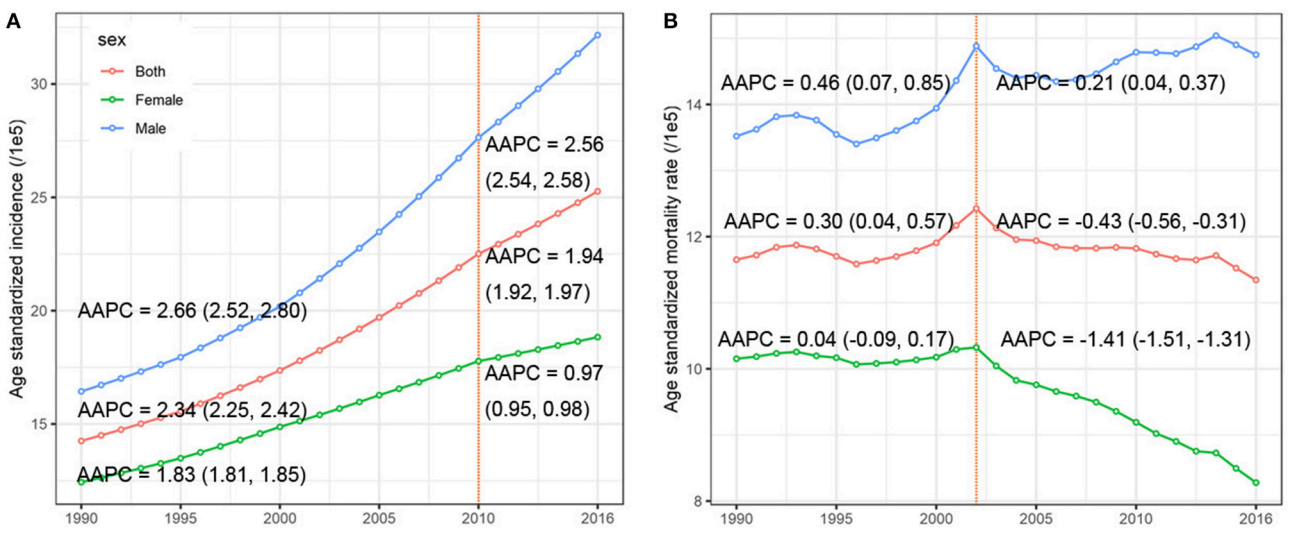


FIGURE 2 | The temporal trends of the **(A)** age-standardized incidence rate, **(B)** age-standardized mortality rate, by sex, from 1990 to 2016. The AAPCs presented in **(A,B)** were derived from a period-specific data: **(A)** 1990–2010 and 2011–2016; **(B)** 1990–2002 and 2003–2016.

TABLE 2 | The deaths and age standardized mortality rate of colorectal cancer in China in 1990 and 2016.

	1990		2016		1990-2016	
	Deaths No. × 10^3 (95% UI)	ASMR (/1e5) No. × 10^3 (95% UI)	Deaths No. × 10^3 (95% UI)	ASMR (/1e5) No. × 10^3 (95% UI)	Change in deaths (%) No. (95% CI)	AAPC in ASMR No. (95% CI)
Overall	81.1 (77.2, 85.7)	11.65 (11.10, 12.28)	167.1 (159.1, 174.5)	11.34 (10.81, 11.83)	106.0 (97.8, 111.6)	−0.04 (-0.13, 0.05)
SEX						
Male	44.4 (42.2, 46.7)	13.52 (12.89, 14.16)	104.2 (99.6, 109.0)	14.75 (14.11, 15.38)	134.7 (126.5, 140.7)	0.42 (0.34, 0.51)
Female	36.7 (34.1, 40.1)	10.15 (9.42, 11.09)	62.9 (57.6, 68.2)	8.28 (7.60, 8.98)	71.4 (63.2, 79.6)	−0.75 (−0.90, −0.60)
AGE (YEARS)						
5–14	0	0	0	0	–	–
15–49	14.5 (13.8, 15.3)	2.25 (2.14, 1.38)	17.6 (16.5, 18.7)	2.36 (2.21, 2.51)	21.4 (14.6, 26.5)	−0.31 (−0.55, −0.07)
50–69	36.3 (34.6, 38.5)	25.20 (23.96, 26.74)	73.7 (70.0, 77.4)	22.98 (21.83, 24.12)	103.0 (90.3, 112.3)	−0.37 (−0.46, −0.27)
70+	30.2 (28.7, 32.2)	80.00 (75.99, 85.08)	75.7 (72.2, 79.0)	90.32 (86.05, 94.14)	150.7 (145.3,156.1)	0.51 (0.38, 0.63)

UI, uncertainty interval; ASMR, age standardized mortality rate; AAPC, average annual percentage change; CI, confidence interval.

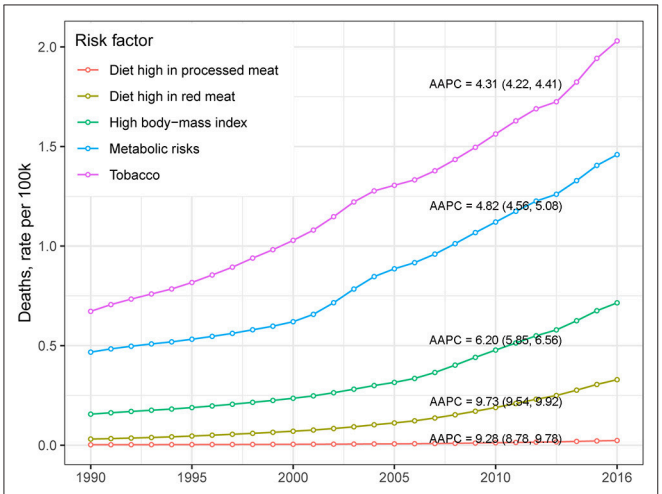


FIGURE 3 | The temporal trends of CRC death rate, stratified by risk factor, in China from 1990 to 2016.

risks, high BMI, diet high in red meat, and diet high in processed meat. In contrast, the most pronounced increases in CRC death rate was found in diet high in red meat and diet high in processed meat.

The annual Disability-Adjusted Life Years (DALYs), Years Lived with Disability (YLDs), and Years with Life Lost (YLLs) of CRC stratified by sex are presented in **Figure 4**. Overall, the disease burden posed by CRC was substantially increased in China during the study period. Specifically, the DALYs, YLDs, and YLLs increased 79.4, 354.4, and 64.5, respectively, between 1990 and 2016.

The results of the forecast of CRC cases and deaths for 2017–2025 based on the data from the GBD Study are shown in **Figure 5**. New CRC cases and deaths will continue to rise in the near future. Cancer cases among males will increase from 243.9 thousand in 2016 to 410.9 thousand (95% CI 398.4, 422.4) in 2025, and the corresponding cancer-related deaths will increase from 104.2 thousand to 153.4 thousand (95% CI 142.2, 164.8) during this period. Among females, cancer cases will increase from 148.9 thousand in 2016 to 231.4 thousand (95% CI 213.2, 253.7) in 2025. Accordingly, the number of deaths caused by CRC will increase from 62.9 thousand to 67.7 thousand (95% CI 63.2, 73.7).

DISCUSSION

Colorectal cancer is one of the most common malignancies diagnosed worldwide (1). In this study, we analyzed the disease burden caused by CRC and temporal trends in CRC incidence and mortality in China. In general, the CRC incidence was relatively lower than that of developed countries (4, 24–26). However, this study showed an upward trend in its incidence, although there has been a downward trend in the mortality of CRC during the past three decades. The CRC incidence and mortality in males were almost twice as high as that of females. The most pronounced increase in the incidence of CRC was observed in younger and middle-aged adults.

Risk factors for CRC have been investigated extensively in the last decade. Previous epidemiological studies have suggested that alcohol consumption, diets high in meat intake, obesity, smoking, and physical inactivity are associated with an increased risk of CRC in various populations (27–31). Targeted prevention strategies for combating CRC have led to a significant decrease in its incidence in several developed countries (32, 33). However, an upward trend has been observed in most developing countries (4). Likewise, in this study, we found that CRC incidence has rapidly increased from 1990 to 2016 and will further increase in China over the next decade. This remarkable increase might be attributed mainly to the upward trend in Western dietary patterns (34), changes in occupational patterns (35), increases in high-risk behaviors (e.g., smoking and excessive calorie intake) (36), and an increase in the aging population (3). Moreover, changes in access to health care and early cancer screenings might contribute to variations in CRC incidence (37). In a report of a longitudinal analysis of dietary patterns of Chinese adults from 1991 to 2009, the authors expressed concern about the

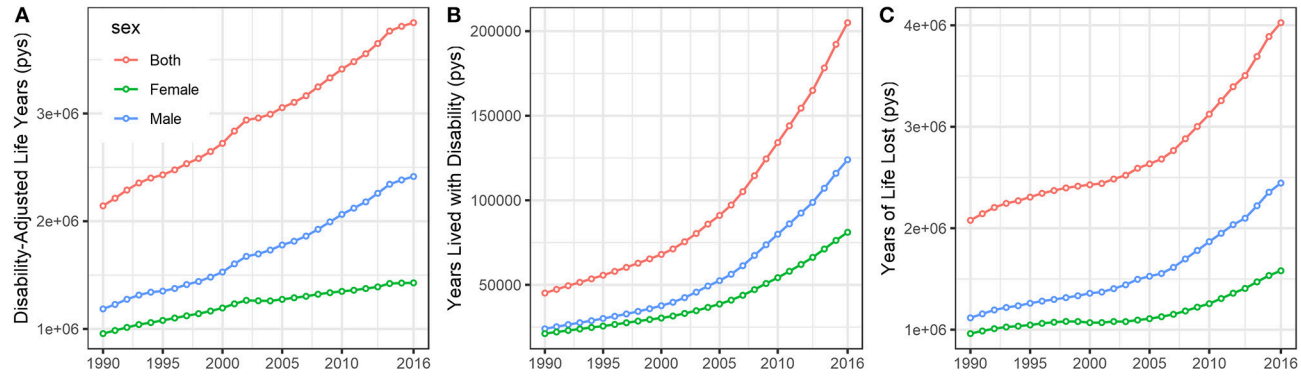


FIGURE 4 | The temporal trends of the **(A)** Disability-Adjusted Life Years (DALYs), **(B)** Years Lived with Disability (YLDs), and **(C)** Years with Life Lost (YLLs) of CRC stratified by sex in China from 1990 to 2016. (pys, person years).

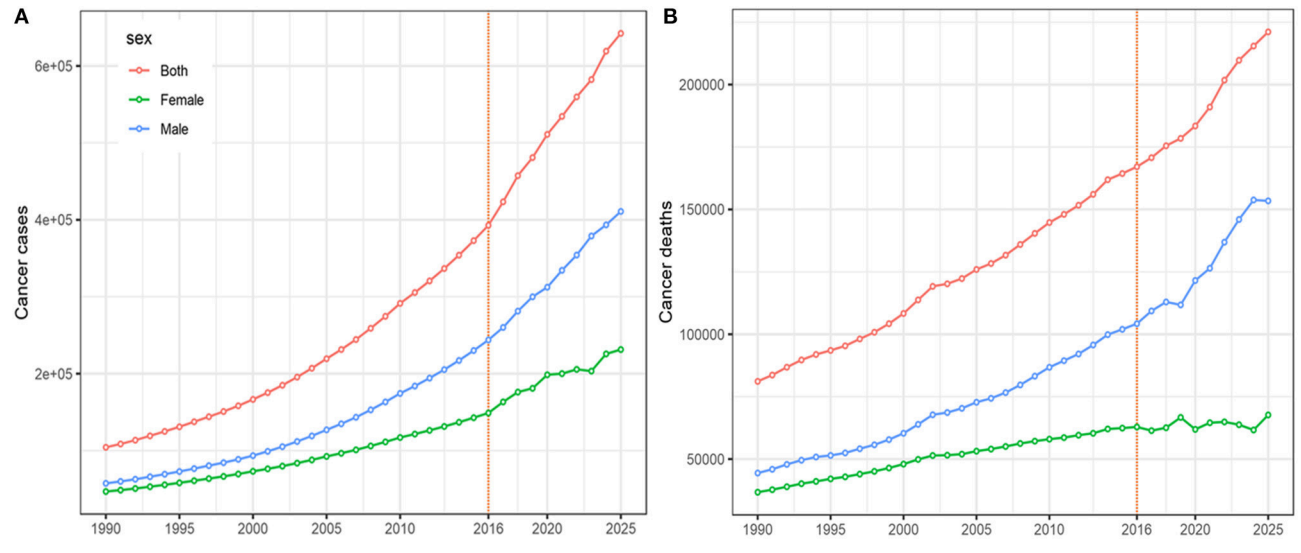


FIGURE 5 | The projections of colorectal cancer cases **(A)** and deaths **(B)**, by sex, from 2017 to 2025 in China.

increasing popularity of the modern high-wheat dietary pattern, which consists of energy-dense foods, in the context of China's rapid economic changes (38). A Westernized diet, characterized by high intake in red meat and processed meat (39), might drive changes in cancer profiles, including an increase in CRC (34), which has been verified by epidemiological studies on immigrants (40, 41). For example, the CRC incidence among Asian migrants to the US has shifted to an intermediate level between their country of origin and their new country of residence (40, 41). The shift in diet pattern and the concurrently increase in CRC incidence rate in Chinese population emphasizes the importance of establishment of healthy diet for CRC prevention. In this regard, the general population might be benefitted more from a traditional Chinese diet or Mediterranean diet pattern (42, 43). Moreover, the prevalence of known risk factors, such as obesity, diabetes, and smoking, are still on the rise (44–47), although measures have been initiated to counter these risks. As a result, the incidence of CRC is expected to increase in the future as predicted in this study if effective interventions are not introduced.

Surprisingly, we found that the most pronounced increase in CRC incidence was observed in people aged 15–49 years. This finding is consistent with those of countries in which similar trends in younger populations have been reported over roughly the same period (48). For instance, in the US, the incidence rates of CRC among people below 50 years of age rose 1.61% per year in men and 1.46% per year in women from 1998 to 2009 (49). As more than 80% of young-onset CRC cases are diagnosed with symptomatic disease, which may be associated with a delay in diagnosis and poor prognosis (50), greater emphasis should be placed on young adults' risk reduction and awareness of CRC symptoms, and greater vigilance by healthcare providers is needed for the early detection of CRC in young patients.

Over the past two decades, the range of CRC screening modalities has expanded, and many population-based programs have been initiated (51). As expected, screening programs

have been more frequently implemented in Western countries with higher rates of CRC and more available resources. Recommendations for CRC screening in the Asia Pacific region have been published and recently updated (52). Currently, those aged 40–74 years in China are screened using the fecal occult blood test, and followed up with a digital rectal exam and colonoscopy, which might influence the temporal trends in CRC, especially in the recent years. However, this program is far from complete and the national registry used to track clinical outcomes captures only 13% of the country's population, increasing the difficulty of planning healthcare services (53). The screening method also have impact on the identification of CRC subtypes. For example, more sigmoidoscopy screening would identify more rectal cases than colonoscopy does, though these screening methods have not widely adopted in China due to their cost and availability. In addition, screening methods with improved diagnostic ability or more sensitive biomarkers, such as methylated *Septin9* (m*SEPT9*), have been proposed and developed (54), though these potential biomarkers might be expensive and also not recommended by the current global guidelines or Asian-pacific guidelines for CRC screening.

Although the incidence of CRC is on the rise, we have observed a mild decrease in the CRC mortality rate, especially in recent years. This decrease might involve at least two aspects. First, the implementation of CRC screening to detect cancer in the earlier stages has demonstrated its effectiveness in populations that have initiated early screening programs. Second, the clinical treatment and management of CRC patients has received substantial improvement. For example, the 5-year survival rate has increased from 49% in the 1960s to 77% in the 2000s among CRC patients (55). The unequilibrium between CRC incidence rate and mortality rate can be partly explained by these reasons. Other factors contributed to this unequilibrium warrant further investigations. Of note, cancer-related deaths have doubled between 1990 and 2016 and are expected to increase in the near future, which might be the results of population expansion and aging. CRC, therefore, is a major public health concern, and poses a heavy disease burden on China.

Our study has limitations. First, we used multiple data sources that represented a diverse population. The data were retrieved from different international agencies; therefore, incompatibilities between these data might exist. Second, changes in risk factors and population structures were not taken into account when we predicted the cancer cases and deaths in future years. Finally, since the diagnostic pattern of CRC depends strongly on the screening compliance in the population and the screening method might also have effect on the disease form, the results presented in our study might be interpreted with cautions.

In summary, our study presents evidence of a steady increase in CRC incidence in China over the past three decades. It also predicts further increases in the near future. The sample's diversity of age, gender, and geography may yield insights for the future development of national programs. For example, the prevention and management of known risk factors, e.g., obesity and smoking, should be improved through effective national polices. Efforts should be made to prioritize CRC prevention in younger adults, as the incidence of CRC has increased in this group, and it could be prevented through the adoption of screening programs for this population.

AUTHOR CONTRIBUTIONS

TM: study design; LZ, FC, GZ, LS, SC, ZZ, and WZ: data collection; LZ and GZ: data analyses; all authors: results interpretations; LZ, FC, GZ, LS, SC, ZZ, and WZ: manuscript writing; TM and WZ: manuscript proofing.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Oncology

ORIGINAL RESEARCH
published: 09 April 2019
doi: 10.3389/fonc.2019.00252

Prognosis Prediction of Colorectal Cancer Using Gene Expression Profiles

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Edited by:
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Specialty section:
This article was submitted to Cancer Epidemiology and Prevention, a section of the journal Frontiers in Oncology

Received: *25 November 2018*
Accepted: *20 March 2019*
Published: *09 April 2019*

Citation:
Pan F, Chen T, Sun X, Li K, Jiang X, Försti A, Zhu Y and Lai M (2019) Prognosis Prediction of Colorectal Cancer Using Gene Expression Profiles. Front. Oncol. 9:252. doi: 10.3389/fonc.2019.00252

Background: Investigation on prognostic markers for colorectal cancer (CRC) deserves efforts, but data from China are scarce. This study aimed to build a prognostic algorithm using differentially expressed gene (DEG) profiles and to compare it with the TNM staging system in their predictive accuracy for CRC prognosis in Chinese patients.

Methods: DEGs in six paired tumor and corresponding normal tissues were determined using RNA-Sequencing. Subsequently, matched tumor and normal tissues from 127 Chinese patients were assayed for further validation. Univariate and multivariate Cox regressions were used to identify informative DEGs. A predictive index (PI) was derived as a linear combination of the products of the DEGs and their Cox regression coefficients. The combined predictive accuracy of the DEGs-based PI and tumors' TNM stages was also examined by a logistic regression model including the two predictors. The predictive performance was evaluated with the area under the receiver operating characteristics (AUCs).

Results: Out of 75 candidate DEGs, we identified 10 DEGs showing statistically significant associations with CRC survival. A PI based on these 10 DEGs (PI-10) predicted CRC survival probability more accurately than the TNM staging system [AUCs for 3-year survival probability 0.73 (95% confidence interval: 0.64, 0.81) vs. 0.68 (0.59, 0.76)] but comparable to a simplified PI (PI-5) using five DEGs (LOC646627, BEST4, KLF9, ATP6V1A, and DNMT3B). The predictive accuracy was improved further by combining PI-5 and the TNM staging system [AUC for 3-year survival probability: 0.72 (0.63, 0.80)].

Conclusion: Prognosis prediction based on informative DEGs might yield a higher predictive accuracy in CRC prognosis than the TNM staging system does.

Keywords: colorectal cancer, prognostic index, gene expression, prognosis prediction, combined predictor

INTRODUCTION

Colorectal Cancer (CRC) is one of the most common malignancies globally (1). In order to guide clinical treatment and predict prognosis, several CRC staging systems have been established, especially the American Joint committee on Cancer (AJCC) tumor-node-metastasis (TNM) system based on anatomical information, which is widely used (2). According to the TNM staging system, the survival of CRC patients is related to the size of primary tumor (T), nearby lymph nodes affected (N), and distant metastasis (M). However, CRC is an etiologically heterogeneous disease involving several distinct biologic pathways, resulting in different survival status even among patients who are at the same TNM stage (3).

Over last few decades we have seen a remarkable advance in the knowledge of CRC biological pathways with an abundance of novel molecular biomarkers having been found to have potentials in prognosis prediction. By applying the quantitative reverse transcription polymerase chain reaction (RT-qPCR) platform, O'Connell et al. selected seven recurrence risk genes among patients with stage II/III colon cancer and developed a recurrence risk score using the seven genes to stratify patients with significantly different recurrence risks (4). Barrier et al. also reported an 80% prognosis prediction accuracy obtained by profiling 30 genes among stage II colon cancer patients (5). Regarding the overall survival, it has been reported that molecular staging based on 43 core genes was 90% accurate in predicting 36-month overall survival, significantly better than Dukes' staging (6). Investigation on prognostic markers for CRC deserves efforts, but data from China are scarce.

The objective of the present study was to build a prognostic index (PI) based on differentially expressed gene (DEG) profiles between tumor and normal tissues and to compare this PI with the TNM staging system regarding their accuracy in prognosis prediction among Chinese CRC patients.

MATERIALS AND METHODS

Flowchart of This Study and DEG Selection

As shown in the flowchart (**Figure 1**), tumor-normal matched tissue samples of CRC were collected at the time of surgery and immediately stored in liquid nitrogen. We applied the RNA-Sequencing (RNA-Seq) approach to identify candidate DEGs among six pairs of tumor and corresponding normal tissues (5 cm from the edge of the tumor). RNA was extracted following the instruction of RNeasy Plant Mini Kit (QIAGEN Inc., Valencia, CA, USA). The total RNA of all the samples was first treated with DNase I to degrade any possible DNA contamination. The mRNA was then enriched using oligo (dT) magnetic beads and mixed with a fragmentation buffer to be fragmented into approximately 200-bp fragments. First-strand cDNA synthesis

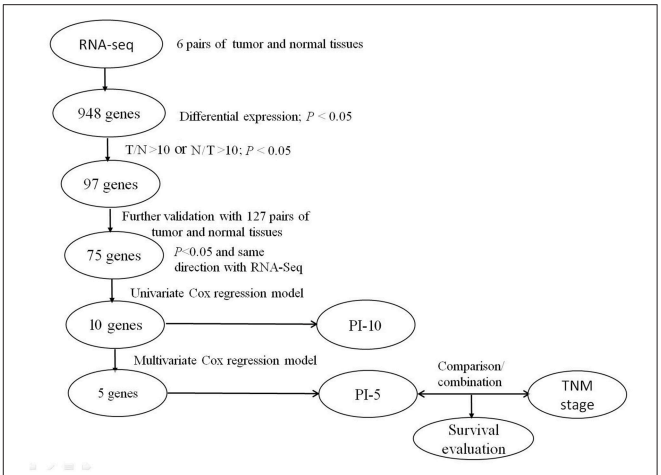

FIGURE 1 | Flowchart of the study.

was performed using random hexamers. Buffer, dNTPs, RNase H, and DNA polymerase I were added to synthesize the second strand. The double-stranded cDNA was purified with magnetic beads. End preparation and 3′-end addition of the nucleotide adenine (A) were performed. Finally, sequencing adaptors were ligated to the fragments. The fragments were enriched by PCR amplification. During the QC step, the Agilent 2100 Bioanalyzer and ABI StepOnePlus Real-Time PCR System were used to qualify and quantify the DNA library. The library products were then sequenced with the Illumina HiSeq 2000.

The levels of gene expressions were calculated using the reads per kilobase million (RPKM) method. Using the method proposed by Audic and Claverie (7), we identified 97 candidate DEGs (differentiated expression $\geq$10 folds, $P < 0.05$) from 948 genes (**Supplementary Table S1**).

Patients and Tumor Samples

Afterwards, we verified the 97 DEGs with the QuantiGene Plex assay performed on 127 pairs of tumor and matched normal tissues. We recruited 127 patients (82 men) diagnosed with CRC and received resection between September 2006 and February 2012. All tumor samples were collected before any systemic chemotherapy. The main patient and tumor characteristics are shown in **Table 1**. Clinically relevant data, including socio-demographic and pathological information (sex, age, tumor location, tumor size, depth of tumor invasion, lymph node metastasis, distant metastasis, TNM stage, and postoperative chemotherapy), were collected by reviewing the medical records. We eventually identified 75 DEGs ($P < 0.05$ and same direction as in RNA-Seq) for further analyses. This study was approved by the ethics committee of Zhejiang University and all the patients provided a written informed consent.

Statistical Analysis

We used univariate and multivariate Cox proportional hazards models to explore the associations between the identified DEGs and the overall survival time after resection.

Abbreviations: CRC, colorectal cancer; DEG, differentially expressed gene; PI, predictive index; ROC, receiver operating characteristics; AUC, area under the curve; TNM, tumor-node-metastasis; CPI, combined predictive index; cfNRI, category-free net reclassification improvement.

TABLE 1 | The main patient and tumor characteristics, stratified by 3-year survival status.

Variables	N	Survivors	Non-survivors	*P*-value*
GENDER				
Male	82	60	22	0.071
Female	42	24	18	
AGE (YEARS)				
≤60	46	35	11	0.127
>60	78	49	29	
LOCATION				
Rectum	71	47	24	0.670
Colon	53	37	16	
MAXMUM DIAMETER				
≤5	80	50	30	0.098
>5	40	31	9	
TNM STAGE				
I	18	16	2	0.091
II	26	19	7	
III	50	29	21	
IV	26	16	10	
DEPTH OF TUMOR INVASION (T)				
T1-T3	60	47	13	0.007
T4	60	33	27	
LYMPH NODE METASTASIS (N)				
N0	55	43	12	0.016
N1-N2	66	38	28	
DISTANT METASTASIS (M)				
M0	97	67	30	0.466
M1	26	16	10	
POSTOPERATIVE CHEMOTHERAPY				
No	48	32	16	0.839
Yes	76	52	24	

**Univariate analysis of categorical variables was performed using χ^2.*

The multivariate model adjusted for sex, age, TNM stage, postoperative chemotherapy, and DEGs. A PI was derived as a linear combination of the products of the DEGs and their coefficients obtained from the univariate and multivariate Cox regressions. All DEGs were mean-centered to ensure that PI of zero corresponds to the survival probability given that all the DEGs are at their medium level, with PI < 0 and PI > 0 indicating the good and poor prognosis, respectively. The predictive performance of the PI was investigated with the area under the receiver operating characteristics (ROC) curves. DEGs-based PI grade was then established according to the cut-off value which maximizes the Youden's index. Furthermore, we developed combined predictors (CPs) for prognosis prediction (1-year, 3-year, and 5-year survival) using logistic regression, which included both the DEGs-based PI grade and tumors' TNM stages. The accuracy of CPs, DEGs-based PI grade and TNM staging system for prognosis prediction was compared by the area under the ROC curve (AUC). Additionally, we used the indicator of category-free net reclassification improvement (cfNRI) to evaluate the effect of prognosis prediction for DEGs-based PI grade.

Statistical analyses were performed using the SAS statistical software, version 9.3 (SAS Institute, Cary, NC, USA) and R version 3.2.2 (R Foundation for Statistical Computing, Vienna, Austria). Two-sided P-value < 0.05 was considered statistically significant.

RESULTS

After controlling for sex, age, TNM stage and postoperative chemotherapy in the multivariate Cox regression model, 10 out of the 75 DEGs showed statistically significant associations with the overall survival time. As shown in **Table 2**, CPNE8, LOC646627, CDKN2A, ATP6V1A, SCARA5, BEST4, and KLF9 were positively associated with the overall survival time, while DNMT3B, GRIN2D, and ANLN were negatively associated with the overall survival. By summing up the products of the 10 DEGs and their Cox regression coefficients, we developed a PI (hereinafter referred to as PI-10), which ranged from −6.280 to 5.694, with the quartiles being −0.956, 0.118, and 1.057, respectively. The ROC curves for PI-10 to predict 1-year, 3-year, and 5-year survival are given in **Figures 2A–C** (blue line)**,** and the AUCs were 0.748, 0.730, and 0.807, respectively.

To shrink the number of DEGs involved in prognosis prediction, we performed a multivariate Cox regression on the 10 DEGs, sex, age, TNM, stage and postoperative chemotherapy, which ended up with 5 independent DEGs, i.e., LOC646627, BEST4, KLF9, ATP6V1A, and DNMT3B (**Table 2**). Thus, we developed a parsimonious PI based on these 5 DEGs (hereinafter referred to as PI-5). In comparison with PI-10, PI-5 yielded improved AUCs for all the three survival intervals of interest (0.720, 0.722, and 0.790, respectively), which however was not statistically significant (**Figures 2A–C**, red line). No significant difference of AUC was found between PI-10 and PI-5.

For PI-5, a cut-off point of −0.053 would maximize the Youden's index, reaching 0.344, 0.348, and 0.509 for all the three survival intervals of interest, respectively (**Supplementary Table S2** and **Supplementary Figure S1**). Subsequently, we categorized the patients into two groups: high grade (PI-5 > -0.053) and low grade (PI-5 ≤ -0.053). The survival probabilities for the patients with low grade were statistically significantly higher than those with high grade (**Figure 3**). The survival time for patients with low grade and high grade was 85.77 ± 3.59 vs. 45.52 ± 3.92 (**Supplementary Table S3**). We further derived combined predictive indexes (CPIs) from logistic models in which PI-5 grade and TNM stage were both included as predictor variables, as shown below: CPI for 1-year survival = PI-5 + 0.301*TNM; CPI for 3-year survival = PI-5 + 0.235*TNM; CPI for 5-year survival = PI-5 + 0.199*TNM.

Figure 4 and **Supplementary Table S4** compare the AUCs among PI-5 grade, TNM stage, and CPI, consistently showing significantly higher AUC for CPI than for PI-5 grade and TNM stage across the 3-year, and 5-year survival intervals ($P < 0.05$). Specifically, PI-5 grade yielded an improvement in the AUC

compared to TNM stage for all the three survival intervals of interest, yet no significant difference was observed for the 1-year and 3-year survival. The AUCs for 1-year, 3-year, and 5-year survival of PI-5 grade and TNM stage were 0.676 vs. 0.634, 0.681 vs. 0.611, and 0.760 vs. 0.637, respectively. Moreover, CPI showed significantly higher AUCs compared to TNM stage, for 3-year and 5-year intervals of interest ($P < 0.05$), reaching 0.719 and 0.801, respectively (referring to AUCs elevation of 17.68 and 25.75%, respectively). Additionally, the cfNRIs (0.295, 0.391, and 0.464 for the three survival intervals, respectively) showed significantly improved predictions by PI-5 grade for all (**Supplementary Table S5**).

TABLE 2 | DEGs statistically significantly associated with the overall survival time.

Gene name	β[a]	SE	*P*-value
UNIVARIATE COX MODEL*			
CPNE8	0.365	0.109	0.001
LOC646627	0.285	0.124	0.022
DNMT3B	−0.756	0.278	0.007
CDKN2A	0.329	0.134	0.014
ATP6V1A	0.450	0.155	0.004
SCARA5	0.260	0.117	0.026
ANLN	−0.343	0.170	0.044
BEST4	0.289	0.108	0.008
KLF9	0.237	0.114	0.037
GRIN2D	−0.443	0.207	0.033
MULTIVARIATE COX MODEL*			
LOC646627	0.263	0.123	0.032
BEST4	0.246	0.102	0.016
KLF9	0.412	0.122	0.001
ATP6V1A	0.613	0.162	0.001
DNMT3B	−0.832	0.291	0.004

**Univariate and multivariate Cox proportional hazard regression models were adjusted by sex, age, TNM, and postoperative chemotherapy.*

[a]PI was derived as a linear combination of the products of the DEGs and their coefficients obtained from the Cox models, with PI < 0 and PI > 0 indicating the good and poor prognosis, respectively.

DISCUSSION

Among a pool of 75 DEGs, we identified 10 DEGs that showed statistically significant associations with CRC survival after surgery. Additionally, we developed a PI based on 5 DEGs, which performed better than the classical TNM staging system for CRC prognosis prediction. We found that it is worthwhile to combine the DEGs-based PI and the long-established TNM staging system given significantly improved predictive accuracy gained by doing so.

The DEGs which we identified in the present study to have statistically significant associations with the survival probability of CRC patients after surgery confirms the findings of previous studies which suggested gene expression profiling to improve accuracy of prognosis prediction (8–10). From the genes included in the PI-5, it has been found that BEST4 is a member of the bestrophin gene family (BEST1, BEST2, BEST3, and BEST4) of anion channels. The BEST4 was predominantly expressed in the colon and weakly in fetal brain, spinal cord, retina, lung, trachea,

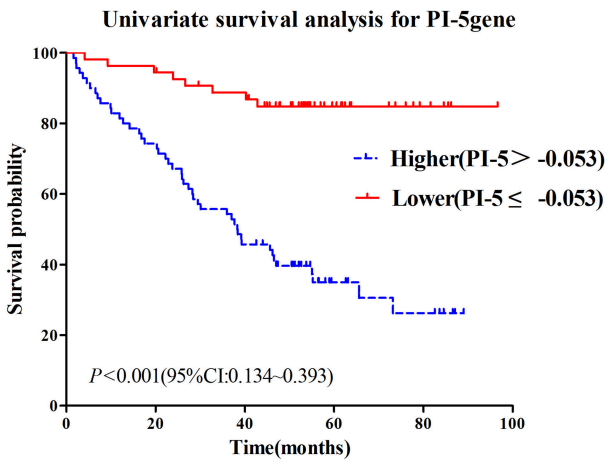

FIGURE 3 | Survival time and survival probability by tumor grade as defined using PI-5: high grade *vs.* low grade.

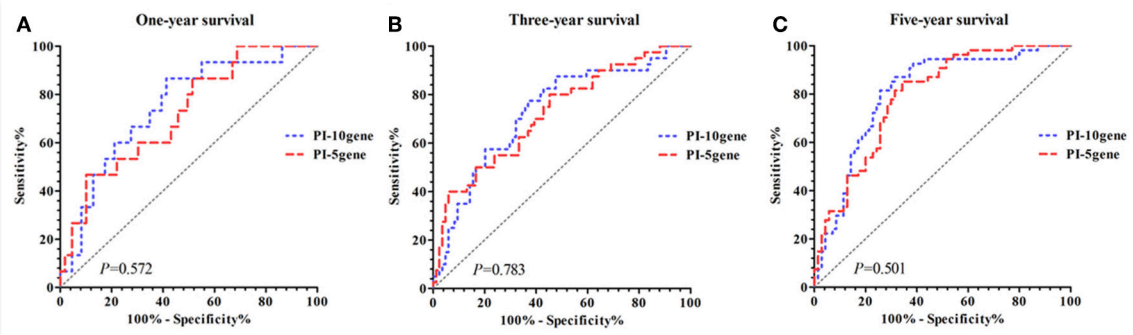

FIGURE 2 | Comparison of predictive performance for PI-10 and PI-5. **(A)** PI-10 vs. PI-5 (1-year survival). **(B)** PI-10 vs. PI-5 (3-year survival). **(C)** PI-10 vs. PI-5 (5-year survival).

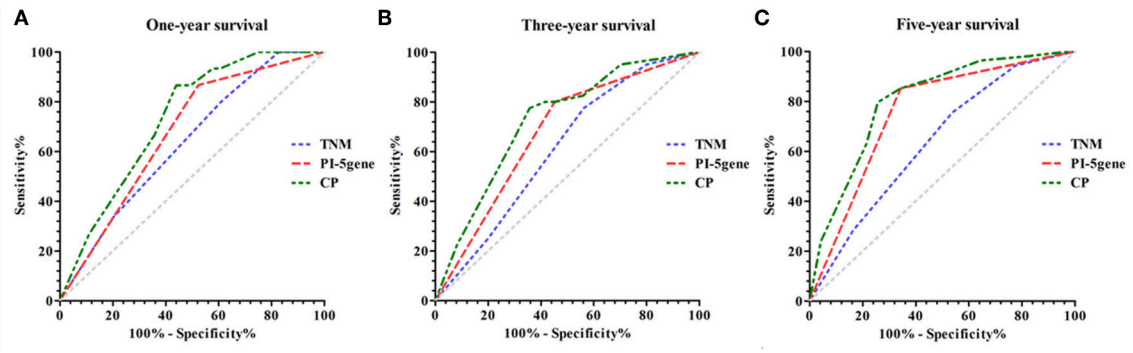

FIGURE 4 | Comparison of predictive performance for PI-5, TNM stage and CPI. **(A)** 1-year survival. **(B)** 3-year survival. **(C)** 5-year survival.

testis and placenta. Significantly down regulation of BEST2 was found in the active lesions of ulcerative colitis. In contrast to BEST2, the expression of BEST4 appeared to be maintained (11). So far, there was little to no research on the mechanism of BEST4 contributing to the development of CRC. In the present study, significant down regulation of BEST4 was found in tumor issues of CRC patients. However, we observed a statistically negative association between BEST4 expression and the survival probability of CRC patients after surgery, suggesting that the role of this gene in CRC prognosis merits further investigation.

DNMT1, DNMT3A, and DNMT3B are the major DNA methyltransferases (DNMTs) that so far have been found in mammals. An established body of knowledge concludes that DNA hypomethylation plays a crucial role in human cancers (12). DNMT3B has been reported to be overexpressed in breast, oral, and colorectal tumor tissues (13–15), while other studies have suggested that DNMT3B and DNMT3A are tumor suppressor genes for lymphoma and lung cancer (16, 17). In the present study, however, we observed a positive association between DNMT3B expression and survival probability among CRC patients after surgery, inconsistent to what we expected, therefore we assume that DNMT3B is likely to have a tumor suppressing effect in colorectal carcinogenesis.

LOC646627 encodes LYPD8 protein, a family member of LY6/PLAUR. LYPD8 can mediate segregation of intestinal bacteria and epithelia cells in the colon to preserve intestinal homeostasis (18). In the present study, LYPD8 was underexpressed in the tumor tissues and was associated with poor prognosis. Chronic inflammation targets the intestinal microbiota and impacts the progression of CRC by inducing the expansion of microbes including *E. coli*, which has carcinogenic effect (19). For CRC patients after resection, intestinal homeostasis can moderate the inflammatory response and thus prevent the occurrence of complications following surgery.

In the present study, we observed an overexpression of ATP6V1A in CRC tumor tissues, which had an adverse effect on prognosis. The ATP6V1A gene encodes a component of vacuolar ATPase (V-ATPase), an enzyme that mediates the acidification of eukaryotic intracellular organelles. Studies have reported overexpressed ATP6V1A in gastric tumor issues and its association with cancer prognosis, suggesting that ATP6V1A might be a target of gastric cancer treatment (20). However, studies investigating the ATP6V1A expression in other tumor tissues are scarce.

It has been reported that KLF9 exhibited low expression in pancreatic cancer, and upregulation of KLF9 may inhibit the progression of pancreatic cancer (21). However, the expression of KLF9 was up-regulated in human ovarian cancer, and KLF9 deficiency significantly inhibited tumor growth in nude mice (22). What was more, some results show KLF9 to be haploinsufficient suppressor of colon tumorigenesis in the ApcMin/+mouse colon by suppressing expression of ISG15, an apoptosis-inhibiting cytokine (23). Contrary to what we found in the present study, KLF9 was low expressed in CRC tumor tissues and was associated with poor prognosis.

The TNM staging system has been widely adopted for prognosis prediction and treatment strategy selection. This staging system relies solely on anatomical information about the size and extent of primary tumor. Since more and more novel promising non-anatomical prognostic factors have been identified, the TNM staging system calls for an evolution so as to remain usable in the era of personalized diagnosis and molecular-targeted therapy. As a response to this need, two genetic biomarkers, namely KRAS gene mutation and 18q loss of heterozygosity, along with other five factors, have been incorporated into the 7th revision of the TNM staging system (24), though the resulting improvement in predictive capacity compared with its predecessor is disputable (25, 26).

The present study is one of the few studies that aimed to build a PI by integrating informative genetic biomarkers. We found that this DEGs-based PI predicted the survival probability among CRC patients after resection more accurately than the classic TNM staging system (AUC for five-year survival probability 0.77 vs. 0.65) and comparable to most of the reported CRC prognosis prediction nomograms based on non-DEGs data (27). A recent study reported that a multi-RNA-based classifier also outperformed the TNM staging system regarding the overall survival (AUC 0.83 vs. 0.74) (28). However, our results still supported the predictive value of the TNM staging system.

The strength of the present study includes its comprehensive search for statistically informative DEGs and thus it provides important insights into their value in clinical decision-making process. However, some limitations of the present study should be noted. First of all, our study was moderate in its sample size and therefore unbiased estimates of model coefficients were difficult to achieve. Secondly, like many other studies, we did not validate our DEGs-based PI externally and its performance may thus be subject to over-optimism.

In conclusion, this study confirms that prognosis prediction based on informative DEGs might yield a higher accuracy than the TNM staging system alone. Therefore, we recommend integration of differentially expressed gene data into the TNM staging system for further improvement in CRC prognosis prediction.

