CANCER IN AFRICANS: THE PAST, THE PRESENT, AND THE FUTURE

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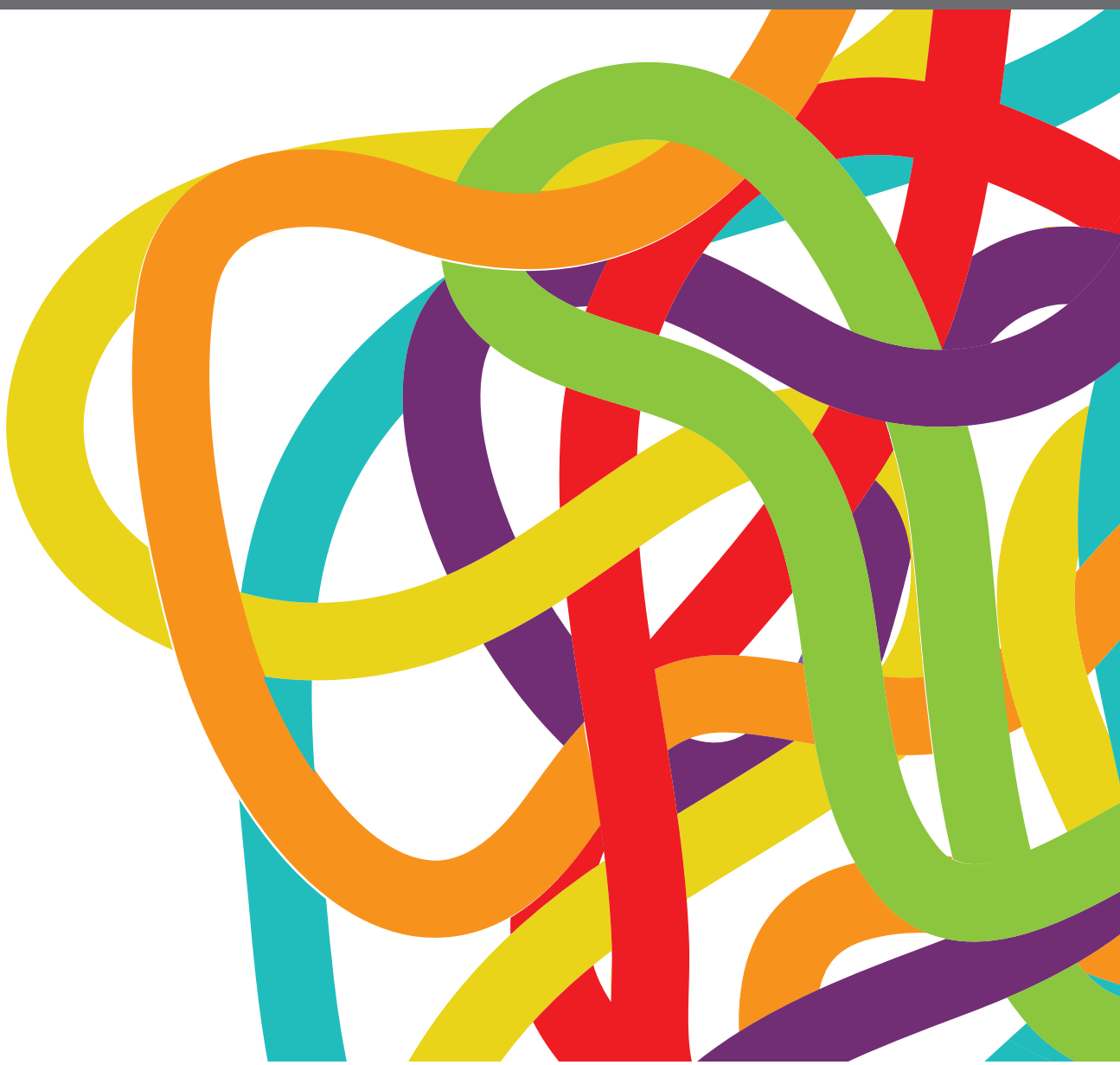

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CANCER IN AFRICANS: THE PAST, THE PRESENT, AND THE FUTURE

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Efavirenz and Lopinavir/Ritonavir Alter Cell Cycle Regulation in Lung Cancer

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Highly active anti-retroviral treatment (HAART) is currently the most effective treatment for HIV/AIDS. Additionally, HIV positive patients receiving HAART have a better health-related quality of life (HRQoL). Cancers previously associated with HIV/AIDS also known as the AIDS defining cancers (ADCs), such as Kaposi's sarcoma and non-Hodgkin's lymphoma have been on the decline since the introduction of HAART. However, non-AIDS defining cancers (NADCs), in particular, lung cancers have been documented to be on the rise. The association between the use of HAART components and lung carcinogenesis is poorly understood. This study aimed at elucidating the effects of two HAART components [efavirenz (EFV), and lopinavir/ritonavir (LPV/r)] on lung cancer. This was achieved through the use of *in vitro* cell biological approaches to assess cell health, including cell viability, Real Time Cell Analysis (RTCA) growth monitoring, evaluation of the cell cycle, and progression to apoptosis, following on drug treatments. At plasma level concentrations, both EFV and LPV/r induced S-phase arrest, while at lower concentrations both drugs promoted the progression of cells into G2/M phase following cell cycle FACS analysis. At higher concentrations although cell viability assays reflected anti-proliferative effects of the drugs, this was not statistically significant. RTCA showed a significant decline in cell viability in response to the highest dose of LPV/r. Dual staining by Annexin V-FITC and PI confirmed significant pro-apoptotic effects were promoted by LPV/r. Both EFV and LPV/r exert double-edged oncogenic effects on MRC-5 and A549 lung cells, acting to either promote cell proliferation or to enhance apoptosis. This is affected by EFV and LPV/r altering cell cycle progression, with a significant S-phase arrest, this being an indication of cellular stress, cytotoxicity, and DNA damage within the cell.

Keywords: efavirenz, lopinavir/ritonavir, cell cycle, lung cancer, cell proliferation, cell death, real-time cell analysis

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INTRODUCTION

HIV infection is a major global concern with increasing prevalence. In 2018, UNAIDS estimated that ∼37.9 million people were living with HIV, 1.7 million people were newly infected, while ∼0.77 million people died from AIDS-related illness. An estimated 23.3 million people were receiving antiretroviral treatment (ART) (1). In total, an estimated 32 million people have died of the disease

since the first cases of AIDS were reported in 1981. Long term effects of HAART exposure on cancer risk are not well-defined. In this regard according to basic and epidemiological research, there might be specific associations of each HAART component with distinct patterns of cancer risk (2). Currently, the human immunodeficiency virus acquired immunodeficiency syndrome (HIV/AIDS) and lung cancer are arising as colliding epidemics and urgent interventions are necessary to combat these leading causes of morbidity and mortality (3). In addition, cancer incidence rates are also shown to be increased in people living with HIV/AIDS (PLWA) compared to the general population (4–7). To date, there is no cure for HIV/AIDS and highly active antiretroviral treatment (HAART) is the most effective treatment regimen (8). Additionally, there has been a decline in cancers previously associated with HIV/AIDS, also known as the AIDS defining cancers (ADCs): including Kaposi's sarcoma, primary central nervous system lymphoma, non-Hodgkin's lymphoma, and cervical cancer. In contrast to this, non-ADCs have been documented to be on the rise in the HAART era, with lung cancer emerging as a leading NADC (6, 9).

Lung cancer is one of the leading NADCs both globally and in South Africa (10). In South Africa, adenocarcinoma is the most common form of lung cancer (10–12). Lung cancer is characterized by high genetic diversity (13). Genetic mutations in Ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS), epidermal growth factor receptor (EGFR), B-RAF (BRAF), and phosphatidylinositol 3-kinase (PI3K) signaling oncogenic pathways have been identified in lung cancer. The aberrant expression of TP53, PTEN, RB1, LKB11, and p16 tumor suppressor genes in lung cancer has also been reported. Other gene targets with genetic alterations in lung cancer include human epidermal growth factor receptor (HER2), Mitogen-activated protein kinase (MEK), Anaplastic lymphoma kinase (ALK), (ROS1) and Fibroblast growth factor receptor 1 (FGFR1) (14–17). Smoking remains one of the significant factors in lung carcinogenesis (16). However, the association between lung cancer and the use of HAART components is poorly understood. The identification of genetic markers in the development and progression of lung cancer has made significant improvements in the understanding of lung cancer molecular pathogenesis and overall patient diagnosis and treatment. In addition, when compared to the same age group in the general population, the risk of developing non-small cell lung carcinoma (NSCLC), the most predominant form of lung cancer, is higher in HIV positive patients (10). While South Africa has the largest HIV epidemic and antiretroviral therapy (ART) program in the world (18, 19), the poor understanding of the relationship between the use of HAART components and tumorigenesis especially lung cancer has placed a burden on public health, globally and in South Africa. This study aimed at determining the effects of two HAART components (EFV and LPV/r) on lung cancer. Cell viability, cytotoxicity assays, cell cycle analysis, and apoptosis assay were performed on treated MRC-5 and A549 cells. Treatment with EFV and LPV/r alters the cell cycle progression, with a significant S-phase arrest, cellular stress, DNA damage, and cytotoxicity.