ETHICS STATEMENT

This study was approved by the ethics committee of Zhejiang University and all the patients provided a written informed consent.

AUTHOR CONTRIBUTIONS

ML and YZ are responsible for the study concept and design. YZ and TC obtained funding. YZ and FP acquired data, analyzed, and interpreted data. KL, XJ, and TC drafted the manuscript, and all authors revised it for important intellectual content. YZ and TC are the guarantors of this work. All co-authors commented the manuscript and approved the submission.

FUNDING

This work was supported by grants from National Key R&D Program of China [grant number: 2017YFC0908200], Key Research-Development Program of Zhejiang Province [grant number: 2017C03013], Joint Key Program of Zhejiang Province-Ministry of Health [grant number: WKJ-ZJ-1714], Key Project of State Bureau of Foreign Affairs [grant number: 20173300013], National Key Research and Development Program of China [grant number: 2017YFC0907004], and Qianjiang Talents Fund of Zhejiang Province [grant number: QJD1602026]. The study sponsors were not involved in the study design, data collection, data analysis, interpretation of results, writing of the manuscript, and in the decision to submit the manuscript for publication.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2019.00252/full#supplementary-material

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Oncology

ORIGINAL RESEARCH
published: 10 April 2019
doi: 10.3389/fonc.2019.00253

Milk Consumption Across Life Periods in Relation to Lower Risk of Nasopharyngeal Carcinoma: A Multicentre Case-Control Study

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Specialty section:
This article was submitted to Cancer Epidemiology and Prevention, a section of the journal Frontiers in Oncology

Received: *15 November 2018*
Accepted: *20 March 2019*
Published: *10 April 2019*

Citation:
Mai Z-M, Lin J-H, Ngan RK-C, Kwong DL-W, Ng W-T, Ng AW-Y, Yuen K-T, Ip DKM, Chan Y-H, Lee AW-M, Ho S-Y, Lung ML and Lam T-H (2019) Milk Consumption Across Life Periods in Relation to Lower Risk of Nasopharyngeal Carcinoma: A Multicentre Case-Control Study. Front. Oncol. 9:253. doi: 10.3389/fonc.2019.00253

Background: The much higher incidence of nasopharyngeal carcinoma (NPC) in men suggests sex hormones as a risk factor, and dairy products contain measurable amounts of steroid hormones. Milk consumption has greatly increased in endemic regions of NPC. We investigated the association between NPC and milk consumption across life periods in Hong Kong.

Methods: A multicentre case-control study included 815 histologically confirmed NPC incident cases and 1,502 controls who were frequency-matched on age and sex at five major hospitals in Hong Kong in 2014–2017. Odds ratios (ORs) of NPC (cases vs. controls) for milk consumption at different life periods were estimated by unconditional logistic regression, adjusting for sex, age, socioeconomic status score, smoking and alcohol drinking status, exposure to occupational hazards, family history of cancer, IgA against Epstein-Barr virus viral capsid antigen, and total energy intake.

Results: Compared with abstainers, lower risks of NPC were consistently observed in regular users (consuming ≥5 glasses of milk [fresh and powdered combined] per month) across four life periods of age 6–12 (adjusted OR 0.74, 95% CI 0.54–0.86), 13–18 (0.68, 0.55–0.84), 19–30 (0.68, 0.55–0.84), and 10 years before recruitment (0.72, 0.59–0.87). Long-term average milk consumption of ≤2.5, >2.5, and ≤12.5, >12.5 glasses per month yielded adjusted OR (95% CI) of 1.00 (0.80–1.26), 0.98 (0.81–1.18), 0.95 (0.76–1.18), and 0.55 (0.43–0.70), respectively (all *P*-values for trend <0.05).

Conclusion: Consumption of milk across life periods was associated with lower risks of NPC. If confirmed to be causal, this has important implications for dairy product consumption and prevention of NPC.

Keywords: milk, nasopharyngeal carcinoma, case-control study, life-course, multiple imputation

BACKGROUND

The etiology of nasopharyngeal carcinoma (NPC) is unclear, and its male predominance has been linked to sex hormones (1). Dairy products are a source of steroid hormones (2) and contain numerous potential antitumor substances.

Using an ecological study design, we found that increasing consumption of dairy products might explain the declining NPC incidence in 48 countries/regions (3, 4). Six case-control studies in other countries/regions [Malaysia (5), Guangzhou (6), Shanghai (7), Taiwan (8), Italy (9), and Maghrebian countries (10)] had examined the association between dairy intake and NPC risk. Three of them measured consumption of milk and cow's milk, while the others measured milk drinking with daily meals, and intake of rancid butter, and milk, and yogurt. Results were mixed with positive, null and negative associations. Milk is not a major component of the East Asian traditional diet but consumption has greatly increased with economic growth and globalization (11). We conducted a multicentre NPC case-control study in Hong Kong to further examine such association.

METHODS

The methods of this case-control study have been detailed elsewhere (12). Briefly, the cases were 815 histologically and/or radiologically confirmed incident NPC patients (response rate 78.4%) recruited in 2014–2017 from five major regional hospitals that treat up to 70% of all NPC cases in Hong Kong. The controls were 1,502 frequency-matched (by 5-year age group and sex; response rate 85.1%) new patients or referrals of a new health complaint in the past 12 months in specialist outpatient clinics, or new inpatients admitted in the past 3 months in the same hospitals. Those with a history of NPC, dementia, or suspected symptoms of NPC such as recent unilateral facial nerve palsy, tinnitus, unilateral hearing loss and epistaxis were excluded. Following the AsiaLymph guideline of the US National Cancer Institute (13), we also specified that no more than 15% of controls had the same specific type of disease. A limited number of specific diagnoses were further excluded, based on a known or suspected relation with vitamin D exposure, and immunological, infectious and/or inflammatory etiology. The disease list of controls is shown in **Supplementary Part I**.

Consumption of Milk and Other Dairy Products

The subjects reported their average monthly consumption of dairy products over four life periods (age 6–12, 13–18, and 19–30, and 10 years before recruitment for fresh and powered milk; age 13–18 and 19–30, and 10 years before recruitment for other dairy products) on a computer-assisted, self-administered questionnaire with satisfactory test-retest reliability (coefficients 0.4–0.8) (12). Dairy consumption was categorized as: (1) milk [fresh and powdered milk combined, in glasses (one glass = 250 ml)], and (2) other dairy products [ice cream, yogurt or cheese, in servings (one cup of ice cream, one cup of yogurt, or 50 g of firm cheese)]. Those who consumed <5 glasses/month of milk (fresh and powdered combined) or ≤8 servings/month of other dairy products (ice cream, yogurt or cheese) were classified as "non-regular users," those who consumed ≥5 glasses/month of milk or >8 servings/month of other dairy products as "regular users," and those who never consumed as "abstainers." To overcome the limitations of conventional approaches by using self-reported exposure, rs4988235 was genotyped as an "instrumental variable" to "unbiasedly" assess the association between dairy intake and NPC risk. Genotyping for the LCT-13910 C/T (rs4988235) polymorphism (14) was conducted using iPLEX assay on the MassARRAY System (Sequenom, San Diego, CA, USA) in 512 NPC cases and 898 controls (data not shown because only one case and three controls had the T allele that was associated with lactase persistence).

Covariates

We also collected information on sex, 5-year age group, socioeconomic status score [range: −1 (lowest) to 13 (highest), calculated by the subject's, and his/her father's and mother's education, housing type at age 10, personal income, and household income], smoking and drinking status, occupational hazards, family history of cancer, and total energy intake. Dietary information was collected with the Semi-Quantitative Food Frequency Questionnaire with about 30 food items (12). The subjects reported how often, on average, they consumed a specified portion size of each food during the preceding year. We calculated total energy intake (residual method) (15) by multiplying the frequency of consumption of each item by its caloric content and summing the products across all foods in a specific period using the China Food Composition Table (2008 No. 2). Our questionnaire had acceptable test-retest reliability (coefficients 0.4–0.8) (12).

Antibody of IgA against Epstein-Barr virus (EBV) viral capsid antigen (VCA-IgA) was measured using a commercial kit (EUROIMMUN AG, Lübeck, Germany) based on the standard method of ELISA. Results were evaluated semi-quantitatively by calculating the ratio of the optical density value of the sample over the optical density value of the calibrator, expressed as relative optical density (rOD). According to the manufacturer's instruction, the serostatus of VCA-IgA was classified as seronegative (rOD value: <1.2) or seropositive (rOD value: ≥1.2).

Statistical Analysis

Case vs. control odds ratios (ORs) for dairy consumption (non-regular/regular users vs. abstainers) were calculated using unconditional logistic regression, with/without adjusting for potential confounders. Odds ratios were calculated for dairy consumption at each life period and as average values across all periods to represent long-term intake. The group-specific confidence interval (CI) for the abstainers' OR of 1.00 was calculated using Plummer's methods to reflect the variance of the log odds (16).

Abbreviations: NPC, nasopharyngeal carcinoma; OR, odds ratio; EBV, Epstein-Barr virus; VCA-IgA, IgA against EBV viral capsid antigen; SNP, single nucleotide polymorphism; rOD, relative optical density.

To assess dose-response effect, a test for linear trend was examined for each categorical exposure. Interaction by sex was tested based on the likelihood ratio test by introducing interaction terms into the crude model.

We predicted missing values of the exposure and confounders (EBV VCA-IgA serostatus: 296 cases/478 controls, smoking status: 6/5, family history of cancer: 124/111 and exposure to occupational hazards: 131/147) based on a flexible additive regression model with predictive mean matching incorporating data on the factors included in the multivariable model (17). As a sensitive analysis, we also conducted a complete case analysis (**Supplementary Table 1**, the results were similar to those with multiple imputation). Statistical analyses were done with R 3.5.1, and all tests were two-sided with $\alpha = 0.05$.

RESULTS

Table 1 shows that the cases were older and had a greater proportion of men, lower socioeconomic status, family history of NPC, ever-smoking, EBV seropositivity, and exposure to any occupational hazards compared with the controls (all *P*-values <0.001). No difference in alcohol drinking status was observed ($P = 0.19$).

Table 2 shows, compared with abstainers, the adjusted ORs (95% CI) of NPC in regular users who consumed ≥5 glasses of milk (fresh and powdered combined) per month were 0.74 (0.61–0.91) at age 6–12, 0.68 (0.54–0.86) at age 13–18, 0.68 (0.55–0.84) at age 19–30, and 0.72 (0.59–0.87) 10 years before recruitment (all *P*-values for trend <0.05). For other dairy products (ice cream, yogurt or cheese), compared with abstainers, the adjusted ORs (95% CI) in non-regular users who consumed ≤8 servings/month were 0.85 (0.74–0.97) at age 13–18, 0.84 (0.73–0.96) at age 19–30, and 0.88 (0.77–1.01) 10 years before recruitment, and in regular users who consumed >8 servings/month were 0.92 (0.74–1.14) at age 13–18, 1.01 (0.81–1.25) at age 19–30, and 1.05 (0.85–1.31) 10 years before recruitment (all *P*-values for trend >0.05).

Figure 1 shows the adjusted ORs for long-term average milk consumption of none, ≤2.5, >2.5 & ≤12.5, >12.5 glasses per month were, respectively, 1.00 (0.80–1.26), 0.98 (0.81–1.18), 0.95 (0.76–1.18), and 0.55 (0.43–0.70) (*P* for trend <0.001). For long-term average consumption of other dairy products, the adjusted ORs (95% CI) for none, ≤2.5, >2.5, and ≤12.5, >12.5 servings per month were, respectively, 1.00 (0.76–1.13), 0.80 (0.65–0.99), 0.84 (0.68–1.02), and 0.92 (0.74–1.14) (*P* for trend 0.89).

TABLE 1 | Characteristics of nasopharyngeal carcinoma (NPC) cases and controls in five regional hospitals in Hong Kong, China 2014–2017.

Characteristics	NPC cases ($N = 815$)		Controls ($N = 1502$)		*P*-value[‡]
	n	%	*n*	%	
Sex					0.001
Men	613	75.2	1028	68.4	
Age at recruitment, years					
Mean (interquartile range)	52.6 (44–59)		51.5 (42–60)		0.05
18–<35	54	6.7	179	11.9	0.001
35–<45	146	17.9	241	16.1	
45–<55	234	28.6	391	26.1	
55–<65	267	32.6	448	29.9	
≥65	114	14.2	243	16.1	
Socioeconomic status score[‡]					
Mean (SD)	3.0 (2.8)		3.7 (3.0)		<0.001
Family history of cancer					<0.001
None	288	35.3	753	50.1	
Had any family member(s) with history of cancer, excluding NPC	269	33.0	561	37.4	
Had any family member(s) with history of NPC	134	16.4	77	5.1	
Don't know	124	15.2	111	7.4	
Exposure to any occupational hazards					<0.001
None	285	35.0	758	50.5	
Ever exposed	399	49.0	597	39.8	
Don't know	131	16.1	147	9.8	
Smoking					<0.001
Never	417	51.2	945	62.9	
Ever	392	48.1	552	36.8	
Refuse to answer	6	0.7	5	0.3	
Alcohol drinking					0.19
Never	512	62.8	977	65.1	
≤210 g/week	203	24.9	377	25.1	
>210 g/week	100	12.3	148	9.9	
EBV VCA-IgA[†]					<0.001
Seronegative	56	10.8	900	88.1	
Seropositive	463	89.2	123	11.9	

[‡]*t-test and Chi-square test were used to compare the mean of continuous factors, and proportions of categorical factors between cases and controls, respectively.* [‡]*Socioeconomic status score ranged from−1 (lowest socioeconomic status) to 13 (highest socioeconomic status), and was calculated by the subject's, and his/her father's and mother's education, personal income, household income and housing type at age 10.* [†]*Epstein-Barr virus viral capsid antibody (EBV VCA-IgA) levels: optical density value <1.2 (seronegative) or ≥1.2 (seropositive). We excluded subjects who had not provided blood, or whose plasma EBV VCA-IgA was not measured.*

DISCUSSION

Consumption of milk but not other dairy products across life periods was associated with lower risks of NPC in Hong Kong. This is consistent with our ecological analysis of international data in which milk consumption was negatively correlated with the incidence of NPC. Results on the association between milk intake and risk of NPC are scarce. Previous case-control studies reported inconsistent results in different types of dairy products in different populations. One showed a negative association (5) and three showed no association in the East (6–8), and two showed a positive association in the West (9, 10). No prospective cohort studies and randomized controlled trials were found. One possible explanation for the negative association between

TABLE 2 | Odds ratios (ORs) and 95% confidence intervals (CI) of nasopharyngeal carcinoma for dairy product consumption in 815 NPC cases and 1,502 controls after multiple imputation‡.

		N cases/controls	Age- and sex-adjusted OR (95% CI)	Multivariable adjusted OR† (95% CI)
MILK (FRESH AND POWDERED COMBINED), GLASSES/MONTH				
At age 6–12	Abstainers	334/543	1.00 (0.86–1.16)	1.00 (0.82–1.22)
	Non-regular users	275/436	1.01 (0.87–1.16)	1.24 (1.00–1.50)
	Regular users	206/523	0.63 (0.53–0.74)	0.74 (0.61–0.91)
	P for trend		<0.001	0.038
At age 13–18	Abstainers	326/515	1.00 (0.86–1.16)	1.00 (0.82–1.22)
	Non-regular users	343/617	0.86 (0.76–0.98)	0.96 (0.82–1.13)
	Regular users	146/370	0.61 (0.50–0.74)	0.68 (0.54–0.86)
	P for trend		<0.001	0.019
At age 19–30	Abstainers	311/467	1.00 (0.86–1.17)	1.00 (0.82–1.22)
	Non-regular users	326/612	0.79 (0.69–0.90)	0.85 (0.72–1.00)
	Regular users	178/423	0.62 (0.52–0.74)	0.68 (0.55–0.84)
	P for trend		<0.001	0.009
10 years before recruitment	Abstainers	316/513	1.00 (0.86–1.16)	1.00 (0.83–1.20)
	Non-regular users	305/535	0.93 (0.80–1.07)	0.97 (0.81–1.16)
	Regular users	194/454	0.70 (0.59–0.82)	0.72 (0.59–0.87)
	P for trend		<0.001	0.019
OTHER DAIRY PRODUCTS (ICE CREAM, YOGURT OR CHEESE), SERVINGS/MONTH				
At age 13–18	Abstainers	205/301	1.00 (0.82–1.22)	1.00 (0.77–1.29)
	Non-regular users	402/836	0.71 (0.63–0.79)	0.85 (0.74–0.97)
	Regular users	208/365	0.84 (0.70–1.00)	0.92 (0.74–1.14)
	P for trend		0.29	0.73
At age 19–30	Abstainers	204/315	1.00 (0.82–1.21)	1.00 (0.76–1.31)
	Non-regular users	400/828	0.75 (0.67–0.84)	0.84 (0.73–0.96)
	Regular users	211/359	0.92 (0.77–1.10)	1.01 (0.81–1.25)
	P for trend		0.68	0.75
10 years before recruitment	Abstainers	202/328	1.00 (0.82–1.21)	1.00 (0.77–1.31)
	Non-regular users	399/821	0.80 (0.72–0.90)	0.88 (0.77–1.01)
	Regular users	214/353	1.01 (0.85–1.21)	1.05 (0.85–1.31)
	P for trend		0.79	0.59

†*Adjusted for sex, age (5-year group), socioeconomic status score (range: −1 [lowest] to 13 [highest], calculated by the subject's, and his/her father's and mother's education, housing type at age 10, personal income and household income), smoking and drinking status (never/ever), occupational hazards (never/ever), family history of cancer (none/NPC/other cancers), IgA against EBV viral capsid antigen VCA (EBV VCA-IgA, seronegative/seropositive), and total energy intake (residual method) at different life periods as appropriate.* ‡*A flexible additive regression model with predictive mean matching incorporating data on the primary outcome (NPC cases vs. controls), exposure (milk or other dairy products intake) and other covariates included in the multivariable model was used to predict missing values of these factors.*

All the risk estimates did not vary (P for sex interaction ranged 0.21–0.99) by sex.

milk intake and NPC is that milk contains estrogen that accounts for over 40% of estrone intake from foods (18). High levels of calcium, fat, protein and folate in milk may also have a role. These nutrients have been found to have anti-cancer effects through various pathways, like inducing apoptosis, anti-inflammation, anti-proliferation and DNA methylation. Further studies are needed to confirm these results.

The strength of the present study included: (1) being the largest series of NPC for investigating the associations with consumption of individual dairy products at different life periods, and (2) having reliable information on dairy consumption as shown in our reliability study (12). However, several limitations should be noted. First, despite adjusting for covariates, residual confounding cannot be excluded. Consumption of fresh fruits or vegetables was associated with NPC risk, but it has not been found to be associated with dairy intake. Indeed, dairy intake was not correlated with consumption of fresh fruits or vegetables in our analysis (data not shown). Therefore, consumption of fresh fruits or vegetables was not regarded as a potential confounder for the association between dairy intake and NPC risk in the present study. Nonetheless, further analysis yielded similar ORs (data not shown) after adjusting for consumption of fruits or vegetables. Mendelian randomization approach using the single nucleotide polymorphism (SNP) of LCT-13910 C/T is recommended, but a larger sample size is needed because of the relatively low frequency of the T allele that can digest milk (i.e., lower prevalence of lactase persistence)

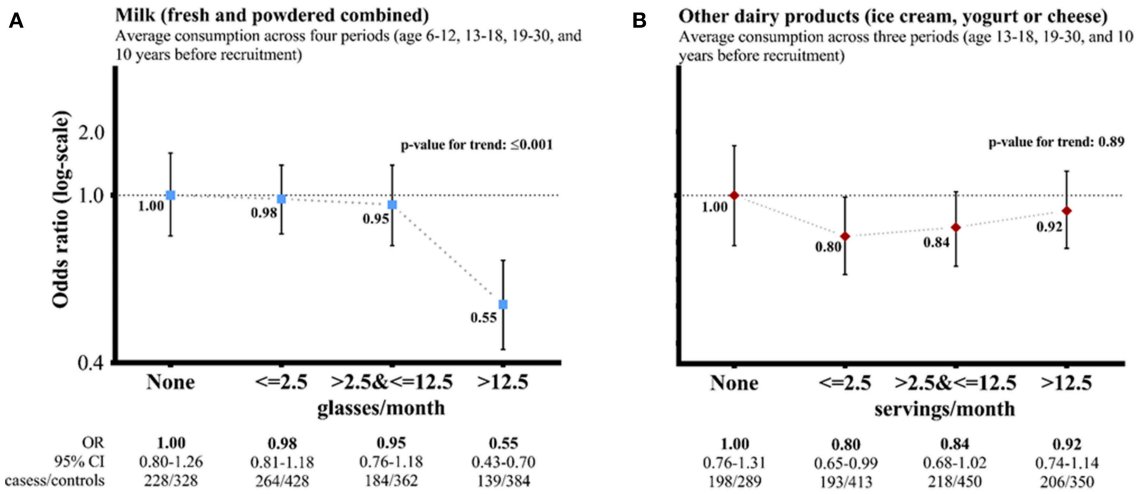

FIGURE 1 | Dairy product consumption and risk of nasopharyngeal carcinoma (cases vs. controls)—adjusted[†] odds ratios (ORs, 95% confidence interval: CI) comparing average consumption across life periods (three categories) vs. none after multiple imputation[‡]. [†]Adjusted for sex, age (5-year group), socioeconomic status score, smoking and drinking status, exposure to any occupational hazard, family history of cancer, IgA against Epstein-Barr virus viral capsid antigen, and total energy intake at different life periods as appropriate. [‡]A flexible additive regression model with predictive mean matching incorporating data on the primary outcome (NPC cases vs. controls), exposure (milk or other dairy products) and other covariates included in the multivariate model was used to predict missing values of these factors. CI: group-specific confidence interval for the multivariable OR, reflecting the variance of the log risk in only that one group. The risk estimates did not vary by sex [*P* for interaction: 0.52 for **(A)** and 0.91 for **(B)**]. **(A)** Milk (fresh and powdered combined). **(B)** Other dairy products (ice cream, yogurt, or cheese).

in our sample and in Chinese populations. Furthermore, as we used hospital-based control subjects, Berkson's bias might exist. Notably, the dairy consumption (milk and others) in our control group was lower than that in the general Hong Kong population, suggesting that our results might underestimate the protection of milk consumption against NPC. The external validity of our results might be limited because hospital-based controls were used. Further studies may recruit population-based controls or use other study designs (Mendelian randomization approach, prospective cohort studies, and randomized controlled trials) which can provide stronger evidence for causation. Another concern is potential information bias, especially as the range of dairy consumption was already limited, which made dose-response relations more difficult to detect. To limit any information bias, we designed and used a computer-assisted self-administered questionnaire to collect information on exposure of interest in the same way from both cases and controls. We also conducted a test-retest reliability study to assess recall error, and found that the questionnaire data of most NPC etiology factors of our NPC case-control had acceptable reliability (fair-to-substantial reliability), even for early life exposure (age 6–12 and 13–18) (12).

CONCLUSIONS

Our data suggest milk intake may be a protective factor of NPC. Such protective association may be attributed to the estrogen, calcium, vitamin D (fortified), or folate in milk, but further research is needed for confirmation. Our result, if confirmed to be causal, has important implications for the consumption of dairy products and prevention of NPC in the East, where consumption of dairy products is generally low.

ETHICS STATEMENT

The Institutional Review Board of the HKU/Hospital Authority HK West Cluster (UW 11-192), the HK East Cluster Research Ethnics Committee (HKEC-2012-043), the Research Ethics Committee of the Hospital Authority Kowloon Central/Kowloon East (KC/KE-13-0115/ER-2), the Research Ethics Committee of the Kowloon West Cluster [KW/EX-13-073(63-11)], and the NTW Cluster Clinical & Research Ethics Committee (NTWC/CREC/1239-13) approved the study. Informed consent was obtained from all individual subjects included in the study.

AUTHOR CONTRIBUTIONS

Z-MM, J-HL, and Y-HC designed and conducted the study in consultation with T-HL. S-YH is the guarantor for the paper. Z-MM analyzed the data, wrote the first draft, and has checked the accuracy and completeness of the references. All authors revised it critically for important intellectual content and contributed to final approval of the paper.

FUNDING

This study was supported by the Hong Kong RGC Area of Excellence Scheme (Grant Number AoE/M-06/08), and the World Cancer Research Fund UK (WCRF UK) and Wereld Kanker Onderzoek Fonds (WCRF NL), as part of the WCRF International Grant Programme (Grant 2011/460). The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or report writing.

ACKNOWLEDGMENTS

The chief acknowledgment is to the subjects who provided information for this study, and the research staff. We thank Thomas SC Chiang for revising the manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2019.00253/full#supplementary-material

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Oncology

ORIGINAL RESEARCH
published: 24 April 2019
doi: 10.3389/fonc.2019.00286

Ultrasound for Breast Cancer Screening in High-Risk Women: Results From a Population-Based Cancer Screening Program in China

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OPEN ACCESS

Edited by:
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Specialty section:
This article was submitted to Cancer Epidemiology and Prevention, a section of the journal Frontiers in Oncology

Received: 07 January 2019
Accepted: 29 March 2019
Published: 24 April 2019

Citation:
Wang Y, Chen H, Li N, Ren J, Zhang K, Dai M and He J (2019) Ultrasound for Breast Cancer Screening in High-Risk Women: Results From a Population-Based Cancer Screening Program in China. Front. Oncol. 9:286. doi: 10.3389/fonc.2019.00286

Background: Ultrasound is an important modality for breast cancer screening. However, the evidence on the effectiveness of ultrasound screening in population-based cancer screening program in lacking. We aimed to evaluate the diagnostic yield of ultrasound screening in a population-based breast cancer screening in China.

Methods: The analyses were conducted in the context of the Cancer Screening Program in Urban China, which recruited 1,938,996 eligible participants aged 40–69 years from 16 provinces in China from 2012 to 2016. We included 72,250 women assessed to be high-risk for breast cancer who undertook ultrasound screening per study protocol. Diagnostic yield according to the Breast Imaging Reporting and Data System (BI-RADS) was evaluated. Risk factors associated with the positive findings of ultrasound were also explored by univariate and multivariable logistic regression analyses.

Results: Overall, there were 9,765 (13.51%) women had positive findings of ultrasound screening, including 8,487 (11.75%), 1,210 (1.67%), and 68 (0.09%) of BI-RADS categories of III, IV, and V, respectively. Younger ages, late age of 1st live birth and short-term breast feeding were found to be positively associated with positive findings under ultrasound in multivariate analyses stratified by menopause status and family history of breast cancer. Multivariable prediction models were constructed and yielded only modest prediction accuracy, with AUCs around 0.55.

Conclusions: We found the diagnostic yield of ultrasound screening for breast cancer in high-risk population was satisfactory. Prediction models based on environmental risk factors had limited prediction accuracy and need to be improved in the future.

Keywords: ultrasound, early diagnosis, breast cancer, cancer screening, risk prediction

INTRODUCTION

With an estimate of 2,088,849 newly diagnosed cases in 2018 worldwide, breast cancer is the most frequently diagnosed cancer for women and is also the leading cause of cancer-related deaths (1). In China, the burden of breast cancer increased dramatically for the past decades, with incidence and mortality of 28.77 per 10,000 and 6.35 per 10,000, respectively in 2014 (2). While advantages in treatment have improved the overall outcomes of breast cancer, evidences from observational studies and randomized controlled trials have clearly demonstrated the effectiveness of breast cancer screening in reducing the mortality of breast cancer (3–5).

In most cancer screening programs, mammography was regarded as main screening method. However, the diagnostic accuracy of mammography for breast cancer was not equal in all women. The overall sensitivity of mammography for detecting breast cancer was around 85%, but it dropped dramatically to 47.8–64.4% for women with dense breast tissue (6). Previous studies have demonstrated that women with dense breast had an elevated risk of breast cancer (7). Therefore, such limitation of mammography may limit the its screening efficacy in population having a high proportion of dense breast. Ultrasound has the potential of detecting small nodules and is also widely accessible and affordable in countries having limited and unbalanced health resources (8–10). The current breast cancer screening guidelines recommended that ultrasound could be served as an auxiliary screening method to mammography (5, 11). However, most previous studies were conducted in western populations, evidences regarding the suitable screening methods in Chinese population are sparse.

Since October 2012, the China government initiated a population-based Cancer Screening Program in Urban China (CanSPUC), in which breast cancer screening is a major component. For the present study, we reported the results of breast cancer screening using ultrasound conducted between October 2012 and October 2016. We aimed to evaluate the diagnostic yield of ultrasound screening in high-risk Chinese populations and to identify risk factors associated with the clinical findings of ultrasound screening.

METHODS

Study Design and Study Population

We performed a cross-sectional study under the framework of Cancer Screening Program in Urban China (CanSPUC). CanSPUC is an ongoing national cancer screening program which was initiated in October 2012. Briefly, residents aged 40–69 years old living in the selected communities of the participating cities were approached by trained staffs by means of phone-calls and personal encounter. After obtaining signed written informed consent, all the eligible participants were interviewed by trained staffs to collect information about their exposure to risk factors and to evaluate their cancer risk using an established risk score system. For the present screening program, to optimize use of the limited healthcare resources and to enhance the detection rate of positive findings, only participants who were assessed to be at high-risk of breast cancer were recommended to undergo subsequent ultrasound and/or mammography intervention at tertiary-level hospital designated by the program at free of charge. The study was approved by the Ethics Committee of National Cancer Center/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College and all participants provided written informed consent.

The overall screening strategy for breast cancer in this program was tailored according the age. For participant aged 40–44 years old, ultrasound was provided firstly and only those with suspicious findings under ultrasound (BI-RADS categories of III, IV, and V) were recommended to take subsequent mammography examination. For participants aged 45–69 years old, both ultrasound and mammography were provided to the participants. For patients with positive findings, further treatment were suggested according the up-to-date clinical guidelines.

For the present analyses, we only used the data of the ultrasound screening conducted in the first 4 years between October 2012 and October 2016, which covered a total of 22 cities in 16 provinces. Overall, there were 1,938,996 eligible participants recruited. After excluding participants of male sex ($N = 903,936$), participants with invalid risk assessment results ($N = 1,055$), and those not at high-risk for breast cancer ($N = 833,542$), 200,462 participants were evaluated to be high-risk for breast cancer. We further excluded 126,426 participants who did not attend ultrasound screening and 1,786 participants with invalid ultrasound results, yielding an overall of 72,250 participants included in the final analyses. A flow-diagram showing the recruitment of study population is shown in the **Figure 1**.

Risk Assessment

Participants were required to undertake risk assessment before clinical intervention. The rationale of the development of the cancer risk score system basically followed the Harvard Risk Index, but the included risk factors, relative risks and exposure rates of risk factors were adjusted according to the characteristics of Chinese population. Briefly, the following factors were included in the risk score system, including Body Mass Index (BMI), age of menarche, total years of menstruation, age of first marriage, total months of breastfeeding, history of breast benign diseases, History of female reproductive system surgery, and family history of breast cancer. Each risk factor was allocated a score by the expert panel based on the magnitude of its association with breast cancer. The cumulative risk scores were calculated and were then divided by the average risk score in general population to get the final individual relative risks. Individuals with relative risks over 1.50 were defined as high-risk for breast cancer.

Clinical Procedures

Screening ultrasound was performed by color Doppler and high-resolution transducers scanning for transverse and sagittal planes by experienced radiologists (attending physician or above having experiences of endoscopy for at least 5 years). Any findings during ultrasound examination were required to be photo documented. Clinical information such as morphology,

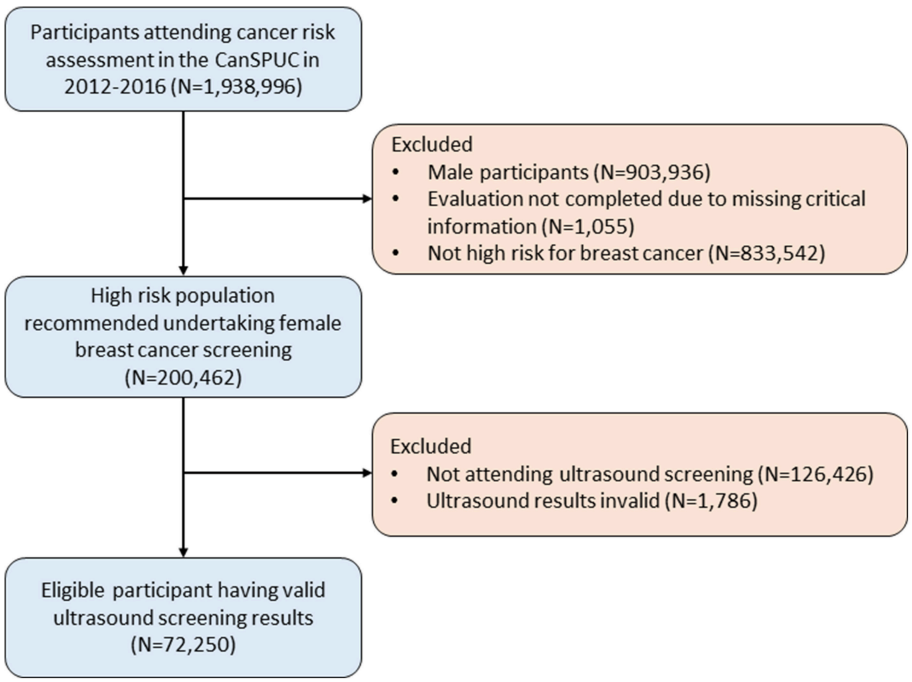

FIGURE 1 | Flow diagram of the study participants in the CanSPUC from 2012 to 2016.

thickness, and structure of gland, features of breast space occupying solid lesions (position, size, margin, echogenicity, etc.) and clinical diagnosis were collected and documented in the data system. In this study, the Breast Imaging Reporting and Data System (BI-RADS) was used to interpret the ultrasound screening results and derive diagnosis reports, with the following categories: I, negative; II, benign; III, probably benign; IV, suspicious malignancy; and V, highly suggestive of malignancy.

For quality and consistency among all study sites, central capacity training programs were conducted annually to ensure that uniform standard of BI-RADS was implemented by radiologists from all participating hospitals. In addition, the images of all positive findings (BI-RADS categories of III, IV, and V) and 1% of randomly selected negative findings (BI-RADS categories of I and II) were centrally reviewed by an expert panel from National Cancer Center. Any discrepancy with the original diagnosis were discussed until consensus were reached.

Data Acquisition

Paper-based standardized documentation forms (epidemiological questionnaire, clinical ultrasound examination forms) were filled by trained study staffs and physicians. Validity of forms were checked and entered into the data management system by trained study staffs. Consistency check was conducted, and mistakes were corrected by retrieving the original records if inconsistencies were identified. Each participant had a unique identification code using to track all the individual's relevant documentation forms. All data were transmitted to the Central Data Management Team, who were responsible data monitoring and subsequent data analyses.

Statistical Analysis

Descriptive statistically analyses regarding the characteristics of the study population were firstly performed. The distribution of risk factors by different BI-RADS categories (I/II, III, and IV/V) were presented. Chi-square tests were used to compare the distribution of risk factors between participants with or without positive findings (BI-RADS category of III-V) under ultrasound screening. Multivariable logistic regression models stratified by the menopause status (pre-menopause or post-menopause) were employed to explore the associations between the risk factors and positive findings of screening ultrasound, and odds ratios (ORs) and their 95% confidence intervals (95% CIs) were also calculated and reported. Receiver operating characteristics (ROC) curves were constructed to estimate the diagnostic accuracy of multivariate logistic model using the selected risk factors for predicting the abnormal findings (BI-RADS II-IV) under screening ultrasound. Area under the curves (AUCs) along with the 95% CIs were also calculated and reported. All statistical analyses were performed with the statistical software R version 3.5.1. All tests were two-sided and *p*-values of 0.05 or less were considered to be statistically significant.

RESULTS

Characteristics of the Study Population

Overall, 72,575 participants having valid ultrasound screening results were included in our analyses. **Table 1** shows the sociodemographic characteristics of the study population. The mean age of the participants was 52.8 years old, with the proportions of 40–49, 50–59, and 60–69 years of 37.4, 41.4, and

TABLE 1 | Study population characteristics among participants having breast ultrasound screening in the CanSPUC in 2012–2016.

Group	*N*	Percentage (%)
AGE (YEARS)		
40–49	27,018	37.4
50–59	29,946	41.4
60–69	15,286	21.2
RACE		
Han	68,600	94.9
Minorities	3,650	5.1
EDUCATION		
Primary school or below	8,271	11.5
Middle/high school	47,099	65.5
College or above	16,519	23.0
MARITAL STATUS		
Single	777	1.1
Married/have married	71,067	98.9
BI-RADS CATEGORY		
I	40,458	56.00
II	22,027	30.49
III	8,487	11.75
IV	1,210	1.67
V	68	0.09

21.2%, respectively. 94.9% of the population were ethnic Han, and most of the participants had an education background equal or higher than middle school, and nearly all the participants had a history of marriage.

Regarding the clinical diagnosis of ultrasound screening, 56.00% of the participants ($N = 40,458$) had negative findings (BI-RADS I). For the participants with abnormal findings, the proportions of BI-RDAS categories of II (benign), III (probably benign), IV (suspicious malignancy), and V (highly suggestive of malignancy) were 30.49% ($N = 22,027$), 11.75% ($N = 8,487$), 1.67% ($N = 1,210$), and 0.09% ($N = 68$), respectively For the nodule findings, the mean sizes for patients with BI-RADS categories of II, III, IV, and V were 5.00, 6.57, 8.58, and 16.31 mm, respectively.

Factors Associated With BI-RADS Diagnosis of Ultrasound

We further explored the association of risk factors with BI-RADS diagnosis of ultrasound screening. Results of univariate analyses are shown in **Table 2**. Overall, women with high BMI (≥24.0), late age of menarche (>13 years old), at stage of pre-menopause, late age of first live birth (≥ 28 years old), and short period of breast feeding (<4 months) were tending to have abnormal findings under ultrasound screening.

As family history of breast cancer was an important risk factor for identifying high-risk population of breast cancer, about one half of high-risk population identified reported to have a family history of breast cancer among 2-degree relatives, and menopause status was an important predeterminant factor defining the weight of risk factors. We therefore conducted multivariable logistic regression analyses stratified by these two factors to explore the association between the risk factors with positive findings under ultrasound screening and detailed results are shown in **Table 3**.

For pre-menopause women without family history of breast cancer within 2-degree relatives, age, BMI, age of 1st live birth and duration of breast feeding were found to be associated with the positive findings of breast ultrasound screening. For instance, compared to women aged of 60–69 years old, women aged of 40–49 years old, and 50–59 years old had higher likelihood to have positive findings of ultrasound, with ORs of 2.18 (1.52–3.28) and 1.92 (1.32–2.90), respectively. For pre-menopause women with family history of breast cancer, age, BMI and total years of menstruation were also found to be associated factors. Similarly, for post-menopause women without or with family history of breast cancer within 2-degree relatives, age were also identified to be a potential risk factor associated with the abnormal findings of ultrasound screening.

Models for Predicting Abnormal Findings Under Ultrasound Screening

By using the above-mentioned risk factors, we further constructed multivariable logistic regression models to predict abnormal findings under ultrasound screening for the four subgroups, and the diagnostic accuracy was evaluated by constructing ROC curves. The results of ROC curves are shown in **Figure 2**. Overall, the four prediction models only yielded poor diagnostic accuracy for prediction women with abnormal findings under ultrasound examinations, with AUCs around 0.55. For instance, for premenopausal women without family history of breast cancer within 2-degree relatives, the AUC was 0.54 (95% CI: 0.53–0.55). Similar AUCs were also observed for the rest three subgroups.

DISCUSSION

We reported here the preliminary results of 72,575 high-risk women who undertook ultrasound screening in a population-based cancer screening program in China. The analyses showed that positivity rate for abnormal findings (BI-RADS III-V) of ultrasound screening in this high-risk population was 13.51%, with BI-RADS categories III, IV, and V of 11.75, 1.67, and 0.09%, respectively. Additionally, we identified several factors including age, BMI, age of first live birth and duration of breast feeding were potentially associated with the positive findings of ultrasound. However, multivariable prediction models using these factors only conferred modest diagnostic performance. To our limited knowledge, this is the first large-scale study reporting the diagnostic findings of ultrasound screening in a population-based cancer screening program in China. The finding of our study provided timely estimate of screening yield of ultrasound in breast cancer screening programs and will be helpful for designing effective breast cancer screening strategies in future.

Ultrasound was suggested to serve as an adjunctive screening method for women having dense breasts (3). Regarding the epidemiology of dense breasts, about 25 million women (about 43.3%) aged 40–74 years are classified as having heterogeneously or extremely dense breasts according to data from Breast

TABLE 2 | Distribution of risk factors among participants with different BI-RADS findings under ultrasound screening in the CanSPUC in 2012–2016.