MATERIALS AND METHODS

ARV Drugs

The ARV drugs for this study were purchased from Toronto Research Chemicals (Toronto, Ontario, Canada), and prepared as stock solutions in pharmaceutical/analytical grade methanol. The mean steady-state peak plasma concentration (Cmax) is the most physiologically relevant concentration for the ARVs because it represents naturally occurring concentration of the drugs following their intake (20). The concentrations used in this study includes the clinically relevant plasma level doses and experimental doses.

Cell Culture

The lung cell lines MRC-5, normal lung fibroblast (ATCC CCL171) and A549, lung adenocarcinoma (ATCC CCL185) were obtained from the American Type Culture Collection (ATCC). MRC-5 and A549 cells were grown in Dulbecco's Modified Eagle Medium (DMEM, Life Technologies, Inc, Rockville, MD) supplemented with 10% heat-inactivated fetal bovine serum (Sigma-Aldrich, St. Louis, MO) and 1% penicillin and streptomycin (GIBCO). Cells were cultured in 25 cm^2 cell culture flasks (Corning, USA) and were kept in a CO_2 incubator at 37°C in a humidified atmosphere with 5% CO_2 in air. For experimental purposes, cells cultured to an exponential growth phase (at ∼70% confluency) were used. Cells were then serum-starved for 24 h to synchronize the cell cycle. The following day, the cells were pharmacologically treated with either EFV at concentrations of 4, 13, 26, or 50 μM, respectively; or with LPV/r at concentrations of 10, 32, 50, or 80 μM, respectively. Treatment was carried out for 24–72 h. Control cells were exposed to growth medium and vehicle only (methanol 0.1% v/v).

Alamar Blue (AB) Cell Viability Assay

The Alamar Blue (AB) cell viability assay was used to measure MRC-5 and A549 cell viability in response to EFV and LPV/r treatment, respectively, and relative to (0.1v/v) methanol, the vehicle control. Confluent cells were trypsinised and harvested by centrifuging; the cell pellets were re-suspended in a small volume of cell culture medium. An aliquot of cells was then counted using an automated cell counter (Bio-Rad) and 2×10^3 cells were seeded in a 96-well-plate). Cells were allowed to attach and grow overnight. Prior to treatment, the cells were serum starved for 24 h to synchronize the cell-cycle. The cells were treated in triplicate with one of the following treatments: a vehicle control consisting of 0.1%; v/v methanol; one of four different concentrations of EFV (4, 13, 26, 50 μM), respectively; and one of four different concentrations of LPV/r (10, 32, 50, 80 μM), respectively. Treatment time was for a period of either 24, 48, or 72 h, respectively. At the end of each treatment phase, AB was added directly into culture media in each well at a final concentration of 10% and incubated for 3–4 h at 37°C in an atmosphere of 5% $C0_2$ in air. The absorbance of test and control wells was measured at 540 and 630 nm, wherein the number of viable cells correlates with the magnitude of dye reduction and is expressed as percentage of AB reduction (21). The calculation

of the percentage of AB reduction (%AB reduction) is as follows, according to the protocol reduced controls are:

$$\%\text{Reduction} = \left\{ \frac{\varepsilon\text{oxid } 630\text{ nm } (\text{sample A450 nm}) - \varepsilon\text{oxid } 540\text{ nm } (\text{sample A630 nm})}{\varepsilon\text{red } 540\text{ nm } (\text{oxidized control A630 nm}) - \varepsilon\text{red } 630\text{ nm } (\text{oxidized control A450 nm})} \right\}_{\times 100}$$

The molar extinction coefficients of AB for the oxidized and reduced controls are:

εoxid 630 nm = 34.798, εoxid 540 nm = 47.619, εred 630 nm = 5.494, and εred 540 nm =104.395 (22).

The values of % AB reduction was corrected for background values of blank wells containing AB and medium only without cells. The % AB reduced corresponded to the percentage of viable cells and was a functional indicator of cell viability in response to ARV drug treatment over 24–72 h.

xCELLigence RTCA Cell Proliferation and Cytotoxicity Assay

Cell proliferation was measured using the xCELLigence Real-Time Cellular Analysis (RTCA) system (ACEA Biosciences), which allows researchers to monitor the cell viability and cell growth continuously at multiple time points. Cells were seeded at a density of 1×10^4 cells per well of the 16-well E-Plate and this was placed on the docking station contained within the incubator. The cells were then left to grow for 24 h with the RTCA instrument taking readings every minute. Following this, cells were treated with EFV (4, 13, 50 μM) or LPV/r (10, 32, 80 μM). A vehicle control consisted of 0.1% v/v methanol. During the treatment phase, the cells were continuously monitored for up to 100 h, with a reading being taken every 15 min. Cell sensor impedance was expressed as an arbitrary unit termed the Cell Index (CI). To eliminate variation between wells, the cell index values were normalized to the value at the beginning of treatment time-point; and thus, a normalized cell index (NCI) was used to determine cell viability.

Cell-Cycle Analysis by FACS

Analysis of the cell cycle distribution in response to ARV treatment was performed by seeding 1×10^5 cells/ml overnight in 25 cm^2 flasks and treating them with one of four different concentrations of EFV (4, 13, 50 μM), or with one of four concentrations of LPV/r (10, 32, 80 μM) for 24–48 h. After treatment cells were fixed with 70% ethanol at −20°C for 1 h. Next, cells were washed twice with PBS, treated with 10 mg/ml RNAse (Sigma) and stained with 25 μl of PI (1 mg/ml), (Sigma) and incubated at 4°C overnight in the dark. All experiments were performed in triplicate. The stained cells were analyzed on the BD Accuri C6 FACS instrument and results were generated and analyzed as histograms (G1, S, and G2 phases) using the BD C6 Accuri software.

Apoptosis Assay Using Annexin V-FITC and Propidium Iodide (PI) Dual Staining

In order to carry out an apoptosis assay by flow cytometry, MRC-5 and A549 cells were seeded at a density of 1×10^5/ml in 25 cm^2 flask overnight before being treated LPV/r at various concentrations for 24–48 h. Camptothecin (CPT) (50 μM) (Sigma) treatment was used as a positive control to induce apoptosis. Determination of apoptotic cell numbers by fluorescent staining was done using the Annexin V FITC/PI apoptosis kit from Santa Cruz Biotechnology, following manufacturer's instructions. Briefly, cells were incubated in triplicate with Annexin V FITC and propidium iodide (PI) in binding buffer for 15 min in the dark; and stained cells were immediately subjected to flow cytometry analyses using the BD C6 Accuri flow cytometer (BD Biosciences).

Statistical Analysis

Results for this study were analyzed using Graph-Pad Prism 5 and expressed as means ± standard error of the mean (SEM). Significant differences were determined using one-way analysis of variance (ANOVA) followed by Tukey's *post-hoc* test. A probability level of $p < 0.05$ was considered significant.

RESULTS

Alamar Blue Assay, Figure 1

The physiological reduction of the Alamar Blue (AB) dye was used here to quantitatively measure both cell proliferation and viability of MRC-5 and A549 cells in either EFV or LPV/r treated and vehicle control cells.

Efavirenz (EFV) Treatment, Figures 1A,B

The reduction of AB was monitored at 24 h intervals (24, 48, and 72 h) and measured spectrophotometrically at 540 and 630 nm. **Figures 1A,B** illustrate the percentage reduction of AB by MRC-5 and A549 cells in response to EFV, respectively. As represented in **Figure 1**, 4 μM EFV did not significantly change cell viability over a 24–72 h treatment period. At 13 μM (physiological dose and indicated by the blue box), the slight increase in cell proliferation at all three-time intervals was not significant. Similarly, a decline in cell proliferation with 26 and 50 μM treatment was also not significant.