Factors	BI-RADS I, II (*N*, %)	BI-RADS III (*N*, %)	BI-RADS IV, V (*N*, %)	*p*-value*
AGE				
40–44	8,766 (14.0)	1,414 (16.7)	177 (13.8)	<0.001
45–49	13,861 (22.2)	2,461 (29.0)	339 (26.5)	
50–54	14,617 (23.4)	2,321 (27.3)	315 (24.6)	
55–59	11,305 (18.1)	1,185 (14.0)	203 (15.9)	
60–69	13,936 (22.3)	1,106 (13.0)	244 (19.1)	
BMI				
<24.0	32,073 (51.4)	4,757 (56.2)	685 (53.7)	<0.001
24.0–27.9	22,911 (36.7)	2,885 (34.1)	452 (35.4)	
≥28.0	7,367 (11.8)	826 (9.8)	139 (10.9)	
AGE OF MENARCHE				
<13	8,872 (16.4)	1,316 (17.8)	199 (17.9)	<0.001
13–16	38,549 (71.4)	5,283 (71.6)	792 (71.3)	
>16	6,569 (12.2)	776 (10.5)	120 (10.8)	
MENOPAUSE STATUS				
Pre-menopause	27,553 (44.1)	3,599 (42.4)	589 (46.1)	<0.001
Post-menopause	34,932 (55.9)	4,888 (57.6)	689 (53.9)	
TOTAL YEAR OF MENSTRUATION				
<30	9,274 (15.0)	1,315 (15.6)	179 (14.2)	<0.001
≥30	52,745 (85.0)	7,107 (84.4)	1,085 (85.8)	
AGE OF FIRST LIVE BIRTH				
<28	43,351 (69.4)	5,936 (69.9)	905 (70.8)	<0.001
≥28	14,368 (23.0)	1,989 (23.4)	278 (21.8)	
Nulliparous	4,766 (7.6)	562 (6.6)	95 (7.4)	
TOTAL MONTHS OF BREASTFEEDING				
≥4 months	47,215 (75.6)	6,380 (75.2)	934 (73.1)	<0.001
<4 months	4,012 (6.4)	469 (5.5)	85 (6.7)	
No feeding	11,258 (18.0)	1,638 (19.3)	259 (20.3)	
FAMILY HISTORY OF BREAST CANCER WITHIN 2-DEGREE RELATIVES				
No	28,829 (46.1)	4,924 (58.0)	633 (49.5)	<0.001
Yes	33,656 (53.9)	3,563 (42.0)	645 (50.5)	

**Chi-square tests comparing the distribution of risk factor groups between participants with or without positive findings (BI-RADS III-V) under ultrasound screening.*

Cancer Surveillance Consortium in the US (12). For Chinese women, the prevalent of dense breast was even higher which therefore further limited the efficacy of mammography in breast cancer screening (13, 14). One previous research in China also demonstrated that ultrasound was superior to mammography for breast cancer screening in high-risk Chinese women (15). Therefore, ultrasound was regarded as an important adjunctive method to mammography for breast cancer screening in Chinese population.

In our study, the detection rate for suspicious malignancy (BI-RADS IV and V) was 0.17%. Our results were in line with previous researches conducted in China (15, 16). As active and passive follow-ups collecting the health outcomes of the participants is still under way in this cancer screening program, sensitivity, specificity, and positive/negative predictive values of the ultrasound for detecting breast cancer cannot be assessed in the current analyses and will be explored in further research.

Previous studies have identified a series of risk factors of breast cancer and risk prediction models based on such risk factors such as the Gail breast cancer risk model have been developed to identified women at high risk for breast cancer for preventive interventions or more intensive surveillance (17–21). In our study, we found BMI, age of menarche, age of first live birth and duration of breast feeding were associated with the positive findings under ultrasound examinations, which were lines with previous studies. As some factors (such as age of menarche) were also included in the risk score system to select high risk population, the magnitude of association might be underestimated. However, such analyses are indispensable to validate pre-included factors and explore new risk factors, with the purpose of further optimizing the risk assessment model for future research.

It deserves to be noted that the overall positivity rate of ultrasound screening was high in a high-risk population in urban China, with around 44% participants having benign or potential

TABLE 3 | Associations between risk factors and positive findings (BI-RADS III-V) of breast ultrasound screening.

Factors	Pre-menopause				Post-menopause			
	Without family history of breast cancer within 2-degree relatives		With family history of breast cancer within 2-degree relatives		Without family history of breast cancer within 2-degree relatives		With family history of breast cancer within 2-degree relatives	
	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
AGE								
60–69	*Ref.*		*Ref.*		*Ref.*		*Ref.*	
40–49	2.18 (1.52–3.28)	<0.001	3.25 (1.89–6.18)	<0.001	1.35 (1.07–1.69)	<0.001	*1.90 (1.56–2.30)*	*<0.001*
50–59	1.92 (1.32–2.90)	<0.001	3.35 (1.94–6.37)	<0.001	1.32 (1.17–1.50)	<0.001	*1.49 (1.36–1.65)*	*<0.001*
BMI								
<24.0	*Ref.*		*Ref.*		*Ref.*		*Ref.*	
24.0–27.9	0.85 (0.78–0.94)	0.001	0.90 (0.82–0.99)	0.037	0.89 (0.79–1.01)	0.083	0.94 (0.86–1.04)	0.237
≥28.0	0.82 (0.71–0.97)	0.018	0.62 (0.52–0.72)	<0.001	0.95 (0.80–1.12)	0.557	0.82 (0.70–0.96)	0.012
AGE OF MENARCHE								
>16	*Ref.*		*Ref.*		*Ref.*		*Ref.*	
13–16	1.01 (0.86–1.19)	0.900	0.97 (0.83–1.15)	0.761	1.05 (0.89–1.23)	0.572	1.11 (0.98–1.29)	0.113
<13	0.93 (0.77–1.12)	0.424	1.16 (0.96–1.41)	0.118	1.19 (0.96–1.48)	0.111	1.11 (0.93–1.32)	0.254
TOTAL YEAR OF MENSTRUATION								
<30	*Ref.*		*Ref.*		*Ref.*		*Ref.*	
≥30	1.01 (0.90–1.13)	0.854	1.27 (1.12–1.45)	<0.001	0.98 (0.82–1.17)	0.788	1.01 (0.85–1.20)	0.944
AGE OF 1ST LIVE BIRTH								
<28	*Ref.*		*Ref.*		*Ref.*		*Ref.*	
≥28	1.11 (1.01–1.24)	0.030	1.02 (0.92–1.14)	0.703	0.90 (0.79–1.04)	0.157	1.02 (0.92–1.14)	0.682
Nulliparous	1.15 (0.63–1.20)	0.633	0.89 (0.67–1.19)	0.444	0.54 (0.34–0.82)	0.005	1.39 (0.75–2.48)	0.283
BREAST FEEDING								
≥4 months	*Ref.*		*Ref.*		*Ref.*		*Ref.*	
<4 months	1.09 (0.61–2.01)	0.778	0.92 (0.67–1.26)	0.589	1.33 (0.84–2.13)	0.231	0.62 (0.33–1.18)	0.147
No feeding	1.12 (1.01–1.24)	0.032	1.06 (0.93–1.20)	0.371	1.08 (0.91–1.28)	0.371	1.03 (0.92–1.15)	0.626

OR, odds ratio; 95% CI, 95% confidence interval.

malignancies. Therefore, the potential harms of ultrasound screening including the consequences of false-positive and false-negative tests, and the occurrence of over-diagnosis cannot be neglected. Further studies addressing the estimates of the positive and negative effects of ultrasound screening in women based on the latest evidence are required to help policy makers in their decision-making about implementation of the breast cancer screening programs. Although ultrasound had several advantages over mammography such as lower cost and easier accessibility especially in resource poor regions, it had barriers in screening programs, such as operator dependence procedure, limited ability to detect calcifications, lack of trained technologist, and limited reproducibility. Further large-scale trials and rigorous health-economic evaluations should be conducted to illustrate whether ultrasound screening is cost-effective in breast cancer screening programs.

Specific strengths and limitations deserve careful attention when interpreting our results. A major strength of our study is the fact that our analyses were the first to illustrate diagnostic yield of ultrasound screening in a large-scale population-based cancer screening program in China. Furthermore, detailed patient information including epidemiological questionnaire and clinical examination data were collected in a standardized manner by trained study staffs to ensure the quality of data. Capacity training and central review of ultrasound reports by an expert panel were also conducted yearly to enhance the consistency and accuracy of clinical diagnoses. Limitations include that the study population was a pre-selected high-risk population using the predefined risk model which was not representative of entire general population of China and therefore selection bias cannot be ruled out. In addition, follow-ups tracing the outcomes of all the participants are undergoing, so evaluation of detection rate of breast cancer or occurrent of interval cancer cannot be evaluate at the current stage.

In summary, in this large-scale cancer screening program in China, we found the diagnostic yield of ultrasound screening for breast cancer in high-risk population was satisfactory. Using environmental risk factors associated with positive findings of ultrasound identified in this study only carried limited prediction accuracy and further improvement by incorporating other effective factors could contribute to the development of a useful risk prediction model for identifying high-risk populations of breast cancer in the future.

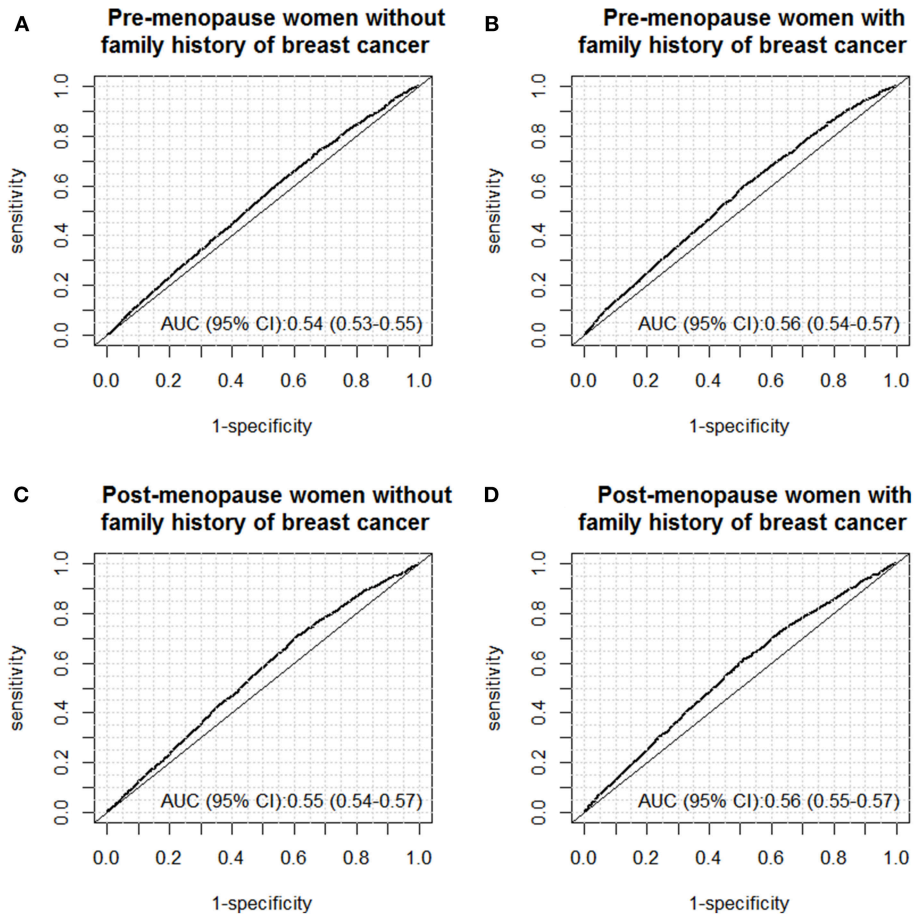

FIGURE 2 | ROC curves of the models for predicting abnormal findings (BI-RADS III-V) under breast ultrasound screening in the following subgroups: **(A)** pre-menopause women without family history of breast cancer; **(B)** pre-menopause women with family history of breast cancer; **(C)** post-menopause women without family history of breast cancer; **(D)** post-menopause women with family history of breast cancer.

ETHICS STATEMENT

The study was approved by the Ethics Committee of National Cancer Center/Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College and all participants provided written informed consent.

AUTHOR CONTRIBUTIONS

JH and MD: conception and design. YW and HC: statistical analyses and drafting of the article. YW, HC, NL, JR, and KZ: data acquisition and data interpretation. All authors revised the manuscript and approved the final version of the manuscript.

FUNDING

The study was partly funded by China Postdoctoral Science Foundation (no. 2017M620683), PUMC Youth Fund and the Fundamental Research Funds for the Central Universities (no. 2017320015).

ACKNOWLEDGMENTS

We sincerely thank all the members of the Screening Program in Urban China program from the National Cancer Center of China, provinces, and external expert panels. We are also grateful to the participants for attending this study.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Oncology

ORIGINAL RESEARCH
published: 26 April 2019
doi: 10.3389/fonc.2019.00312

Is Epstein-Barr Virus Infection Associated With Thyroid Tumorigenesis?—A Southern China Cohort Study

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OPEN ACCESS

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Specialty section:
This article was submitted to Cancer Epidemiology and Prevention, a section of the journal Frontiers in Oncology

Received: 22 January 2019
Accepted: 05 April 2019
Published: 26 April 2019

Citation:
Yu S-T, Ge J-N, Li R-C, Wei Z-G, Sun B-H, Jiang Y-M, Luo J-Y, Liu H and Lei S-T (2019) Is Epstein-Barr Virus Infection Associated With Thyroid Tumorigenesis?—A Southern China Cohort Study. Front. Oncol. 9:312. doi: 10.3389/fonc.2019.00312

Background: Epstein-Barr virus (EBV) is associated with many epithelial malignancies. A few reports on the association between EBV and thyroid tumorigenesis have been investigated. However, the conclusion is highly contradictory. We aimed to explore the role of EBV in thyroid nodule development and its clinical significance in a cohort from southern China.

Method: We conducted a retrospective data abstraction study of patients who underwent thyroidectomy between December 2017 and June 2018. We retrospectively analyzed the clinicopathological parameters and EBV infection status (serological antibodies and *in situ* hybridization).

Result: The cohort comprised 384 patients with newly diagnosed thyroid diseases, including 261 papillary thyroid carcinomas, 87 nodular goiters, 21 follicular adenomas, 12 follicular thyroid carcinomas, and 3 medullary thyroid carcinomas. Forty-two (10.9%) patients were identified as being serological antibody positive. However, there was no association between the clinicopathological parameters and serological antibody positivity. Additionally, none of the patients showed EBER expression in thyroid normal/cancer cell nuclei in *in situ* hybridization.

Conclusion: In this study, no correlation between EBV and thyroid diseases was found in a cohort from southern China.

Keywords: thyroid cancer (TC), Epstein-Barr virus (EBV) infection, EBER = EBV-encoded small RNA, EBV-specific capsid antigen (VCA/IgA), EBV-specific early antigen (EA/IgA), Southern China

INTRODUCTION

Epstein-Barr virus (EBV) is a well-known human tumor virus with a very high prevalence in the population, especially for children and youth. EBV serum (IgG) is positive in an estimated 95% of the world's population (1). EBV infection is associated with epithelial and lymphoid malignancies, including nasopharyngeal carcinoma (NPC), gastric cancer, Hodgkin's lymphoma and Burkitt's lymphoma (2, 3). Although EBV has B-lymphocyte tropism, it can also infect T lymphocytes,

myocytes, and epithelial cells in the oropharynx and stomach (4). Once EBV infects a host cell, it starts to induce a lytic or latent infection with diverse genes expressed. EBV nuclear antigens (EBNA 1, 2, 3A, 3B, 3C, and LP), the latent membrane proteins (LMP 1, 2A, and 2B) and two small noncoding RNAs (EBV-coded small RNA, EBER-1, and EBER-2) are expressed during the infection (3). These genes collaborate to induce tumorigenesis by causing systematic inflammation, suppressing the antitumoral immune system, and preventing anoikis.

Whether EBV infects the thyroid gland remains controversial. To date, only a handful of reports on the association between EBV and thyroid tumorigenesis have been investigated. Stamatiou et al. (5) summarized publications regarding the EBV detection rate in thyroid cancer specimens from 2001 to 2015. The conclusion was inconclusive because the results were highly contradictory, ranging from negative to 100% positive for EBV infection.

Therefore, in the current study, we explored the role of EBV in thyroid nodule development and its clinical significance in a cohort from southern China.

METHODS

Patient Selection and Sample Collection

A total of 384 patients, including 261 with papillary thyroid carcinomas, 87 with nodular goiters, 21 with follicular adenomas, 12 with follicular thyroid carcinomas, and 3 with medullary thyroid carcinomas, who underwent thyroidectomy between December 2017 and June 2018 at the Department of General Surgery, Nanfang Hospital, Southern Medical University, were identified as being eligible for the study. All the data were extracted from the database of the Department of General Surgery, Nanfang Hospital, Southern Medical University. The patients included in the study met the following criteria: (1) primary thyroid neoplasms (including thyroid cancer, nodular goiter and follicular adenoma) confirmed by post-operative pathological results, (2) no history of thyroid/neck surgery, (3) no previous diagnosis of nasopharyngeal carcinoma (NPC) or other EBV-related disease, (4) no history of neck radiotherapy, (5) exclusion of cervical metastatic cancer, parathyroid neoplasms and Graves' disease, and (6) sufficient medical history. All patients were invited to donate a 5-mL blood sample for storage when they received preoperative examinations in our department. Blood samples were allowed a maximum of 6 h at room temperature before serologic analysis. The serum samples were then separated and divided into 4 tubes. All the serum samples were stored at −80°C at the Department of General Surgery, Nanfang Hospital, Southern Medical University. All the tissues samples were formalin fixed and paraffin embedded (FFPE) and then were cut into 4-μm-thick sections. All the clinicopathological parameters were recorded and evaluated according to the criteria of the American Joint Committee on Cancer (AJCC, 8th edition). This study was approved by the ethical committee of Nanfang Hospital, Southern Medical University. Informed consents were obtained from all involved patients when they were admitted to the hospital to provide their test results/specimens for future medical research.

In situ Hybridization

The EBER-ISH (*in situ* hybridization) test is the most commonly employed method and is regarded as the gold standard to diagnose EBV-infected diseases. We evaluated the presence of EBV in thyroid cells by *in situ* hybridization (ISH) analysis using EBV-encoded small RNA (EBER) probes, PNA probe/FITC (code Y5200), and the PNA ISH detection kit (code K5201) (Dako, Denmark) on the FFPE samples. The protocol was performed according to the manufacturer instructions. Briefly, the slides were baked at 60°C for 2 h and then were deparaffinized using a standard protocol. Deparaffinized slides were boiled for 20 min in pH 6.0 citric buffer for antigen retrieval. The slides were further permeabilized by Protease III treatment at room temperature for up to 10 min. The slides were then hybridized at 40°C for 2 h, followed by amplification and detection by adding Amp 1–4. The primary antibody incubation time at 4°C varied from 12 to 16 h. The substrate was incubated for 60 min, followed by counterstaining with eosin and mounting using Aquamount (Dako). EBER expression was graded from negative (–) to slight (+), moderate or intensive (+ + +), where most cells express EBER-RNA. The procedure was conducted by an experienced pathologist. Additionally, ISH was performed individually using 3 slides in each patient. Previously known cases of EBV-positive Hodgkin's lymphoma and NPC were used as positive controls. Because there is no established cutoff for EBER in solid malignancies other than NPC or gastric cancer (6), we referred this cutoff (>5%) of dark-brown staining of the tumor nucleus as positive for EBER transcript expression.

Serologic Antibody Analysis

Serologic antibody analysis was conducted in all patients using EBV-specific capsid antigen (VCA/IgA) antibodies and EBV-specific early antigen (EA/IgA) antibodies. Serological analysis was performed on July 2018 at the Department of Laboratory Medicine, Nanfang hospital, Southern Medical University because VCA-IgA and EA-IgA were routinely tested for screening NPC in Southern China institutions (7–9). Validation of the serological data was existing results from patients who underwent these two tests at our hospital between December 2017 and June 2018. Positive groups were patients with NPC, and healthy controls were the normal population from the medical examination center. Their ages and sex were matched with those in the thyroid disease cohort (1:1). EBV-specific VCA/IgA antibodies and EA/IgA antibodies were measured by ELISA (Euroimmun, Lubeck, Germany). The levels of these seromarkers were measured photometrically, according to the manufacturers' instructions. Additionally, these results were standardized by calculating the ratio of the optical density (OD) of the sample over that of the reference control (rOD). If the rOD value was >1, the sample was regarded as positive (10, 11). Either VCA/IgA or EA/IgA was positive, and the patients were regarded as serological antibody positive.

Statistical Analysis

All continuous variables were expressed as medians (Percentile 25, Percentile 75). Statistical analysis was conducted using SPSS 22.0 (SPSS, Inc, Chicago, IL, USA). To explore the relationship between EBV and the clinical pathological features in PTC, such as gender, age, and tumor size, chi-squared test and Fisher's exact test were used as appropriate; $p < 0.05$ considered as statistically significant.

RESULTS

The clinicopathological features are presented in **Table 1**. The median age of the included patients was 45 years (40, 61). The median number of resected lymph nodes in Level VI (for those with central neck dissection) and Level II-Vb (for those with lateral neck dissection) were 11.5 (7, 15) and 40 (25.75, 58.75), respectively. Two hundred eighty eight patients came from Guangdong Province (Southern China), and 96 patients came from other provinces of China (**Figure 1**).

Serological Antibodies

The VCA/IgA and EA/IgA antibodies were tested in all 384 patients with thyroid nodules as well as the population of positive/normal controls. There were 42 (10.9%) patients who were either VCA/IgA antibody or EA/IgA antibody positive or positive for both antibodies; therefore, these patients were designated as the serological antibody positive group. Comparing all clinicopathological features between the serological antibody groups, none of these parameters with statistical significance were identified (**Supplementary Tables 1, 2**). The number of patients with available serological results was 857 and 6,923 for NPC (positive control) and normal population (healthy control). After matched ages and sex, 384 NPC patients (positive control) and 384 healthy controls (normal population) were compared with those of the thyroid cohort (**Supplementary Table 3**). There were 311 (81.0%) patients who were serological positive in positive controls, and 40 (10.4%) patients with positive serological tests in the normal population.

In situ hybridization

None of the samples analyzed by ISH showed EBER expression in thyroid normal/cancer cell nuclei, even those with positive results in serological antibody analysis (**Figure 2**).

DISCUSSION

EBV has been revealed to be associated with the development of many cancers, such as gastric cancer, NPC, and Hodgkin's lymphoma (3). Chronic inflammation induced by EBV infection may play a significant role in the progression of cancer (4). However, the relationship between thyroid tumorigeneses and EBV has not been fully elucidated with conflicting results. The preliminary investigation of EBV in thyroid lymphoma was inspired by EBV persistently infecting B lymphocytes, contributing to lymphoma formation. In 2003, Shimakage et al. first reported EBV infection in other types of thyroid malignancies with a Japanese cohort (12). Shimakage et al. (12) explored the potential involvement of EBV expression in the progression of thyroid cancer by examining different thyroid neoplasm specimens, ranging from PTC to anaplastic thyroid carcinoma (ATC). The specimens were subjected to PCR, RT-PCR, ISH and indirect immunofluorescence staining. The results showed that mRNA and protein were positive for all carcinoma specimens and their expression was prominent in ATCs. For benign nodules, they showed no signal or very few signals during ISH. A similar result was conducted by Moghoofei et al. (13). The authors determined EBV infection by qRT-PCR and revealed that the EBV detection rate in PTC was similar to that of the healthy control. However, EBV positivity was associated with the tumor stage. Additionally, based on the PCR result, several inflammatory factors, such as Survivin, CD44, NF-kappaB, and IL-6, were higher expressed in the EBV-positive groups, and the mRNA expression of EBER1 and EBER2 was higher in thyroid tumor group. The reports from Almeida et al. (14) and Homayouni et al. (15) conducted similar

TABLE 1 | Baseline characteristics of all thyroid neoplams patients.

Characteristics	*n*	%
Gender	All = 384	
Male	153	39.8
Female	231	60.2
Pathology		
Nodular goiter	87	22.7
Follicular adenoma	21	5.5
Papillary thyroid carcinoma	261	68
Follicular thyroid carcinoma	12	3.1
Medullary thyroid carcinoma	3	0.8
VCA/IgA positivity	29	7.6
EA/IgA positivity	19	4.9
Serological positivity	42	10.9
EBER positivity	0	0
T stage	Thyroid carcinoma = 276	
1	216	78.3
2	30	10.9
3	21	7.6
4	9	3.3
N stage		
0	102	37
1a	117	42.4
1b	57	20.6
M stage		
0	270	97.8
1	6	2.2
AJCC stage		
1	232	84.1
2	26	9.42
3	12	4.3
4	6	2.2
Strap muscles invasion	24	8.7
Multifocality	57	20.7
Bilaterial	36	13
Hashimoto's thyroiditis	36	13

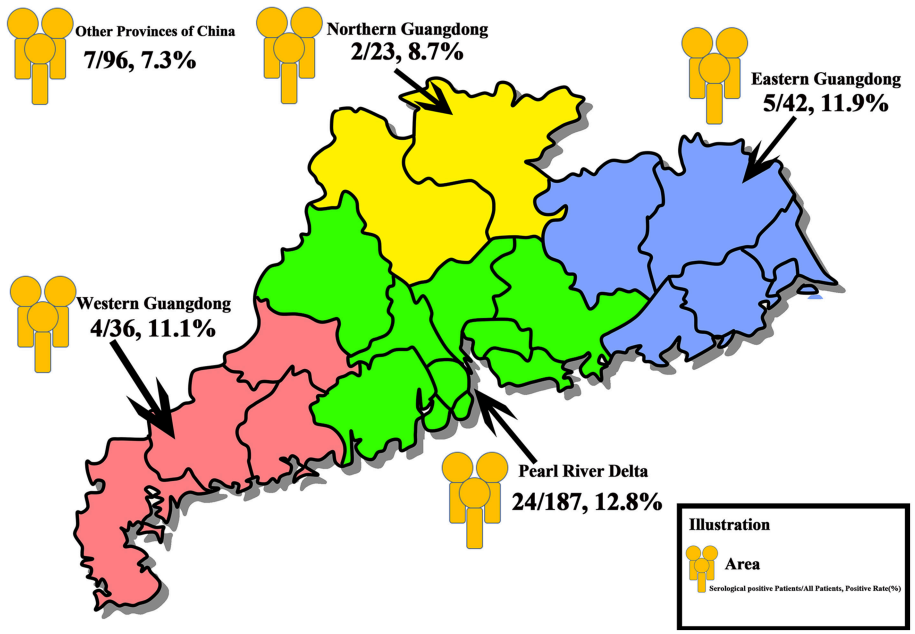

FIGURE 1 | Overall distribution of the study population. In total, 384 patients with thyroid disease who underwent thyroidectomy were recruited. A total of 187 patients were from the Pearl River Delta (Green Area), 24 (12.8%) patients were positive for serological antibodies test, 42 patients were from the Eastern Guangdong (Blue Area), 5 (11.9%) patients were serological positive, 36 patients and 23 patients were from the Western (Red Area) and the Northern (Yellow Area) Guangdong, with a positive rate of 11.1% (4 patients) and 8.7% (2 patients), respectively. Ninety-six patients were from other provinces of China, and 7 patients (7.3%) were serologically positive.

results based on PCR and the ISH test in Brazilian and Iranian populations, respectively.

However, negative results for the association between thyroid tumors and EBV infection have been obtained. Despite a few infiltrating lymphoid cells in 1 (2.2%) of the specimens, none of the 45 PTCs were positive for the EBER-ISH test in a Japanese cohort (16). Bychkov et al. (17) reported that 1 of 20 thyroid cancer tissues contained single EBER-positive inflammatory cells. Cancer cells and normal thyroid tissues were consistently negative for ISH. Additionally, Tsai et al. (18) reported a negative association between benign tumors and EBV infection using ISH or PCR or Southern hybridization in a Taiwanese population.

To the best of our knowledge, this is the first study regarding EBV and thyroid neoplasms based on serological antibodies and ISH analyses in a cohort from the southern part of China. Similar to other southeast Asia neighbors, EBV is highly prevalent in southern China. Additionally, NPC, which has a closer relationship with EBV infection, is more endemic than in any part of the world, especially in Guangdong Province and Hong Kong (19). VCA/IgA and EA/IgA antibodies could reflect the status of recent viral infection and, therefore, are widely used biomarkers for screening NPC in the southern China population (8). In the current study, the positivity rate of serological antibody analysis was 10.9%, which is similar to that in previous national population-based studies conducted in the 1970s (7–9). However, there is no meaningful results between the clinicopathological parameters and VCA/IgA or EA/IgA antibodies. Furthermore, we failed to detect EBER signals in thyroid cancer cells or normal thyroid cells based on the ISH test. Interestingly, two PTC patients who were serological test positive with concurrent NPC were enrolled in this cohort. These two patients had pathologically confirmed NPC by preoperative fiber-laryngoscopy accidentally. They all received radiotherapy for NPC after thyroidectomy and were disease-free at the last follow up. However, we failed to detect the EBER signal in thyroid cancer cells, although the NPC specimens showed nasopharyngeal carcinoma cells that were positive for ISH. Our data indicated that the EBV detection rate in thyroid tumor samples from the southern China population is extremely low, consistent with previous EBER-based reports but differed from PCR-based tests. Several reasons may explain our findings. First, there is difference in populations and geography among different studies. Second, diversity in EBV detection techniques and their interpretation may cause conflicting results. Bychokov A believed that not only viral load assays (PCR or qPCR) but histochemical assays (ISH, immunofluorescence and immunohistochemistry) should be implied to ensure the precise tissue detection of EBV (17). A false-positive result may be received because any tissue containing B lymphocytes may have traces of EBV DNA (20). Additionally, there is no standard for the interpretation of EBV positivity for the ISH EBER test in thyroid tumors. Previous studies with positive results, which set a cutoff of few (<5%) EBER-positive cancer cells and even a single EBER-positive cancer cell are questionable because, in hematological malignancy, <1% of the EBER-positive background lymphocytes are usually regarded as negative during evaluation (20).

Indeed, in the current study, there are many limitations. First, the retrospective study nature may cause inevitable bias. Second, serological antibody analysis only involved VCA/IgA and EA/IgA

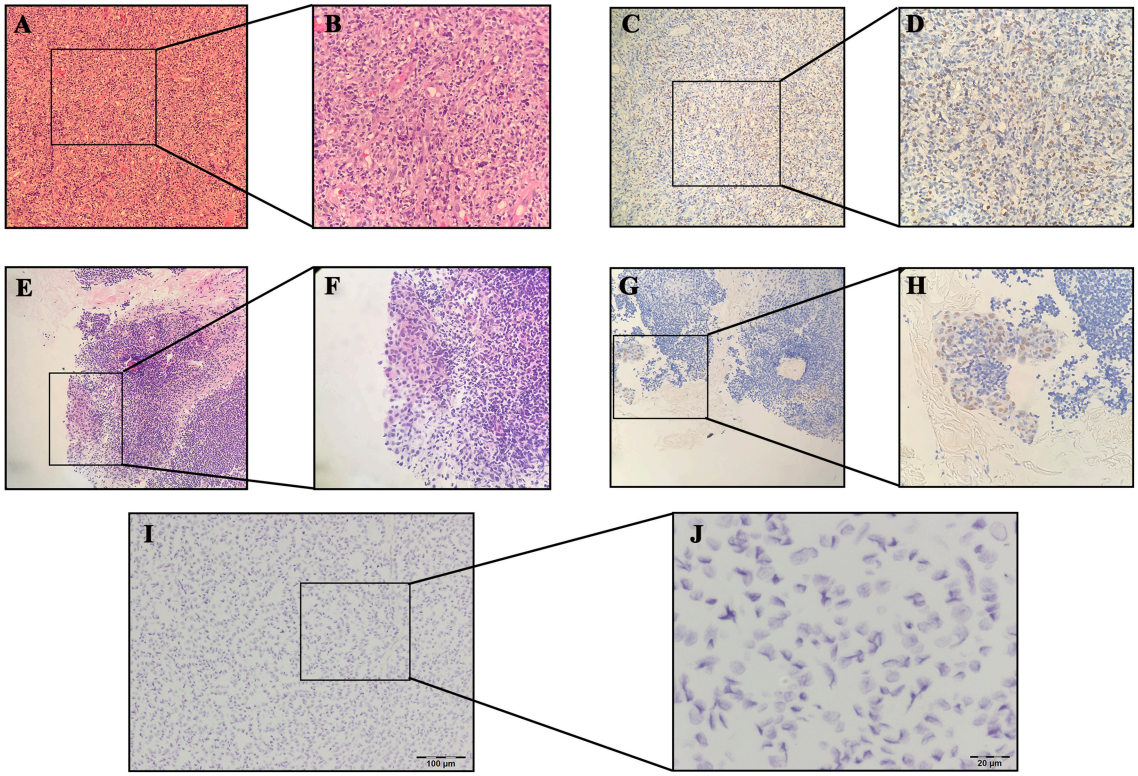

FIGURE 2 | Results of hematoxylin-eosin (HE) staining of Hodgkin's lymphoma: **(A)** 10×; **(B)** 40×; Results of EBER-*in situ* hybridization (ISH) of Hodgkin's lymphoma: **(C)** 10×; **(D)** 40×; Results of HE staining of nasopharyngeal carcinoma (NPC): **(E)** 10×; **(F):** 40×; Results of EBER-ISH of NPC: **(G)** 10×; **(H)** 40×; Results of EBER-ISH of papillary thyroid carcinoma: **(I)** 10×; **(J)** 40×.

antibodies and may be insufficient for EBV viral load evaluation; more comprehensive assays should be included in further studies. Third, only the EBER-based ISH test was included in the current study. Other viral detection methodologies, such as the LMP-1 immunohistochemical test, could be implied to better evaluate EBV infection in a future study.

CONCLUSION

We found no correlation between EBV and thyroid diseases in our study based on the current evidence. Future studies are warranted to reveal the significance of EBV in thyroid tumor developments.

ETHICS STATEMENT

This study was approved by the ethics committee of Nanfang Hospital, Southern Medical University. Informed consents were obtained from all enrolled patients.

AUTHOR CONTRIBUTIONS

S-TL and HL designed the study. S-TY, J-NG, and R-CL conducted the analysis. S-TY and R-CL drafted the manuscript. Z-GW, B-HS, Y-MJ, and J-YL participated in result interpretation. All authors have read the manuscript and approved for publication.

ACKNOWLEDGMENTS

S-TY received a grant from President Foundation of Nanfang Hospital, Southern Medical University (No. 2018C024). The funders played no role in study design, collection, analysis, interpretation of data, writing of the report, or in the decision to submit the paper of publication. They accept no responsibility for the contents. We thank Professor Shuang Wang (Department of Pathology, Nanfang Hospital, Southern Medical University) and Dr. Yan Li (Department of Pathology, Sun Yat-sen University Cancer Center) for pathological guidance. We also thank Dr. Huasong Zhang (Department of Otolaryngology, Sun Yat-sen Memorial Hospital, Sun Yat-sen University) for plotting the figures in this paper.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2019.00312/full#supplementary-material

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Oncology

ORIGINAL RESEARCH
published: 21 May 2019
doi: 10.3389/fonc.2019.00370

Contribution of Hepatitis B Virus Infection to the Aggressiveness of Primary Liver Cancer: A Clinical Epidemiological Study in Eastern China

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OPEN ACCESS

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Reviewed by:
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Specialty section:
This article was submitted to Cancer Epidemiology and Prevention, a section of the journal Frontiers in Oncology

Received: 22 March 2019
Accepted: 23 April 2019
Published: 21 May 2019

Citation:
Yang F, Ma L, Yang Y, Liu W, Zhao J, Chen X, Wang M, Zhang H, Cheng S, Shen F, Wang H, Zhou W and Cao G (2019) Contribution of Hepatitis B Virus Infection to the Aggressiveness of Primary Liver Cancer: A Clinical Epidemiological Study in Eastern China. Front. Oncol. 9:370. doi: 10.3389/fonc.2019.00370

Background and aims: The contribution of hepatitis B virus (HBV) infection to the aggressiveness of primary liver cancer (PLC) remains controversial. We aimed to characterize this in eastern China.

Methods: We enrolled 8,515 PLC patients whose specimens were reserved at the BioBank of the hepatobiliary hospital (Shanghai, China) during 2007–2016. Of those, 3,124 who received primary radical resection were involved in survival analysis. A nomogram was constructed to predict the survivals using preoperative parameters.

Results: Hepatocellular carcinoma (HCC), intrahepatic cholangiocarcinoma (ICC), and combined hepatocellular cholangiocarcinoma (CHC) accounted for 94.6, 3.7, and 1.7%, respectively. The rates of HBV infection were 87.5, 49.2, and 80.6%, respectively. HBV infection was significantly associated with 10-year earlier onset, more cirrhosis, higher α-fetoprotein, higher carbohydrate antigen 19-9 (CA19-9), more microvascular invasion (MVI), lower neutrophil-to-lymphocyte ratio (NLR), and lower platelet-to-lymphocyte ratio (PLR) in HCC. HBV infection was also associated with 7-year earlier onset, more cirrhosis, higher α-fetoprotein, more MVI, and lower PLR in ICC. In the multivariate Cox analysis, high circulating HBV DNA, α-fetoprotein, CA19-9, NLR, tumor size, number, encapsulation, Barcelona Clinic Liver Cancer (BCLC) stage, and MVI predicted an unfavorable prognosis in HCC; only CA19-9 and BCLC stage, rather than HBV-related parameters, had prognostic values in ICC. A nomogram constructed with preoperative HBV-related parameters including HBV load, ultrasonic cirrhosis, and α-fetoprotein perform better than the current staging systems in predicting postoperative survival in HCC.

Conclusion: HBV promotes the aggressiveness of HCC in Chinese population. The contributions of HBV to ICC and other etiological factors to HCC might be indirect *via* arousing non-resolving inflammation.

Keywords: primary liver cancer, hepatitis virus, radical resection, prognosis, aggressiveness

INTRODUCTION

Primary liver cancer (PLC), comprising hepatocellular carcinoma (HCC, 70–90%), intrahepatic cholangiocarcinoma (ICC, 10–20%), and rare histotypes including combined hepatocellular cholangiocarcinoma (CHC), is the second leading cause of cancer death in men and the sixth leading cause of cancer death in women worldwide (1, 2). The incidence rates of PLC remain highest in Asia. China alone accounts for half of global PLC (1). Over decades, the mortalities increased in Europe and America and decreased in East Asia (1).

Of global PLC, 56% were attributable to hepatitis B virus (HBV) and 20% to hepatitis C virus (HCV) (3). Chronic HBV infection is the major cause of HCC in Asian and African countries (4). Although HCV infection is the leading cause of HCC in most European and American countries, the contribution of HBV is increasing possibly because of immigration. Aflatoxin B1 exposure, alcohol consumption, sugar consumption, and diabetes also contribute to the development of HCC (1–5). Aflatoxin B1 exposure or smoking increases the occurrence of HCC caused by other factors (6, 7). Infection with *Opisthorchis viverrini* and *Clonorchis sinensis*, hepatolithiasis, and primary sclerosing cholangitis are associated with cholangiocarcinoma (8). Chronic infection with HBV or HCV also increase the risk of ICC (9, 10). However, it remains to be identified whether the risk factors promote the development of HCC or ICC directly or indirectly *via* inducing inflammation.

The association of etiological factors and PLC prognosis remains controversial. Data from Australia and the United States indicate that HBV-related HCC has a better prognosis than HCV-related HCC, which is hardly repeated in other populations (11–15). Thus, we performed this large epidemiological study to clarify the contribution of HBV infection to the aggressiveness of major PLC histotypes.

MATERIALS AND METHODS

Patient Enrollment

From January 1st 2007 to March 31st 2016, 8,515 consecutive PLC patients who received hepatectomy at the Eastern Hepatobiliary Surgery Hospital (Shanghai, China) and had their removed tissues reserved in the BioBank were enrolled in this study. Their diagnoses were pathologically confirmed. Radical resection was defined as follows: (i) complete resection of all tumor nodules, with the cut surface free of cancer cells by pathologic examination; (ii) no macroscopic tumor thrombosis in the portal vein (main trunk or two major branches), hepatic veins, or bile duct; (iii) number of tumor nodules not exceeding three; (iv) serum α-fetoprotein (AFP), if positive, declined to undetectable level 2 months after surgery; and (v) no extrahepatic metastasis. Six months after surgical treatment, patients were regularly followed up through telephone by the same team of professional staff, followed by another five sequential follow-ups at the time point of 1, 2, 3, 4, and 5 years after surgery. In the telephone interview, we collected data including survival situation, treatment(s) received after surgery, as well as the exact date of death in case of death. The final date of follow-up was August 31st, 2017. Patients who survived were censored at their last follow-up. Patients with microvascular invasion (MVI) were recommended to receive postoperative transcatheter arterial chemoembolization (TACE) as previously described (16). Patients with imaging evidence of tumor recurrence were recommended to receive second resection or radiofrequency ablation (RFA) (17).