Lopinavir/Ritonavir (LPV/r) Treatment, Figures 1C,D

Cell proliferation and viability following LPV/r treatment is shown in **Figures 1C,D** for the MRC-5 and A549 cell lines, respectively. When compared to the control cells, the 10 μM LPV/r treatment, was shown to have insignificantly increased proliferation, while at 32 μM there was a slight but insignificant decrease in proliferation. Concentrations of 50 and 80 μM LPV/r, decreased MRC-5 cell viability (see **Figure 1C**), but these effects of LPV/r on MRC-5 cell viability were not significant. A change in AB% reduction in A549 cells was observed following treatment with a range of LPV/r concentrations: at 10 μM LPV/r, the cells proliferated relative to the vehicle control cells. A decline in AB% reduction occurred with the 32 μM LPV/r treatment at all three

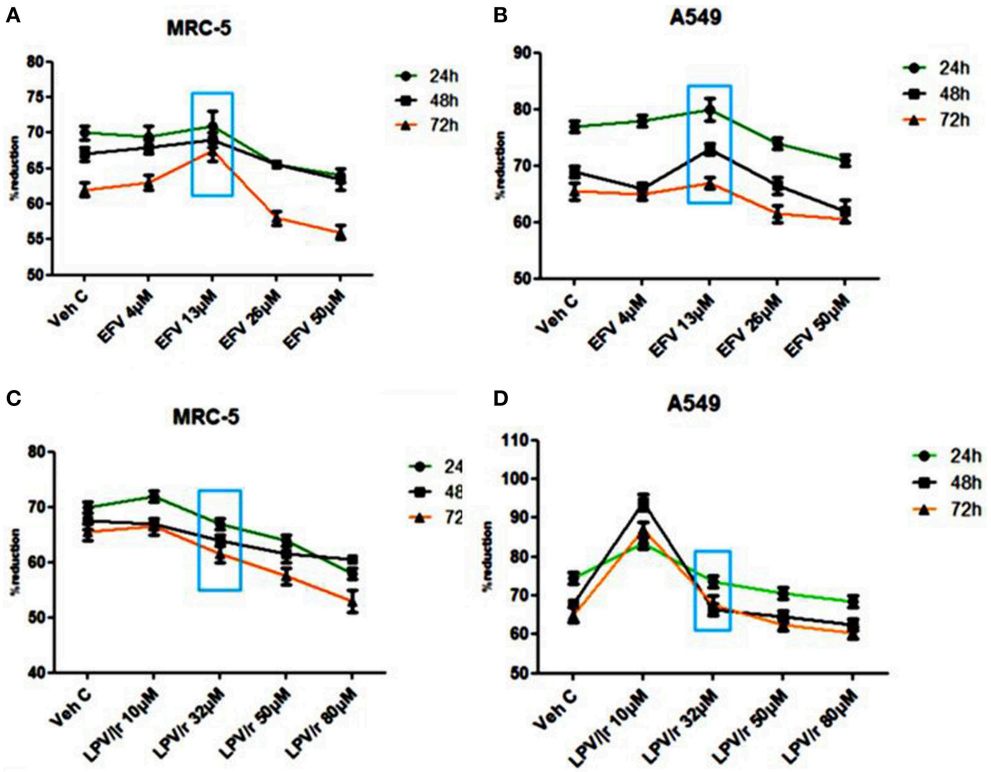

FIGURE 1 | Alamar blue assay analysis. **(A)** The percentage (%) of AB reduction representing the MRC-5 cell viability. **(B)** The A549 cell viability in response to the EFV drug treatment. **(C)** The MRC-5 cell viability in response to LPV/r drug treatment relative to control. **(D)** The representation of the A549 cell viability in response to the LPV/r cytotoxic effects. A–D represent treatments vs. control at 24, 48, and 72 h, the blue box indicates the most relevant physiological dose, and effects on cell viability are statistically insignificant, with $p > 0.0.5$. The graphs are a representative of three independent experiments, which were done in triplicate each.

time points. While treatment with both 50 and 80 μM LPV/r had an anti-proliferative effect on the A549 cells, the observed changes were however statistically not significant.

Real-Time Cell Analysis (RTCA) of Cytotoxicity Using xCELLigence, Figure 2

The potential cytotoxic effects of EFV on the MRC-5 and A549 cells were determined by plotting the growth curves acquired as a function of cell index (normalized to 1) vs. time (h) over a period of ∼100 h. Since the cell index is proportional to cell viability, the greater the cell index, the better the cell viability. Based on the preceding AB data, three of the four ARV concentrations were further selected for the cytotoxic, cell viability, and proliferation assays using RTCA. For these evaluations, both cell lines were treated with one of three concentrations of EFV (4, 13, 50 μM), respectively; or one of three concentrations of LPV/r (10, 32, 80 μM), respectively. To further analyse the effects of EFV and LPV/r on cell proliferation in a time dependent manner, the slope function of the curve was used. This function describes the steepness, incline, gradient, or changing rate of a curve within the given time period; and provides a measure for parameters of cell proliferation, cell adhesion, receptor activation, cytotoxicity, and other indicators of cell behavior. Here, the slope function was used to determine the rate of change of the cell index (CI) or normalized cell index (NCI) for the cells following drug treatment.

EFV Treatment Response in MRC5, Figures 2A,E

With reference to **Figure 2A**, following treatment at 24 h, all MRC5 cells whether treated with either EFV or with methanol (vehicle control) they continued to proliferate. Additionally, cells treated either with 4 μM or 13 μM EFV proliferated more than the vehicle control cells. In contrast to this, cells treated with 50 μM EFV had a decreased cell proliferation. The slope function of MRC-5 cells treated with either 4 μM or 13 μM EFV, indicated an increase in cell proliferation and growth after 24 h of treatment (**Figure 2E**). A steady decline in cell proliferation was noted at 48 h, this being more evident at 72 h, indicating the onset cell detachment/cell death. Treatment of MRC-5 cells with 50 μM EFV resulted in a slight increase in cell proliferation and growth at 24 h, with further growth at 48 h; followed by a steep decline in cell proliferation at 72 h.

A549 Cell Response to EFV, Figures 2B,F

After 24 h of exposure to the vehicle control and lower concentrations of EFV, A549 cells grew and proliferated steadily. At 48 h after treatment the vehicle control continued to proliferate steadily, while the cells treated with 4 and 13 μM