Data Collection

Demographical information, pathological examinations (including nodule number, capsule integrity of tumor, and MVI), and results of the latest pre-operative laboratory examinations [including serum AFP, carbohydrate antigen 19-9 (CA19-9), HBV parameters, routine blood test, and liver function tests] were extracted from electronic medical records. Child–Pugh score and Barcelona Clinic Liver Cancer (BCLC) stage were determined as previously described (18, 19).

Statistical Analysis

HBV infection was defined if a patient was seropositive for HBsAg and/or HBV DNA (20). Occult HBV infection (OBI) was defined as the presence of HBV DNA in a patient seronegative for HBsAg (21). Neutrophil–lymphocyte ratio (NLR) and platelet–lymphocyte ratio (PLR) were calculated as neutrophil count and platelet count divided by lymphocyte count, respectively. Their cutoff values were determined using X-tile software (http://www.tissuearray.org/rimmlab/xtile.html, RRID: SCR_005602). Cutoff values of AFP, CA19-9, total bilirubin, direct bilirubin, and albumin were the same as those in previous studies (22, 23). Categorical variables including the positive rates of hepatitis B surface antigen (HBsAg) were compared by χ^2 test or Fisher's exact test when appropriate. Continuous variables with skewed distribution were compared by Kruskal–Wallis ANOVA for multiple group comparison and Mann–Whitney *U*-test for double group comparison. The Bonferroni correction was applied for multiple comparisons. The Kaplan–Meier method was applied to estimate overall survival (OS), and the log-rank test was performed to compare the difference between survival curves. A Cox proportional hazard model was applied to calculate the hazard ratio (HR) and its 95% confidence interval (CI) for each variable. Significant variables in the univariate Cox analysis were introduced into the multivariate Cox model to determine the factors that independently contributed to postoperative survival. Our cohort was randomly dichotomized into a training cohort and a validation cohort. A Cox model utilizing pre-operatively available variables was fitted in training cohort. A final model was selected by a backward stepwise selection procedure following the Akaike information criterion (24). A nomogram was formulated by applying the *rms* package

Abbreviations: AFP, α-fetoprotein; BCLC, Barcelona Clinic Liver Cancer; CA19-9, carbohydrate antigen 19-9; CHC, combined hepatocellular cholangiocarcinoma; CI, confidence interval; HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; HR, hazard ratio; HCC, hepatocellular carcinoma; ICC, intrahepatic cholangiocarcinoma; IQR, interquartile range; MVI, microvascular invasion; NLR, neutrophil-to-lymphocyte ratio; OS, overall survival; PLR, platelet-to-lymphocyte ratio; PLC, primary liver cancer; RFA, radiofrequency ablation; TACE, transcatheter arterial chemoembolization.

TABLE 1 | Comparison of demographical and clinical characteristics between HCC patients with HBV infection and those without HBV infection.

Variable	Patients without HBV infection ($N = 1,010$)[a]	Patients with HBV infection ($N = 6,976$)[a]	P[b]
Gender			
Female	139 (13.8)	916 (13.1)	0.580
Male	871 (86.2)	6,060 (86.9)	
Age			
Medium (IQR)	62 (54–68)	52 (45–60)	<0.001
≤40	50 (5.0)	825 (11.9)	
40–60	404 (40.0)	4,526 (65.2)	
>60	556 (55.0)	1,586 (22.9)	
Cirrhosis (ultrasound)			
No	788 (82.9)	3,518 (53.4)	<0.001
Yes	163 (17.1)	3,071 (46.6)	
Cirrhosis (pathology)			
No	767 (76.2)	3,559 (51.2)	<0.001
Yes	239 (23.8)	3,393 (48.8)	
AFP (ng/mL)			
Negative (<20)	477 (47.9)	2,389 (34.7)	<0.001
Positive (≥20)	519 (52.1)	4,495 (65.3)	
CA19-9 (U/mL)			
Negative (<37)	873 (89.6)	5,251 (79.3)	<0.001
Positive (≥37)	101 (10.4)	1,373 (20.7)	
HBeAg			
Negative	1,007 (99.7)	4,880 (70.3)	<0.001
Positive	3 (0.3)	2,059 (29.7)	
HBcAb			
Negative	144 (14.3)	1 (0.0)	<0.001
Positive	866 (85.7)	6,936 (100.0)	
HBV DNA (copies/mL)			
Undetectable (<500)	849 (100.0)	2,812 (41.5)	<0.001
Detectable (≥500)	0 (0)	3,957 (58.5)	
Total bilirubin (μmol/L)			
≤20	846 (85.4)	5,700 (83.6)	0.150
>20	145 (14.6)	1,121 (16.4)	
Direct bilirubin (μmol/L)			
≤7	786 (79.3)	5,183 (76.0)	0.021
>7	205 (20.7)	1,638 (24.0)	
Albumin (g/L)			
>35	951 (96.9)	6,362 (95.1)	0.009
≤35	30 (3.1)	330 (4.9)	
NLR			
≤3.3	813 (80.4)	5,742 (83.0)	0.042
>3.3	198 (19.6)	1,176 (17.0)	
PLR			
≤117	541 (53.6)	4,540 (65.3)	<0.001
>117	468 (46.4)	2,417 (34.7)	
Child–pugh score			
A	920 (98.9)	6,333 (98.9)	0.991
B	11 (1.1)	72 (1.1)	
BCLC stage			
0	29 (2.9)	334 (4.8)	<0.001
A	326 (32.6)	2,575 (37.2)	
B	526 (52.5)	2,920 (42.2)	
C	120 (12.0)	1,093 (15.8)	
Tumor diameter (cm)			
<3	149 (14.9)	1,518 (22.0)	<0.001
≥3	848 (85.1)	5,393 (78.0)	
Tumor number			
Single	858 (86.1)	5,560 (80.5)	<0.001
Multiple	138 (13.9)	1,345 (19.5)	
Tumor encapsulation			
No	232 (23.1)	1,689 (24.4)	0.364
Yes	771 (76.9)	5,220 (75.6)	
MVI			
No	670 (66.9)	4,297 (62.0)	0.003
Yes	332 (33.1)	2,632 (38.0)	

[a] *Data are presented as number (%), unless otherwise indicated. Some data do not sum up to the total number for the existence of missing data.*

[b] *For age (continuous variable) and BCLC stage (rank variable), Mann–Whitney U test was conducted. For other variables (categorical variables), chi-square test was conducted.*

HCC, Hepatocellular carcinoma; HBV, hepatitis B virus; IQR, interquartile range; AFP, α-fetoprotein; CA19-9, carbohydrate antigen 19-9; HBeAg, hepatitis B e antigen; HBcAb, hepatitis B core antibody; NLR, neutrophil-lymphocyte ratio; PLR, platelet-lymphocyte ratio; BCLC, Barcelona Clinic Liver Cancer; MVI, microvascular invasion.

in R (25). The performance of nomogram was measured by the concordance index (C-index) and calibration plots with 1,000 bootstraps. Comparisons of the prediction power between the nomogram and independent prognostic factors or clinical staging systems were performed using the rcorrp.cens package in Hmisc in R and were evaluated by the C-index (26). The accuracy of the nomogram was validated in the validation cohort. All statistical analyses were two-sided and performed using SPSS V21.0 for Windows (http://www-01.ibm.com/software/uk/analytics/spss/, RRID: SCR_002865) and RStudio V3.9.2 (http://www.rstudio.com/RRID:SCR_000432). $P < 0.05$ was considered as statistically significant.

RESULTS

Patients were from almost all provinces of mainland China except Tibet. Patients from eastern China accounted for 93.9% (**Supplementary Figure 1**). They are self-reported Chinese with a median age of 53 years [interquartile range (IQR), 46–61 years]. Patients were predominantly male, with a male-to-female ratio of 6.15. Of the 8,515 patients, 8,056 (94.6%) had HCC, 314 (3.7%) had ICC, and 145 (1.7%) had CHC. The proportions of patients seropositive for HBsAg, HBV DNA, and anti-HCV antibody were 87.3, 51.7and 1.7% in HCC, 49.2, 33.8, and 1.8% in ICC, and 80.6, 41.5, and 1.4% in CHC, respectively. OBI accounted for 0.2% in HCC. Compared with ICC patients, HCC

TABLE 2 | Comparison of demographical and clinical characteristics between ICC patients with HBV infection and those without HBV infection.

Variable	Patients without HBV infection ($N = 157$)[a]	Patients with HBV infection ($N = 152$)[a]	P[b]
Gender			
Female	72 (45.9)	27 (17.8)	<0.001
Male	85 (54.1)	125 (82.2)	
Age			
Medium (IQR)	61 (56–68)	54 (47–61)	<0.001
≤40	5 (3.2)	8 (5.3)	
40–60	65 (41.4)	105 (69.1)	
>60	87 (55.4)	39 (25.7)	
Cirrhosis (ultrasound)			
No	135 (91.2)	78 (54.5)	<0.001
Yes	13 (8.8)	65 (45.5)	
Cirrhosis (pathology)			
No	143 (92.9)	83 (54.6)	<0.001
Yes	11 (7.1)	69 (45.4)	
AFP (ng/mL)			
Negative (<20)	138 (89.6)	98 (65.3)	<0.001
Positive (≥20)	16 (10.4)	52 (34.7)	
CA19-9 (U/mL)			
Negative (<37)	71 (46.4)	77 (54.2)	0.180
Positive (≥37)	82 (53.6)	65 (45.8)	
HBeAg			
Negative	157 (100.0)	107 (70.4)	<0.001
Positive	0 (0.0)	45 (29.6)	
HBcAb			
Negative	41 (26.1)	0 (0.0)	<0.001
Positive	116 (73.9)	162 (100.0)	
Total bilirubin (μmol/L)			
≤20	140 (90.3)	126 (84.6)	0.129
>20	15 (9.7)	23 (15.4)	
Direct bilirubin (μmol/L)			
≤7	130 (83.9)	118 (79.2)	0.293
>7	25 (16.1)	31 (20.8)	
Albumin (g/L)			
>35	148 (96.7)	137 (97.2)	1.000
≤35	5 (3.3)	4 (2.8)	
NLR			
≤3.3	101 (64.3)	100 (65.8)	0.788
>3.3	56 (35.7)	52 (34.2)	
PLR			
≤117	65 (41.4)	91 (59.9)	0.001
>117	92 (58.6)	61 (40.1)	
Child–pugh score			
A	146 (95.4)	141 (100.0)	0.029
B	7 (4.6)	0	
BCLC stage			
0	1 (0.7)	2 (1.3)	0.573
A	35 (23.0)	28 (18.8)	
B	80 (52.6)	82 (55.0)	
C	36 (23.7)	37 (24.8)	
Tumor diameter (cm)			
<3	8 (5.3)	16 (10.9)	0.079
≥3	142 (94.7)	131 (89.1)	
Tumor number			
Single	128 (85.3)	119 (81.0)	0.313
Multiple	22 (14.7)	28 (19.0)	
Tumor encapsulation			
No	135 (90.0)	120 (81.6)	0.039
Yes	15 (10.0)	27 (18.4)	
MVI			
No	134 (87.0)	108 (73.0)	0.002
Yes	20 (13.0)	40 (27.0)	

[a]Data are presented as number (%), unless otherwise indicated. Some data do not sum up to the total number for the existence of missing data. Some percentages do not sum up to 100 because of rounding.

[b]For age (continuous variable) and BCLC stage (rank variable), Mann–Whitney U test was conducted. For other variables (categorical variables), chi-square test was conducted.

patients had a higher male-to-female ratio, higher proportions of AFP positivity, HBsAg positivity, and HBV DNA positivity, and a lower proportion of CA19-9 positivity. Compared with HCC patients, CHC patients had higher proportions of CA19-9 seropositivity, NLR (>3.3), and PLR (>117). These data are shown in **Supplementary Table 1**.

Demographical and Clinical Characteristics Between Primary Liver Cancer Patients With Hepatitis B Virus Infection and Those Without Hepatitis B Virus Infection

Compared with HCC patients without HBV infection, HCC patients with HBV infection were 10 years younger and had higher proportions of positive AFP (≥20 ng/ml), positive CA19-9 (≥37 U/ml), the presence of liver cirrhosis, high direct bilirubin (>7 μmol/L), advanced BCLC stage, and the presence of MVI and lower proportions of NLR (>3.3) and PLR (>117; **Table 1**). Compared with ICC patients without HBV infection, those with HBV infection were 7 years younger and had a higher male-to-female ratio, higher proportions of positive AFP, cirrhosis, and MVI and lower proportions of PLR (>117) and advanced Child–Pugh score (B vs. A; **Table 2**). Similarly, CHC patients with HBV infection were 9 years younger and had higher proportions of AFP positivity and cirrhosis, and lower proportions of NLR (>3.3) and PLR (>117) than those without HBV infection (**Supplementary Table 2**).

Postoperative Survival

Patients who received first radical resection at the study hospital ($n = 5,602$) were invited to join in the survival analysis. Of those, 2,478 (1,932 refused to be followed-up and 546 were lost in the follow-up) were excluded from survival analysis. The

TABLE 3 | Univariate and multivariate Cox regression analysis of prognostic factors for postoperative survival in HCC patients.

Variable	No. (%) of participants (n = 2,963)[a]	Univariate analysis HR (95% CI)	*P*	Multivariate analysis HR (95% CI)[b]	*P*
Gender					
Female	382 (12.9)	1			
Male	2,581 (87.1)	1.17 (0.98–1.41)	0.080		
Age					
<40	324 (10.9)	1			
40–59	1,800 (60.7)	0.85 (0.70–1.03)	0.848		
≥60	839 (28.3)	0.81 (0.66–1.00)	0.814		
Cirrhosis (ultrasound)					
No	1,602 (57.6)	1			
Yes	1,181 (42.4)	1.10 (0.97–1.24)	0.136		
Cirrhosis (pathology)					
No	1,643 (55.5)	1			
Yes	1,319 (44.5)	1.08 (0.96–1.21)	0.211		
HBV DNA (copies/mL)					
<500	1,433 (50.6)	1		1	
≥500	1,397 (49.4)	1.55 (1.38–1.75)	<0.001	1.35 (1.18–1.55)	<0.001
AFP (ng/mL)					
≤20	1,092 (37.4)	1		1	
>20	1,826 (62.6)	2.03 (1.78–2.32)	<0.001	1.68 (1.45–1.95)	<0.001
CA19-9 (U/mL)					
≤37	2,284 (81.4)	1		1	
>37	521 (18.6)	1.34 (1.16–1.54)	<0.001	1.25 (1.07–1.47)	0.005
HBsAg					
Negative	382 (13.1)	1			
Positive	2,543 (86.9)	1.34 (1.12–1.61)	0.002		
HBeAg					
Negative	2,170 (74.2)	1			
Positive	755 (25.8)	1.21 (1.07–1.38)	0.004		
Anti-HCV					
Negative	2,769 (98.1)	1			
Positive	54 (1.9)	0.64 (0.40–1.01)	0.058		
Total bilirubin (μmol/L)					
≤20	2,467 (85.5)	1			
>20	417 (14.5)	1.16 (0.98–1.36)	0.078		
Direct bilirubin (μmol/L)					
≤7	2,237 (77.6)	1			
>7	647 (22.4)	1.17 (1.02–1.34)	0.027		
Albumin (g/L)					
>35	2,707 (94.4)	1			
≤35	161 (5.6)	1.31 (1.04–1.66)	0.023		
NLR					
≤3.3	2,477 (83.6)	1		1	
>3.3	485 (16.4)	1.55 (1.34–1.79)	<0.001	1.42 (1.20–1.68)	<0.001
PLR					
≤117	1,915 (64.7)	1			
>117	1,047 (35.3)	1.41 (1.25–1.59)	<0.001		
Tumor diameter (cm)					
<3	665 (22.6)	1		1	
≥3	2,282 (77.4)	2.20 (1.85–2.62)	<0.001	1.37 (1.08–1.74)	0.010

(Continued)

TABLE 3 | Continued

Variable	No. (%) of participants (n = 2,963)[a]	Univariate analysis HR (95% CI)	*P*	Multivariate analysis HR (95% CI)[b]	*P*
Tumor number					
Single	2,339 (79.4)	1		1	
Multiple	608 (20.6)	1.69 (1.48–1.93)	<0.001	1.28 (1.10–1.48)	0.002
Tumor encapsulation					
No	523 (17.7)	1		1	
Yes	2,440 (82.3)	0.63 (0.55–0.72)	<0.001	0.63 (0.54–0.73)	<0.001
Child–pugh score					
A	2,665 (99.0)	1			
B	28 (1.0)	1.98 (1.23–3.20)	0.005		
BCLC stage					
0&A	1,355 (46.0)	1		1	
B	1,592 (54.0)	2.15 (1.89–2.43)	<0.001	1.54 (1.30–1.82)	<0.001
MVI					
No	2,037 (69.1)	1		1	
Yes	911 (30.9)	2.07 (1.83–2.33)	<0.001	1.66 (1.46–1.90)	<0.001
Post-operative tace					
No	1,359 (45.9)	1			
Yes	1,604 (54.1)	1.20 (1.06–1.35)	0.003		
Reoperation					
No	2,764 (93.3)	1		1	
Yes	199 (6.7)	0.49 (0.38–0.64)	<0.001	0.48 (0.36–0.65)	<0.001
Post-operative RFA					
No	2,716 (91.7)	1		1	
Yes	247 (8.3)	0.67 (0.54–0.82)	<0.001	0.67 (0.54–0.84)	<0.001

[a] *Some data do not sum up to the total number for the existence of missing data. Some percentages do not sum up to 100 because of rounding.*

[b] *The final model selection was carried out by a backward stepwise selection procedure with the Akaike information criterion. Only significant ($P < 0.05$) covariates in univariate analysis were included.*

CI, confidence interval; HR, hazard ratio; TACE, transcatheter arterial chemoembolization; RFA, radiofrequency ablation.

remaining 3,124 patients were included in survival analysis (**Supplementary Figure 2**). The median follow-up time was 1.18 years, with an IQR of 0.78–2.35 years. **Supplementary Table 3** shows the baseline characteristics of patients involved in survival analysis and those not involved. Of the 3,124 patients, 1,443 died of this malignancy during follow-up, with the 1-, 3-, and 5-year survival rates of 79.7, 47.5, and 28.6%, respectively. Postoperative 1-, 3-, and 5-year survival rates of patients with each histotype are shown in **Supplementary Table 4**. Multivariate Cox regression analysis indicated that serum HBV DNA (≥500 copies/ml), AFP (>20 ng/ml), CA19-9 (>37 U/ml), NLR (>3.3), tumor size (≥3 cm in diameter), multiple tumor nodules, incomplete tumor capsule, later more advanced BCLC stage, and MVI independently predicted shorter OS in HCC. Second resection and RFA independently improved OS (**Table 3**). CA19-9 (>37U/ml), multiple tumor nodules, and BCLC were significantly associated with shorter OS in univariate Cox analysis, while CA19-9 and more advanced BCLC stage were independently associated with OS in ICC (**Supplementary Table 5**). To further clarify the effect of HBV parameters on the aggressiveness of HCC, multivariate Cox regression analysis was conducted in HBV-positive HCC patients. It was found that HBV DNA (≥500 copies/ml) was significantly associated with shorter OS (**Supplementary Table 6**), indicating that HCC patients with active HBV replication had shorter OS than those with inactive HBV replication.

Predication for Postoperative Prognosis Using Preoperative Parameters

To evaluate if HBV-related clinical parameters harvested preoperatively could predict postoperative prognosis, we developed a nomogram using the independent prognostic factors. HCC patients with radical resection (n = 2,963) were randomly dichotomized into the training cohort (n = 1,482) and validation cohort (n = 1,481). All demographical and clinical characteristics were balanced between the training cohort and validation cohort except CA19-9 (**Supplementary Table 7**). Multivariate Cox analysis in the training cohort showed that preoperative ultrasound cirrhosis, AFP, BCLC stage, HBV DNA, and tumor size were independently associated with OS (**Supplementary Table 8**). A nomogram that integrated all independent prognostic factors in the training cohort is shown in **Figure 1A**. The C-index for survival prediction of the nomogram in the training cohort was 0.699 (95% CI, 0.669–0.729). The calibration plot for the probability of postoperative OS showed

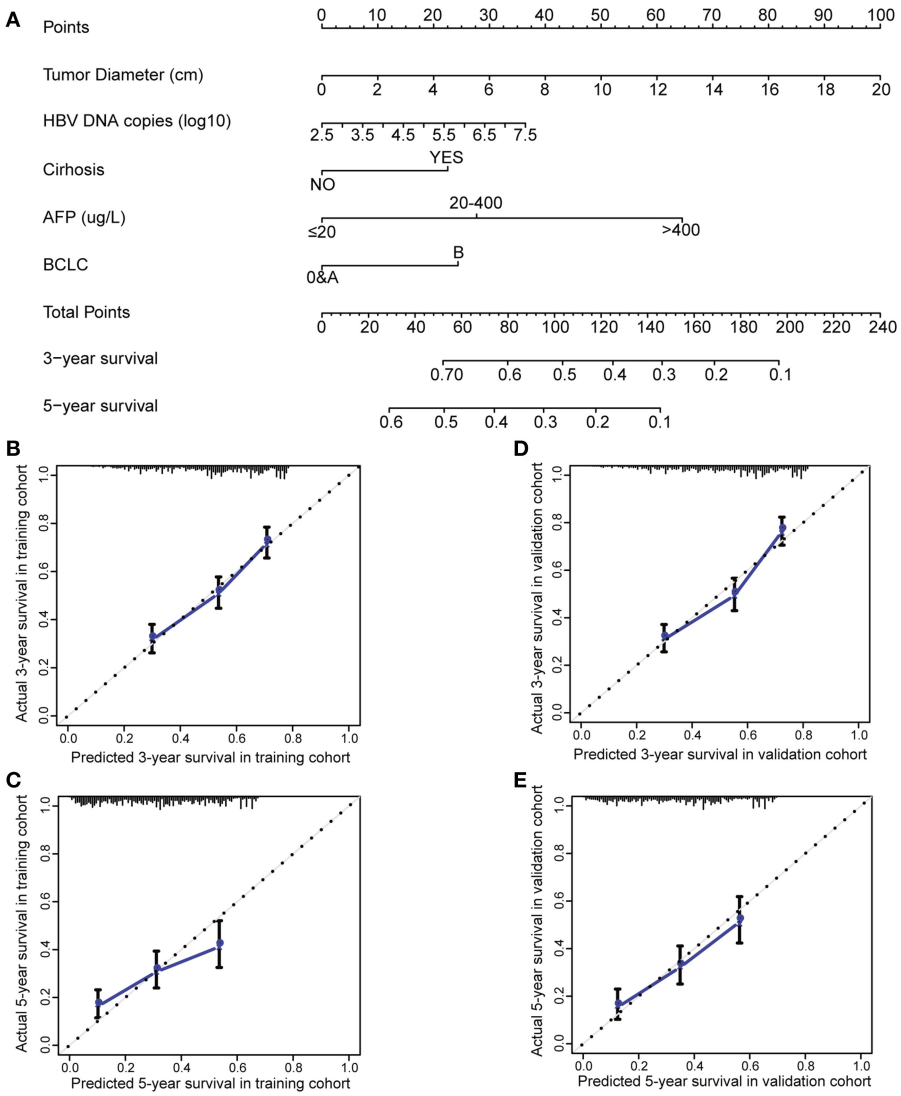

FIGURE 1 | Preoperative nomogram and the calibration curve for predicting postoperative survival of patients with HCC. **(A)** The nomogram. To use this nomogram, a patient's value is located on each variable axis, and a line represents the number of points received for each variable value. The sum of these numbers is located on "Total Points" axis, and a line is drawn downward to the survival axes to determine the likelihood of postoperative 3- or 5-year survival. **(B)** The calibration curve for predicting postoperative 3-year survival in the training cohort. **(C)** The calibration curve for predicting postoperative 5-year survival in the training cohort. **(D)** The calibration curve for predicting postoperative 3-year survival in the validation cohort. **(E)** The calibration curve for predicting postoperative 5-year survival in the validation cohort. AFP, alpha-fetoprotein; BCLC, Barcelona Clinic Liver Cancer; HCC, hepatocellular carcinoma.

good agreement between the prediction by nomogram and actual observation (**Figures 1B**,**C**). The results were faithfully replicated in the validation cohort. The C-index in the validation cohort was 0.700 (95% CI, 0.670–0.730), and a calibration curve showed a good agreement between prediction and actual observation in the probability of 3- and 5-year survivals (**Figures 1D**,**E**). The C-index was 0.644, 0.638, 0.597, and 0.546 by tumor size, AFP, MVI, and incomplete tumor capsule, respectively, which were significantly lower than that by the nomogram ($P < 0.001$ for each comparison). We then compared the accuracy between the nomogram and each of the clinical staging systems including American Joint Committee on Cancer (AJCC) Staging Manual, 7th ed., Okuda, Chinese University Prognostic Index (CUPI), Groupe d'Etude et de Traitement du Carcinome Hepatocellulaire Prognostic classification (GETCH), and BCLC (16, 27–31). The AJCC 7th, Okuda, CUPI, GETCH, and BCLC systems showed good stratification for the postoperative prognosis of HCC patients in both the training cohort and the validation cohort (**Supplementary Figure 3**). In the training cohort, the C-index of the nomogram was significantly higher than the AJCC 7th (0.644, $P < 0.001$), Okuda (0.564, $P < 0.001$), CUPI (0.514, $P < 0.001$), GETCH (0.611, $P < 0.001$), and BCLC

(0.608, $P < 0.001$). Thus, the nomogram resulted in more accurate prediction for postoperative prognosis of HCC than the prevailing prognostic factors and well-established clinical staging systems.

DISCUSSION

In this study, we found that HBV infection was associated with 10-year earlier onset and higher proportions of positive AFP, positive CA19-9, the presence of liver cirrhosis, high direct bilirubin, advanced BCLC stage, and the presence of MVI in HCC. AFP, whose expression can be driven by HBV X protein, plays a critical role in promoting the stemness of HCC cells (32). Liver cirrhosis represents anti-inflammatory immune responses to hepatitis B flares or hepatic injuries caused by other chronic inflammation (33). HBV, especially its evolved forms generated during chronic infection and its integrated forms, directly promotes the development of HCC (34–36). This may explain why HBV-related HCC occurs 10 years earlier and is more aggressive than HCC caused by other etiological factors. HBV was inversely associated with NLR and PLR, the well-established inflammatory factors (37, 38), indicating that the non-HBV etiological factors may cause HCC *via* inducing non-resolving inflammation. Interestingly, the HBV-related parameters including HBV DNA, AFP, CA19-9, BCLC stage, and MVI predicted an unfavorable postoperative prognosis independently. Furthermore, the nomogram constructed with HBV-related parameters harvested preoperatively including HBV DNA, liver cirrhosis, AFP, and BCLC stage accurately predicted an unfavorable postoperative prognosis. The prediction power is better than the current clinical staging systems. These evidences indicate that HBV infection promotes the aggressiveness of HCC, at least in the HBV endemic areas. The non-HBV etiological factors promote the development of HCC possibly *via* inducing non-resolving inflammation.

Surprisingly, HBV infection was also associated with 7-year earlier onset, more cirrhosis, higher AFP, more MVI, and lower PLR and Child–Pugh score in ICC. We believe that the changes in these clinical parameters in ICC reflect the role of HBV in generating inflammatory background from which ICC develops, rather than direct etiological role of HBV in ICC. Compared to HCC patients, ICC patients had significantly lower proportions of positive HBsAg, positive HBV DNA, the presence of liver cirrhosis, positive AFP, and the presence of MVI and lower male-to-female ratio, the HBV-related parameters. By contrast, ICC patients had higher proportions of NLR (>3.3) and PLR (>117). These data indicate that HBV-caused inflammation, rather than HBV itself, play a major role in inducing ICC in HBV-infected subjects. CA19-9 and BCLC stage were not associated with HBV infection in ICC, but they predicted an unfavorable prognosis in ICC independently. These data indicate that HBV infection is not related to the aggressiveness of ICC. HBV promotes the development of ICC indirectly *via* inducing non-resolving inflammation. Antiviral treatment reduces the risk of ICC (9, 10), possibly *via* reducing the non-resolving inflammation caused by active HBV infection.

HBV infection accounted for 87.5% of HCC, while HCV infection only accounted for 1.7%. This difference might be fundamentally related to the genetic predispositions. The genotypes and/or allele of *HLA-DQ*, *HLA-DP*, and *NFKBIA* single-nucleotide polymorphisms (SNPs) that significantly increased the risk of chronic HBV infection are more frequent in Chinese population than in European population (http://www.hapmap.org/) (39–42). These genetic predispositions in Chinese population facilitate chronic transformation of HBV infection, possibly *via* weakening the corresponding antiviral immune function. HBV that evolved in chronic inflammatory microenvironment promotes the occurrence and aggressiveness of HCC. Instead, the C/C genotype of a SNP (rs12979860) of the *IL28B* gene, which is strongly associated with spontaneous clearance of HCV, is more frequent in Chinese population than in African or European population (http://www.hapmap.org/) (43). Thus, HCV might be more apt to cause HCC-inducing non-resolving inflammation in Western populations than in Chinese population. Non-resolving inflammation caused by chronic HCV infection might promote the aggressiveness of HCC, especially in Western populations.

Our study has several limitations. First, selection bias cannot be avoided in a single center. Second, severity of cirrhosis, family history, exposure to aflatoxin, metabolic syndrome, dietary changes, alcohol consumption, and cigarette smoking were not included because these data were not intact in their medical records. Third, compared with patients lost to follow-up, patients who were successfully followed-up had higher AFP level, higher Child–Pugh score, higher BCLC stage, larger tumor diameter, lower albumin level, larger tumor diameter, higher proportion of multiple tumor nodules, tumor encapsulation, and MVI, most of which were associated with shorter OS in HCC, so the survival rates of HCC should be underestimated. Fourth, the effects of postoperative radiotherapy, chemotherapy, stereotactic radiation, percutaneous ethanol injection, antiviral treatment, and targeted therapy as well as their combinations were not evaluated because of very small sample size. Fifth, prognosis prediction analysis was not carried out in ICC or CHC because of small sample sizes. Sixth, HBV replication was not found to be significantly associated with the progression of ICC, which might also be related to insufficient power due to small sample size.

Conclusively, this large epidemiological study demonstrates that HBV infection contributes to the aggressiveness of HCC in China and possibly in other HBV-endemic areas. The contribution of HBV to the aggressiveness of ICC and other risk factors to the aggressiveness of HCC might be indirect *via* arousing non-resolving inflammation.

DATA AVAILABILITY

All datasets generated for this study are included in the manuscript and/or the **Supplementary Files**.

ETHICS STATEMENT

This study conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the ethics committee of Eastern Hepatobiliary Surgery Hospital.

AUTHOR CONTRIBUTIONS

FY, LM, and WL contributed to the data organization, data analyses, and data interpretation. YY, JZ, MW, SC, and FS contributed to the patient enrolment, surgical treatment, and follow-up. XC, HZ, and HW conducted data collection and analyses. WZ performed surgical treatment as well as construction and maintenance of the database and BioBank. GC contributed to the study design, supervision, and writing of the manuscript.

FUNDING

This study was supported by the National Key Basic Research Program of China (973 Program) (grant numbers 2015CB554000 to GC and 2014CB542102 to WZ), the National Natural Science Foundation of China (grant numbers 81520108021, 91529305, and 81673250 to GC, 81502882 to XC, and 81521091 to HW), the State Key Infection Disease Project of China (grant numbers 2017ZX10203208 to WZ and 2017ZX10201201-006-001 to HZ), and the National Human Genetic Resources Sharing Service Platform (grant number 2005DKA 21300 to WZ).

ACKNOWLEDGMENTS

The authors acknowledge the medical staff including Fengqiu Chen, Yuanping Tao, Jutang Li, and Huifen Li for their excellent work in conducting follow-up study and managing the clinical information.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2019.00370/full#supplementary-material

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Oncology

ORIGINAL RESEARCH
published: 22 May 2019
doi: 10.3389/fonc.2019.00375

Electronic Health Record-Based Screening for Major Cancers: A 9-Year Experience in Minhang District of Shanghai, China

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OPEN ACCESS

Edited by:
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Reviewed by:
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Xiangqian Guo, Henan University, China

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Specialty section:
This article was submitted to Cancer Epidemiology and Prevention, a section of the journal Frontiers in Oncology

Received: *14 February 2019*
Accepted: *23 April 2019*
Published: *22 May 2019*

Citation:
He D, Xu W, Su H, Li W, Zhou J, Yao B, Xu D and He N (2019) Electronic Health Record-Based Screening for Major Cancers: A 9-Year Experience in Minhang District of Shanghai, China. Front. Oncol. 9:375. doi: 10.3389/fonc.2019.00375

Background: An electronic health record (e-HR) system has been developed in Minhang District of Shanghai, China, since 2005, making it convenient for local health institutions to provide integrative and comprehensive health care and management for major diseases.

Methods: In 2008, an e-HR-based cancer prevention program was initiated to screen multiple cancers, including colorectal, gastric, liver, lung, cervical, and breast cancers, and provide subsequent health education and health management to cancer patients and high-risk individuals. This study was designed in prospective analysis, based on the constructive analysis of key information, observation of cancer screening and healthcare processes and organizations, and stages of cancers detected by the e-HR-based programs.

Results: From 2008 to 2016, health education was conducted for over 5 million attendances, and more than 3 million screening tests were performed for eligible residents over 40 years old. A total of 2,948 cancer cases were detected, accounting for 13.3% of all newly diagnosed cancers in the district during the 9-year period. Thirty point seven percent detected cancer cases were at the early stage, significantly higher than the 22.9% in cases identified by e-HR-based follow-up and 13.8% in cases diagnosed due to signs or symptoms. More than 136,000 residents were identified as individuals at high risk of cancer and subject to sustainable clinical follow-up and health management.

Conclusions: The successful application of e-HR system in cancer prevention and control in Minhang district of Shanghai, China, implies that the system may act as an extendable and sustainable infrastructure for comprehensive health care and services for a broad spectrum of diseases and health events.

Keywords: electronic health record, cancer screening, health management, major cancer, information platform

INTRODUCTION

In China, a vertical networking system for non-communicable disease prevention and control has been well-established, in which national, provincial, municipal, and local Health Commissions; the Centers for Disease Control and Prevention (CDC); offices for specific disease control; hospitals; and Community Healthcare Service Centers (CHSC) are supposed to work together to fight against non-communicable diseases (1). At the county/district level, a tree-like structured healthcare system was also established, with local CDC, CHSC, and medical centers working as functional units for comprehensive healthcare services under the administration of the local Health Commission (**Figure 1**). Due to lacking health information sharing platform, however, health information and health records could not be exchanged and shared among these executive institutions. As a result, health services offered by different institutions, from primary to tertiary, were not effectively integrated.

Cancer care provided at the county/district level is a typical example of separated public service delivery: health education and screening programs are usually organized by local CDCs, while clinical diagnosis and treatment of cancers are performed by local medical centers and community-based check-up and post-treatment services are provided by CHSCs (2). Due to a lack of a referral system, these institutions remain distinct and independent from each other. Only in limited areas where the cancer registry system is well-established will medical centers report newly diagnosed cancer cases to a local cancer registry system, from which the local CDC and CHSCs can be alerted of occurrence of the disease and then provide standardized health care to the patients (3). However, this happens in the absence of detailed feedbacks and technical supports from medical centers for specific and individualized patient care.

To solve the problem, Minhang District, one of the 18 administrative districts in Shanghai, China, established an electronic health record (e-HR) system in 2005. The system has been used to comprehensively deliver various health services and improve accessibility and quality of services (4). In 2008, Minhang district initiated a comprehensive cancer screening program based on the e-HR system (5), aimed to improve the effectiveness and efficacy of cancer screening with a seamless interface of government machinery.

In this article, we introduced the 9-year experience of the e-HR-based cancer screening and health management for detected cancer cases and individuals at high risk and thus provide recommendations for seamless service delivery in the real world.

Abbreviations: e-HR, electronic health records; CDC, Centers for Disease Control and Prevention; CHSC, Community Healthcare Service Centers; FOBT, fecal occult blood test; RE, rectal exam; TAA, tumor-associated antigens; AFP, alpha-fetoprotein; UT, ultrasonic testing; LDCT, low-dose computerized tomography; CBE, clinical breast examination; TTM, thermal texture maps; MAM, mammography.

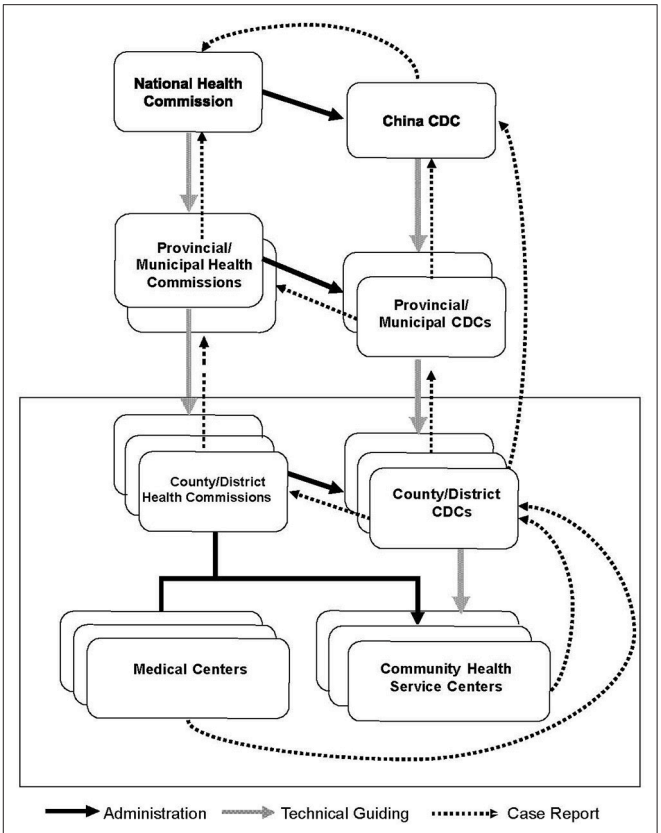

FIGURE 1 | Institutional infrastructure of disease control system and cancer care services in China. Frame: Functional units of county/district-level institutions that practice the basic services.

MATERIALS AND METHODS

This study was chosen as a prospective design, based on the constructive analysis of key information, organizations, and observations of cancer screening, stages of cancers detected by the e-HR-based programs, and healthcare processes for high-risk population.

The study material was the process and results of the e-HR-based screening programs for six major cancers, i.e., colorectal cancer, gastric cancer, liver cancer, lung cancer, cervical cancer, and breast cancer, among residents in Minhang District of Shanghai, China. All data for this study were extracted from the established e-HR system. The study was approved by the Institutional Review Board of Minhang District CDC (NO: EC-P-2012-002). Verbal informed consent was obtained from each participant of the cancer screening program.

Electronic Health Record System in Minhang District

The Minhang e-HR system is a comprehensive information platform integrating primary care, medical or clinical records, vaccine inoculation, and other public health activities (**Figure 2**). It is administrated by the Health Commission of Minhang District and executed by the CDC of Minhang District. All

CHSCs and medical centers in the district were organized and interlinked within the system.

All residents in Minhang District have a medical care card implanted with a microchip, through which their medical records and healthcare information are recordable and accessible by responsible doctors in all local health institutions, including CHSCs and medical centers in the district. The information is also accessible by staff in CDC by logging in the e-HR system. There are three major functions of the e-HR system: (1) to report and evaluate medical records, (2) to feedback clinical results and conduct follow-up, and (3) to provide mutual referral service.

The Minhang e-HR system was established in 2005 and covered 93.05% ($n = 830{,}400$) of the local permanent residents and 30.5% ($n = 334{,}800$) of a migrant population in 2014 (6). So far, the e-HR system has included information for over 2.6 million people, covering almost all residents in the district.

Comprehensive Electronic Health Record-Based Cancer Prevention Programs

The cancer prevention programs in Minhang District are a series of comprehensive healthcare services provided based on the established e-HR system. Three major modules were included in the e-HR system to promote accessibility and implementation of the early detection of cancers: (1) health education on cancer prevention. Usually, training courses on cancer prevention were delivered by general practitioners in CHSCs for residents aged over 40 years. These residents were asked to record their attendance in any courses in the e-HR system by scanning their medical care card; (2) free health check-up programs, including health check-up for senior residents over 60 years, "two cancer" (breast and cervical cancer) screening for vulnerable women population, and health check-up for rural residents. The subjects could be identified through the e-HR system; and (3) opportunistic screening in all local clinics and medical centers. Physicians involving in the program were reminded by the e-HR system automatically to provide free cancer screening for those who had certain risk factors, related symptoms, or claims.

Application of Electronic Health Record System in Cancer Risk Assessment

In all health institutions, the recruited residents were asked to answer whether they had (1) cancer-related symptoms like changed shape/property of feces, phlegmatically blood feces, abdominal pain, hematemesis, anemia, cough or expectoration sputum, abnormal vaginal secretions (women only), abnormal nipple discharge (women only), etc.; (2) precancerous lesions, such as digest duct polyps, adenomas, gastric intestinal metaplasia, atrophic gastritis, or cervical intraepithelial neoplasia (women only), etc.; (3) family history of the six major cancers; (4) occupational exposures to radon, arsenic, chromium, nickel, or asbestos; (5) cigarette smoking; (6) infections with HBV, HCV, HPV, or other cancer-related pathogens; (7) infertility (women only); and (8)

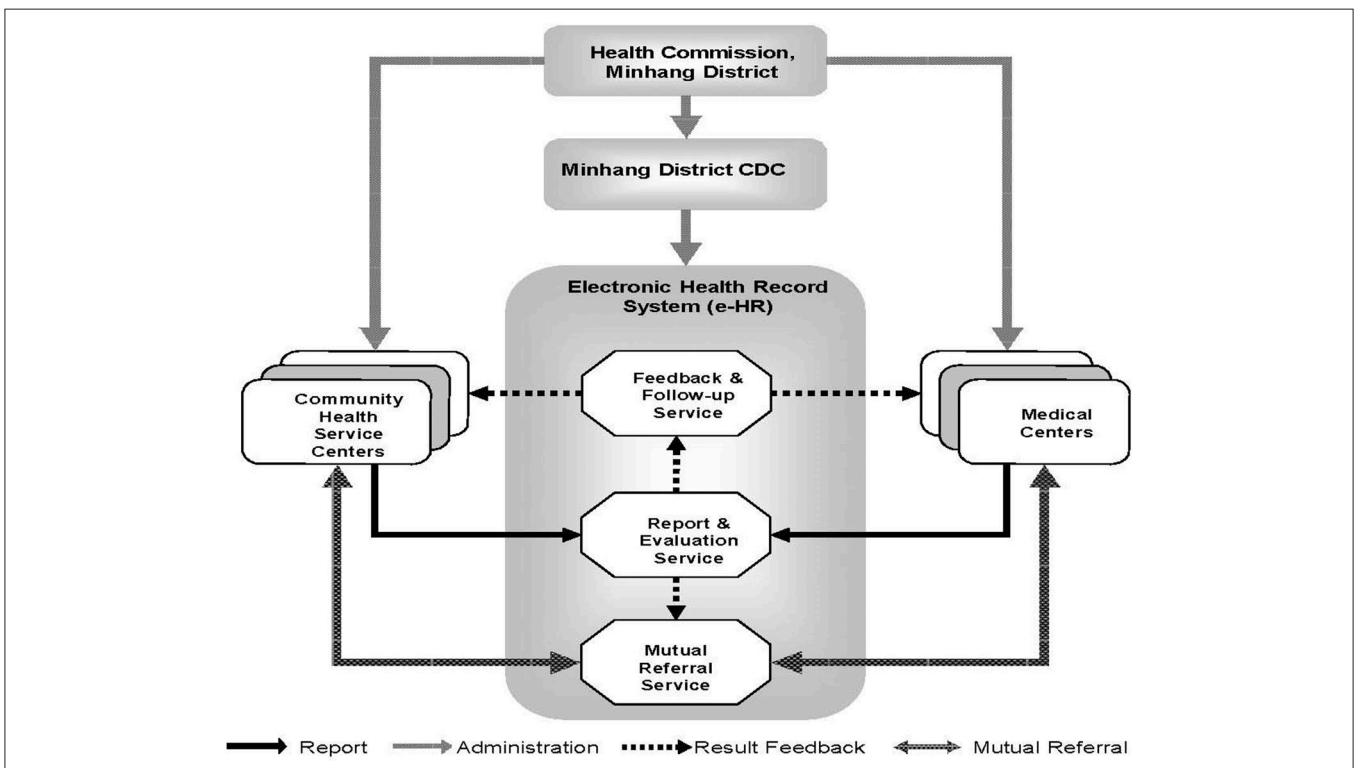

FIGURE 2 | Administrative and institutional infrastructure of electronic health record (e-HR)-based early detection and continuous health management for six major cancers in Minhang district of Shanghai, China.

use of estrogens or oral contraceptives (women only).These information were entered into the e-HR system, based on which individuals' risks for the six major cancers could be evaluated by all health institutes involved according to the criteria predefined based on the guidelines (7, 8) or previous studies (9–15).

Application of Electronic Health Record System in Cancer Screening

Individuals with positive results in risk assessment were referred to receiving respective free initial screening tests. The testing results, both negative and positive, were entered into the e-HR system by staffs in CHSCs and could be accessed by staff in Minhang CDC through logging in the system and by doctors in secondary or tertiary medical centers using patients' medical care cards implanted with a microchip.

Referral, Follow-Up, and Information Exchange

A mutual referral and information exchange mechanism between CHSCs and secondary or tertiary medical centers was also established within the framework of e-HR system (**Figure 2**). For example, if a resident was negative in cancer screening tests but evaluated to be at high risk of cancer, he/she would be referred to secondary or tertiary medical centers in Minhang District for further clinical examinations. On the other hand, if a resident was positive in cancer screening tests, he/she would be visited by a CHSC doctor within 1 month for community-based primary care and a physician in medical centers for clinical follow-up and medical care. The whole process and results of questionnaire-based risk assessment, screening, community and clinical follow-up, and health management were electronically recorded and centralized in the district-level database and were available for all health institutions in the district.

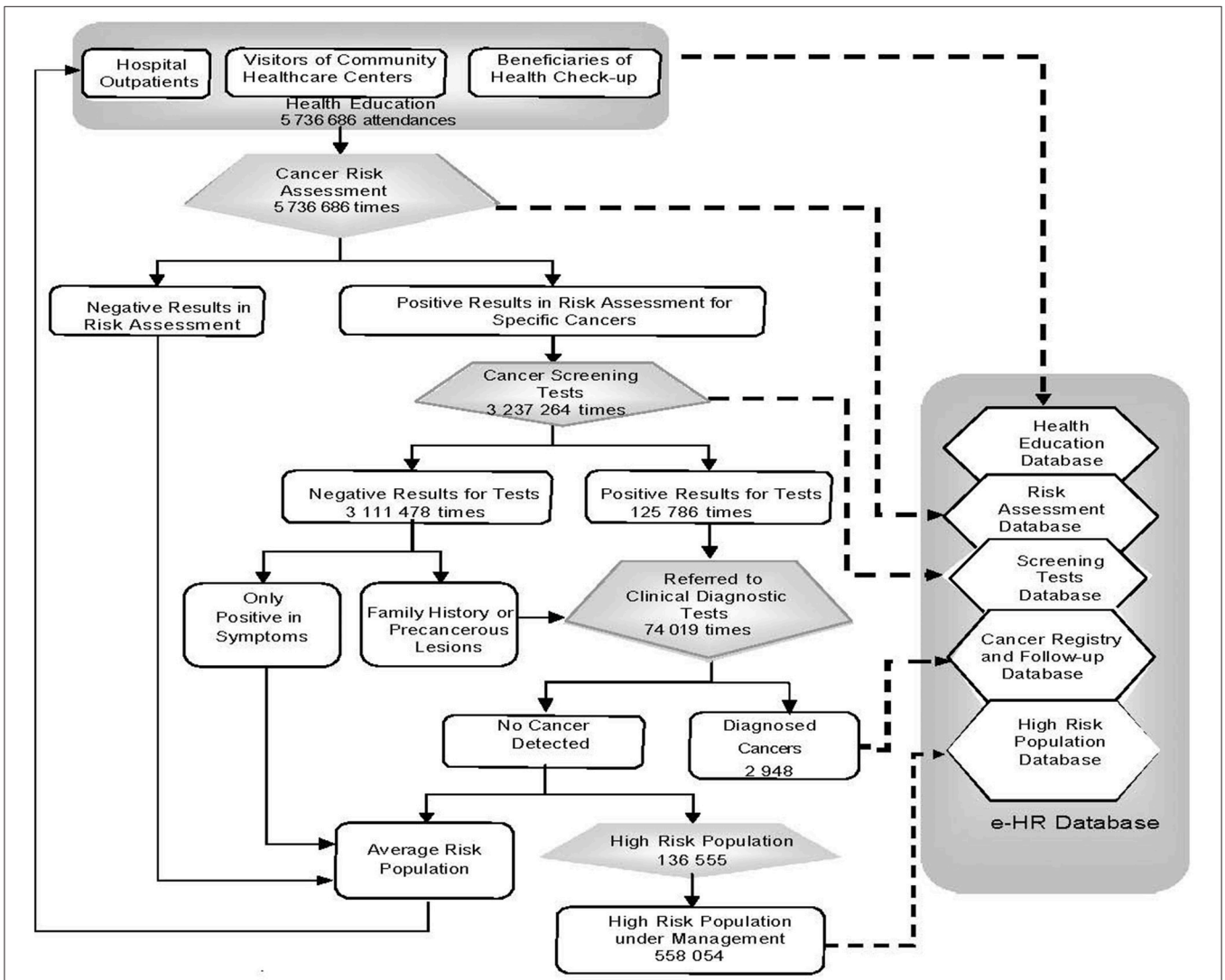

FIGURE 3 | Reporting process of e-HR-based early detection for six major cancers in Minhang District of Shanghai, China, 2008–2016. Solid arrow: resident flow; dashed arrow: information flow.

For newly diagnosed cancer cases, an effective referral mechanism ensures information exchange between local and municipal Cancer Registry System. Once a permanent resident in Minhang District was diagnosed with cancer in hospitals in the district, his/her information would be reported to the local Cancer Registry System first and then to the Shanghai Municipal Cancer Registry. On the contrary, if a permanent resident of Minhang District was first diagnosed with cancer outside the district, his/her information would be reported to the Shanghai Municipal Cancer Registry System first and then was recognized and added to the e-HR database and local Cancer Registry by the CDC of Minhang District. Thereby, the information of all cancer cases could be accessible for designated continuous clinical follow-up and health management by local institutes.

Continuous Health Management for High-Risk Individuals

Targeted residents who met either of the following two criteria were identified as high-risk individuals: (1) with a positive result in initial screening tests but a negative result in diagnostic tests and (2) with a family history of any cancer or a precancerous lesion. For this population, doctors at CHSCs were designated to provide primary care and follow-up services per month, which include health education, behavioral interventions and routine clinical tests, and if necessary, referral advices for further qualified diagnosis and clinical care in medical centers (**Figure 3**).

Data Analysis

Descriptive analyses were performed by presenting the number and percentage of residents in each subgroup. Chi-square tests were used to compare proportions of early-stage cancers among those screen-detected, identified by follow-up or diagnosed by clinic visits. The trend analysis was conducted by using *p*-values for row mean score differences in Cochran–Mantel–Haenszel statistics. A *p*-value of <0.05 was considered as statistically significant. SPSS 11.0 software was used in all data analyses.

RESULTS

Table 1 presents the criteria to identify eligible subjects for initial cancer screening tests based on e-HR system. All residents over 40 years in Minhang district were eligible for colorectal cancer and gastric cancer screening programs. For liver cancer and lung cancer screening, only those with positive results in risk assessment were recruited. For cervix uteri cancer and breast cancer screening, all women over 40 years receiving free health check-up were included, while only those with positive results in risk assessment were recruited in CHSCs and medical centers.

As shown in **Table 2**, subjects identified with high risk of respective cancers received fecal occult blood test (FOBT) and/or rectal exam as initial screening tests for colorectal cancer, had FOBT for gastric cancer, alpha-fetoprotein (AFP) and ultrasonic testing for liver cancer, chest X-ray for lung cancer, Pap smears for cervix uteri cancer, and clinical breast examination (CBE) and thermal texture maps (TTMs) for breast cancer. These tests, as well as tumor-associated antigen test, mammography, low-dose computerized tomography, colonoscopy, gastroscopy, or colposcopy, were further provided for those with negative results in diagnostic tests or those with a family history of cancer or precancerous lesion as a continuous health management service.

TABLE 1 | Criteria used to define high-risk individuals in electronic health record (e-HR)-based risk assessment in Minghang District of Shanghai, China.

Cancer site	Criteria used to define high-risk individuals	
	In CHSCs and medical centers	In health check-up
Colorectum	Over 40 years	
Stomach	Over 40 years	
Liver	Positive in risk assessment†	
Lung	Positive in risk assessment†	
Cervix uteri ‡	Positive in risk assessment†	Over 40 years
Breast ‡	Positive in risk assessment†	Over 40 years

CHSC, Community Healthcare Service Center.
†With related family history, risk behaviors, or symptoms.
‡Only for women.

During the period of 2008 to 2016, the proportion of targeted residents receiving questionnaire-based risk evaluation increased from 3.1 to 22.2% for colorectal cancer (p for trend < 0.01), from 1.6 to 23.0% for gastric cancer (p-trend < 0.01), from 7.3 to 20.5% for liver cancer (p trend < 0.01), and from 1.8 to 21.2% for lung cancer (p trend < 0.01) (**Table 3**). The proportion also increased for cervix uteri cancer and breast cancer from 2008 to 2014 but decreased in 2015.

A total of 24,278 residents over 40 years old were identified at high risk of colorectal cancer, 13,503 for stomach cancer, 9,988 for liver cancer, 44,779 for lung cancer, 20,603 for cervix uteri cancer, and 23,404 for breast cancer. All these subjects were registered into the high-risk population management database and offered with regular community-based primary care mentioned earlier.

As shown in **Table 4**, a total of 2,948 cancer cases were detected through the e-HR system, accounting for 13.3% of all 22,182 newly diagnosed cancer cases in Minhang District. The proportions of early-stage cancers through identified e-HR system, both by screening and by subsequent follow-up, were significantly higher than those through regular medical practices (all $p < 0.0001$).

DISCUSSION

In Minhang district of Shanghai, China, with over 950,000 residents and over 530,000 residents aged more than 40 years (http://www.shmh.gov.cn/, accessed on Jan 16, 2019), an infrastructure for e-HR-based cancer screening was well-established and a series of screening programs have been implemented effectively to detect major cancers over the past several years, particularly for colorectal cancer, gastric cancer, liver cancer, lung cancer, cervical cancer, and breast cancer. This, to the best of our knowledge, is the first e-HR-based comprehensive health practice in cancer prevention at district/city level, which is regarded as a local level exploration for healthcare reform in China. Currently,

TABLE 2 | Initial screening tests used in e-HR-based cancer screening programs in Minhang District of Shanghai, China.

Cancer site	Initial screening tests used in subgroups			Continuous health management services[†]
	Visitors of CHSCs	Outpatients in medical centers	Beneficiaries of health check-up	
Colorectum	FOBT	FOBT + RE	FOBT	Colonoscopy[‡]
Stomach	FOBT	FOBT	FOBT	Gastroscopy
Liver	AFP + UT	AFP + UT	AFP + UT	AFP + UT
Lung	Chest X-ray	Chest X-ray	Chest X-ray	Chest X-ray or LDCT
Cervix uteri	Pap smear	Pap smear	Pap smear	Pap smear + colposcopy
Breast	CBE + TTM	CBE + TTM	CBE + TTM	MAM or UT

CHSC, Community Healthcare Service Center; FOBT, fecal occult blood test; RE, rectal exam; AFP, alpha-fetoprotein; UT, ultrasonic testing; LDCT, low-dose computerized tomography; CBE, clinical breast examination; TTM, thermal texture maps; MAM, mammography.

[†] *Only for individuals with a negative result in diagnostic tests or with a family history of any cancer or having a precancerous lesion.*

[‡] *FOBT or RE for some subjects.*

the system has achieved a capability of offering relevant services for over 20% of population aged 40 years or above each year.

Comprehensive e-HR systems have been successfully applied in many European countries (16–18) and partly in the United States such as Kaiser Permanente (19) and Veterans Affairs Health Care (20). The successful models of organizing and operating e-HR systems provide platforms for cancer screening in resource-rich settings (21–25). In China, a middle-income country, the e-HR system has been used to identify patterns of non-communicable diseases (26), evaluate effect of an intervention in patients with chronic obstructive pulmonary disease (27), and improve cardiovascular care and outcome (28). The present program, taking advantages of the e-HR system, successfully identified a higher proportion of early-stage cancers than regular medical services, offering an example of applying the e-HR system as a feasible comprehensive cancer care system in resource-limited settings.

Our results also suggest that the e-HR system in Minhang District is not only a surveillance system but also a useful platform for health education and health promotion. Health education as a primary prevention strategy is delivered to all community participants at the very beginning, followed by disease screening as the secondary prevention and where appropriate, post-treatment follow-up and health management as the tertiary service. Furthermore, the e-HR system seems useful for identifying health needs in local settings. For instance, the present program has reached a fairly comparable coverage for colorectal, breast, and cervical cancers with screening programs in the United States (29), but only limited screening services were provided for liver cancer and lung cancer due to the relatively high cost but low sensitivity of the initial screening tests like AFP test, ultrasonic, and chest X-ray examinations.

Evidently, the e-HR system has the potential to extend the accessibility of healthcare services in the general population by coordinating and integrating various healthcare services efficiently. In this case, health services for cancer screening, from risk evaluation, early detection of cancer cases, to post-treatment follow-up and health management, were provided efficiently by multiple institutes based on the e-HR system.

Experiences and lessons also can be learned from this program for sustaining a public health system, which may balance increasing challenges and health needs. The e-HR system requires a team approach to input, analyze, and implement huge data. The doctors in CHSCs and medical centers act as the driving force behind the system, but advanced practice clinicians, nurses, quality coordinators, information technology support, and many others should collaborate to make it successful. In most developing countries, however, limited investment in public health and shortage of medical resources remain a big issue. However, the investment in e-HR system will save public health resources in the long run. As a typical example, Minhang District is a rapidly developing region with limited public health budget. Local annual budget for public health and disease prevention is only 100 Yuan RMB per person (1 US dollar equals about 6.8 Yuan RMB), which cannot cover a universal screening for all kinds of chronic diseases, including cancer. Based on the e-HR system, however, the present early cancer detection and continuous service program costs only about 10% of the annual public health budget for risk assessment, screening, subsidies for clinical check-ups, and health management for high risk people. With possibly increased public health budget in the coming years in Minhang District, it is very likely and foreseeable to extend the program from cancer to other non-communicable diseases. Moreover, the e-HR data can be accessed and used by any researchers once their applications are approved by local Health Commissions, which provide valuable opportunities for further scientific researches.

There are several limitations of this study. First, we did not compare the characteristics of participants and non-participants of the cancer screening program. The potential differences between the two subpopulations may have biased our results. Second, we did not take the sensitivities and specificities of cancer screening methods used in the population

TABLE 3 | Cancers and high-risk individuals identified in e-HR-based cancer screening programs in Minhang District of Shanghai, China, 2008–2016.

Cancer site	Calendar year	All subjects		Participants of e-HR-based cancer screening programs						
		No. of eligible residents	No. of incident cancers	% of participants for risk assessment	No. of subjects for screening tests	No. of subjects with positive results	Cancer cases detected	Proportion of detected cancers (%)	No. of high-risk individuals	No. of high-risk individuals receiving health management**
COLORECTUM										
	2008	491,853	476	3.1	15,351	924	34	7.1	1,151	–
	2009	511,366	567	18.6	76,407	1,647	84	14.8	3,225	1,065
	2010	469,691	569	24.6	115,630	2,854	119	20.9	1,838	3,574
	2011	549,318	527	21.3	117,039	1,883	98	18.6	1,337	7,765
	2012	563,525	512	34.4	193,654	5,678	164	32.0	4,260	11,153
	2013	577,892	639	32.3	186,391	7,350	183	28.6	5,075	10,800
	2014	592,322	598	29.5	174,581	6,888	160	26.8	3,109	20,409
	2015	606,961	640	22.2	134,790	6,181	124	19.4	2,322	21,555
	2016	623,788	628	22.2	138,445	8,861	112	17.8	1,961	18,446
STOMACH										
	2008	491,853	417	1.6	8,035	206	12	2.9	924	–
	2009	511,366	393	15.5	79,342	781	49	12.5	1,198	686
	2010	469,691	403	25.6	120,104	2,190	86	21.3	1,402	3,634
	2011	549,318	385	22.3	122,557	1,522	73	19.0	1,126	8,251
	2012	563,525	331	36.2	203,723	4,000	106	32.0	2,325	9,697
	2013	577,892	454	33.6	194,343	5,234	126	27.8	1,862	18,485
	2014	592,322	420	30.6	181,057	5,190	127	30.2	1,676	23,261
	2015	606,961	462	23.4	142,288	4,552	65	14.1	1,127	11,200
	2016	623,788	483	23.0	143,236	8,619	84	17.4	1,863	10,462
LIVER										
	2008	491,853	251	7.3	15,181	52	14	5.6	481	–
	2009	511,366	242	16.4	3,448	15	9	3.7	2,152	291
	2010	469,691	280	24.0	6,376	99	10	3.6	695	3,479
	2011	549,318	214	20.4	2,335	24	9	4.2	914	5,463
	2012	563,525	153	36.0	3,367	87	10	6.5	1,673	8,275
	2013	577,892	242	31.2	2,651	36	4	1.7	1,435	10,913
	2014	592,322	264	28.3	2,243	27	14	5.3	760	5,213
	2015	606,961	265	20.5	2,061	35	3	1.1	672	7,864
	2016	623,788	270	20.5	2,104	41	6	2.2	1,206	7,678

(Continued)

TABLE 3 | Continued

Cancer site	Calendar year	All subjects		Participants of e-HR-based cancer screening programs						
		No. of eligible residents	No. of incident cancers	% of participants for risk assessment	No. of subjects for screening tests	No. of subjects with positive results	Cancer cases detected	Proportion of detected cancers (%)	No. of high-risk individuals	No. of high-risk individuals receiving health management**
LUNG										
	2008	491,853	609	1.8	1,867	43	15	2.5	593	–
	2009	511,366	677	16.1	56,215	215	79	11.7	3,787	436
	2010	469,691	667	24.5	30,622	237	89	13.3	4,346	4,221
	2011	549,318	577	22.0	20,868	265	45	7.8	3,473	13,021
	2012	563,525	547	37.8	25,605	1,246	54	9.9	8,152	18,799
	2013	577,892	899	32.0	22,350	949	48	5.3	6,909	8,423
	2014	592,322	984	28.6	19,258	633	71	7.2	6,144	4,868
	2015	606,961	1070	21.9	19,441	695	55	5.1	5,410	37,498
	2016	623,788	923	21.2	19,473	1,152	45	4.9	5,965	36,105
CERVIX UTERI†										
	2008	245,435	44	16.8	2,727	60	1	2.3	4,810	–
	2009	255,753	67	18.8	15,309	134	13	19.4	3,261	6,322
	2010	266,289	66	23.9	53,763	1,439	16	24.2	691	8,948
	2011	275,492	62	21.4	39,001	3,379	10	16.1	1,338	3,430
	2012	282,763	79	29.3	48,349	4,485	22	27.8	1,506	4,110
	2013	290,167	74	25.3	41,384	1,736	22	29.7	1,767	5,839
	2014	297,616	124	23.6	45,698	2,132	42	33.9	1,730	7,544
	2015	305,206	140	14.8	29,018	1,364	27	19.3	2,591	18,757
	2016	314,008	145	18.2	41,706	1,374	50	34.5	2,909	20,430
BREAST†										
	2008	245,435	333	16.9	6,407	1,796	24	7.2	2,799	1,862
	2009	255,753	318	18.3	10,258	2,493	21	6.6	5,683	9,196
	2010	266,289	356	25.2	58,237	6,841	47	13.2	3,930	15,329
	2011	275,492	361	21.7	45,393	7,804	62	17.2	1,602	11,839
	2012	282,763	371	28.5	47,580	4,585	58	15.6	2,340	12,531
	2013	290,167	380	23.4	40,154	1,976	41	10.8	3,564	16,601
	2014	297,616	413	21.7	44,402	1,424	50	12.1	979	16,526
	2015	305,206	362	13.5	27,876	1,035	27	7.5	1,006	23,522
	2016	314,008	395	16.9	37,564	1,318	29	7.3	1,501	22,278
Total			22,182				2,948		136,555	

†*Only for women; Data in 2008 were incomplete for cervix uteri cancer.*

TABLE 4 | Comparison of cancer cases detected by e-HR-based programs and by clinical visits in Minhang District of Shanghai, China, 2008–2016.

Cancer site	Identified in cancer screening programs			Identified by subsequent follow-up			Diagnosed by clinical visits			*p*-values for x^2 tests
	No. of cases	No. of cases at early stage†	%	No. of cases	No. of cases at early stage†	%	No of cases	No. of cases at early stage†	%	
Colorectum	1,078	311	28.8	1,677	254	15.1	2,401	182	7.6	<0.0001
Stomach	728	150	20.6	939	135	14.9	2,081	276	13.3	<0.0001
Liver	79	16	20.3	200	15	7.5	1,902	120	6.3	<0.0001
Lung	501	79	15.0	1,524	198	13.0	4,928	566	11.5	<0.0001
Cervix uteri†	203	168	82.8	329	264	80.2	269	57	21.2	<0.0001
Breast†	359	182	50.7	871	401	46.0	2,059	681	33.1	<0.0001
Total	2,948	906	30.7	5,540	1,267	22.9	13,640	1,882	13.8	

† *Cases with TNM staging 0–II for colorectal, gastric, liver, and lung cancers, at stage 0–IIa under FIGO 2,000 classification for cervical cancer, and with TisN0M0/T1N0M0 and primary tumor diameter ≤2 cm for breast cancer.*

into consideration. Several methods with low validity such as CBE, TTM, AFP, and chest X-ray were used in the program, leading to unnecessary costs. Finally, cost-effective analysis of the program was not conducted due to lack of financial data for e-HR system building, limiting our ability to evaluate the system.

CONCLUSIONS

In conclusion, the e-HR system in Minhang District enables local health institutions to provide integrative and comprehensive health care and management for cancers. The successful application of an e-HR system in cancer prevention and control implies that the system may act as an extendable and sustainable infrastructure for comprehensive health care and services for a broad spectrum of diseases and health events.

DATA AVAILABILITY

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The study was approved by the Institutional Review Board of Minhang District CDC (NO: EC-P-2012-002). Verbal informed consent was obtained from each participant of the cancer screening program.

AUTHOR CONTRIBUTIONS

DH and WX drafted the manuscript. DX and NH conceived and designed the study. DH, WX, HS, and WL made substantial contributions to the study design. DH, JZ, and BY are responsible for study coordination. DH and DX are responsible for data quality control. DH and BY are responsible for data wrangling. DH is responsible for data analysis. All authors contributed to the revision of the manuscript and approved the final manuscript.

FUNDING

This study was supported by the National Natural Science Foundation of China (grant no. 81161120407) and the Natural Science Foundation of Shanghai, China (12ZR1448700). The funding institutions provided purely financial support and have no access to the data and no intervention in data analysis or manuscript preparation.

ACKNOWLEDGMENTS

The authors sincerely appreciate the support from the Health Commission of Minhang District for establishment and implementation of the e-HR system and the hard work and contributions of all health professionals in Minhang District, Shanghai, with particular gratefulness to Dr. Su Xu for his leadership in these efforts.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Oncology

ORIGINAL RESEARCH
published: 04 June 2019
doi: 10.3389/fonc.2019.00399

Colorectal Cancer Screening Modalities in Chinese Population: Practice and Lessons in Pudong New Area of Shanghai, China

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Background: Parallel test of risk stratification and two-sample qualitative fecal immunochemical tests (FITs) are used to screen colorectal cancer (CRC) in Shanghai, China. This study was designed to identify an optimal initial screening modality based on available data.

Methods: A total of 538,278 eligible residents participated in the program during the period of January 2013 to June 2017. Incident CRC was collected through program reporting system and by record linkage with the Shanghai Cancer Registry up to December 2017. Logistic regression model was applied to identify significant factors to calculate risk score for CRC. Cutoff points of risk score were determined based on Youden index and defined specificity. Sensitivity, specificity, and positive predictive values (PPVs) were computed to evaluate validity of assumed screening modalities.

Results: A total of 446 CRC were screen-detected, and 777 interval or missed cases were identified through record linkage. The risk score system had an optimal cutoff point of 19 and performed better in detecting CRC and predicting long-term CRC risk than did the risk stratification. When using a cutoff point of 24, parallel test of risk score, and FIT were expected to avoid 56 interval CRCs with minimal decrease in PPV and increase in colonoscopy. However, the observed detection rates were much lower than those expected due to low compliance to colonoscopy.

Conclusions: Risk score is superior to risk stratification used in the program, particularly when combined with FIT. Compliance to colonoscopy should be improved to guarantee the effectiveness of CRC screening in the population.

Keywords: colorectal cancer, screening, risk assessment, risk score, fecal immunochemical tests

OPEN ACCESS

Edited by:
Tianhui Chen,
Zhejiang Academy of Medical Sciences, China

Reviewed by:
Hamideh Salimzadeh,
Tehran University of Medical Sciences, Iran
Hao Liu,
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Specialty section:
This article was submitted to Cancer Epidemiology and Prevention, a section of the journal Frontiers in Oncology

Received: *26 February 2019*
Accepted: *29 April 2019*
Published: *04 June 2019*

Citation:
Wu W, Wang Y, Jiang H, Yang C, Li X, Yan B, Zhou Y, Xu W and Lin T (2019) Colorectal Cancer Screening Modalities in Chinese Population: Practice and Lessons in Pudong New Area of Shanghai, China. Front. Oncol. 9:399. doi: 10.3389/fonc.2019.00399

INTRODUCTION

Colorectal cancer (CRC) is one of the most common cancers globally, leading to over 1.8 million new cases and 881,000 deaths in 2018 (1). In China, CRC ranks second in incidence and fourth in death of all cancers (http://gco.iarc.fr/, access date: April 4, 2019). The rapid increasing incidence and mortality of the disease (2) and the proven effectiveness of screening in CRC prevention and control (3) motivate the Chinese government to perform and scale up population-based CRC screening around the country.

Population-based CRC screening has been implemented in many countries as a National Cancer Screening Program (4). Multiple methods were used in these programs, mainly stool-based tests like guaiac-based fecal occult blood test, fecal immunochemical test (FIT), and stool DNA testing, and direct visualization tests such as flexible sigmoidoscopy, colonoscopy, double-contrast barium enema, CT colonography, and video capsule colonoscopy (5). In resource-limited settings, serial use of risk assessment and FIT were conducted to improve cost-effectiveness of screening (6). In Jiashan County of Zhejiang Province of China, however, parallel use of a questionnaire-based risk assessment and two-sample qualitative FITs were conducted as an initial screening method to increase sensitivity of screening. It was reported that in Chinese population, the sensitivity and specificity of one positive qualitative FIT were 90.4 and 53.8%, respectively, for CRC, and those of two positive qualitative FITs were 80.8 and 75.1%, respectively (7). The pilot study in Jiashan County showed that the parallel test modality performed well in detecting early colorectal neoplasms, and the positive predictive value (PPV) reached 2.7% (8, 9).

Based on the evidence, the Shanghai government launched a pilot community-based CRC screening project in 2008. Three-year practice using a similar screening protocol of Jiashan County showed a great improvement in detection of early-stage CRC (10). In 2013, a large-scale screening program was launched as a major public health service project, making Shanghai one of the earliest cities in China to undertake mass screening of CRC. So far, three rounds of screening have been performed, and the results of the first round validated the effectiveness of parallel use of risk stratification and FIT (11). The screening modality with a high sensitivity, however, has led to a high false positive rate and thus low compliance to further colonoscopy examination (12, 13), limiting the effectiveness of screening.

In this study, we took advantage of the database developed in screening practice in Pudong New Area of Shanghai, China, to optimize the risk assessment tool and seek an optimal initial screening protocol for CRC in this population.

MATERIALS AND METHODS

Study Participants

Almost all guidelines recommend CRC screening for asymptomatic individuals between ages of 50 and 75 years (5, 14, 15) as mortality benefit is greatest for patients aged 50–70 years. However, in Shanghai, one of the most aging cities in China, the service was also provided to residents aged 76–79 years old to achieve equity in health care (10). Therefore, the inclusion criteria were defined as follows: (1) permanent residents of Shanghai, (2) living in Pudong New Area of Shanghai, (3) aged 50–79 years, and (4) beneficiaries of the basic medical insurance of Shanghai.

The first round of screening was conducted in 2013, the second round covered 3 years from January 2014 to December 2016, and the third round was planned from January 2017 to December 2019. Through community mobilization, a total of 538,278 eligible volunteers attended initial screening of CRC during the period of January 1, 2013 to June 30, 2017 and were included in this analysis.

This study was approved by the Medical Ethics Committee of the Center for Disease Control and Prevention in Pudong New Area of Shanghai, China, and oral consent was obtained from each participant of the screening program.

Screening Procedure

A two-stage sequential screening was designed and conducted in all 15 districts of Shanghai in 2013. A questionnaire-based risk assessment and two-sample qualitative FIT were used as initial screening.

Risk Stratification

The participants were regarded as positive in risk assessment if they had one of the following events: (1) a history of any cancer; (2) a history of polyps; (3) a family history of CRC in a first-degree relative and/or at least two of the following events: (a) chronic coprostasis, (b) chronic diarrhea, (c) phlegmatically blood feces, (d) serious unhappy life events such as death among first-degree relatives, (e) chronic appendicitis or appendectomy, and (f) chronic cholecystitis or cholecystectomy.

Fecal Immunochemical Test

Two stool samples were collected with an interval of 1 week by community healthcare staff and tested in a local hospital by contracted experienced technicians. Three different parts were taken from each stool sample and then mixed and washed by special buffer solution. Each sample was collected in a tube, including about 5 ml moist stool content. A qualitative FIT test was conducted in 5 min after collection using colloidal gold assay (monoclonal antibody), with a positivity threshold of 100 ng/ml of sample solution. FIT test kits were purchased from Shanghai Lijun Medical Co. Ltd., China.

Colonoscopy

Individuals with a positive FIT test or a positive risk assessment were regarded as positive in the first stage and were invited to undergo a colonoscopy as the second stage of screening. Colonoscopies were required to be performed in one of the 13 designated hospitals, where polyps and adenomas were removed once diagnosed. The risk assessment and FITs were administered free to participants, but colonoscopy was paid by basic medical insurance of Shanghai.

Abbreviations: AUC, area under receiver operating characteristic curve; CI, confidence interval; CRC, colorectal cancer; FIT, fecal immunochemical test; PPV, positive predictive value; ROC, receiver operating characteristic curve.

Data Collection

To evaluate the effectiveness of the CRC screening program, we took all subjects as members of a prospective cohort. A 12-digit barcode was assigned to each participant at recruitment to follow screening results. Baseline demographic information and risk factors were collected through in-person interview using a structured questionnaire. The barcode appeared on the fecal collect tube, and when participants returned the tube, the FIT results were entered into the reporting system by scanning the barcode. The results of colonoscopic and histopathologic examinations were entered using the same barcode in designated hospitals and submitted monthly by the local community healthcare staff to the Center for Disease Control and Prevention in Pudong New Area of Shanghai through an internet-based reporting system.

Newly diagnosed CRCs were obtained from the program reporting system as screen-detected cancers and supplemented by record linkage with the Shanghai Cancer Registry up to December 31, 2017 using unique ID numbers (**Figure 1**). Interval CRC was defined as those detected within 2 years after a negative initial screening test, while missed cases referred to those detected within 2 years after a positive initial screening test.

Quality Control

The process of the screening program was supervised by the staff in the Center for Disease Control and Prevention in Pudong New Area of Shanghai who organized annual training for physicians, planned progress of the screening program, monitored screening tests, and supervised data collection and data entry. The final database was double-checked and verified to improve quality. Field quality control was conducted by community health care staff who were motivated by subsidies according to workload and quality assessment.

Statistical Analysis

Positive rate was calculated as the number of subjects positive in the respective screening test divided by the number of all participants of the test. Observed detection rates were calculated as the number of screen-detected CRC divided by the number of all participants, while expected detection rates were calculated as the number of prevalent CRC (screen-detected, interval, and missed CRC) divided by the number of all participants.

Fisher exact test was used to test the differences in positive rates and detection rates. *Kappa* coefficients were used to evaluate consistency of stratified risk with FIT results. Logistic regression model for prevalent CRC cases was fitted by backward selection with age, sex, education, and risk factors listed in the questionnaire to identify significant factors to construct CRC risk score. Risk score was calculated by multiplying the β-coefficients of the significant variables by 10 and rounding to the nearest integer (16). Receiver operating characteristic (ROC) curve was obtained by plotting sensitivity against 1-specificity to evaluate performance of risk score and risk stratification used in the program. The optimal cutoff point of risk score was identified based on Youden index, which was at the maximum sum of the sensitivity and specificity-1 (16). The cutoff point at the same specificity of risk stratification was also used to compare PPVs of the two risk assessment methods.

In order to testify the stability of the present model, we developed a model in randomly selected 90% of the overall sample according to the above-mentioned analysis method and validated in the remaining 10% of the sample. The above progress was repeated 10 times. Significant risk factors in 10 subgroups were identical to those in the whole samples, and the areas under ROC curve (AUC) ranged from 0.644 to 0.664 for risk score. Sensitivity, specificity, and PPV were computed to evaluate validity of assumed screening modalities.

Person-years of observation was used to calculate overall incidence [95% confidence intervals (CIs)] of CRC by subgroups. The period of observation was further split into two intervals (within 2 years and $\geq$2 years of screening) to calculate incidence (95% CI) of CRC during each period. Sensitivity analysis was performed by defining interval and missed CRCs as those detected within 3 years after an initial screening test.

All statistical analysis was performed in the Statistics Analysis System version 9.4 (SAS 9.4).

RESULTS

Demographic Characteristics of the Participants

In the program, a total of 538,278 residents participated in the screening program, accounting for 39.7% of all eligible residents (**Table 1**). More women and individuals aged 60–69 years participated in the program. Among all subjects, 55,264 (10.0%) were stratified as high-risk individuals, and 70,273 (13.1%) were positive in at least one FIT. As a result, a total of 115,247 (21.0%) participants positive in risk assessment or in FIT were considered as positive in the initial screening test and were advised to have a further colonoscopy examination. The positive rate increased with age and was higher in men and in the residents with college education or higher ($p < 0.0001$). Of all positive subjects in initial screening tests, only 27,097 (23.5%) had a colonoscopy examination, whereas 588 negative subjects had colonoscopy for unknown reasons.

Comparison of Risk Stratification and Risk Score in Detecting Colorectal Cancer

Risk score developed in this study included age group (50–54 years: score 0; 55–59 years: score 6; 60–64 years: score 9; 65–69 years: score 11; 70–74 years: score 14; 75–79 years: score 16), sex (women: score 0; men: score 6), chronic diarrhea (never: score 0; ever: score 3), phlegmatically blood feces (never: score 0; ever: score 12), polyps (never: score 5; ever: score 0), serious unhappy life events (never: score 0; ever: score 3), and family history of CRC (never: score 0; ever: score 6).

The score ranged from 0 to 49, with an optimal cutoff point of 19. The cutoff point increased to 24 at the similar specificity of risk stratification used in the program (89.7%). The risk score performed better in detecting CRC than risk stratification, with AUC being 0.655 vs. 0.526 for risk stratification (**Figure 2**).