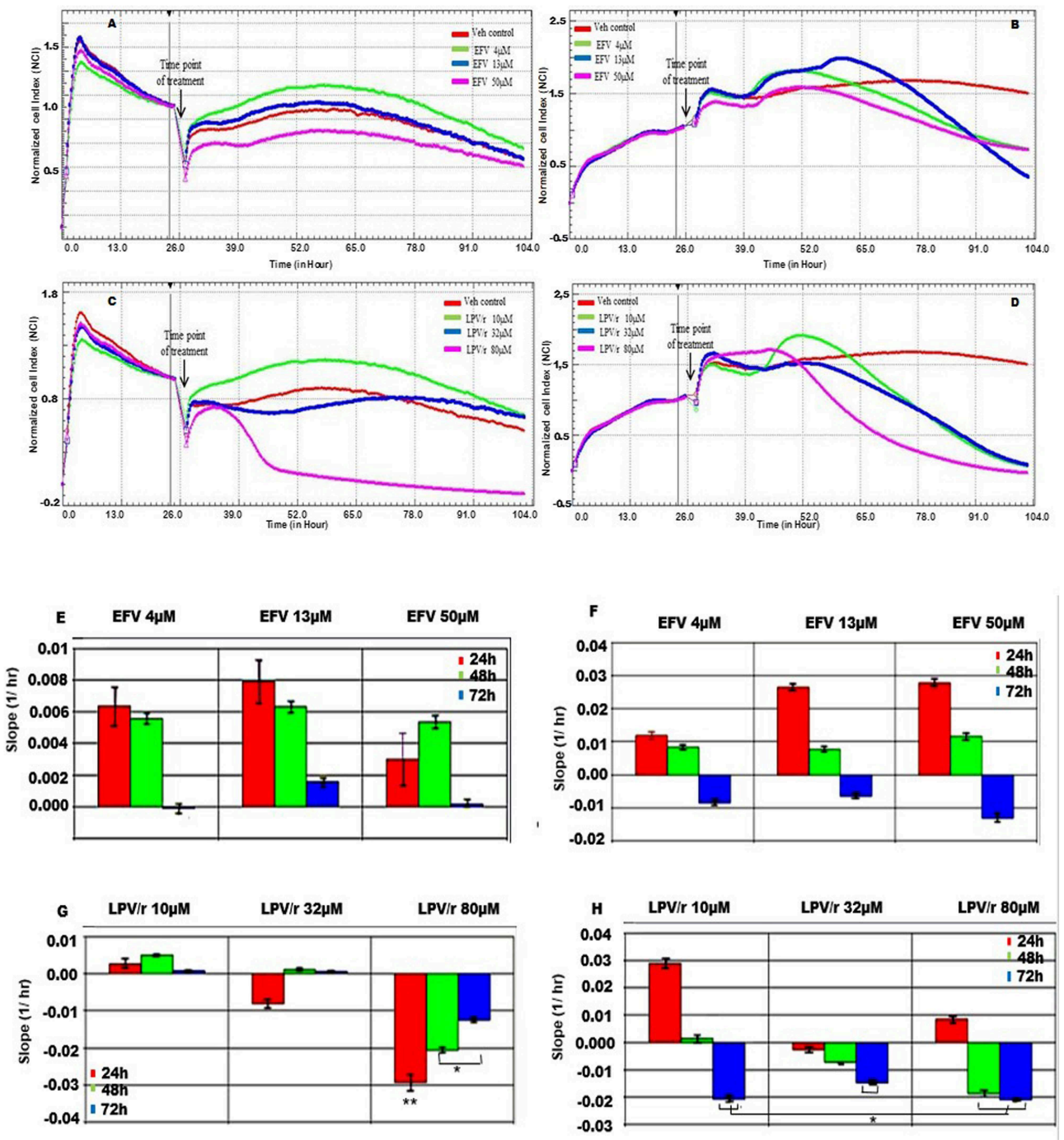

FIGURE 2 | MRC-5 and A549 cell proliferation in response to EFV and LPV/r. **(A)** Cell growth curve of MRC-5 cells treated with EFV **(B)** Growth curves representative of A549 cells treated with EFV. **(C)** MRC-5 growth curves representing cells treated with LPV/r. **(D)** Growth curves for A549 cells treated with LPV/r. The curves were plotted as a function of normalized CI vs. time in ARV treated vs. control. **(E)** The slope function of MRC-5 cells representing the response to EFV treatment over a 24 h time. **(F)** The slope function representing the response of A549 cells to EFV drug treatment at 24 h time intervals. **(G)** The slope function demonstrating MRC-5 cell response to LPV/r drug treatment, monitored at 24 h intervals. **(H)** A slope function representing A549 cells treated with LPV/r at 24 h intervals. The slope function represents the rate of cell detachment, and thus cell death for each of the drug concentrations. Results represent three independent experiments done in triplicate each. Error bars denote SEM; $*p < 0.05$; $**p < 0.01$.

EFV showed a decrease in cell viability (**Figure 2B**). Cells treated with 50 μM EFV proliferated slowly compared to the vehicle control cells, indicating an anti-proliferative effect of 50 μM EFV on the A549 cells. The slope function plot for cell response to EFV reflected a slight increase in cell proliferation for 4, 13, and 50 μM, respectively, after 24 h of treatment (**Figure 2F**). At 48 h

there was decreased cell proliferation, with a marked decline at 72 h in cell viability for each of the three drug concentrations.

MRC-5 Cell Response to LPV/r, Figures 2C,G

The MRC-5 vehicle control cells continued to grow and proliferate steadily. In comparison, MRC-5 cells treated with 10 μM LPV/r increased in proliferation compared to the vehicle control (**Figure 2C**). However, at a concentration of 32 μM LPV/r the cells neither increased nor decreased their proliferation, which indicated cell-cycle arrest. In contrast to this, treatment of MRC-5 cells with 80 μM LPV/r was clearly cytotoxic to the cells, indicated by an abrupt peak of the normalized CI immediately after drug treatment, followed by a rapid decline in cell viability. The slope-function plot reflected the growth trends of the real time growth curves (**Figure 2G**). At a concentration of 10 μM LPV/r the cells continued to grow progressively for 24 and 48 h after treatment, followed by a decline in cell viability after 72 h.When the cells were treated with 32 μM of LPV/r, there was a slight decrease in cell viability 24 h after treatment, followed by a slight increase in cell proliferation at 48 h; and this remained steady even after 72 h of drug exposure. At 24 h following 80 μM LPV/r treatment, there was a marked decline in cell viability. This decrease in cell viability persisted at 48 and 72 h after treatment.

A549 Cell Response to LPV/r, Figures 2D,H

The A549 cells were monitored before and after drug treatments at 24 h post seeding (refer to **Figure 2D**). When compared to the control cells, A549 cells treated with 10 μM and 80 μM LPV/r showed a proliferative effect, followed by a rapid decline in cell viability. The 32 μM treated cells in contrast, displayed a cell-cycle arrest (observed from the time point of treatment), after which there was a decrease in cell viability. The slope function plot for cell response to LPV/r revealed an apparent increase in A549 cell proliferation for cells treated with 10 μM LPV/r at 24 h, while there was a decline in cell viability when cells were treated with 32 μM LPV/r at 24 h. This steady decrease in cell viability for cells treated with 32 μM remained consistent even after periods of 48 and 72 h. There was an initial increase in proliferation for cells treated with 80 μM LPV/r (**Figure 2H**), followed by an abrupt decline in cell viability at 48 and 72 h.

RTCA Demonstrates the Pro-and-Anti-proliferative Effects of EFV and LPV/r

The label free RTCA assay was particularly sensitive to and indicative of the window period of the drug efficacy. This was reflected by the growth curves and further analyzed by the slope function, showing the associated decline in CI, and therefore in cell viability. At lower concentrations EFV had the effect of stimulating cell proliferation in both the MRC-5 and A549 cells, relative to vehicle control cells. Subsequently, proliferation (CI) decreased at higher concentrations with the occurrence of cellular detachment from the culture substrate. However, while there was no clear distinction here in the growth and proliferation patterns between treated and vehicle control cells, there were nevertheless differences observed in the decreases and increases in the proliferation rates between the vehicle control and treated cells. This finding suggests that although EFV treatment does seem to influence cell proliferation, it may not necessarily alter cellular health. Similar to EFV, LPV/r at low concentrations stimulated cell proliferation in both MRC-5 cells and excessively so in A549 cells, followed by cell death. An intermediate dose, caused cell-cycle arrest in both cell types, while high concentrations led to a significant increase in cell death, preceded by increased cell proliferation.

Cell-Cycle Analysis by FACS, Figure 3

Since RTCA analysis demonstrated some effects of EFV and LPV/r on the cell-cycle, flow cytometry was employed to quantify DNA content and thus the particular stage of the cell-cycle treated cells were in, relative to the vehicle control cells. Here, the scope of this analysis was to determine the regulatory effects of ARV's on the cell-cycle in lung cells. Prior to drug treatment and cell-cycle analysis, cells were serum-starved overnight to synchronize the cell-cycle at G0/G1. Results are a represented in **Figure 3** as bar graphs, where data is expressed as mean ± standard error of the mean (SEM).

FACS Analysis of EFV Treated MRC-5 Cells

About 73% of the vehicle control cells were located in the G0/G1 phase of the cell-cycle, at both 24 and 48 h. Relative to this, the percentage of cells in G0/G1 decreased with increased drug concentration at 24h, decreasing to about 60% (4 μM), 10.5% (13 μM), and 21% at 50 μM. At 48 h however, 54% cells treated with 4 μM were in G0/G1, before decreasing to 10% (13 μM) and 16.4% (50 μM). In association with this, the percentage of cells undergoing DNA synthesis in S-phase, began to significantly increase, from 3% in (normal) control cells, to 10% (4 μM), 60% (13 μM), and peaking at about 70% (50 μM), at both the 24 and 48 h time points. While about 20% of control cells were in G2/M, this percentage increased to ∼28–30% when cells were treated with 4 μM EFV; and decreased again to 18–19% of cells treated with 13 μM EFV; and further to about 5–6% of cells following treatment with 50 μM EFV (see **Figures 3A,B**).