The factors for risk assessment were not well-consistent with FIT results, with an agreement ranging from 80.9 to 86.0% and a Kappa coefficient from 0.01 to 0.03 ($p < 0.0001$). The low agreement with FIT was also observed for overall risk assessment,

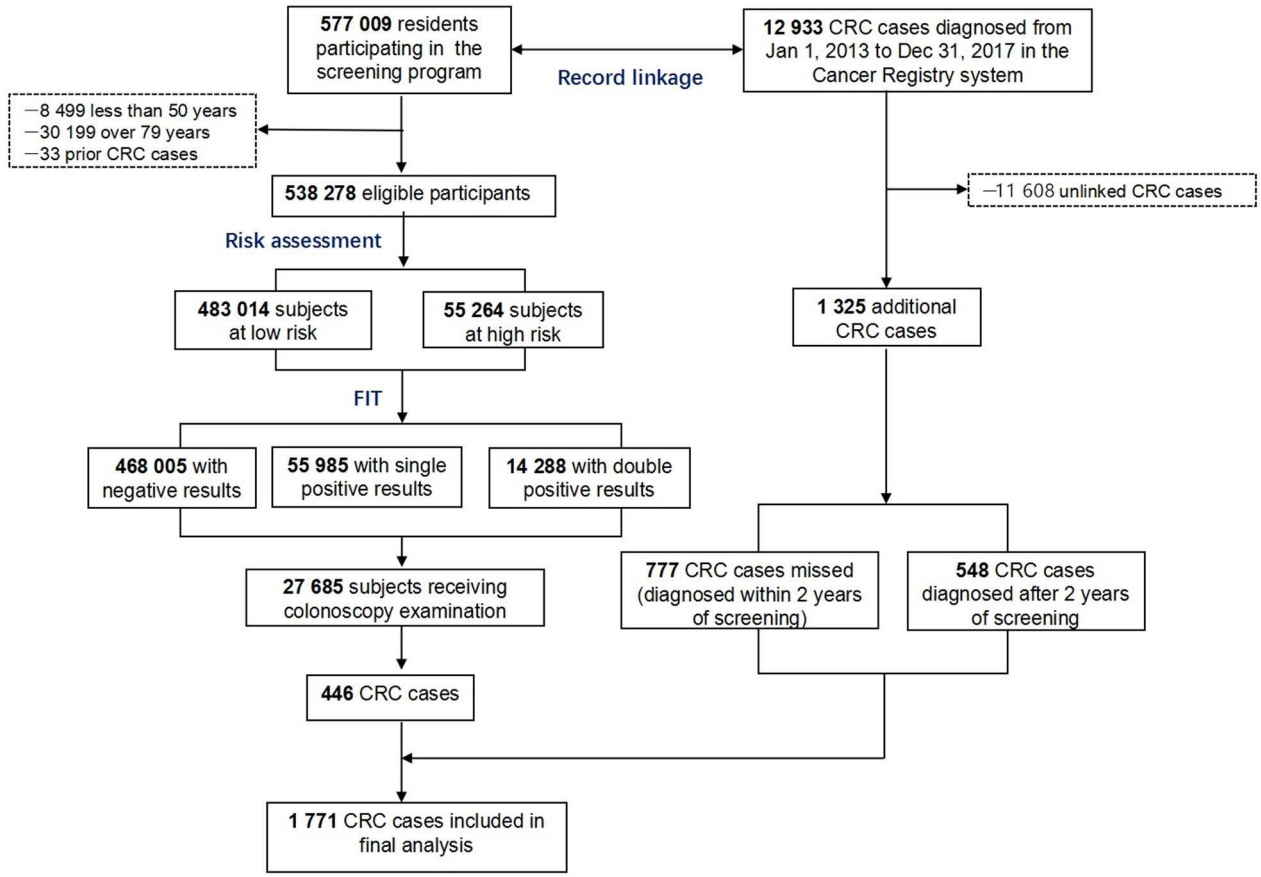

FIGURE 1 | Flow chart for subject recruitment and data collection.

TABLE 1 | Positive rates of screening tests and compliance to colonoscopy by baseline demographic characteristics of participants.

Demographic characteristics	No. of residents	Participants of initial screening, *n* (%)	Risk assessment positive, *n* (%)	FIT positive, *n* (%)	Initial screening positive, *n* (%)	Attended colonoscopy, *n* (%)
All subjects	1,356,068	538,278 (39.7)	55,264 (10.0)	70,273 (13.1)	115,247 (21.0)	27,685 (24.0)
Sex						
Men	663,664	219,698 (33.1)	20,943 (9.5)	32,232 (14.7)	48,660 (22.1)	12,473 (25.6)
Women	692,404	318,580 (46.0)	34,321 (10.8)	38,041 (11.9)	66,587 (20.9)	15,212 (22.8)
Age group (years)						
50–54	234,537	42,784 (18.2)	3,492 (8.2)	3,937 (9.2)	6,982 (16.3)	1,804 (25.8)
55–59	277,152	94,275 (34.0)	8,498 (9.0)	10,669 (11.3)	17,770 (18.8)	4,865 (27.4)
60–64	291,245	141,133 (48.5)	14,097 (10.0)	18,106 (12.8)	29,703 (21.0)	8,029 (27.0)
65–69	207,614	148,444 (71.5)	15,932 (10.7)	20,748 (14.0)	33,585 (22.6)	7,858 (23.4)
70–74	117,743	75,643 (64.2)	8,895 (11.8)	11,052 (14.6)	18,139 (24.0)	3,803 (21.0)
75–79	227,077	35,999 (15.9)	4,350 (12.1)	5,761 (16.0)	9,068 (25.2)	1,326 (14.6)
Education						
No formal education	–	24,777	2,255 (9.1)	3,196 (12.9)	5,013 (20.2)	1,286 (25.7)
Primary school	–	159,868	11,713 (7.3)	19,736 (12.3)	29,221 (18.3)	8,444 (28.9)
Middle or occupational school	–	313,951	34,580 (11.0)	41,470 (13.2)	69,792 (22.2)	16,093 (23.1)
College or above	–	39,682	6,716 (16.9)	5,871 (14.8)	11,221 (28.3)	1,862 (16.6)

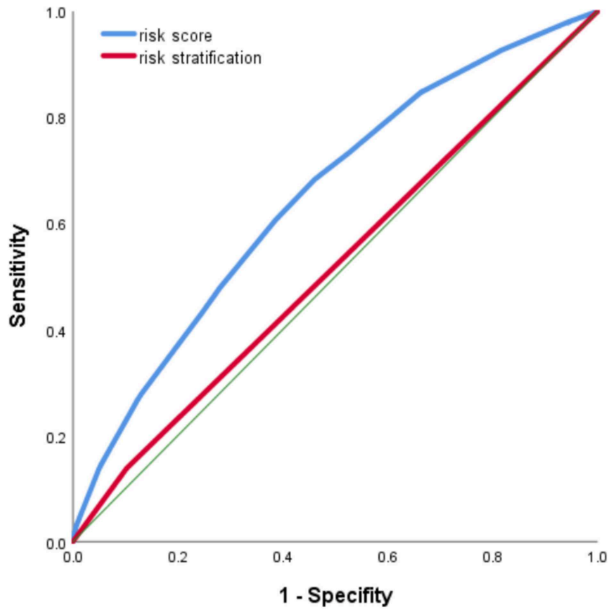

FIGURE 2 | Receiver operating characteristic (ROC) curve of risk score in identifying colorectal cancer (CRC) cases.

with an agreement of 80.5% and a Kappa coefficient of 0.06 with risk stratification ($p < 0.001$), and an agreement of 54.7% and a Kappa coefficient of 0.04 with risk score ($p < 0.001$) (**Table 2**).

Detection Rates of Colorectal Lesions by Subgroups

A total of 446 CRC cases were screened and reported, and as many as 777 missed or interval cases were identified through record linkage with the Shanghai Cancer Registry possibly due to low compliance to colonoscopy. Detection rates, both observed and expected, were significantly higher in high-risk individuals defined by risk stratification, risk score, and FIT and were the highest (20.8/1,000 and 38.7/1,000, respectively) among subjects with high-risk score and positive double FIT. Detection rates of precancerous lesions (advanced adenoma, small tubular adenoma, serrated adenoma, villous adenoma, hamartoma, high- and low-grade dysplasia, tubular villous adenoma, etc.) were also higher in high-risk subjects defined by risk stratification, risk score, and FIT (**Table 3**).

As shown in **Figure 3**, CRC incidence was 81.5/100,000 among subjects with high-risk score only, significantly higher than 34.2/100,000 among those with low-risk score and negative double FIT. Detection rates and incidence of CRC doubled among subjects with high-risk score and any FIT positive than in those with any FIT positive only.

Incidence of Colorectal Cancer Along Follow-Up Time

As shown in **Table 4**, risk stratification, risk score, and FIT performed well in predicting CRC risk, with significant higher incidence of CRC after 2 or 3 years of initial screening in positive subjects. With the least number of interval CRC cases, parallel use of FIT and risk score performed better than modality used in the program in identifying individuals at high risk of CRC.

Figure 4 presents incidence of CRC along with years of follow-up until December 2017 by results of risk score and FIT. A peak in incidence was observed within 6 months of screening, and then the incidence decreased within 2–3 years of screening. Thereafter, the incidence increased with the follow-up time in each group.

Validity of Assumed Screening Modalities in Detecting CRC

As presented in **Table 5**, if all positive subjects received further colonoscopy and diagnostic examinations, the initial screening

TABLE 2 | Consistency in results of risk assessment and FIT in CRC screening.

Risk assessment	FITs		Agreement	Kappa	*P* value
	Any positive	Double negative			
Items for risk assessment					
Chronic diarrhea					
Ever	4,261	20,715			
Never	66,012	447,290	83.9	0.02	< 0.0001
Chronic coprostasis					
Ever	5,497	26,617			
Never	64,776	441,388	83.0	0.03	< 0.0001
Phlegmatically blood feces					
Ever	2,077	7,320			
Never	68,196	460,685	86.0	0.02	< 0.0001
Chronic appendicitis/appendectomy					
Ever	6,598	35,749			
Never	63,675	432,256	81.5	0.02	< 0.0001
Cholecystitis or cholecystectomy					
Ever	6,903	39,362			
Never	63,370	428,643	80.9	0.02	< 0.0001
Serious unhappy life events					
Ever	1,373	6,481			
Never	68,900	461,524	86.0	0.01	< 0.0001
History of any cancer					
Ever	1,814	9,484			
Never	68,459	458,521	85.5	0.01	< 0.0001
Colon polyps					
Ever	2,375	8,736			
Never	67,898	459,269	85.8	0.02	< 0.0001
CRC in first degree relatives					
Positive	2,516	11,856			
Negative	67,757	456,149	85.2	0.02	< 0.0001
Overall risk assessment					
Risk stratification					
High risk	10,290	44,974			
Low risk	59,983	423,031	80.5	0.06	< 0.0001
Risk score					
≥19	37,065	210,749			
<19	33,208	257,256	54.7	0.00	< 0.0001

CRC, colorectal cancer; FIT, fecal immunochemical test.

TABLE 3 | Detection rates of colorectal lesions by initial screening results.

Methods of screening	No. of subjects	Attended colonoscopy, *n* (%)	Precancerous lesions[a]		CRC			
			No. of detected cases	Detection rate (1/1,000)	No. of detected CRC	Detection rate (1/1,000)	No. of prevalent CRC[b]	Expected detection rate (1/1,000)
Risk stratification								
Low risk	483,014	18,356 (3.8)	2,537	5.3	366	0.8	1,035	2.1
High risk	55,264	9,329 (16.9)	908	16.4	80	1.4	188	3.4
Risk score								
<19	290,464	13,831 (4.8)	1,370	4.7	134	0.5	383	1.3
≥19	247,814	13,854 (5.6)	2,075	8.4	312	1.3	840	3.4
FIT								
Double negative	468,005	6,353 (1.4)	545	1.2	19	0.0	404	0.9
Single positive	55,985	16,250 (29.0)	2,034	36.3	191	3.4	384	6.9
Double positive	14,288	5,082 (35.6)	866	60.6	236	16.5	435	30.4
Any positive	70,273	21,332 (30.4)	2,900	41.3	427	6.1	819	11.7
Risk stratification and FIT								
Low risk and double FIT (–)	423,031	588 (0.1)	77	0.2	7[c]	–	350[c]	–
Single FIT positive only	47,953	13,583 (28.3)	1,726	36.0	163	3.4	324	6.8
Double FIT positive only	12,030	4,185 (34.8)	734	61.0	196	16.3	361	30.0
High risk only	44,974	5,765 (12.8)	468	10.4	12	0.3	54	1.2
High risk and single FIT (+)	8,032	2,667 (33.2)	308	38.3	28	3.5	60	7.5
High risk and double FIT (+)	2,258	897 (39.7)	132	58.5	40	17.7	74	32.8
Risk score and FIT								
Risk score < 19 and double FIT (–)	257,256	3,190 (1.2)	221	0.9	4[c]	–	129[c]	–
Single FIT positive only	27,192	8,343 (30.7)	827	30.4	66	2.4	139	5.1
Double FIT positive only	6,016	2,298 (38.2)	322	53.5	64	10.6	115	19.1
Risk score ≥19 only	210,749	3,163 (1.5)	324	1.5	15	0.1	275	1.3
Risk score ≥19 and single FIT (+)	28,793	7,907 (27.5)	1,207	41.9	125	4.3	245	8.5
Risk score ≥19 and double FIT (+)	8,272	2,784 (33.7)	544	65.8	172	20.8	320	38.7

[a] *Including advanced adenoma, small tubular adenoma, serrated adenoma, villous adenoma, hamartoma, high- and low-grade dysplasia, and tubular villous adenoma.*
[b] *Including screened CRC, missed CRC, and/or interval CRC diagnosed within 2 years after initial screening among positive subjects.*
[c] *Potential interval CRC.*

modality used in the program, i.e., parallel test of FIT and risk stratification, would detect 873 CRC cases, with a sensitivity of 71.4%, specificity of 78.7%, and PPV of 0.76%. One hundred thirty-two colonoscopy examinations were required to detect one CRC case.

We further evaluated validity of assumed risk score-based screening modality. Parallel test of FIT with risk score using the optimal cutoff point of 19 detected more CRC cases than parallel tests of FIT with risk stratification, but at the cost of decreased PPV (0.39%) and doubled colonoscopy examinations for each detected CRC. When using 24 as the cutoff point of risk score, parallel test of FIT with risk score was expected to avoid 56 interval CRCs with a minimal decrease in PPV and an increase in colonoscopy per detected CRC.

DISCUSSION

In this CRC mass screening program provided by the Chinese government as a major public health service (17), the main findings include the following: (1) risk assessment was

FIGURE 3 | Performance of combined use of fecal immunochemical test (FIT) with risk score.

TABLE 4 | Incidence and 95% CI of CRC by initial screening results.

Screening methods	No. of subjects	No. of CRC cases	Incidence (95%CI) (1/100,000)	Incidence (95% CI)		Incidence (95% CI)	
				Within 2 years of screening	After 2 years of screening	Within 3 years of screening	After 3 years of screening
Risk stratification							
Low risk	483,014	1,526	103.9 (98.8, 109.2)	52.5 (47.9, 57.6)	177.0 (166.7, 188.0)	54.5 (50.5, 58.9)	300.4 (281.2, 320.8)
High risk	55,264	245	146.4 (129.2, 165.9)	92.1 (74.9, 113.3)	222.4 (190.0, 260.4)	84.7 (70.4, 101.9)	388.6 (327.7, 460.9)
Risk score							
<19	290,464	563	64.1 (59.0, 69.6)	30.5 (26.1, 35.7)	112.4 (102.0, 124.0)	34.5 (30.5, 39.2)	183.8 (164.7, 205.1)
≥19	247,814	1,208	159.5 (150.7, 168.7)	87.1 (78.8, 96.2)	260.7 (243.5, 279.1)	84.6 (77.6, 92.3)	450.6 (418.4, 485.3)
FIT							
Negative	468,005	792	55.6 (51.8, 59.6)	9.8 (7.9, 12.2)	120.6 (112.1, 129.9)	17.6 (15.3, 20.2)	206.4 (190.5, 223.8)
Single positive	55,985	485	285.0 (260.7, 311.6)	227.2 (199.4, 258.9)	366.7 (324.6, 414.3)	201.9 (179.4, 227.3)	615.3 (537.4, 704.4)
Double positive	14,288	494	1,184.9 (1,084.8, 1,294.3)	936.3 (823.7, 1,064.3)	1,158.8 (1,380.1, 1,760.7)	823.9 (732.5, 926.7)	2,733.4 (2,391.4, 3,124.4)
Any positive	70,273	979	462.1 (434.0, 492.0)	369.6 (337.3, 405.0)	594.5 (545.4, 648.0)	325.8 (299.7, 354.1)	1,012.0 (920.2, 1,112.9)
Parallel test of risk stratification and FIT							
Negative	423,031	707	54.9 (51.0, 59.1)	8.3 (6.5, 10.7)	121.1 (112.1, 130.8)	16.2 (13.9, 18.8)	208.7 (191.8, 227.0)
Positive	115,247	1,064	305.6 (287.8, 324.6)	234.8 (214.7, 256.8)	406.2 (374.6, 440.6)	210.8 (194.4, 228.6)	684.9 (626.1, 749.3)
Parallel test of risk score and FIT							
Negative	257,256	266	34.2 (30.3, 38.6)	3.3 (2.0, 5.4)	78.6 (69.5, 89.0)	9.6 (7.5 12.4)	133.4 (116.4, 152.9)
Positive	281,022	1,505	175.6 (166.0, 184.7)	105.4 (96.8, 114.8)	274.3 (257.6, 292.0)	101.5 (94.2, 109.4)	465.9 (434.9, 499.2)

complementary to FIT in identifying CRC cases, supporting parallel test of the two methods in the population; (2) the compliance rate was as low as 23.5% in positive subjects, indicating the urgency to optimize initial screening modality in the population; (3) risk score system developed in this study performed better in detecting CRC than risk stratification used in the program, indicating potential benefits by using risk score; and (4) parallel use of FIT and risk assessment performed well in predicting long-term risk of CRC, suggesting that subjects positive in initial screening should be followed up extensively even if they are negative in colonoscopy examinations.

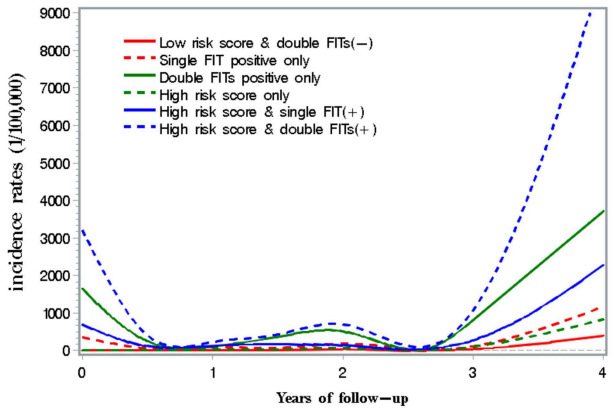

FIGURE 4 | CRC incidence along follow-up time by combined use of FIT with risk score.

Selection of CRC screening modality depends not only on validity of the modality in target population but also on feasibility, affordability, compliance, and clinical capacity of screening, particularly in resource-limited settings (5). In Shanghai CRC screening program, FIT, the most widely used qualitative CRC screening method, was used to identify high-risk individuals using a cutoff value of fecal hemoglobin (Hb) ≥ 100 ng/ml (20 μg Hb/g feces) based on evidence from Chinese (18) and other populations (4, 19, 20). In a meta-analysis including 17 studies, the median fecal Hb positivity cutoff was found to be 20 μg Hb/g feces, with a range of 10–200 μg Hb/g feces (21). The detection threshold resulted in high specificity but low sensitivity in our population and thus a large number

TABLE 5 | Validity of used and assumed initial screening methods.

Screening modality	No. of subjects	No. of CRC	Sensitivity (%)	Specificity (%)	PPV (%)	Colonoscopy per detected CRC	Sensitivity analysis[a]				
							No. of CRC	Sensitivity (%)	Specificity (%)	PPV (%)	Colonoscopy per detected CRC
Risk stratification											
Low risk	483,014	1,035					1,237				
High risk	55,264	188	15.4	89.7	0.34	294	213	14.7	89.7	0.39	259
Risk score											
Risk score < 19	290,464	383					459				
Risk score ≥ 19	247,814	840	68.7	54.0	0.34	295	991	68.3	54.0	0.40	250
Risk score < 24	472,651	883					1,061				
Risk score ≥ 24	65,627	340	27.8	87.8	0.52	193	389	26.8	87.8	0.59	169
FIT											
Negative	468,005	404					567				
Single positive	55,985	384	48.7	89.3	0.69	146	421	42.6	89.3	0.75	132
Double positive	14,288	435	51.8	97.1	3.04	33	462	44.9	97.0	3.23	31
Any FIT positive	70,273	819	67.0	86.9	1.17	86	883	60.9	86.9	1.26	80
Parallel test of risk stratification and FIT											
Negative	423,031	350					498				
Positive	115,247	873	71.4	78.7	0.76	132	952	65.7	78.6	0.83	121
Parallel test of risk score and FIT											
Risk score cutoff point 19											
Negative	257,256	129					184				
Positive	281,022	1,094	89.5	47.8	0.39	257	1,266	87.3	47.8	0.45	222
Risk score cutoff point 24											
Negative	413,631	294					420				
Positive	124,647	929	76.0	77.0	0.75	134	1,030	71.0	77.0	0.83	121

[a] *Sensitivity analysis by defining interval and missed CRC as those diagnosed within 3 years after initial screening tests.*
PPV, positive predictive value.

of interval CRCs, which are usually considered as a failure of detection due to the lack of diagnostic tools with perfect sensitivity and specificity (22).

Combined use of risk stratification and FIT has been performed to achieve higher accuracy than FIT only (23). The importance of risk assessment in initial screening was also supported by Steele et al. (24), who found that interval CRCs were less likely to bleed. Considering that FIT can detect bleeding lesions while questionnaire-based risk assessment helps to identify individuals with lesions not bleeding (25), parallel test of the two methods was developed in 2006 in China as an initial screening modality to improve sensitivity of CRC screening (9) and recommended to the whole country (8). The observed low consistency of risk factors with FIT, as well as the greatly improved sensitivity, strongly supports parallel test of risk assessment and FIT in the population.

In this study, we developed a risk score system based on long-standing risk factors like age, sex, history of any cancer, and family history of CRC that perform well in long-term risk prediction (26), and specific intestinal symptoms such as diarrhea, constipation, mucus bloody stool, and intestinal polyps that had better short-term predictive values for CRC (27, 28). The risk score system was superior to currently used risk stratification in detecting malignant and precancerous lesions and in predicting long-term risk of CRC, but at the cost of almost doubled colonoscopy per detected CRC. It is of note that sensitivity of qualitative FIT was much lower in this study than in a previous report (7). Therefore, the parallel test screening modality should be optimized to trade off validity, compliance to colonoscopy, and clinical capacity of screening by adjusting cutoff point for risk score and by improving stool-based test.

In this study, only 23.5% positive subjects had colonoscopy, lower than 39.8% in the whole population of Shanghai (11). In addition to subpopulation disparity, compliance to colonoscopy in this study may have been underestimated due to the lack of information beyond the 13 designated hospitals. Nevertheless, low compliance to colonoscopy is common around the world, regardless of age, sex, and ethnicity (29), making a large number of missed cases a bigger challenge than interval cases. Validity of screening modality, particularly specificity, has been associated with compliance to colonoscopy (30). Lower specificity of the risk score-based screening modality may further decrease the compliance. Given the low compliance to colonoscopy, the numbers of detected neoplasms in each category of the new risk score strategy may be greatly underestimated. In this study, compliance to colonoscopy was 16.9% among high-risk individuals defined by risk stratification, triple of 5.6% in subjects with high-risk score, indicating potential benefits of using risk score even at the current level of compliance. When we improved specificity of risk score at same level of risk stratification by increasing its cutoff point to 24, we found that the risk score-based screening modality may detect additional 56 CRCs at the cost of additional 9,400 colonoscopy examinations, supporting utility of the risk score system. Moreover, medical insurance, lower educational attainment, discomfort during colonoscopy, fear of complications, and lack of information on colonoscopy procedures were also barriers to colonoscopy screening (31–33), and should be overcome to increase compliance to colonoscopy.

There are several strengths of this study. First, the large sample size makes it possible to evaluate performance of multiple assumed screening modalities. Second, the risk score system was developed with a comprehensive range of risk variables such as age, sex, history of cancers, and intestinal symptoms. All the information are easy to collect (26), ensuring feasibility of the system in the "real world." Moreover, the record linkage with the Cancer Registry and the Vital Statistics enabled us to collect all CRC cases and to calculate person-years of observations accurately, through which we found that the incidence of CRC decreased sharply after an incidence peak and began to increase between 2 and 3 years after screening, supporting the use of the period to define interval CRC and missed CRC (20, 24, 34). Finally, sensitivity analysis was conducted by defining interval or missed cases as linked CRC diagnosed within 3 years after initial screening. Similar results provide further evidence for our conclusions.

Several limitations should be considered. First, we did not collect information on lifestyle factors such as smoking, alcohol use, red meat intake, and physical activities, which have been included in multiple risk score systems (26, 35). It is possible that these unmeasured confounders may have biased the associations of collected risk factors with the risk of CRC and thus the weighing of each factor in the system. We could not compare the risk score system developed in this study with others due to the lack of lifestyle information to calculate risk score within other systems. Second, we may have underestimated the incidence of CRC in this population because of the lagging in cancer registry. Furthermore, the screening value of risk score system developed in this study was just validated internally. External validation study is needed to verify the extrapolation and generalization of the system. Finally, the follow-up time was not long enough to observe long-term predictive value of the risk score system, in which a longer follow-up is warranted.

CONCLUSIONS

In conclusion, quantitative risk score-based modality may help to improve effectiveness of CRC screening and has potential of scaling up in the population. Cutoff points of risk score should be optimized and stool-based test should be improved for large-scale usage in Chinese population. The effect of the parallel screening modality on improving compliance to colonoscopy and early detection of CRC, as well as its cost-effectiveness in view of society, warrant further evaluations.

DATA AVAILABILITY

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

This study was approved by the Medical Ethics Committee of the Center for Disease Control and Prevention in Pudong New Area of Shanghai, and oral consent was obtained from each participant of the screening program.

AUTHOR CONTRIBUTIONS

WW and YW drafted the manuscript. TL and WX conceived and designed the study. CY and BY made substantial contributions to the study design. CY and YZ were responsible for study coordination. YW and BY contributed to data quality control. HJ and XL contributed to data analysis. All authors contributed to the revision of the manuscript and approved the final manuscript.

FUNDING

This study was supported by the Key Discipline Project in Health System of Pudong New Area of Shanghai (PWZxk2017-28) and the Shanghai Municipal Commission of Health and Family Planning (15GWZK0801).

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Oncology

ORIGINAL RESEARCH
published: 17 July 2019
doi: 10.3389/fonc.2019.00662

Incidence and Mortality of Sarcomas in Shanghai, China, During 2002–2014

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OPEN ACCESS

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Specialty section:
This article was submitted to Cancer Epidemiology and Prevention, a section of the journal Frontiers in Oncology

Received: *15 January 2019*
Accepted: *05 July 2019*
Published: *17 July 2019*

Citation:
Pingping B, Yuhong Z, Weiqi L, Chunxiao W, Chunfang W, Yuanjue S, Chenping Z, Jianru X, Jiade L, Lin K, Zhengdong C, Weibin Z, Chen F and Yang Y (2019) Incidence and Mortality of Sarcomas in Shanghai, China, During 2002–2014. Front. Oncol. 9:662. doi: 10.3389/fonc.2019.00662

Background: Sarcomas are a heterogeneous group of rare but deadly malignant tumors. The aim of this study was to comprehensively describe the incidence and mortality of sarcomas in Shanghai during 2002–2014.

Method: Data were from Shanghai Cancer Registry. All new cases diagnosed with sarcomas and all death records where the cause of death listed as sarcomas were included. The characteristics of sarcomas incidence and mortality were analyzed. Age-standardized rates (ASRs) were adjusted by the world standard population. The trends were assessed by Joinpoint analysis.

Results: A total of 9,440 incident cases were identified. The ASR was 3.4/10^5 for all sarcomas combined. Incidence of sarcomas overall was similar in females (3.5/10^5) as in males (3.4/10^5). Except for sarcomas "Not Otherwise Specified" (NOS), the most common histological subtype was gastrointestinal stromal sarcoma (GISS) (14.8%), which was followed by fibrosarcoma (7.2%), lipoblastoma (6.7%), leiomyosarcomas (6.5%), and osteosarcoma (5.3%). Among those incident cases, 87.9% were located in soft tissue sarcomas (STS) and 12.1% in bone and joint (bone sarcomas). The ASRs for STS and bone sarcomas were 2.8/10^5 and 0.6/10^5, respectively. Incidence rates for all STS combined rose exponentially with age, while bone sarcomas had the highest incidence at age 0–19.

There were 4,279 deaths during 2002–2014 with the ASR of 1.3/10^5. Age-adjusted mortality due to sarcomas was slightly higher in males (1.5/10^5) than females (1.2/10^5). Except for sarcomas NOS, leiomyosarcomas was the most common subtype, comprising 9.9% of deaths due to sarcomas, followed by lipoblastoma (6.4%) and osteosarcoma (6.3%). The ASRs of mortality for STS and bone sarcomas were 1.0/10^5 and 0.2/10^5, respectively.

For both males and females, the age-standardized incidence for STS and bone sarcomas did not change meaningfully over the study period. In contrast, age-standardized STS mortality in females increased by 2.3% per year (95% CI: 0.3, 4.4%), but was unchanged in males. No meaningful trends in bone sarcomas mortality were observed for either males or females.

Conclusion: This population-based study was the first report of epidemiology of sarcomas in Shanghai according to anatomic site and histologic type. The diversity and rarity of sarcomas suggested more detailed data are warranted.

Keywords: sarcoma, incidence, mortality, epidemiology, population-based cancer registry

INTRODUCTION

Sarcomas, a heterogeneous group of rare malignant tumors arising from mesenchymal cells, account for about 1% of all new malignancies diagnosed (1, 2). These tumors can occur at any age and in almost any anatomic site. In relation to the anatomy, there have two types of common and distinct sarcomas: sarcomas from bone and joint (bone sarcomas) and soft tissue sarcomas (STS). Based on the histology, more than 50 distinct histological sarcoma subtypes exist according to the classification of the World Health Organization (WHO) updated in 2002 (3). It's difficult to obtain the precise estimates of sarcomas and sarcoma subtypes. The patterns of incidence and mortality of sarcomas have little been studied (1, 4, 5). Sarcomas, although relatively rare, are quite deadly and disproportionately affect younger population. STS are reported to account for, respectively, 0.7–1% and 4–8% of all adult and pediatric malignant tumors, and bone sarcomas for, respectively, 0.2% and 5% in most comprehensive reviews (1, 6–8).

Sarcomas can originate from any organ, tissue, bone, or cartilage. STS diagnoses predominate over bone sarcoma diagnoses with about 4:1 incidence ratio (5). A study on sarcomas of all types combined from RARECARE project showed that 84% were STS and 14% were bone sarcomas, of which age-standardized incidence of STS was $4.2/10^5$ and that of bone sarcomas was $0.8/10^5$ in Europe (4). In Surveillance, Epidemiology, and End Results (SEER) program data, STS also occurred much more frequently than bone sarcomas, which accounted for nearly 87% and 13% in 2008, respectively (1). No population-based mortality data have been reported before.

The causes of most sarcomas are unknown. Environmental factors, including ionizing radiation, occupational exposure to certain chemicals such as herbicide, have been associated with increased risk of specific types of sarcomas. Several heritable syndromes are associated with the development of some sarcomas [e.g., heritable retinoblastoma, neurofibromatosis 1, Li-Fraumeni syndrome (LFS)] (5, 9).

The aim of this paper is to examine incidence, mortality, and the temporal trends for sarcomas in Shanghai from 2002 through 2014, based on a population-based cancer registry, according to anatomic site and histologic type/subtype, using the most recent criteria of the WHO classification (10). These population-based data will be important in furthering our understanding of the morphologic and genetic diversity of sarcomas.

METHODS

Population-based cancer incidence and mortality data were derived from the Shanghai Cancer Registry (SCR), a member of the International Association of Cancer Registries (IACR). SCR has been a regular contributor to the *Cancer Incidence in Five Continents* (*CI5*) published by the IARC and the data have been published in the last seven volumes of *CI5*. Details of the cancer registry have been previously described (11, 12). Briefly, the SCR has formed standard system to collect, process, and report cancer incidence data. A standardized notification card, which includes information on name, date of birth, gender, address, occupation, primary site of cancer, histopathology, incidence date, basis of cancer diagnosis, and reporting hospital is used for reporting cancer cases. Death certificates have been used to gather information on unregistered cancer patient and all death cases due to sarcoma based entirely on vital statistics records during the time period have been included in SCR. The data for incidence and mortality of sarcomas during this period were complete in this study. The completeness of coverage of the Registry is very high with death certification only (DCO) <1%. Sarcomas cases (including second primary cancers with 0.5%) from soft tissues or bone, diagnosed among residents of Shanghai during 2002–2014, were included in this study.

Primary site and histological type were coded according to the third edition of the International Classification of Disease for Oncology (ICD-O-3) and then categorized into major histological types and subtypes of sarcoma as shown in **Table 1**. In brief, the cancers described in this manuscript include all sarcomas from soft tissue and from bone, including ICD-O-3 M codes 8800-8935, 8910, 8920, 8936, 8940, 8950-8959, 8963-8964, 8990-8991, 9020-9044, 9120-9133, 9150, 9170, 9180-9251, 9260-9261, 9364-9372, 9540-9581 combined with all ICD-O-3 T codes (C00-C80). There were 546 cases from C49 with M 8000-8004 included in this study. Finally, a total 9,440 incident cases and 4,279 death cases that met these criteria were included in the study. The percentage of histologically verified cases (MV%) was 94.4% and the death certificate only (DCO) % was 0.15%.

The corresponding population data of Shanghai urban areas were retrieved from the Shanghai Municipal Bureau of Public

TABLE 1 | Histological group of sarcomas by ICD-O-3 code.

Histological group	ICD-O-3 codes
Sarcoma NOS	M8800-8806, M8000-8004 located in C49
Osteosarcoma	M9180-9195
Chondrosarcoma	M9220-9243
Ewing's sarcoma & PNET	M9260, 9261, 9364, 9471, 9473, 9474
Giant cell sarcoma	M9250-9252
Lipoblastoma	M8850-8858
Fibrosarcoma	M8810-8815
Malignant fibrohistiocytoma	M8830
Dermatofibrosarcoma protuberans	M8832,8833
Vascular sarcoma	M8710, 9120-9133, 9150, 9170
Rhabdosarcoma	M8900-8920
leiomyosarcomas	M8890-8896
Gastrointestinal stromal sarcoma	M8936
Ameloblastoma	M9270, 9290, 9310, 9330
Malignant peripheral nerve sheath tumor (MPNST)	M9540-9571
Synovial sarcoma	M9040-9043
Stromal sarcoma	M8930-8935
Clear cell sarcoma	M8964,9044
Myxosarcoma	M8840
Malignant mesenchymoma	M8990
Embryonic sarcoma	M8991
Kaposi's sarcoma	M9140
Granulosa cell sarcoma	M9580
Alveolar soft part sarcoma (ASPS)	M9581

NOS, not otherwise specified; PNET, primitive neuroectodermal tumors.

Security every year. Between 2002 and 2014, Shanghai had a total population of inhabitants 179,955,231. The study was approved and the need for consent was waived by the institutional review board (IRB) of Shanghai Municipal Center for Disease Control and Prevention. In this study, only data in annual cancer report was used and no information to identify individual subjects was included.

The relative frequency was calculated as the percentage contribution of each particular group or subgroup to the total case series. The incidence rate was the number of new cases divided by the population at risk and was expressed as the number per 100,000 at risk and was age-adjusted by the direct method using the weight of the 1960 world standard population (13). The annual percent changes (APCs), representing the average percent increase or decrease in cancer rates per year over a specified period of time, were obtained using the joinpoint regression analysis. The joinpoint analysis has been widely applied to detect the changes points (joinpoints) and determine the trends between join points, which involves fitting a series of joined straight lines on a logarithmic scale to the trends in annual age-standardized rates (ASRs) (14). The allowed maximum number of joinpoints was one over 13 years as at least 5 years was required for each segment. We used a Joinpoint regression model implemented in the Joinpoint Regression Program (Version 4.5.0.1), which was developed by the Surveillance, Epidemiology, and End Results Program of the US National Cancer Institute (15).

RESULTS

Incidence

During 2002–2014, 4,503 (47.7%) males and 4,937 (52.3%) females were diagnosed with sarcomas. The crude annual incidence rate (CR) was $5.3/10^5$ and the ASR was $3.4/10^5$. Incidence of sarcomas overall was similar in females ($3.5/10^5$) as in males ($3.4/10^5$). The number of cases, percent distribution, age distribution, and incidence rates according to the histological group were shown in **Table 2**. About 3.9% of sarcomas occurred in children and adolescents (0–19 years), while majority (60.9%) occurred in the 20–64 years age group. The remaining 35.2% occurred in the elderly aged over 65 years. The most common histological subtype was gastrointestinal stromal sarcoma (GISS, malignant GISTs, 14.8%), which was followed by fibrosarcoma (7.2%), lipoblastoma (6.7%), leiomyosarcoma (6.5%), and osteosarcoma (5.3%). It should be noted that 31.2% of total cases were sarcomas "Not Otherwise Specified" (NOS). Kaposi sarcoma was very rare in Shanghai with only 14 cases during this whole period. In females, the incidence of stromal sarcoma (ASR $0.2/10^5$), the fifth common subtype, was apparently higher than in males (ASR $0.02/10^5$).

Incidence rates of sarcomas overall increased with age following a modest peak in adolescents. Incidence rates for all STS combined rose exponentially with age, while bone sarcomas had the highest incidence at age 0–19. Among the histological categories, osteosarcoma was the most frequent at age 0–19, with the peak of incidence of $0.5/10^5$. Rhabdosarcoma was the second frequent at this age group. Liposarcoma rates were very low in children and adolescents and then rose exponentially before peaking in the elderly. GISS incidence cases were rare before 40 years old and had the highest incidence at age 65+ group. Leiomyosarcoma incidence rates in females had two peaks at age group of 45–49 and 75–79, respectively.

As **Table 3** shown, among those newly diagnosed cases, 87.9% were located in STS and 12.1% in bone and joint (bone sarcomas).The CR and ASR were $4.6/10^5$ and $2.8/10^5$ for STS, $0.6/10^5$ and $0.6/10^5$ for bone sarcomas, respectively. About one-third (32.2%) of STS were located in the connective, subcutaneous and other soft tissues (ICD-10: C49), followed by digestive organs (31.4%), and female genital organs (8.6%).

For STS, except for the sarcomas NOS, the most common histological subtype was GISS (16.8%), which was followed by fibrosarcoma (7.7%), lipoblastoma (7.5%), leiomyosarcoma (7.3%), and malignant peripheral nerve sheath tumor (MPNST) (5.1%). For bone sarcomas, osteosarcoma (43.0%), giant cell sarcoma (17.4%), and chondrosarcoma (16.1%) were the most three subtypes.

Mortality

There were 4,279 death cases with sarcomas in Shanghai during 2002–2014, 2,191 (51.2%) for males and 2,088 (48.8%) for females, respectively. The crude annual mortality rate was $2.4/10^5$ and the mortality rate adjusted by the world standard population

TABLE 2 | Incidence of sarcomas by age, gender, and histologic type, Shanghai, 2002–2014.

Histologic group	Male								Female							
			Incidence rates (1/100,000)								Incidence rates (1/100,000)					
	N	%	Ages 0–19	Ages 20–44	Ages 45–64	Ages 65+	CR	ASR*	*N*	%	Ages 0–19	Ages 20–44	Ages 45–64	Ages 65+	CR	ASR*
Sarcoma NOS	1,390	30.8	0.13	0.49	1.8	4.9	1.5	0.88	1,552	31.4	0.15	0.64	2.1	4.4	1.7	0.96
Osteosarcoma	259	5.8	0.58	0.27	0.22	0.23	0.29	0.34	239	4.8	0.41	0.22	0.25	0.28	0.27	0.29
Chondrosarcoma	91	2.0	0.08	0.06	0.13	0.13	0.10	0.08	102	2.1	0.08	0.07	0.16	0.14	0.11	0.09
Ewing's sarcoma and PNET	48	1.1	0.12	0.05	0.04	0.02	0.05	0.08	41	0.83	0.11	0.05	0.03	0.01	0.05	0.07
Giant cell sarcoma	111	2.5	0.06	0.17	0.12	0.06	0.12	0.11	109	2.2	0.06	0.17	0.13	0.06	0.12	0.10
Lipoblastoma	360	8.0	0.01	0.14	0.56	1.1	0.40	0.23	274	5.6	0.02	0.14	0.44	0.58	0.30	0.17
Fibrosarcoma	364	8.1	0.09	0.28	0.48	0.86	0.40	0.27	314	6.4	0.04	0.25	0.45	0.60	0.35	0.23
Malignant fibrohistiocytoma	201	4.5	0.02	0.07	0.26	0.72	0.22	0.13	161	3.3	0.02	0.08	0.19	0.48	0.18	0.10
Dermatofibrosarcoma protuberans	251	5.6	0.09	0.32	0.34	0.20	0.28	0.21	154	3.1	0.08	0.22	0.17	0.14	0.17	0.14
Vascular sarcoma	98	2.2	0.02	0.02	0.12	0.39	0.11	0.06	94	1.9	0.02	0.05	0.13	0.23	0.10	0.06
Rhabdosarcoma	91	2.0	0.33	0.03	0.08	0.11	0.10	0.18	62	1.3	0.18	0.05	0.04	0.08	0.07	0.11
Leiomyosarcomas	168	3.7	0.00	0.06	0.24	0.56	0.19	0.10	441	8.9	0.02	0.25	0.77	0.79	0.49	0.28
Gastrointestinal stromal sarcoma	663	14.7	0.00	0.13	0.97	2.40	0.74	0.39	732	14.8	0.00	0.13	1.2	2.1	0.81	0.40
Ameloblastoma	4	0.09	0.00	0.00	0.01	0.00	0.00	0.00	4	0.08	0.00	0.00	0.00	0.01	0.00	0.00
Malignant peripheral nerve sheath tumor (MPNST)	217	4.8	0.01	0.16	0.30	0.51	0.24	0.15	221	4.5	0.06	0.17	0.34	0.35	0.25	0.17
Synovial sarcoma	44	0.98	0.04	0.05	0.06	0.04	0.05	0.05	43	0.87	0.01	0.05	0.06	0.05	0.05	0.04
Stromal sarcoma	30	0.67	0.00	0.01	0.04	0.12	0.03	0.02	274	5.6	0.02	0.24	0.46	0.35	0.30	0.20
Clear cell sarcoma	12	0.27	0.01	0.02	0.01	0.02	0.01	0.01	18	0.36	0.01	0.01	0.02	0.04	0.02	0.02
Myxosarcoma	22	0.49	0.00	0.01	0.03	0.09	0.02	0.01	27	0.55	0.00	0.02	0.05	0.03	0.03	0.02
Malignant mesenchymoma	57	1.3	0.01	0.02	0.08	0.18	0.06	0.04	54	1.1	0.01	0.03	0.09	0.11	0.06	0.03
Embryonic sarcoma	1	0.02	0.01	0.00	0.00	0.00	0.00	0.00	3	0.06	0.02	0.00	0.00	0.00	0.00	0.01
Kaposi's sarcoma	10	0.22	0.00	0.01	0.01	0.04	0.01	0.01	4	0.08	0.00	0.00	0.01	0.01	0.00	0.00
Granulosa cell sarcoma	5	0.11	0.00	0.00	0.01	0.02	0.01	0.00	8	0.16	0.00	0.01	0.02	0.00	0.01	0.01
Alveolar soft part sarcoma (ASPS)	6	0.13	0.02	0.01	0.00	0.00	0.01	0.01	6	0.12	0.01	0.02	0.00	0.00	0.01	0.01
Total	4,503	100.0	1.6	2.4	5.9	12.6	5.0	3.4	4,937	100.0	1.3	2.9	7.1	10.9	5.5	3.5

**Adjusted by the world standard population. CR, crude rate; ASR, age-standardized rate; NOS, not otherwise specified; PNET, primitive neuroectodermal tumors.*

TABLE 3 | Incidence of sarcomas by age, gender, and primary site, Shanghai, 2002–2014.