FACS Analysis of EFV Treated A549 Cells

Approximately 80% of the vehicle control cells were located in the G0/G1 phase of the cell-cycle, at both 24 and 48 h. Relative to this, the percentage of cells in G0/G1 decreased with increased drug concentration at 24 h, reducing to about 58% when treated with 4 μM EFV, 33% when treated with 13 μM EFV and 13% when treated with 50 μM EFV. At 48 h however, 80% of cells treated with 4 μM EFV remained in the G0/G1 stage, before decreasing to 30% (13 μM) and 22% (50 μM). In relation to this, a significant increase in the proportion of cells in S-phase with increasing EFV dose was observed. This S-phase increase ranges from 11% in vehicle control cells, to 15, 40, and 55% in cells treated with 4, 13, and 50 μM treatment with EFV at 24 h. This trend was also noted at 48 h, when cells were treated with 4 μM EFV, some 80% of cells remained at G0/G1. In addition, there was an increased G2/M population when cells were treated with 4 μM EFV at both time points, from 4.6 to 16.1% at 24 h and 2.7 to 10% at 48 h (see **Figures 3C,D**). This however was not statistically significant.

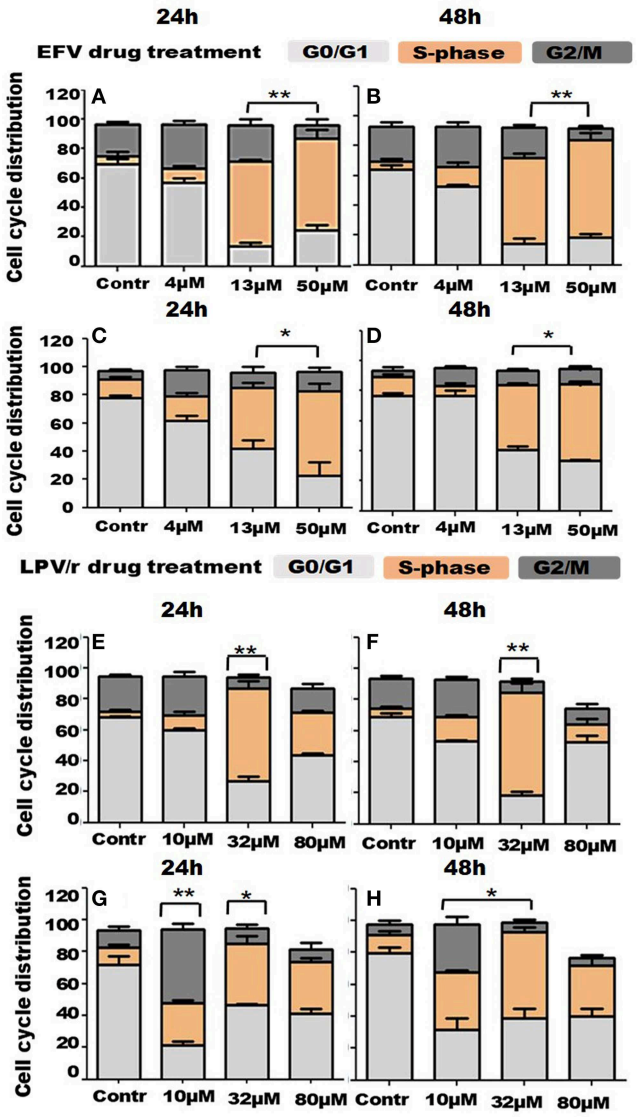

FIGURE 3 | Cell cycle distribution in response to ARV drugs. **(A,B)** Show bar graphs of EFV treated MRC-5 cells at 24 and 48 h. **(C,D)** Demonstrate bar graphs of A549 cells treated with EFV at 24 and 48 h. **(E,F)** Illustrate bar graphs of MRC-5 cells treated with LPV/r at 24 and 48 h, while **(G,H)** are bar graphs of A549 LPV/r treated cells at 24 and 48 h. The increase in S-phase, (S-phase arrest) observed here is statistically significant with $p < 0.0$ 1. A significant increase in G2/M with $p < 0.05$ in A549 10 μM LPV/r treated cells is also evident. Error bars denote SEM; $*p < 0.05$; $**p < 0.01$.

FACS Analysis of LPV/r Treated MRC-5 Cells

At 24 and 48 h some 60–70% of the vehicle control cells were located in the G0/G1 phase of the cell- cycle. Relative to this, the percentage of cells in G0/G1 decreased with increased drug concentration at both 24 and 48 h, dropping to about 51–52% following treatment with 10 μM LPV/r and 16–23% following treatment with 32 μM LPV/r. However, at the highest concentration of LPV/r treatment (80 μM), the percentage of cells in G0/G1 increased in the range of 45–57%. The percentage of cells in S-phase increased from ∼3% at 24 and 48 h in control cells to 65 and 72%, respectively, at 24 and 48 h, with the 32 μM LPV/r treatment. At 80 μM LPV/r, the percentage of cells synthesizing DNA decreased markedly to 26% at 24 h and 8% at 48 h. For G2/M phase, the proportion of cells increased marginally from about 30% to about 20% at 24 and 48 h, respectively, when treated with 10 μM LPV/r. At higher concentrations, the cell percentages decreased to below those at the levels of the control (see **Figures 3E,F**).

FACS Analysis of A549 Cells Treated With LPV/r

After drug treatment, the percentage of cells in G0/G1 decreased from 78 and 82% in vehicle control, to 19 and 24% when treated with 10 μM LPV/r. An increase in G2/M phase from 8 and 4% in vehicle control cells, to 42 and 22% when cells were treated with 10 μM LPV/r for the 24 and 48 h time points, respectively. When treated with 32 μM LPV/r this increased again to about 49 and 32%, at 24 and 48 h, respectively. At the highest concentration of LPV/r these percentages remaining in a similar range at both time periods. The relative stability of these proportions at the upper concentrations of LPV/r signifies an S-phase arrest. A sub-G0/G1 population was detected in response to 80 μM LPV/r, indicating cell-death (see **Figures 3G,H**).

Both EFV and LPV/r Alter the Cell-Cycle Stages

FACS analysis more precisely determined the effects of the ARV drugs, EFV, and LPV/r on cell-cycle stages. In summary, at low concentrations and at each time point, the ARVs effectively stimulated an increase in the percentage of cells in the G2/M phase in normal and cancerous cells. At higher concentrations, an S-phase arrest occurred, which is usually preceded by DNA damage. This results in cells with damaged DNA being unable to proceed to the G2 phase. Thus, it would seem that at higher concentrations LPV/r causes irreparable DNA damage, potentially leading to apoptosis. At the maximum ARV concentrations used here, cell viability was reduced, leading to the detection of a sub-G0/G1 cell population.

The Effect of ARVs on Apoptosis, Figure 4

The ability of LPV/r to induce programmed cell death is demonstrated by LPV/r having a cytotoxic effect on both the normal MRC-5 and cancerous A549 cells; whereas EFV in comparison did not seem to predispose cells to apoptosis. Further to this, the demonstration of a sub-G0/G1 population after LPV/r treatment prompted additional investigation of the cytotoxic/apoptotic effects of LPV/r.

Induction of Apoptosis by LPV/r

Following 32 and 80 μM LPV/r drug treatments, including Camptothecin (CPT) (50 μM) as positive control, FACS analysis using Annexin FITC and PI staining was used to quantify and analyse apoptosis in the lung cell lines. Control and treated cells were labeled with both Annexin FITC and PI. The control-unstained cells were used as a reference blank, the control-stained cells, the negative control, while Annexin-FITC single staining and PI single staining were used for compensation and setting up of quadrants. These results are represented in histograms and bar-graphs (**Figure 4**).