ICD-O	Primary sites	Male								Female							
				Incidence rates (1/100,000)								Incidence rates (1/100,000)					
		N	%	Ages 0–19	Ages 20–44	Ages 45–64	Ages 65+	CR	ASR	*N*	%	Ages 0–19	Ages 20–44	Ages 45–64	Ages 65+	CR	ASR
C00-14	Lip, oral cavity, and pharynx	40	0.89	0.02	0.03	0.06	0.09	0.04	0.03	15	0.30	0.01	0.01	0.02	0.04	0.02	0.01
C15-26	Digestive organs	1,280	28.4	0.00	0.31	1.9	4.5	1.4	0.76	1,361	27.6	0.04	0.30	2.0	4.1	1.5	0.77
C16	Stomach	669	14.9	0.00	0.13	0.93	2.56	0.74	0.39	798	16.2	0.01	0.14	1.2	2.6	0.89	0.44
C30-39	Respiratory system and intrathoracic organs	210	4.7	0.05	0.06	0.32	0.64	0.23	0.14	119	2.4	0.04	0.10	0.18	0.17	0.13	0.09
C40-41	Bone and Joint	582	12.9	0.77	0.61	0.65	0.62	0.65	0.63	558	11.3	0.61	0.57	0.65	0.66	0.62	0.57
C44	Skin	274	6.1	0.09	0.34	0.34	0.32	0.30	0.23	178	3.6	0.10	0.23	0.19	0.24	0.20	0.16
C47	Peripheral nerve and autonomic nerve system	159	3.5	0.02	0.13	0.21	0.36	0.18	0.12	157	3.2	0.06	0.12	0.24	0.26	0.17	0.12
C48	Retropheritoneum and peritoneum	233	5.2	0.00	0.07	0.36	0.74	0.26	0.14	308	6.2	0.00	0.15	0.52	0.65	0.34	0.19
C49	Connective, subcutaneous, and other soft tissues	1,480	32.9	0.47	0.74	1.8	4.6	1.6	1.1	1,227	24.9	0.30	0.69	1.5	3.3	1.4	0.83
C50	Breast	7	0.16	0.00	0.00	0.01	0.03	0.01	0.00	116	2.4	0.01	0.10	0.22	0.10	0.13	0.08
C51-57	Female genital organs	–	–	–	–	–	–	–	–	718	14.5	0.05	0.49	1.3	0.96	0.80	0.49
C53-55	Uterus	–	–	–	–	–	–	–	–	618	12.5	0.03	0.43	1.2	0.78	0.69	0.42
C60-63	Male genital organs	75	1.7	0.07	0.03	0.09	0.22	0.08	0.06	–	–	–	–	–	–	–	–
C64-68	Urinary tract	61	1.4	0.05	0.02	0.08	0.17	0.07	0.06	67	1.4	0.05	0.02	0.11	0.14	0.07	0.06
C69-72	Eye, brain, and other parts of the central nervous system	10	0.22	0.04	0.01	0.01	0.01	0.01	0.02	11	0.22	0.03	0.02	0.00	0.01	0.01	0.03
C73-75	Thyroid and other endocrine glands	10	0.22	0.00	0.00	0.01	0.04	0.01	0.01	15	0.30	0.00	0.01	0.02	0.04	0.02	0.01
C76, C77, C80	Other sites, lymph nodes, and unknown primary site	82	1.8	0.04	0.03	0.09	0.29	0.09	0.06	87	1.8	0.02	0.06	0.13	0.17	0.10	0.06

CR, crude rate; ASR, age-standardized rate.

was $1.3/10^5$. Age-adjusted mortality due to sarcomas was slightly higher in males ($1.5/10^5$) than females ($1.2/10^5$).

Leiomyosarcoma was the most common subtype, comprising 9.9% of all death cases, followed by lipoblastoma (6.4%) and osteosarcoma (6.3%). About 3.6% of sarcomas death cases occurred in children and adolescents (0–19 years), and osteosarcoma composed of 40.8% in this age group. Majority occurred in the 65+ group with 55.5% of total death cases (**Table 4**).

Among those death cases, 88.1% were located in STS and 11.9% in bone and joint. The CR and ASR of mortality were $2.1/10^5$ and $1.0/10^5$ for STS, $0.3/10^5$ and $0.2/10^5$ for bone sarcomas, respectively. About 39.4% of STS death cases were from connective, subcutaneous, and other soft tissues (ICD-10: C49), followed by digestive organs (24.0%), and retropheritoneum and peritoneum (7.9%) (**Table 5**).

Trends

Figure 1 showed the trends in the incidence for all sarcomas combined, STS and bone sarcomas during 2002–2014. No significant incidence trend for all sarcomas combined was observed in males and females, with an APC of 0.3% (95% CI: −0.9, 1.4%) and −0.2% (95% CI: −1.3, 1.0%) in ASRs by Joinpoint regression, respectively. The trends of incidence rates for STS and bone sarcomas were not significant for both genders. Further analysis showed that the trends of top five subgroups showed that incidence rates continued to decline during 2002–2014 for leiomyosarcomas, fibrosarcoma, and MPNST, while the ASR of lipoblastoma stabilized and GISS increased significantly (data not shown).

The trend in the mortality of total sarcomas during 2002–2014 increased significantly with APC 2.7% (95% CI: 0.7, 4.7%) for females, while it was not significant for males with APC 1.1% (95% CI: −1.1, 3.5%). Further analysis found that the significant rising trend only existed in the ASRs for female STS with APC 2.3% (95% CI: 0.3, 4.4%), but not for female bone sarcomas during the entire time period, as shown in **Figure 2**. In female, the morality rates increased but not significantly for lipoblastoma (APC 5.7, 95% CI: −0.2, 11.1%).

DISCUSSION

The results presented in this paper gave for the first time a comprehensive analysis focusing the incidence and mortality of sarcomas in Shanghai. The recent 2002 WHO criteria were used to recategorize histologic subtypes of sarcomas in the present study to facilitate comparison with other studies. Total 9,440 cases were diagnosed during 2002–2014 in SCR, of which 12.1% were bone sarcomas and 87.9% were soft tissue sarcomas. The annual ASR for all sarcomas combined was $3.4/10^5$, and the ASRs for STS and bone sarcomas were $2.8/10^5$ and $0.6/10^5$, respectively.

Sarcomas account for about 1% of all malignant tumors and the total incidence ∼2–4 per 100,000 population (1, 2). Data from *CI5* showed that sarcoma incidence rates were comparable throughout much of the world (16). Previous studies that examined the incidence of sarcomas combined with STS and bone sarcomas were limited and there were marked variations in the distribution of subtypes. Population-based data on incidence of sarcomas in Europe was investigated in a study from RARECARE project (4), covering 27,908 incident cases diagnosed during 1995–2002 in the EU27 countries with crude incidence of $5.6/10^5$, of which 84% were soft tissue sarcomas and 14% were bone sarcomas, similar to our findings.

The ASR of STS in Shanghai ($2.8/10^5$) were comparable with the report from Austrian National Cancer Registry (ASR $2.4/10^5$) (17) and were lower than the findings from SEER program in the USA (total $5.0/10^5$ with US 2000 standard population) (18) and from RARECARE project in Europe (total ASR $4.2/10^5$) (4). A previous report in Beijing on STS, only including the cases diagnosed with sites of C47 and C49, showed that the incidence was lower than our findings, with CR and ASR $1.2/10^5$ and $0.9/10^5$, respectively (19). Bone sarcomas were relatively rare and the ASR ($0.6/10^5$) in Shanghai was similar to the finding from RARECARE project in Europe (ASR $0.8/10^5$) (4) and a report in Taiwan (ASR $0.67/10^5$) (7).

The histological and molecular classification of sarcomas has been revised with the progress of new techniques, such as immunohistochemistry, multiplex PCR and sequencing, which would be reflected by the distribution and trends of histological subgroups. In this study, the three most frequent histological subtypes among STS were GISS, fibrosarcoma, and lipoblastoma, respectively. RARECARE project showed that leiomyosarcoma was the most frequent type, whereas others showed that the most common histology was liposarcoma (4). There has been a significant steadily increasing numbers of GISS in Shanghai since 2010 accounting for about range of 14.8–18.5% of all sarcomas. GISS were registered with six cases only in 2002 and varied a range of 0.4–5.9% before 2010. One possible reason was that GISS had been diagnosed as sarcomas NOS. The total proportion of sarcomas NOS in this study was 31.2% during the entire period and the percent declined significantly since 2010, accounting for about 20%. The "*Chinese consensus guidelines for diagnosis and management of gastrointestinal stromal tumor*" was published in 2008 and then it has received more attention with several revised edition. The application of techniques of immunohistochemistry and molecular pathology resulted in more GISS identification. Another possible explanation was that the incidence rates of leimyosarcomas decreased significantly during this period. It was reported that before using immunohistochemistry, some GISS might had been identified as leiomyosarcoma (20).

Age is an important determinant of sarcoma occurrence. Incidence of STS increases more dramatically after 50 years old. Generally, malignant bone tumors have a stable incidence rate across all ages. However, in adolescents and young adults, there is a noticeable increase (1). Similar age patterns were found in this study. A considerable variation in incidence patterns of sarcomas by histologic subtypes in this study was observed, which supported the notion that these tumors are etiologically distinct and should be considered separately in studies of potential risk factors, in accord with previous epidemiologic studies (18).

During 2002–2014, the trends in the ASRs of incidence for all sarcomas combined, STS and bone sarcomas were not significant for males and females. An upward trend in the incidence of STS overall and for females was seen in Osaka,

TABLE 4 | Mortality of sarcomas by age, gender, and histologic type, Shanghai, 2002–2014.

Histologic group	Male								Female							
			Mortality rates (1/100,000)								Mortality rates (1/100,000)					
	N	%	Ages 0–19	Ages 20–44	Ages 45–64	Ages 65+	CR	ASR*	*N*	%	Ages 0–19	Ages 20–44	Ages 45–64	Ages 65+	CR	ASR*
Sarcoma NOS	888	40.5	0.09	0.17	0.90	4.1	0.99	0.52	867	41.5	0.07	0.21	0.78	3.5	0.96	0.46
Osteosarcoma	153	7.0	0.29	0.12	0.14	0.26	0.17	0.18	117	5.6	0.20	0.10	0.08	0.23	0.13	0.13
Chondrosarcoma	32	1.5	0.01	0.02	0.04	0.09	0.04	0.02	32	1.5	0.00	0.01	0.02	0.13	0.04	0.02
Ewing's sarcoma & PNET	37	1.7	0.06	0.04	0.04	0.02	0.04	0.05	30	1.4	0.06	0.05	0.01	0.03	0.03	0.05
Giant cell sarcoma	20	0.91	0.00	0.02	0.02	0.06	0.02	0.01	16	0.77	0.01	0.02	0.02	0.03	0.02	0.01
Lipoblastoma	157	7.2	0.00	0.03	0.19	0.66	0.17	0.09	118	5.7	0.00	0.02	0.14	0.44	0.13	0.06
Fibrosarcoma	121	5.5	0.02	0.04	0.12	0.53	0.13	0.08	106	5.1	0.01	0.01	0.10	0.46	0.12	0.05
Malignant fibrohistiocytoma	121	5.5	0.01	0.01	0.13	0.60	0.13	0.07	94	4.5	0.00	0.02	0.08	0.40	0.10	0.05
Dermatofibrosarcoma protuberans	22	1.0	0.01	0.01	0.02	0.10	0.02	0.01	15	0.72	0.00	0.00	0.01	0.08	0.02	0.01
Vascular sarcoma	68	3.1	0.00	0.01	0.07	0.33	0.08	0.04	41	2.0	0.01	0.00	0.06	0.13	0.05	0.02
Rhabdosarcoma	56	2.6	0.20	0.02	0.04	0.08	0.06	0.10	45	2.2	0.06	0.04	0.03	0.10	0.05	0.05
Leiomyosarcomas	151	6.9	0.00	0.03	0.14	0.74	0.17	0.09	274	13.1	0.00	0.08	0.40	0.81	0.30	0.15
Gastrointestinal stromal sarcoma	148	6.8	0.00	0.01	0.11	0.84	0.16	0.08	104	5.0	0.00	0.01	0.07	0.52	0.12	0.04
Ameloblastoma	1	0.05	0.00	0.00	0.00	0.01	0.00	0.00	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Malignant peripheral nerve sheath tumor (MPNST)	113	5.2	0.02	0.04	0.12	0.48	0.13	0.07	94	4.5	0.03	0.04	0.11	0.28	0.10	0.06
Synovial sarcoma	24	1.1	0.01	0.02	0.04	0.04	0.03	0.02	24	1.2	0.01	0.03	0.04	0.03	0.03	0.02
Stromal sarcoma	13	0.59	0.00	0.00	0.02	0.05	0.01	0.01	64	3.1	0.00	0.01	0.08	0.22	0.07	0.03
Clear cell sarcoma	7	0.32	0.00	0.01	0.01	0.02	0.01	0.00	10	0.48	0.01	0.01	0.01	0.03	0.01	0.01
Myxosarcoma	13	0.59	0.00	0.00	0.01	0.07	0.01	0.01	6	0.29	0.00	0.00	0.01	0.01	0.01	0.00
Malignant mesenchymoma	35	1.6	0.00	0.00	0.05	0.14	0.04	0.02	22	1.1	0.01	0.01	0.03	0.06	0.02	0.01
Embryonic sarcoma	0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1	0.05	0.01	0.00	0.00	0.00	0.00	0.00
Kaposi's sarcoma	4	0.18	0.00	0.00	0.00	0.02	0.00	0.00	2	0.10	0.00	0.00	0.00	0.01	0.00	0.00
Granulosa cell sarcoma	3	0.14	0.00	0.00	0.00	0.02	0.00	0.00	2	0.10	0.00	0.00	0.01	0.00	0.00	0.00
Alveolar soft part sarcoma (ASPS)	4	0.18	0.01	0.00	0.00	0.02	0.00	0.00	4	0.19	0.00	0.01	0.00	0.00	0.00	0.00
Total	2,191	100.0	0.72	0.60	2.2	9.3	2.4	1.5	2,088	100.0	0.49	0.67	2.1	7.5	2.3	1.2

**Adjusted by the world standard population. CR, crude rate; ASR, age-standardized rate; NOS, not otherwise specified; PNET, primitive neuroectodermal tumors.*

TABLE 5 | Mortality of sarcomas by age, gender, and primary site, Shanghai, 2002–2014.

ICD-O	Primary sites	Male								Female							
				Mortality rates (1/100,000)								Mortality rates (1/100,000)					
		N	%	Ages 0–19	Ages 20–44	Ages 45–64	Ages 65+	CR	ASR	*N*	%	Ages 0–19	Ages 20–44	Ages 45–64	Ages 65+	CR	ASR
C00-14	Lip, oral cavity, and pharynx	24	1.1	0.00	0.01	0.03	0.09	0.03	0.01	6	0.29	0.00	0.01	0.00	0.03	0.01	0.00
C15-26	Digestive organs	501	22.9	0.00	0.07	0.47	2.5	0.56	0.27	405	19.4	0.02	0.06	0.28	1.91	0.45	0.19
C16	Stomach	206	9.4	0.00	0.02	0.15	1.2	0.23	0.11	194	9.3	0.00	0.03	0.09	1.00	0.22	0.08
C30-39	Respiratory system and intrathoracic organs	152	6.9	0.03	0.03	0.20	0.56	0.17	0.09	74	3.5	0.02	0.05	0.12	0.13	0.08	0.05
C40-41	Bone and Joint	286	13.1	0.37	0.21	0.29	0.60	0.32	0.29	225	10.8	0.23	0.16	0.17	0.60	0.25	0.19
C44	Skin	36	1.6	0.01	0.01	0.02	0.21	0.04	0.02	26	1.3	0.00	0.00	0.02	0.13	0.03	0.01
C47	Peripheral nerve and autonomic nerve system	78	3.6	0.00	0.03	0.08	0.32	0.09	0.05	77	3.7	0.05	0.04	0.08	0.21	0.09	0.06
C48	Retropheritoneum and peritoneum	161	7.4	0.00	0.03	0.21	0.65	0.18	0.09	175	8.4	0.00	0.05	0.25	0.53	0.19	0.10
C49	Connective, subcutaneous and other soft tissues	805	36.7	0.21	0.17	0.75	3.8	0.89	0.52	681	32.6	0.10	0.20	0.53	2.83	0.76	0.37
C50	Breast	3	0.14	0.00	0.00	0.00	0.01	0.00	0.00	40	1.9	0.00	0.01	0.06	0.11	0.04	0.02
C51-57	Female genital organs	–	–	–	–	–	–	–	–	290	13.9	0.01	0.06	0.48	0.76	0.32	0.16
C53-55	Uterus	–	–	–	–	–	–	–	–	238	11.4	0.00	0.05	0.42	0.58	0.26	0.13
C60-63	Male genital organs	36	1.6	0.05	0.01	0.03	0.12	0.04	0.03	–	–	–	–	–	–	–	–
C64-68	Urinary tract	43	2.0	0.02	0.01	0.06	0.16	0.05	0.03	30	1.4	0.02	0.00	0.03	0.10	0.03	0.02
C69-72	Eye, brain, and other parts of the central nervous system	9	0.41	0.03	0.01	0.01	0.01	0.01	0.02	9	0.43	0.02	0.02	0.00	0.01	0.01	0.02
C73-75	Thyroid and other endocrine glands	6	0.27	0.00	0.00	0.01	0.02	0.01	0.00	10	0.48	0.00	0.00	0.01	0.04	0.01	0.01
C76, C77, C80	Other sites, lymph nodes and unknown primary site	51	2.3	0.01	0.01	0.04	0.26	0.06	0.03	40	1.9	0.02	0.01	0.05	0.12	0.04	0.02

CR, crude rate; ASR, age-standardized rate.

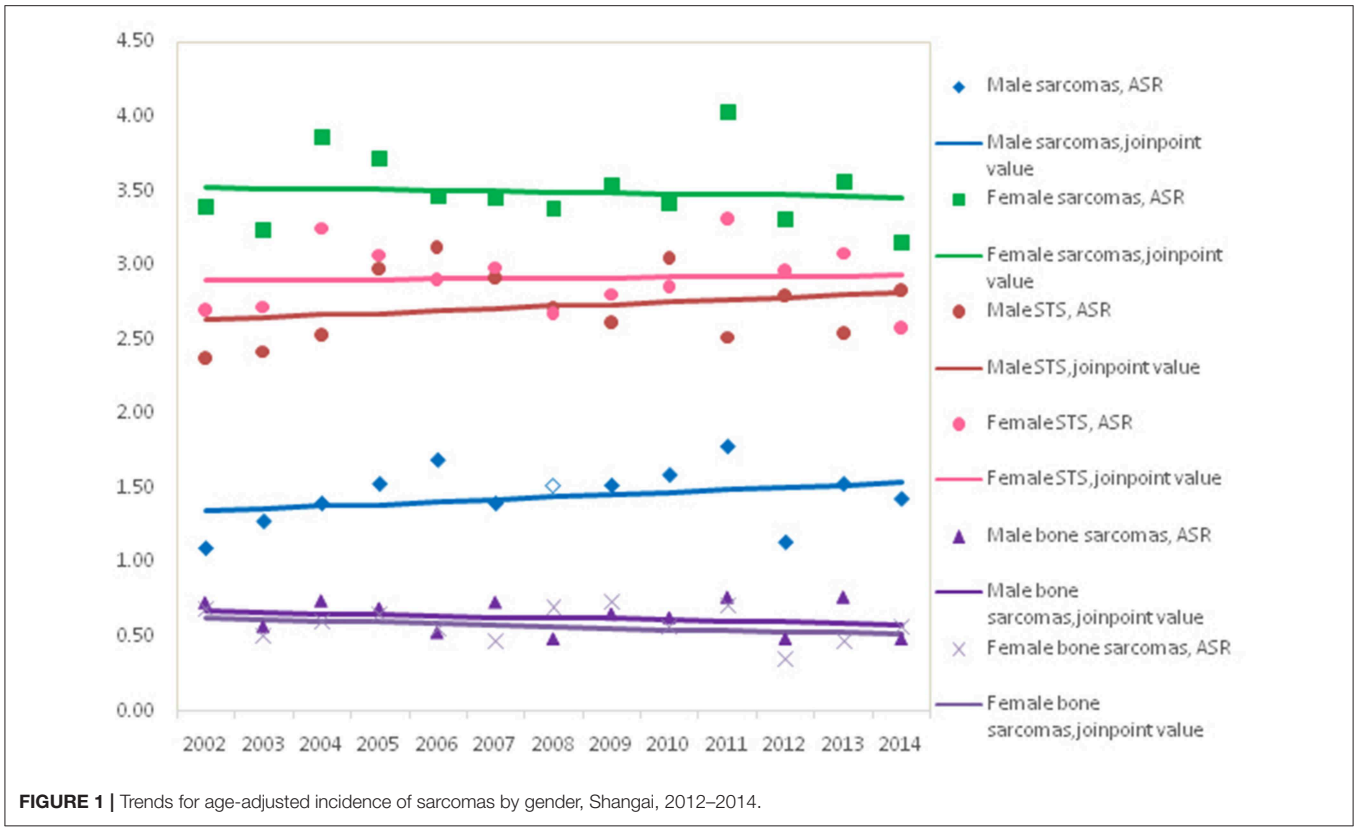

FIGURE 1 | Trends for age-adjusted incidence of sarcomas by gender, Shangai, 2012–2014.

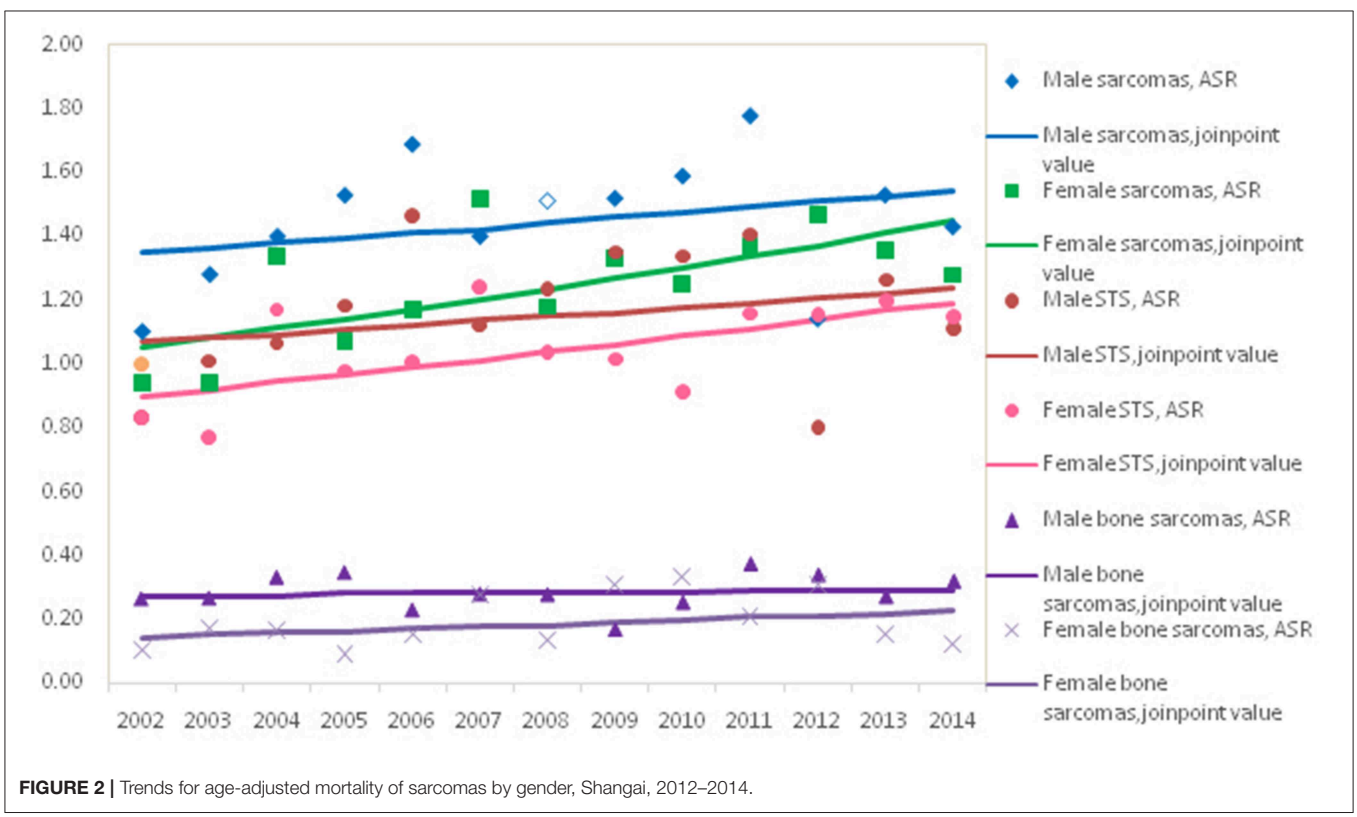

FIGURE 2 | Trends for age-adjusted mortality of sarcomas by gender, Shangai, 2012–2014.

Japan during 1978–2007 (21). However, a population-based epidemiologic study in Austria for the period 1984–2004 (17) has not confirmed the increasing incidence rates of STS. It mentioned that different inclusion criteria (such as Kaposi's sarcoma and dermatofibrosarcoma) and classifications in the various studies would explain the increase of incidence in some studies rather than true increase of STS due to new or accumulated risk factors (17).

The causes of most sarcomas are unknown. Both genetic and environmental factors likely contribute to the etiology of sarcomas (1, 8, 21). The rarity of the disease combined with the diverse number of subtypes make sarcomas difficulty to study and the epidemiology and etiology of sarcomas are not well-understood. Environmental factors that increase sarcoma risk include radiation exposure and chemical carcinogens (8). Ionizing radiation exposure, especially by means of radiotherapy for a previous cancer, has been shown to be strongly associated with secondary sarcoma development (1, 22). There was an increasing incidence of second sarcomas among cancer survivors, and one may speculate a relation to the intensified use of cytotoxic treatment of the preceding malignancy (23). Other risk factors include occupational exposure to certain chemicals, including herbicides such as phenoxyacetic acids. HIV-positive individuals have an increased risk for Kaposi's sarcoma. Several familial cancer syndromes confer sarcoma pre-disposition, such as the LFS (8). No study has been implemented in Shanghai about the risk factors of sarcomas and these data serve to illustrate the complexity of sarcomas.

To our knowledge, no population-based study to date has evaluated the mortality of sarcomas for all types combined and the histological subtypes. In this study, the ASR for mortality of sarcomas combined was $1.3/10^5$ and the ASRs of mortality for STS and bone sarcomas were $1.0/10^5$ and $0.2/10^5$, respectively. For STS, except for the sarcomas NOS, leiomyosarcomas was the most common subtype among death cases of sarcomas and majority occurred in the old over 65+ years. For bone sarcomas, about 40% of cases occurred in children and adolescents (0–19 years).

No substantial changes were found in the mortality rates of sarcomas combined and bone sarcomas for males and females. However, a modest significant increase in average annual mortality rates (APC, 2.3%; 95% CI: 0.3, 4.4%) was observed for STS among females, not males. The mortality increasing of female STS was maybe due to in part to the distribution change of subtypes and the poor survival of lipoblastoma in females. One should be noticed that some of sarcoma subgroups were very rare and varied a lot with a wide range of 95% CI. A study from a medical unit in UK showed that there had been no significant change in 1 year mortality rate of STS during 1985–2010, and TNM stage was a useful predictor (24). Survival studies on sarcomas and its subgroups are warranted.

This study included the sarcomas at all sites including skin and visceral sarcomas, and not only bone and soft tissue tumors. SCR was a population-based registry and has been a regular contributor to the *CI5*. The quality of data in this report was high with 94.4% of MV. Otherwise, there were several potential limitations in our study. The use of new techniques may be systematically under- or over-represented, which influenced the patterns and the trends. There was evidence that the *gold standard* pathologic diagnosis was not consistently reliable for sarcomas and the chances to misclassify the histology for a pathologist also existed (8, 25, 26) Although this study focused on the data after 2002 and some advent of ancillary technologies, such as immunohistochemistry and molecular genetics/molecular cytogenetics, had been applied in Shanghai, there were over 30% of sarcomas NOS in this study. It's a good point that the proportion of sarcomas NOS reduced to about 20% after 2010. In addition, as previously described, some of the sarcoma subtypes are uncommon and generalizations concerning incidence and mortality rates are difficult to make.

The diversity and rarity of sarcomas suggested that a cooperative networking in prevention, diagnosis, therapy, and research for this rare type of cancers was warranted. With the ongoing collection of the sarcoma cases, accumulative detailed information may reveal more subtle etiologic clues and provide more evidence for decision on effective therapy.

ETHICS STATEMENT

The study was approved and the need for consent was waived by the institutional review board (IRB) of Shanghai Municipal Center for Disease Control and Prevention. In this study, only data in annual cancer report was used and no information to identify individual subjects was included.

AUTHOR CONTRIBUTIONS

FC, YY, BP, ZY, and LW contributed conception and design of the study. WChunx, WChunf, and BP collected and organized the database. BP and WChunx performed the statistical analysis. BP and ZY draft the manuscript. SY, ZC, XJ, LJ, KL, CZ, and ZW revised and made the decision to submit for publication. All authors contributed to manuscript revision, read, and approved the submitted version.

FUNDING

This work was supported by 3 year Action Plan on Public Health, Phase IV, Shanghai, China (15GWZK0801).

ACKNOWLEDGMENTS

We thank Dr. Zheng Ying and Zhou Changming at Shanghai Tumor Hospital affiliated to Fudan University for helpful comments on pathology classification. We thank the staffs who continuously provide case reports and case follow-up in all related hospitals and district CDC. We thank the staffs in the department of Vital Statistics for the help of death information.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Oncology

ORIGINAL RESEARCH
published: 20 August 2019
doi: 10.3389/fonc.2019.00786

Trends of Postoperative Radiotherapy for Completely Resected Non-small Cell Lung Cancer in China: A Hospital-Based Multicenter 10–Year (2005–2014) Retrospective Clinical Epidemiological Study

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Objectives: The role of postoperative radiotherapy (PORT) in the treatment of patients with completely resected non-small cell lung cancer (NSCLC) is not clear. Few study explored the trends of the PORT use. In this study, we examine the status of PORT use of completely resected NSCLC in mainland China.

Methods: From 2005 to 2014, patients with primary lung cancer from eight hospitals across seven geographic regions of mainland China were selected. Then patients with

OPEN ACCESS

Edited by:
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Specialty section:
This article was submitted to Cancer Epidemiology and Prevention, a section of the journal Frontiers in Oncology

Received: *04 January 2019*
Accepted: *05 August 2019*
Published: *20 August 2019*

Citation:
Men Y, Wang L, Zhang Y, Gao S, Li J, Wu N, Yang B, Liu S, Ren J, Huang Y, Wang D, Liao X, Xing X, Du L, Yang L, Liu Y, Zhang Y, Wei D, Liu Y, Zhang K, Qiao Y, Shi J, Chen W, Dai M and Hui Z (2019) Trends of Postoperative Radiotherapy for Completely Resected Non-small Cell Lung Cancer in China: A Hospital-Based Multicenter 10–Year (2005–2014) Retrospective Clinical Epidemiological Study. Front. Oncol. 9:786. doi: 10.3389/fonc.2019.00786

staged I–IIIA NSCLC receiving radical surgery were enrolled in this study. The chi-square test was used to compare differences in the use of PORT among the groups of different age, regions and stages. The Cochran-Armitage trend test was used to identify the trend in the PORT use from 2005 to 2014.

Results: Totally, 2,253 out of 7,184 patients were with staged I–IIIA NSCLC receiving completely resection. Only 122 patients (5.42%) received PORT. During this decade, the use of PORT declined significantly ($p = 0.0002$). In high socio-economic areas, the percentage of PORT use was 7.43%, which was significantly higher than 1.34% in the low socio-economic areas ($p < 0.0001$). Age was also associated with PORT use ($p = 0.0747$). For N0-1 and N2 NSCLC, the proportions of PORT use were 4.01 and 10.22%, respectively ($p < 0.0001$). And in N0-1 or N2 NSCLC, the proportions both decreased significantly during this decade ($p = 0.009$ and 0.026, respectively). For stage I, IIA, IIB and IIIA, the proportions who received PORT were 2.59, 4.65, 5.49, and 10.29%, respectively ($p < 0.0001$). Modern radiation techniques were widely used, but the volumes and doses varied widely. The proportions of using IMRT and EPID/IGRT increased after 2012.

Conclusions: In China, the use of PORT was less than developed countries and had a declined trend. The use of PORT was related to disease stages, patients' age and geographic location. Both in N0-1 and N2 diseases, the use of PORT declined. Proper education of radiation doctors was urgently needed.

Keywords: NSCLC, postoperative radiotherapy, trend, epidemiology, multicenter

INTRODUCTION

Based on 2008 estimates, throughout the world, lung cancer accounts for 13% of the total cases of cancer and 18% of the cancer-related deaths (1). In China, lung cancer remains the most common incident cancer and the leading cause of cancer death (2).

Non-small cell lung cancer (NSCLC) accounts for 80–85% of all cases of lung cancer. Surgery remains the most important treatment for stage I/II and IIIA NSCLC. However, the local-regional recurrence is common, occurring in approximately 20% of patients with stage I disease and in up to 50% of patients with stage III disease (3–7). Postoperative radiotherapy (PORT) may reduce local-regional recurrence. However, a landmark meta-analysis study on PORT published in 1998 concluded that PORT was detrimental (HR 1.21, $p = 0.001$) (8). But many radiation oncologists remain skeptical about this results because of the toxicities, especially therapy-related deaths caused by suboptimal, outdated irradiation equipment and techniques, and the unacceptable radiation doses (8, 9). In recent years, the modern treatment techniques, 3D planning, linear accelerators have developed rapidly which could make radiotherapy more effective and less toxic (10–13). In 2004, chemotherapy became standard of care when the International Adjuvant Lung Cancer Trial (IALT) demonstrated that in comparison to surgery alone, cisplatin-based adjuvant therapy improved survival in patients with resected NSCLC (14). Given the above, the role of PORT has remained controversial for decades.

In the aspect of epidemiologic study about PORT in NSCLC, only one study examined the temporal trends based on Surveillance, Epidemiology, and End Results (SEER) Program published in 2006 (15). Up to now, few study examined the trends in the use of PORT especially in China. Besides, the emerging of new evidences of PORT and the development of radiation technologies may broaden the application of radiotherapy. Thus, we design this hospital-based multicenter retrospective clinical epidemiological study to illustrate the shift of PORT use of completely resected NSCLC in mainland China from 2005 to 2014.

METHODS

Study Design

This study was a hospital-based multicenter 10 year (2005–2014) retrospective clinical epidemiological study of randomly selected primary lung cancer cases via medical chart review.

Data Collection

Hospital selection, case sampling, and data collection methods have been previously described in detail (16). China was stratified into seven geographic regions (north, northeast, central, south, east, northwest, and southwest) according to the traditional administrative district definition. Eight hospitals from these seven regions were selected to provide cases. In each hospital, 1 month each year from 2005 to 2014 was randomly selected to review the entire cases except for January and February.

According to the case report forms (CRF), well-trained clerks coded and categorized the selected data, and then send the data to National Office of CanSPUC for the data check.

The study protocol was approved by the Institutional Review Board of the Cancer Hospital of Chinese Academy of Medical Sciences.

Patient Selection

Patients included in this study must meet the following inclusion criteria: (1) pathologically confirmed primary NSCLC, (2) patients must receive radical operation of lung cancer, staged I–IIIA, (3) inpatient admission date within the selected month in the study hospital, (4) patient characteristics and treatment (surgery, chemotherapy, or radiotherapy) were recorded.

Patient characteristics collected contained general information (age, sex, and smoking history), surgical approach, pathology, pathological stage, and the use of chemotherapy and radiotherapy. Pathological stage of cancer was categorized according to the seven edition of American Joint Committee on Cancer (AJCC) TNM System. The details of radiotherapy technology, target volume, dose, and the sequence of radiotherapy and chemotherapy were also collected.

Statistical Analysis

The chi-square test was used to compare differences of the use of PORT among different age, region and stage groups. Then, independent factors were identified using Logistic regression analysis with 95% confidence interval (95% CI) for variables with a $p < 0.05$ in chi-square test. The Cochran-Armitage trend test was used to identify the trend in use of PORT from 2005 to 2014. Statistical significance was assessed by using 2-tailed tests with an alpha level of 0.05. SAS statistical software (version 9.4, SAS Institute Inc, Cary, NC) was used to analyze the data.

RESULTS

From 2005 to 2014, a total number of 7,184 lung cancer patients across the seven geographic regions were collected. Of these patients, 4,211 patients were pathologically diagnosed as NSCLC. After matching the aforementioned criteria, 2,253 patients with staged I–IIIA NSCLC receiving radical surgery were enrolled in this study.

Table 1 presents the characteristics of the enrolled patients. In general, most patients (75.2%) were ≤65 years old. The majority (70.7%) of the patients were male. More than half patients had a history of smoking. Squamous carcinoma and adenocarcinoma accounted for 91.1% of all patients. There were 927 (41.1%) patients with stage I disease, 733 (32.6%) with stage II, and 593 (26.3%) with stage III. Totally, 688 (30.5%) patients received chemotherapy.

Overall, 122 patients received PORT, which occupied 5.42% of all the patients. From 2005 to 2014, the proportion of using PORT decreased significantly, from 6.57% in 2005 to 3.09% in 2014 ($p = 0.0002$). In 2013, the proportion was only 1.73% (**Figure 1**).

As in our previous study, we had measured the area-level socioeconomic status (SES) (16). According to all the indicator

TABLE 1 | The characteristics of staged I–IIIA NSCLC patients received surgery.