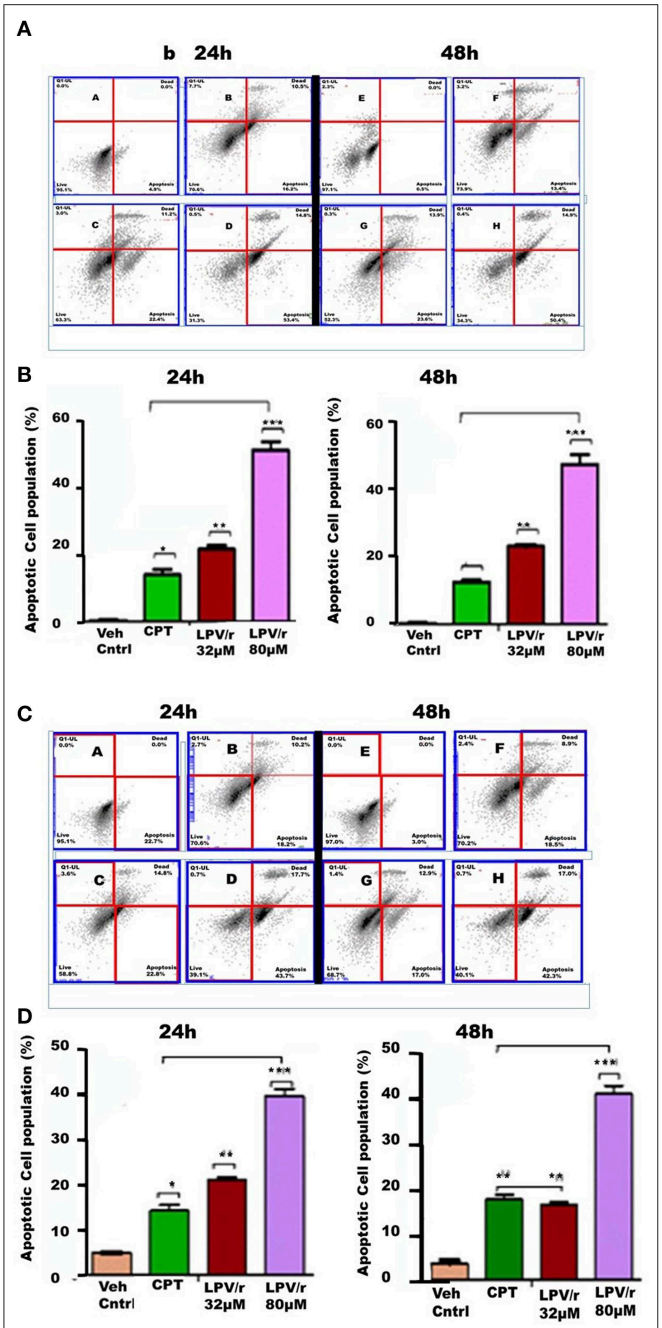

FIGURE 4 | FACS apoptosis analysis of MRC-5 and A549 cells in response to LPV/r. **(A,B)** Show cytograms and bar graphs of MRC-5 cells treated with LPV/r, while **(C,D)** represent cytograms and bar graphs of LPV/r treated A549 cells. Error bars denote standard error of the means (SEM); $*p < 0.05$; $**p < 0.01$; $***p < 0.001$. Results represent three independent experiments which were done three times independently.

LPV/r Drug Treatment Induces Cell Death (Apoptosis and Necrosis) in a Dose Dependent Manner

LPV/r at both, of 32 and 80 μM, induced apoptotic effects on normal and cancerous lung cells, acting to increase the percentage of cells undergoing apoptosis with an increasing LPV/r concentration. However, with this a significant coupled cellular necrosis occurred in both MRC-5 and A549 cells. As represented in **Figures 4A,B**, treatment of MRC-5 cells with 32 μM LPV/r led to a slightly higher degree of apoptosis, compared to the CPT treated MRC-5 cells; whilst this effect was only evident at 24 h in A549 cells, **Figures 4C,D**. With 80 μM LPV/r treatment although there was a doubling in the percentage of cells undergoing apoptosis compared to 32 μM treated cells (**Figure 4**), necrotic cell death nevertheless, did not increase with increasing LPV/r concentrations.

DISCUSSION

The cellular responses to antiretroviral treatment (ART) were assessed in real time to quantitate cell proliferation and to effectively determine cellular response to the pharmacological treatments. The ARVs acted to decrease cell viability in a dose-dependent manner in both cell lines. Notably, however, the two-plasma level equivalent EFV concentrations increased cell proliferation, while only the lowest LPV/r treatment caused a proliferative increase. Moreover, the most physiologically relevant LPV/r dose resulted in growth arrest in lung cancer cells. Thus, depending on concentration and at specific window periods of treatment, both EFV and LPV/r can exert either pro- or anti-tumorigenic effects on cells. The cell-cycle is normally a tightly regulated process with multiple control points at different phases of cell growth, with the failure or improper functioning of these check points potentially leading to either abnormal cell proliferation or apoptosis. In association with increased cell proliferation, subsequent cell-cycle analyses showed a significant increase in S-phase in response to ARV treatments; with an apoptosis inducing effect of one of the ARVs (LPV/r). However, it was noted that besides apoptosis, LPV/r treatment additionally triggered necrotic cell death in a time-dependent manner.

To date, several studies including (23, 24) have revealed the cytotoxic effects of EFV against several cancer cells including colorectal and pancreatic cancer, but to our knowledge, no study yet has shown the anti-proliferative effects of EFV on lung epithelial cancer cells in relation to the primary lung fibroblast cells. Notably, our study demonstrates the anti-proliferative effects rather than the cytotoxic effects of EFV on lung cells, particularly against the A549 cancer cells and sparing the normal fibroblast MRC-5 cells, as Hecht et al. (23) demonstrated (23). Jin et al. (25) also revealed that EFV increased the expression of CASP3 and BAX, thereby reducing the proliferation of neuronal stem cells (25). EFV also causes morphological changes in cells. EFV has been shown to cause apoptosis in the Human Squamous Cell carcinoma from Uterine Cervix (HCS-2) cells and a change was observed in morphological features such as rounding-up of cells, retraction of filopodia, blebbing, and maintenance of plasma membrane integrity- characteristic features of apoptosis (26).

The protease inhibitor (PI) lopinavir is used for the treatment of HIV infections (27–35). Lopinavir has been shown to induce proteotoxic and oxidative stress, and also suppress NF-κB activity

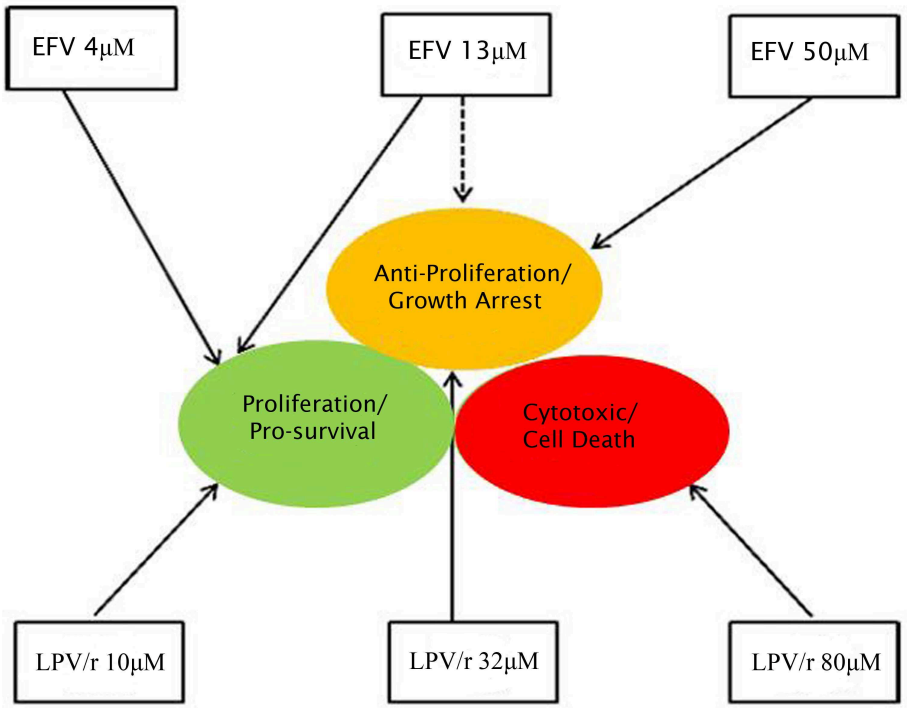

FIGURE 5 | Diagrammatic representation of the effects of EFV and LPV/r at low and high doses. Both EFV and LPV/r exhibit pro-survival effects at low doses, while anti-proliferative and cytotoxic effects are observed at high doses. The solid arrows represent the effects of the drugs on cellular health, while the dashed line shows partial/dual effect. At a high dose, EFV is anti-proliferative, arresting cellular growth, while low doses favor survival modes, as also observed with low LPV/r dose. In contrast, moderate (plasma-level) and high LPV/r doses have anti-proliferative and cytotoxic properties on the cells.

(36–38) The apoptotic and anti-tumor properties of LPV have been previously reported (39). Bissinger et al. (30) showed that LPV induced apoptosis in erythrocytes, accompanied by cell shrinkage and phospholipid scrambling (30). Okubo et al. (40) also showed the anti-proliferative properties of lopinavir/ritonavir (LPV/r) in combination against urological cancer cells. This study used 40/10 μM ratio of LPV/r over 48 h, and indicated that LPV/r treatment induced endoplasmic reticulum (ER) stress and kills urological cancer cells (40). Lopinavir was also shown to inhibit melanoma cell proliferation, induce morphological changes, apoptosis, and reactive oxygen species production, (41). A previous study revealed the anti-proliferative and cytotoxic effects of LPV/r at 20 μM) over 72 h in ovarian cancer. This was accompanied by G1 cell cycle arrest in ovarian cancer cells. LPV/r treatment in this cancer inhibited AKT signaling and this resulted in the inhibition of migration and invasion of ovarian cancer cells, and induction of apoptosis (42).

Based on these observations, it is proposed here that both EFV and LPV/r alter the cell-cycle progression of both normal and cancerous cells. In particular they lead to an arrest at the S-phase inhibiting further progression through the cycle, with LPV/r having the ability to inducing apoptosis. The apoptotic inducing properties of LPV/r merit further investigations not only as an ARV drug, but also as a potential anti-cancer treatment. However, a current limitation of LPV/r is its ability to not only kill tumor cells, but also to eliminate normal healthy cells. On the other hand, while an S-phase arrest is evident from both EFV and LPV/r treated cells, DNA damage usually precedes S-phase arrest. It follows then that both EFV and LPV/r could potentially be causing damage to the genomic DNA, with an arrest at S-phase, during which time there may be an attempt to either repair the damaged DNA or an induction of premature senescence, or even cell death. While the S-phase arrest is induced in the A549 lung cancer cells, it is also evident in the normal MRC-5 cells. This observation implicates both EFV and LPV/r as inducing stress on the DNA, with cells attempting to establish defense mechanisms by blocking the progression to G2/M phase. However, prolonged and constitutive stress effects of these ARVs on normal cells eventually exhaust the cells' repair mechanisms, and this may lead to uncontrolled cell proliferation and tumorigenesis. Furthermore, the cytotoxic effects of EFV on tumor cells such as colorectal cancers were shown by Hecht et al. (23), while primary fibroblast were unaffected. In addition, LPV/r's cytotoxic effects as a potential treatment for cancer was previously reviewed by Maksimovic-Ivanic et al. (43). The limitation of this study is the short exposure time (24–72 h) of lung cells to the ARVs, while in a clinical setting, patients on HAART have been exposed to these drugs for many years. In view of the double-edged properties of these drugs reported on in the present study, using patient samples may aid in a

better understanding of these findings. The great potential of repositioning EFV and LPV/r for the treatment of cancer is of paramount significance, as the repurposing of current drugs provide economic benefit as well as helping to fulfill the need for new cancer treatments.

SUMMARY

A model summarizing the pro- and anti-proliferative effects of EFV and LPV/r is represented in **Figure 5**. In this model, treatment of A549 and MRC-5 cells with various concentrations of EFV and LPV/r leads to either proliferative effects with lower concentrations, or a growth arrest with higher EFV concentrations and mid-level concentrations of LPV/r. Finally, treatment with higher concentrations of LPV/r led to cytotoxic effects on both cell lines.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available on request from the corresponding author.

AUTHOR CONTRIBUTIONS

RM and CP conceived and initiated this project. All experiments described in this manuscript were performed by RM who then generated all figures of this paper. RM, CP, RH, and ZD all contributed to the writing of this paper. RH generated the schematic summary of this manuscript, while ZD further edited this manuscript. All authors contributed to the article and approved the submitted version.

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Awareness of Cervical Cancer and Attitude Toward Human Papillomavirus and Its Vaccine Among Ghanaians

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Background: Cervical cancer (CC) is the fourth most commonly diagnosed cancer among women. Ghana is a low-middle- income country with annual diagnosed cases of 3,151 and 2,119 deaths. The high prevalence rate of cervical cancer in Ghana is mainly due to ineffective preventive measures and insufficient knowledge about the disease. Therefore, our objective was to evaluate the level of knowledge and awareness of cervical cancer and attitude toward human papillomavirus and its vaccine among Ghanaians.

Methods: This descriptive cross-sectional survey on the awareness of cervical cancer and attitude toward human papillomavirus and its vaccine was carried out from March 2019 to February 2020. SPSS v. 23.0 was used in the data analysis. The participants' demographic characteristics, knowledge of cervical carcinoma, human papillomavirus vaccine and HPV, and the likelihood to be vaccinated were represented as percentages and frequencies. The difference between males and females was assessed using the chi-square test. The logistic regression analysis was used to evaluate the relationship of possible related indicators with the willingness to receive the HPV vaccine. A $p < 0.05$ was considered statistically significant.

Results: A total of 1,376 participants were involved in the final analysis. Among the 1,376 participants involved in this survey, 1,240 participants (90.1%) representing 456 males (33.1%) and 784 females (57.0%) were aware of the terminology "cervical cancer" with a significant $p = 0.001$. When stratified by gender, women had significantly greater knowledge, compared to men in terms of "cervical cancer being common in middle age (35–50) females" (75.5 vs. 67.5%, respectively, $p \leq 0.001$). When stratified by gender, women had significantly greater knowledge of human papillomavirus (54.5 vs. 43.6%, respectively, $p < 0.001$) and the human papillomavirus vaccine (39.3 vs. 33.1%, respectively, $p = 0.019$) compared to men.

Conclusion: Majority of the respondents had poor knowledge regarding cervical cancer risk factors, symptoms, HPV, and its vaccine. Hence, this indicates a wakeup call for government to increase the awareness and knowledge level via the media and health professionals.

Keywords: Ghana, cervical cancer, vaccines, human papillomavirus, awareness

INTRODUCTION

Carcinoma of the cervix (CC) is the fourth most commonly diagnosed cancer among women with an annual new registered case of 569,847 and 311,365 deaths worldwide (1, 2). Human papillomaviruses (HPV) have been shown to be one of the most common pathogens transmitted through sexual contact in the cervix, and chronic infections of the cervix with high-risk human papillomavirus is required before cervical cancer can develop or occur (3). The HPV-18 and HPV-16 genotypes cause about 70% of the worldwide cervical cancer cases (4). When measured/estimated by sites, the cervix accounts for about 90% of human papillomavirus attributable global cancers, with two-thirds of that occurring in low and middle-income nations (5). This is primarily attributed to a lack of health insurance coverage in screening programs and a well-established nationwide screening system. Nevertheless, the WHO guide on the control of cervical cancer stated that the success of cervical cancer prevention and control mainly depends on cervical carcinoma screening programs (CCSP) and human papillomavirus vaccinations (6). The highest morbidity rates of cervical cancer were recorded in South–Eastern and South Central Asia, South-America, and sub-Saharan Africa (7). The age-standardization rates (ASR) per 100,000 women annually in West-Africa vary from 53.6 in Guinea, 39.5 in Ghana, 33.0 in Nigeria, 30 in Togo, and 28.6 in Burkina Faso in comparison to the 15.2 globally (8). The level of awareness and knowledge of cervical cancer etiology and HPV vaccination in women, to a great extent, influences their participation in screening and vaccination programs. Ghana is a low-middle-income country with annual diagnosed cases of 3,151 and 2,119 deaths, according to the 2018 ICO/IARC summary reports (9). These statistics, however, are prone to underestimate the actual nation's disease burden, as there exist imparity in the event of cervical cancer screening for females with different geographical and demographical indicators across the nation (10). In addition, most women in rural areas may not avail themselves during cervical cancer screening and HPV vaccination due to lack of knowledge, insufficient funding for health service, and high poverty rates (11). It has been generally acknowledged that health disparities are largely influenced by sociodemographic factors like welfare, unemployment, education, social and health care services, work environment, housing, and living (12). The high uptake and effective implementations of the HPV vaccines depend on the general public comprehension of HPV infections, and their ability to understand the efficacy of the HPV vaccines in preventing cervical cancer (13–16). Some studies have shown that encouragement from close relatives can influence the participation of women in cervical cancer screening and consenting to the children's vaccination. Ndejjo et al. (17) reported that Ugandan women who knew someone who had previously participated in the screening program would avail themselves to be screened. Furthermore, Anyebe et al. (18) and Cunningham et al. found that the willingness of women participating in cervical cancer screening was influenced by their husbands' or partners' decision in helping or encouraging them (18, 19). White et al. (20) also reported that most women in Zambia discuss their screening decisions with their close relatives or people within their immediate social circle. These pieces of evidence indicate that women who receive encouragement from their family, friends, or partners are more likely to participate in the screening program. Studies by Chao et al. (21) and Spencer et al. (22) also demonstrated the effect of women's attitudes in relation to human papillomavirus vaccination uptake in their children. They observed that daughters whose mothers undertook screening were more prone to get the vaccination than those whose mothers did not test or wanted to avoid screening personally. Hence, it was realistic to conclude that females who undergo screening are more willing to have their children vaccinated. Additionally, variables like cultural and religious values were reported to affect health practices. Modibbo et al. (23) noticed that those religious beliefs were a barrier to cervical screening. Consequently, a study by Masika et al. (24) discovered that certain religious beliefs were against vaccination. Past studies on HPV in Ghana focused on the prevalence rate and genotype. Domfeh et al. (25) reported a prevalence rate of 10.7% in 75 women seen in the outpatient department. Yar et al. (26) also reported a prevalence rate of 76.6% in 107 women who tested HIV negative and 42.0% in 100 women who tested HIV positive. Thus, no study has been conducted on the awareness of HPV and its vaccines. Furthermore, given that Cervarix vaccine has been introduced in Ghana, research on the perception of women in relation to human papillomavirus vaccinations are extremely important to assess the effects of past educational programs and further aid the decision-making process to promote these HPV vaccines. We, therefore, conducted this study to assess cervical cancer and HPV awareness, HPV vaccine, and the readiness of both men and women to receive these vaccinations since HPV can cause throat cancer, anal cancer, and genital warts in men. We hypothesized that females who are aware of cervical cancer are most likely to participate in vaccination and screening programs. We also hypothesized that religious beliefs have negative influence on the willingness to receive HPV vaccination.