	All ($N = 2,253$)	PORT ($N = 122$)	Non-PORT ($N = 2,131$)
	No. (%)	No. (%)	No. (%)
Age, years			
≤65	1694 (75.2)	100 (81.9)	1594 (74.8)
>65	559 (24.8)	22 (18.1)	537 (25.2)
Sex			
Male	1592 (70.7)	92 (75.4)	1500 (70.4)
Female	661 (29.3)	30 (24.6)	631 (29.6)
Smoking history			
Yes	1310 (58.2)	81 (66.4)	1229 (57.7)
No	909 (40.3)	40 (32.8)	869 (40.8)
Unknown	34 (1.5)	1 (0.8)	33 (1.5)
Pathology			
Squamous carcinoma	972 (43.2)	67 (54.9)	905 (42.5)
Adenocarcinoma	1079 (47.9)	47 (38.5)	1032 (48.4)
Adenosquamous carcinoma	86 (3.8)	2 (1.6)	84 (3.9)
Large cell carcinoma	21 (0.9)	0 (0.0)	21 (1.0)
Others	95 (4.2)	6 (4.9)	89 (4.2)
Surgical method			
Wedge Resection	59 (2.6)	3 (2.5)	56 (2.6)
Segmental Resection	55 (2.4)	3 (2.5)	52 (2.4)
Lobectomy	1911 (84.8)	98 (80.2)	1813 (85.1)
Pneumonectomy	119 (5.3)	9 (7.4)	110 (5.2)
Unknown	109 (4.9)	9 (7.4)	100 (4.7)
Stage			
I	927 (41.1)	24 (19.6)	903 (42.4)
IIA	387 (17.2)	18 (14.8)	369 (17.3)
IIB	346 (15.4)	19 (15.6)	327 (15.3)
IIIA	593 (26.3)	61 (50.0)	532 (25.0)
Stage, T			
T1	489 (21.7)	18 (14.8)	471 (22.1)
T2	1326 (58.9)	81 (66.4)	1245 (58.4)
T3	438 (19.4)	23 (18.9)	415 (19.5)
Stage, N			
N0	1327 (58.9)	41 (33.6)	1286 (60.3)
N1	417 (18.5)	29 (23.8)	388 (18.2)
N2	509 (22.6)	52 (42.6)	457 (21.5)
Chemotherapy			
No	1541 (68.4)	40 (32.8)	1501 (70.4)
Neoadjuvant	5 (0.2)	0 (0.0)	5 (0.2)
Adjuvant	683 (30.3)	80 (65.6)	603 (28.3)
Unknown	24 (1.1)	2 (1.6)	22 (1.1)
Hospital			
Hunan	229 (10.2)	3 (2.5)	226 (10.6)
Shanxi	383 (17.0)	28 (23.0)	355 (16.7)
Liaoning	804 (35.7)	75 (61.6)	729 (34.2)
Zhejiang	91 (4.0)	6 (4.9)	85 (4.0)
Yunan	314 (13.9)	0 (0.0)	314 (14.7)
Gansu	32 (1.4)	0 (0.0)	32 (1.5)
Anhui	283 (12.6)	5 (4.1)	278 (13.0)
Guangxi	117 (5.2)	5 (4.1)	112 (5.3)

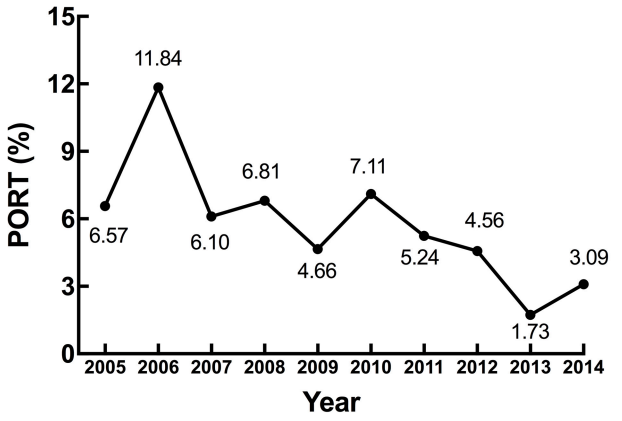

FIGURE 1 | Trend of PORT use for patients from 2005 to 2014.

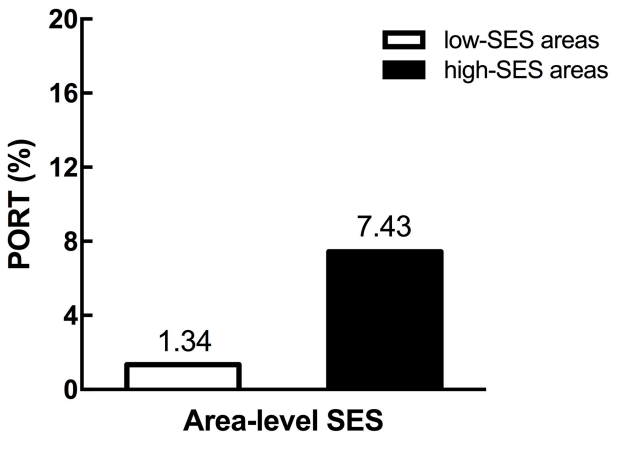

FIGURE 2 | The proportion of PORT use according to area-level SES.

variables, the eight provinces were grouped into low- and high-SES areas. High-SES areas included Hunan, Shanxi, Liaoning, and Zhejiang provinces, while low-SES areas included Yunnan, Gansu, Anhui, and Guangxi provinces. As shown in **Figure 2**, the percentages of PORT use were 1.34% in low—SES areas and 7.43% in high-SES areas, respectively, and the difference was statistically significant ($p < 0.0001$).

Next, we explored the association between different characteristics (including age and stage) and PORT use. Patients ≤65 years old had an overall PORT use rate of 5.90%, which dropped to 3.94% for those older than 65 years old (shown in **Figure 3A**). The difference was marginally significant ($p = 0.0747$). Additionally, there was no significant difference between PORT use and T stage ($p = 0.1262$, shown in **Figure 3B**). However, the PORT use elevated significantly with the increasing of N stage ($p < 0.0001$, shown in **Figure 3C**). The proportion of patients receiving PORT was 4.01% in N0-1 and 10.22% in N2, respectively. A similar trend was also found for TNM stage, with the proportion of 2.59, 4.65, 5.49, and 10.29% for stage I, IIA, IIB, and IIIA, respectively ($p < 0.0001$, shown in **Figure 3D**). When analyzing the trend of PORT use in different N stage patients during this decade, the proportions in N0-1 and N2 diseases both decreased significantly. For N0-1 diseases, the p-value was 0.009. For N2 diseases, the difference was significant ($p = 0.026$), and the proportion decreased especially after 2006 (**Figure 4**).

Multivariate analysis revealed that period, SES, N stage, and TNM stage, except age, were all independent factor for PORT use (**Table 2**).

During the period of 2005 to 2014, modern radiation techniques had been widely used. In simulation, CT/4D-CT scans were commonly performed with a proportion of 68.0%. IMRT and 3D-CRT were applied in 80 patients (65.6%). In order to ensure the position accuracy, EPID and IGRT were recommended. But the survey illustrated the poorly utilization of these techniques (26.2%). The prescribed doses for PORT were delivered <45 Gy in 41 patients (33.6%), 45–54 Gy in 24 patients (19.7%) and >54 Gy in 45 patients (36.9%) (**Table 3**).

Then, we compared the differences of applied radiation techniques between 2005–2011 and 2012–2014. Simulation was performed using CT/4D-CT in most patients and with a stable application. The prescribed doses for PORT were also stable, but a little increase of prescription of 45–54 Gy could be observed. Importantly, we found the use of IMRT and position verification methods increased significantly ($p = 0.014$, 0.003, respectively) (**Table 4**).

DISCUSSIONS

Our study is the first geographically representative epidemiologic study of PORT in NSCLC patients in China. The results showed a declined trend of PORT use from 2005 to 2014. The use of PORT was correlated with stage, especially N stage, rather than T stage. Moreover, patients' age and geographic location also affected the use of PORT. What's more, even in N2 NSCLC, as well as N0-1 NSCLC, the PORT use emerged a declined trend. PORT is the main potential treatment to further reduce local-regional recurrence of resected NSCLC. In the aspect of epidemiologic study, by now, only one study examined the temporal trends of PORT which based on Surveillance, Epidemiology, and End Results (SEER) Program. On the other hand, in the world cancer report published in 2014, it estimated that 35.8% of world's new lung cases would occur in China. And China as one of developing countries, in the health system, has many differences from the USA. Thus, it's necessary to design a survey to learn the Chinese epidemiological situation of the use of PORT in NSCLC.

Although PORT is a valuable treatment for NSCLC, it's not routinely recommended for all patients with resected NSCLC. The results of the 1998 meta-analysis showed that NSCLC patients receiving PORT were detrimental (17). In the USA, based on the research published in 2006, the use of PORT showed a declined trend (15). Similarly, based on our study, in mainland China the use of PORT for NSCLC also has substantially declined from 2005 to 2014. The TNM stage is the main factor affecting the choice of treatment, including PORT. Yet in the survey of USA, the relation between T or TNM stage with the use of PORT was not analyzed. As observed in our study, the use of PORT increased with the increase of TNM stage. When the T and N

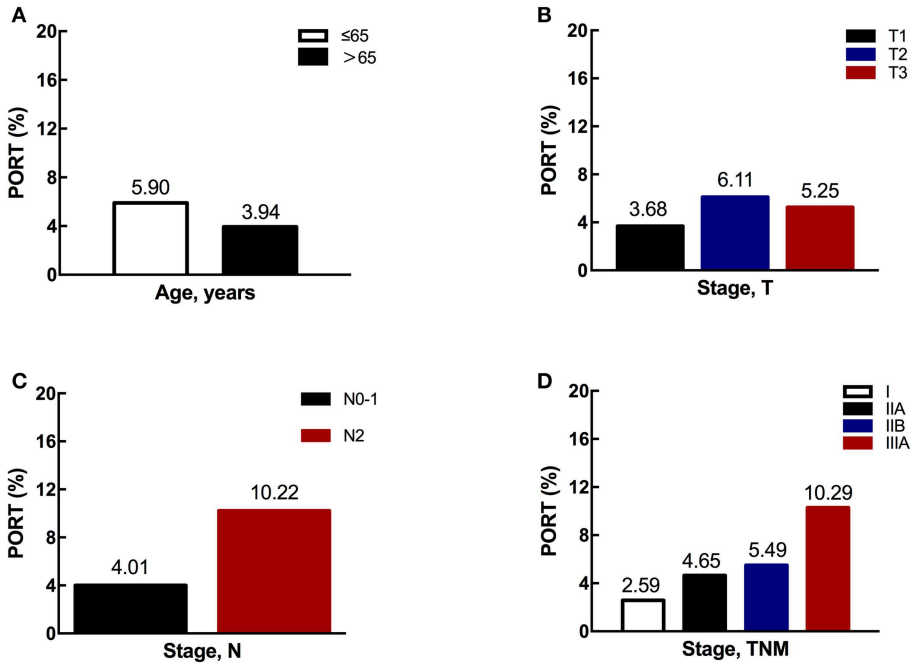

FIGURE 3 | The proportions of PORT use according to different characteristics. **(A)** The proportion of PORT use according to age. **(B)** The proportion of PORT use according to T stage. **(C)** The proportion of PORT use according to N stage. **(D)** The proportion of PORT use according to TNM stage.

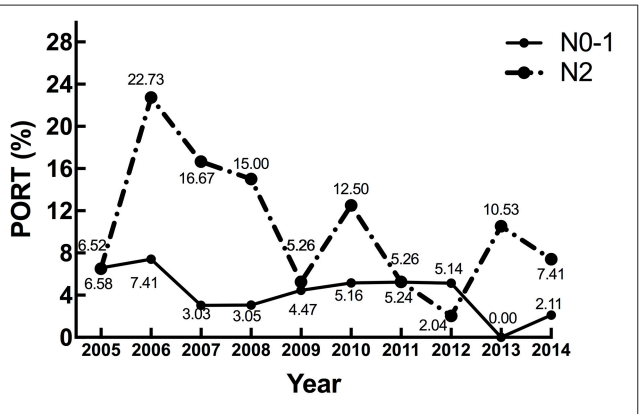

FIGURE 4 | The trends of PORT use for different N stage patients from 2005 to 2014.

TABLE 2 | Multivariate analysis of independent factors for PORT use.

	OR	95%CI
Period		
2005–2009	1	–
2010–2014	0.606	0.419–0.874
SES		
High-SES	1	–
Low-SES	0.169	0.088–0.325
Age		
≤65	1	–
<65	0.653	0.407–1.047
Stage, N		
N0-1	1	–
N2	2.722	1.874–3.954
Stage, TNM		
I	1	–
IIA	1.835	0.984–3.421
IIB	2.186	1.182–4.043
IIIA	4.314	2.658–7.002

stages were considered separately, N stage was more important influencing the selection of PORT in NSCLC. Our results also showed that the T stage was not associated with the choosing of PORT. But with growing of the N stage, the proportions of PORT use were increased simultaneously.

In NCCN guideline and CSCO guideline, PORT is not recommended for patients with pathologic stage N0-1 disease, because it has been associated with increased mortality, at least when using older RT techniques. But PORT is preferred for N2 disease since it appears to improve survival significantly as an adjunct to postoperative chemotherapy in non-randomized analyses. A postoperative multimodality evaluation, including a consultation with a radiation oncologist, is recommended to assess benefits and risks of adjuvant radiotherapy in patients with N2 disease. Our survey showed that the trend of PORT in N0-1 diseases had declined, which was conformal with the recommendation in the aforementioned guidelines. In N2 diseases, the trend hypothetically should be increased or keep stable, but the realistic result was opposite. The possible reasons to explain the result may as follows: Firstly, although PORT is an

TABLE 3 | The techniques and delivery of PORT.

	No.	Percentage (%)
Simulation		
X ray	31	25.4
CT[a]	81	66.4
4D-CT[b]	2	1.6
Unknown	8	6.6
Planning		
2D-RT[c]	39	31.9
3D-CRT[d]	69	56.6
IMRT[e]	11	9.0
Unknown	3	2.5
Position verification system		
No	8	6.6
EPID[f]	5	4.1
IGRT[g]	27	22.1
Unknown	82	67.2
Target volume		
Bronchial stump	45	36.9
Involved lymph node stations	19	15.6
Bronchial stump + Involved lymph node stations	2	1.6
Others	56	45.9
Total Dose		
<45 Gy	41	33.6
45–54 Gy	24	19.7
>54 Gy	45	36.9
Unknown	12	9.8

[a] *CT, Computed Tomography.*
[b] *4D-CT, 4-Dimensional Computed Tomography.*
[c] *2D-RT, 2-Dimensional Radiation Therapy.*
[d] *3D-CRT, 3-Dimensional Conformal Radiation Therapy.*
[e] *IMRT, Intensity-Modulated Radiation Therapy.*
[f] *EPID, Electronic Portal Imaging Device.*
[g] *IGRT, Image-Guided Radiation Therapy.*

TABLE 4 | Changes of applied radiation techniques between 2005–2011 and 2012–2014.

	2005–2011 (*N* = 97)		2012–2014 (*N* = 25)		*p*
	No.	Percentage (%)	No.	Percentage (%)	
Simulation					0.172
X ray	22	22.7	9	36.0	
CT/4D-CT	67	69.1	16	64.0	
Unknown	8	8.2	0	0.0	
Planning					0.014
2D-RT	30	30.9	9	36.0	
3D-CRT	60	61.8	9	36.0	
IMRT	5	5.2	6	24.0	
Unknown	2	2.1	1	4.0	
Position verification system					0.003
No	6	6.2	2	8.0	
Yes	19	19.6	13	52.0	
Unknown	72	74.2	10	40.0	
Total Dose					0.546
<45 Gy	35	36.1	6	24.0	
45–54 Gy	17	17.5	7	28.0	
>54 Gy	36	37.1	9	36.0	
Unknown	9	9.3	3	12.0	

option for N2-NSCLC patients, the evidence is far from enough. The benefit still needs to be confirmed by randomized clinical trials (RCTs). Up to now, there has been three such phase III RCTs: CALGB 9734 failed because of slow accrual; LUNGART and the other phase III multicenter RCT (NCT00880971) from our center are both ongoing now. Therefore, many radiation oncologists may remain skeptical about the use of PORT for N2 disease. Secondly, with the developing of medical insurance system in China, more NSCLC patients have the opportunity to receive surgery, which leads to the increase of pN2 population. Thirdly, resource shortage of radiation still exists in China. The comparison of the absolute value of the use of PORT for patients with N2 disease between China and America may also confirm this: in our study the proportion was only 10.22%, but in America even in 2002, it was 37% (15). Finally, it may be related to the lack of awareness and education about the standards of PORT and the excessive caution of side effects such as radiation induced pulmonary/cardiac toxicities.

Our study also showed the variation in PORT use with patient age and geographic location. Elder patients had less PORT than younger patients. In American study, they also demonstrated that age was highly predictive of PORT use. Despite of an effective treatment modality, PORT may cause a few adverse events including pulmonary and cardiac toxicities. Besides, after radical surgery, the tolerance of patient for radiation toxicities reduces. Patients older than 65 years usually have some complications which must take into consideration when choosing alternative treatments such as PORT. Thus, compared with younger ones, patients above 65 years old had less opportunity to undertake PORT, though it was not an independent factor. The area-based SES judging from seven indicators accurately reflected the multidimensional character of regional socioeconomic position. As a part of our results, the high-SES areas had a high percentage of PORT use. In the surveys conducted by Yin et al. (18), which investigated the changes of radiation oncology in mainland China, the equipment of radiotherapy had grown remarkably, and advanced techniques had been implemented very quickly from 1997 to 2011. But resources based on the population were still far less than the recommendation of the World Health Organization. Rural and remote areas were much less well-equipped which may due to financial problems. When diagnosed as malignant tumors, people living in mainland China traditionally seek for surgery and chemotherapy rather than radiotherapy, though well-educated patients may also consult radiation oncologists as well. Low-income populations often could not afford the high cost of radiotherapy. From above, we can explain that the PORT use is influenced by the economic strength and educational level.

In order to reduce potential pulmonary/cardiac toxic effects, PORT should be delivered with modern techniques such as

CT-based 3D-CRT or IMRT planning, with which target volumes and normal tissue constraints can be precisely defined. In patients with locally advanced NSCLC treated with concurrent chemotherapy, IMRT significantly reduced the rate of high grade pneumonitis and improved higher overall survival compared to 3D-CRT (19). In our survey, we found that the simulation and planning of modern radiation treatment were widely used. When the techniques using before and after 2011 were compared, the use of IMRT was significantly increased and more position verification methods were applied, which implied the improvement of radiation in China. As recommended in NCCN guideline, the CTV includes the bronchial stump and high-risk draining lymph node stations. Previous studies showed that large variability was observed in routine target definition for PORT (20, 21). According to Lung ART study, routine CTVs varied up to 3-fold between clinicians (21). However, this variance was significantly reduced when clinicians were uniform trained. Therefore, the need for standardization must be emphasized. Total dose is another important issue of radiotherapy. Standard doses pointed in guidelines are 50 to 54 Gy in 1.8 to 2 Gy fractions. Corso et al. (22) found that PORT with doses of 45 to 54 Gy remained significantly associated with improved OS. Doses up to 54 Gy are improper unless having nodal extracapsular extension of microscopic positive margins. In our survey, although 45 to 54 Gy was prescribed more often after 2011, the doses in clinical practice seemed to be higher than the recommendation. About 37% patients received PORT dose more than 54 Gy.

There are some limitations in our survey. Firstly, all patients included in our study were ethnically Chinese. Secondly, selection bias may exist as we used the data from the leading public cancer hospital of the province which may not represent the whole population of the area. Thirdly, we used the convenience sampling instead of random sampling methods. Fourthly, the data quality is largely dependent on the clinician's documentation and the records on radiation of some patients were incomplete.

CONCLUSIONS

In China from 2004 to 2015, the application of PORT, in both N0-1 and N2 diseases, was declined. Patients' age, geographic location, and disease stages were all affected the choice of PORT. Proper education of radiation oncologists was urgently needed. Although modern radiation techniques were gradually applied during the past years, the optimal radiation volume and dose should be emphasized.

ETHICS STATEMENT

This retrospective study was approved by the Institutional Ethics Review Board at the Institutional Review Board of the Cancer Hospital and Institute of Chinese Academy of Medical Sciences & Peking Union Medical College. Informed consent was exempted by the board due to the retrospective nature of this research. Patient records were anonymized and de-identified prior to analysis.

AUTHOR CONTRIBUTIONS

JS, MD, and ZH: study concepts and design. YeZ, SG, JL, NW, BY, SL, and JR: CRF design and data management. YH, DWa, XL, XX, LD, LY, YuqL, YoZ, DWe, and YunL: data collection. YM and LW: data analysis and interpretation, statistical analysis, manuscript preparation, and manuscript editing. KZ, YQ, and WC: program supervision. YM, LW, JS, MD, and ZH: manuscript reviewing and approving. All authors read and approved the final manuscript.

FUNDING

This work was supported by National Key R&D Program of China (2017YFC1311000, 2017YFC1311002, 2017YFC0907900, 2017YFC0907901, 2017YFC1308700, 2017YFC1308705); Clinical Application Project of Beijing Municipal Commission of Science and Technology (Z171100001017114); Beijing Hope Run Special Fund of Cancer Foundation of China (LC2016L03); CAMS Key lab of translational research on lung cancer (2018PT31035); National Key Public Health Program of China (Cancer Screening Program in Urban China); National Natural Science Foundation of China (81402740, 81773521).

ACKNOWLEDGMENTS

The authors thank all the members of the LuCCRES Group (Clinical Characteristics and Medical Service Utilization of Lung Cancer in China, 2005–2014: a Multicenter Retrospective Epidemiologic Survey) from the National Cancer Center of China and eight field provinces.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Oncology

ORIGINAL RESEARCH
published: 22 October 2019
doi: 10.3389/fonc.2019.01098

The Current Situation of Esophageal Cancer Staging and Perioperative Strategies Determination in Central and Southern China: A Cross Sectional Survey

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Purpose: We aim to investigate the current esophageal cancer staging according to the 7th edition TNM classification for esophageal carcinoma proposed by American Joint Committee on Cancer (AJCC) among oncology-related physicians in China.

Methods: A specifically-designed 14-item questionnaire was distributed to 366 doctors who were working with esophageal cancer patients. We collected and analyzed the feedbacks and explored the possible associations within different departments, including thoracic surgery, the internal medicine of gastroenterology, oncology, and/ radiotherapy in eight different hospitals from central and southern China.

Results: Among all the responses, 31.42% of them were from thoracic surgery department, 40.44% were from oncology and/or radiation therapy and 28.14% were from the internal medicine of gastroenterology, respectively. Surprisingly, in total 66.12% of all the physicians were unaware that the 7th edition of esophageal carcinoma TNM classification was released in 2009; only 21.86 and 16.67% of physicians recognized cervical nodes and celiac nodes as regional lymph nodes. Furthermore, 67.21% physicians didn't know that tumor location, histologic grade, and histopathology were accepted as new prognostic factors in the latest TNM system; and 51.37% physicians could not determine the correct TNM classification of esophagogastric junction cancers. Intriguingly, over 50% of them could still design appropriate perioperative strategies.

Conclusions: The 7th edition of the TNM classification for esophageal carcinoma is poorly recognized and understood in central and southern China, which might contribute to the relatively low rates of appropriate perioperative procedures applied for esophageal cancer patients.

Keywords: esophageal cancer, TNM, perioperative strategies, central and southern China, current situation

OPEN ACCESS

Edited by:
Paolo Boffetta, Icahn School of Medicine at Mount Sinai, United States

Reviewed by:
Satyanarayana Alleboina, University of Tennessee Health Science Center (UTHSC), United States
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Specialty section:
This article was submitted to Cancer Epidemiology and Prevention, a section of the journal Frontiers in Oncology

Received: *15 February 2019*
Accepted: *07 October 2019*
Published: *22 October 2019*

Citation:
Lu D, Liu X, Feng S, Dong X, Shi X, Ren P, Diao D, Wu H, Xiong G, Wang H, Li M, Rao S, Molena D, Wu AJ and Cai K (2019) The Current Situation of Esophageal Cancer Staging and Perioperative Strategies Determination in Central and Southern China: A Cross Sectional Survey. Front. Oncol. 9:1098. doi: 10.3389/fonc.2019.01098

INTRODUCTION

Esophageal cancer is the eighth most common cancer worldwide with an estimated incidence of 6.5 per 100,000 in 2012 (3.2% of all cancer occurrence), and the sixth most common cause of cancer death with a roughly mortality of 5.7 per 100,000 (4.9% of all cancer-related death). Mortality variation shows apparent geographical difference with the highest one occurring in Eastern Asia (14.1 per 100,000) (1). Particularly, esophageal cancer is the 5th most common cancer (22.16 per 100,000) and 4th most common cause (16.64 per 100,000) of cancer related death in China (2); indicating China has a more severe esophageal cancer burden compared to other regions.

The prognosis for early stage esophageal cancer patients is significantly superior to that of intermediate and late stage patients. However, the overall survival of esophageal cancer in China is very low due to the undeveloped early detection of esophageal cancer via endoscopy thus the majority of patients are diagnosed as the intermediate or late stages (3). Importantly, the poor prognosis might also be caused by clinicians' limited knowledge of esophageal cancer, for example, the TNM staging system, which is extremely important for corresponding treatments planning.

We realized that many physicians and surgeons from esophageal related departments, including thoracic surgery, the internal medicine of gastroenterology, oncology, and radiotherapy departments, with different levels of experience, were not fully aware of neither the 7th edition of TNM classification of esophageal carcinoma which was proposed by American Joint Committee on Cancer (AJCC) in 2009 (4), nor the 2nd edition of Chinese Guidance for Standardized Therapy for Esophageal Carcinoma (5). To our knowledge, there is no cross-sectional survey on the awareness of the esophageal cancer TNM staging system or perioperative strategies determination among Chinese clinicians so far. Therefore, we performed the current survey to explore the possible correlations between recognition of TNM system and perioperative procedures planning, with the ultimate goal to promote standard diagnosis and treatment for esophageal cancer in China.

METHODS

Questionnaire

To obtain the first-hand data regarding current situation of esophageal cancer Staging and perioperative strategies in central and southern China, we carried out this cross-sectional study by generating a specific questionnaire. The questionnaire was developed by Di Lu, Siyang Feng, and Kaican Cai with help of twelve esophageal cancer experts and optimized based on two semi-structured pilot surveys. It was composed with 14 items and modified over ten times, which made it more acceptable to the responders. Finally, the 13th edition (**Supplementary Data Questionnaire 1**) with three sections and fourteen questions was applied in the current study and described as follows. Section 1: TNM staging (awareness of the 7th edition TNM staging system; classification of cervical nodes; classification of celiac nodes; awareness of new factors of the staging system; distinguishing between esophageal cancer and gastric cancer); Section 2: perioperative therapy (POT), (2 cycles of neo-adjuvant chemotherapy, 4 cycles of adjuvant chemotherapy, chemotherapy protocol (paclitaxel or platinum-based) for squamous cell cancer, dose of neo-adjuvant radiation); and Section 3: general opinion and access to staging system updates.

Selection of Hospitals and Examinees

The survey was initiated in Guangdong province and most clinicians were from central and southern China. Particularly, the Chaoshan area, one of the representative areas with the highest incidence of esophageal cancer in China was enrolled in the current survey. The survey was carried out from May 2016 to December 2016, in total eight medical centers from Guangdong, Hunan, Henan, and Shanxi Provinces were selected, including Sun Yat-sen University Cancer Center, Nanfang Hospital of Southern Medical University, General Hospital of Guangzhou Military Region, Gaozhou People's Hospital, Shantou Central Hospital, Xiangya Hospital of Central South University, The First Affiliated Hospital of Zhengzhou University, and the Cancer Hospital of Shanxi Province. The examinees were from department of thoracic surgery, the internal medicine of gastroenterology, oncology, and radiation therapy, ranging from intern to professor. Informed consent was obtained from every responder before the survey.

Data Collection

After he or she had agreed that the survey was anonymous and data were independently collected and would be published, each examinee was asked to complete the survey immediately upon receipt of the questionnaire without access to the internet, textbooks, and their colleagues, under the supervision of inspectors, to make sure the survey was firsthand and unbiased. The survey was carried out in an anonymous and independent fashion. Questionnaires were considered as valid if all questions were addressed properly according to the request.

Statistical Analysis

The results were presented as counts or percentages. Statistical analysis was performed using SPSS version 22.0 (SPSS Inc., Chicago, IL, USA). Frequency tables were generated for relevant variables. Differences among several groups were analyzed by the chi-squared test. A two-sided p-value <0.05 was considered as significant in all analyses.

RESULTS

In total of 401 questionnaires were distributed and 366 validated questionnaires were analyzed; the response rate was 91.3%. 31.42% of them were from the department of thoracic surgery, 40.44% from oncology (including radiation therapy) and 28.14% from the internal medicine of gastroenterology. Approximately a quarter of the examinees (25.14%) were senior attendings and professors, 25.41% were junior attendings, 21.58% were residents, and the rest were interns (27.87%). The regional details for all the examinees were summarized and presented in **Table 1**.

TABLE 1 | The current situation of esophageal cancer staging in central and southern China (*$P < 0.05$).

Variables	Total (%)	Aware-ness of the 7th edition (%)	*P* value	Correct answer of cervical nodes (%)	*P* value	Correct answer of celiac nodes (%)	*P* value	Aware-ness of adding factors to the staging system (%)	*P* value	Correct distinguishment of esophageal cancer from gastric cancer (%)	*P* value
Overall	100	38.25		21.86		16.67		32.79		48.63	
Area			0.33		<0.01*		<0.01*		<0.01*		<0.01*
Guang-dong	68.58	41.04		28.69		19.52		31.08		44.22	
Shanxi	13.66	36.00		2.00		4.00		24.00		64.00	
Hunan	2.19	37.50		25.00		62.50		75.00		87.50	
Henan	15.57	28.07		8.77		8.77		42.11		49.12	
Academic Level			0.015*		0.316		0.422		<0.01*		0.011*
Intern	27.87	26.47		23.53		15.69		38.24		36.27	
Resident	21.58	41.77		25.32		16.46		39.24		46.84	
Junior attending	25.41	48.39		13.98		16.13		31.18		53.76	
Senior attending and professor	25.14	38.04		25.00		18.48		22.83		58.70	
Department			<0.01*		<0.01*		<0.01*		<0.01*		0.011*
Thoracic surgery	31.42	59.13		17.39		25.22		43.48		58.26	
Oncology	40.44	22.97		29.05		14.86		25.00		48.65	
Gastro-enterology	28.14	36.89		16.50		9.71		32.04		37.86	

Regarding TNM staging, only 38.25% of the examinees knew this widely accepted staging system. Moreover, 33.88% of the examinees did not realize the 7th edition was the latest version by the time when this survey was conducted and 53.01% of them did not know this version was released in 2009. There were no differences between different areas regarding the low awareness of the TNM system, however, our data suggested that examinees from different academic levels ($p = 0.015$) or departments ($p < 0.01$) had significant difference in awareness of the latest TNM staging system. Of note, junior attendings knew the system best compared to other levels and surgeons from thoracic department were more familiar with the system than other physicians.

Overall, only 21.86 and 16.67% of all clinicians considered cervical nodes and celiac nodes as regional lymph nodes and there was a clear variation among examinees from different areas and departments ($p < 0.01$). Moreover, 67.21% were not aware that the new prognostic factors including tumor location, histological grade, and histopathology were added to the latest version of TNM system, not surprisingly, there was also significant difference among people from different areas, academic levels and departments regarding the new factors ($p < 0.01$).

Only 48.63% physicians could distinguish esophageal cancer staging from gastric cancer staging when they classified esophagogastric junction cancers according to the 7th edition of the TNM staging discipline. When we further analyzed the data in details, we found that difference of region, academic level, and department could induce significant difference in distinguishing esophageal cancer staging from gastric cancer staging.

In addition, we also analyzed the method to access the 7th edition updates of these examinees, and the three major resources were academic literature (47.81%), textbooks (45.36%), and conferences (35.25%) (**Figure 1**). Surprisingly, we observed only <9% examinees got the updates from smart phone application, suggesting a lot of effort should be taken to improve this most efficient method to remind physicians with the latest updates.

As for perioperative therapy choice (**Table 2**), 53.28% of the examinees preferred two cycles of neo-adjuvant chemotherapy, 64.21% preferred four cycles of postoperative

FIGURE 1 | Proportion of access to the 7th edition of the esophageal cancer TNM staging system updates.

TABLE 2 | Summary of peri-operative therapy decision for esophageal cancer patients in central and southern China (*$P < 0.05$).

Variables	Total (%)	2 cycles of neo-adjuvant chemo-therapy (%)	*P* value	4 cycles of adjuvant chemo-therapy (%)	*P* value	Chemo-therapy protocol (paclitaxel and platinum-based) for squamous cell cancer (%)	*P* value	Dose of neo-adjuvant radiation (%)	*P* value
Overall	100	53.28		64.21		62.30		65.57	
Area			0.020*		0.227		<0.01*		<0.01*
Guangdong	68.58	49.00		65.74		61.35		63.35	
Shanxi	13.66	64.00		66.00		60.00		72.00	
Hunan	2.19	75.00		25.00		25.00		25.00	
Henan	15.57	59.65		61.40		73.68		75.44	
Academic level			<0.01*		0.663		0.314		0.220
Intern	27.87	35.29		60.78		65.69		66.67	
Resident	21.58	51.90		67.09		53.16		60.76	
Junior attending	25.41	62.37		67.74		58.06		72.04	
Senior attending and professor	25.14	65.22		61.96		70.65		61.96	
Department			<0.01*		<0.01*		0.068		<0.01*
Thoracic surgery	31.42	71.30		74.78		51.30		49.57	
Oncology	40.44	32.43		48.65		69.59		66.89	
Gastro-enterology	28.14	63.11		74.76		64.08		81.55	

adjuvant chemotherapy, and 65.57% preferred a dose of 40 Gy for neo-adjuvant radiotherapy with suitable circumstances, these results were consistent with Chinese guideline and high-level randomized clinical trials results in this area (6). For pre- or postoperative adjuvant chemotherapy, 62.3% of examinees would choose paclitaxel and platinum rather than 5-fluorouracil and platinum for esophageal squamous cancer patients, furthermore, 70.49% of clinicians selected neo-adjuvant radio-chemotherapy but not chemotherapy alone to treat patients with local lymph node metastasis. When we looked into these results in more details, we frequently observed significant difference in selecting therapy procedures among physicians from different regions, academic levels and departments, indicating a standard treatment reference is lacking for esophageal cancer patients, which might partially explain the poor prognosis for this prevalent cancer in China.

Taken together, our survey revealed that the awareness of the 7th edition TNM staging system and POT decision making was significant different among different regions in central and southern China. Interestingly, physicians from different departments also showed marked difference in recognition of TNM system and POT determination, while thoracic surgeons usually performed better than clinicians from other departments according to our cross-sectional study.

DISCUSSION

Unlike many other cancers such as lung or colon cancer which drives massive research interest and dramatic improvement of treatment in last few decades, esophageal cancer is still one of the leading causes of cancer-related death with poor prognosis in China. One reason for this phenomenon is that most esophageal cancer patients in China are only diagnosed as locally advanced disease or unsuitable for radical resection at the first-time visit due to the lack of early detection via endoscopy (7, 8), therefore it is very challenging and difficult in most cases for esophageal cancer treatment. Endoscopic technologies have always been one of the most popular research interest for early detection (9). For instance, Lugol's iodine chromoendoscopy (LCE) was proved to be a useful tool to diagnose squamous cell neoplasia in high-risk individuals with a sensitivity of 46% and a specificity of 90% in 190 high-risk subjects (10). Importantly, a recent randomized trial showed that the overall accuracy of narrow band imaging (NBI) and LCE in detecting high grade dysplasia (HGD) or invasive squamous cell carcinoma was comparable (91.2 and 90.5%, respectively), but NBI was significantly more time-saving (11). In addition, the American Society for Gastrointestinal Endoscopy meta-analysis found that the pooled specificity and sensitivity of confocal laser endomicroscopy for diagnosing HGD were satisfying (77.3 and 90.3% respectively) (12). Besides these newly developed

technologies, trans-nasal endoscopy (TNE) was identified to be more popular than conventional endoscopy in 63% of cases (13) without sacrificing sensitivity as compared to LCE (14, 15). Alternatively, apart from classical endoscopy methods, esophageal cell collection devices such as Cytosponge™ in diagnosing Barrett's Esophagus (BE) could achieve satisfying sensitivity and specificity (94 and 79.9%, respectively) (16). Moreover, several studies had shown that miRNAs in peripheral blood could efficiently distinguish BE patients from healthy individuals (17), whereas circulating tumor DNA (ctDNA) could be detected in the scenario of advanced esophageal cancer (18). Interestingly, a panel of breath volatile organic compounds was also applied to determine esophagogastric cancer (19). However, most of these newly developed technologies were not widely practiced for early esophageal cancer detection in Chinese population, thus we advocate a significant effort was required to promote these technologies in China.

Secondly, most research and therapy progress in esophageal cancer therapy is carried out in Western countries, yet there is a huge difference in esophageal cancer subtypes distribution between Western and Eastern countries. The majority of esophageal cancer patients in Western countries are adenocarcinoma, while in China over 90% esophageal cancer patients are squamous (20). Recent studies suggested that these two subtypes of esophageal cancer might be completely different in terms of histopathology, risk factors, and prognostic factors (21), therefore similar treatment might bring different therapeutic effects. Furthermore, it is still uncertain which genetic mutations are major driver of esophageal cancer, thus the establishment of *in vivo* system to recapitulate esophageal cancer development is still lacking, making the preclinical evaluation of targeted therapy or immunotherapy for esophageal cancer unfeasible.

Nevertheless, we believe other reasons, particularly in clinical, might contribute to the poor prognosis of esophageal cancer in China. As we frequently noticed divergence in diagnosis and therapy selection for esophageal cancer patients, we carried out the first cross sectional survey on the recognition of the 7th edition of the esophageal cancer TNM staging system and the current situation of perioperative therapy decision making in central and southern China. To our surprise, less than half of the examinees were aware of the 7th TNM staging system although more than half physicians could make the correct perioperative decision. The 7th edition of the TNM staging system for esophageal cancer is established based on large population study and identifies cervical and celiac lymph nodes as regional nodes. However, given the fact that the esophagus involves multiple regions, it might be confused to classify lymphatic metastasis when the primary tumor and metastatic lymph nodes are located in different regions. Indeed, according to our results, nearly half of the examinees thought that the 7th edition of the TNM staging system for esophageal cancer was debatable, as this latest staging system is more relying on patient data without highlighting the anatomic or biologic properties of esophageal cancer, therefore it is not completely convincing to all clinicians. The awareness of standard TNM staging system can influence correct therapy decision, indeed, our data suggested different levels physicians from different regions or departments showed inconsistence in recognition of the staging system, therefore the ratio of correct POT decision was also quite variable. As the 8th edition of the TNM staging system was released in October 2016 and has been practically used in clinical since January 2018, we advocate the promotion of this latest TNM system as soon as possible to improve the diagnosis and treatment of esophageal cancer in China.

We also admitted several limitations in the present survey. First, the selection of medical centers was not randomized and did not cover the entire country, though enrolled examinees were from those areas with high incidence of esophageal squamous cell cancer. Second, the sample size of the respondents might not be large enough to represent the accurate awareness and therapy decision making in China. Finally, the questionnaire included updates to the 7th edition and perioperative therapy for esophageal cancer but did not with full details of end-stage esophageal cancer therapy, which therefore does not represent the entire spectrum of treatment for esophageal cancer.

CONCLUSIONS

In central and south China, the 7th edition of the AJCC TNM staging system has not been well-accepted and applied, and the current state of decision making for esophageal cancer is not satisfying. The promotion of standardized diagnosis and treatment for esophageal cancer is urgently required.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the ethical committee of Nanfang Hospital, Southern Medical University, with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the name of committee.

AUTHOR CONTRIBUTIONS

DL and KC designed the study. DL and XL were primarily responsible for analyzing the data and writing the manuscript. DL, SF, and KC designed the questionnaire. XD, XS, PR, and DD were responsible for data collection. HWu, GX, HWa, and ML recruited most of the participants. SR, DM, and AW revised the manuscript. All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

This study was financially supported by the Science and Technology Planning Project of Guangdong Province (2017B020226005) and the President Foundation of Nanfang Hospital, Southern Medical University (2016B018).

ACKNOWLEDGMENTS

We thank Prof. Yuji Tachimori from Kawasaki Saiwai Hospital, Kawasaki, Japan, Prof. Side Liu, Fachao Zhi, Wangjun Liao, and Dr. Yuanyuan Wang from Nanfang Hospital of Southern Medical University, Prof. Lanjun Zhang from Sun Yat-sen University Cancer Center, Prof. Guibin Qiao, Weimin Zhang, and Yagang Zhao from General Hospital of Guangzhou Military Region, Prof. Maosheng Wang and Wanli Lin from Gaozhou People's Hospital, Prof. Junhui Fu from Shantou Central Hospital, Prof. Chunfang Zhang from Xiangya Hospital of Central South University, Prof. Song Zhao from The First Affiliated Hospital of Zhengzhou University, and Prof. Rongsheng Zhang, Lu Wen, and Hongwei Wang from the Cancer Hospital of Shanxi Province for supporting us in collecting questionnaires.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2019.01098/full#supplementary-material

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