MATERIALS AND METHODS

Study Population

This descriptive cross-sectional survey on the awareness of cervical cancer and attitude toward human papillomavirus and its vaccine was carried out from March 2019 to February 2020. The study population included (i) a Ghanaian resident, either male or female, (ii) must be 18 years and above, (iii) not deaf and dumb, and (iv) women with no history of HPV vaccination. The target population of men and women mostly resided in either Accra, Kumasi, or Takoradi. These three cities were chosen due to their population density and the availability of cervical cancer screening programs. The questionnaires were designed after a comprehensive review of literature from past studies and then approved by experts (27–29). The soundness and legitimacy of the questionnaire were further verified by a review panel of two experts each in oncology, gynecology and obstetrics, and research methodology prior to the pilot survey. Three questions associated with symptoms and signs were modified, and two questions not related to the topic were deleted according to the comments from the expert. Afterwards, a pilot study was conducted with 30 respondents on the pre-final template to determine the questionnaire's clarity. Findings from the current and pilot study demonstrated that Cronbach's alpha was >0.70. Cronbach's alpha evaluates the internal reliability or consistency of a given dataset. The questionnaire-based survey was undertaken after all participants had given written consent, with their anonymity, and confidentiality maintained. The exclusion criteria included women diagnosed with cervical cancer, women with some gynecological condition, and participants who did not provide their consent. The sample size was determined using the minimum sample size formula; thus, "$n = Z^2\ P(1 - P)/d^2$; where, n = sample size; $Z = z$ statistic for a level of confidence. For the level of confidence of 95%, which is conventional, the Z-value is 1.96. P = expected prevalence or proportion (in proportion of one; if 46%, $P = 0.46$), and d = precision (in proportion of one, if 5%, $d = 0.05$)" (16). The calculated sample size was 382 using an expected proportion or prevalence (p) of 46%; $P = 0.46$ (13), considering a 95% confidence interval (CI) and a 5% marginal error. To cover for heterogeneity in the targeted population and further ensure that maximum responses were received, we increased the sampling size and targeted about 1,500 participants. Simple random sampling was used to attain the targeted sample size.

Data Collection

The selection of group and the designing of our questionnaire was based on (30) Theory of Triadic Influence (TTI) and McLeroy et al. (31) Social Ecological Model (SEM). The Theory of Triadic Influence considers a "3 × 3 frameworks with environmental streams of influence, interpersonal, and intrapersonal crossed by proximal, distal, and ultimate levels of influence." The Social-Ecological Model (SEM) considers public policy, community, institutional, interpersonal, and intrapersonal as levels of influence for health-related attitudes. Many theoretical concepts are shared by these frameworks, even though these frameworks differ in variable and structure interaction thus, integrating them in this study. Each question in this survey was adapted and modified from previously published articles, and experts' opinions and was written in English in clear and straightforward language.

To aid the respondents answer the questions easily and quickly, the questions covered in the questionnaires, was categorized into sociodemographic, knowledge on cervical cancer, knowledge of HPV vaccine and HPV, the willingness to receive the HPV vaccination themselves and also having their children vaccinated, the rationale for not being willing to be vaccinated and the acceptability to pay for the human papillomavirus vaccination by themselves and interview quality evaluation. The cervical cancer section was subcategorized into (a) knowledge about cervical cancer, (b) knowledge about cervical cancer symptoms, and (c) knowledge about risk factors of cervical cancer. Knowledge of cervical cancer was evaluated if a participant responded that they were aware of cervical carcinoma by stating that one has heard or knows about cervical cancer. The participant's knowledge regarding the risk factors ("Can HPV infection cause cervical cancer," "long term use of oral contraceptives pills," "smoking," "unprotected sexual practices," "multiparity," "Immunocompromised/HIV-AIDS," "early age at marriage") and symptoms ("lower abdominal pain," "bleeding after sexual intercourse," "bleeding in between periods," "vaginal discharge with foul smell," "weight loss," "post-menopausal bleeding," and "asymptomatic") of cervical carcinoma was evaluated. A 30-point score was used to evaluate cervical cancer knowledge. Five points were allocated to every sub-section of knowledge; hence participants were required to range between 0 and 30 scores. One point was allocated to each true response, and zero points to the incorrect response. Participants who responded only "Yes" to the questionnaire's first statement, "Do you know about cervical cancer?" were assigned a knowledge score. Participants' level of knowledge while calculating the knowledge score was categorized using Bloom's cut off point (32). Participants who had from 24 to 30 point were regarded as having excellent knowledge with right answers of 80–100%, participants who scored from 18 to 23 point were regarded as having moderate knowledge with right answers of 60–79%, and participants who scored <18 points were lastly regarded as having poor knowledge with right answers of less than 60%. Information on cervical carcinoma was given to all participants to bridge the knowledge disparity after the end of the cervical cancer sub-section.

HPV awareness was evaluated with the phrase, "Have you heard of HPV?" Participants who responded "yes" to this statement were regarded to have knowledge about HPV. The knowledge on HPV vaccine was evaluated in the same manner. Some previous studies have reported these questions (29). Other relevant questions such as, "Is HPV infection a sexually transmitted infection?," "Is a persistent infection of high-risk HPV the leading cause of cervical cancer and other HPV cancer types?," "Can the HPV vaccine prevent cervical cancer and other HPV cancer types?," and "Must the HPV vaccination be received before the first sexual intercourse?" were preliminary used in evaluating participants' knowledge concerning human papillomavirus and its vaccine. Similar questions used by past

studies (28) in evaluating the HPV vaccination acceptability by asking, "Are you willing to vaccinate your current or future children both male and female?," "Are you willing to vaccinate yourself?," and "Will you accept that you pay for the HPV vaccination by yourself?" were also used in this study. Specific questions contained three possible outcomes (don't know, no, yes); however, the "don't know" option was regarded as an incorrect response.

Data Analysis

SPSS v. 23.0 was used in the data analysis. The participants' demographic characteristics, knowledge of cervical carcinoma, human papillomavirus vaccine and HPV, and likelihood to be vaccinated were represented by percentages and frequencies. The difference between males and females was assessed using the chi-square test. The logistic regression analysis was used to evaluate the relationship of possible related indicators with the willingness to receive the HPV vaccine. Indicators in the univariate model were integrated into a multivariate logistic regression, in which confidence intervals of 95% and the adjusted odds ratio were estimated. A stratified assessment was conducted to determine whether gender affected the factors correlated with the willingness to be vaccinated. A $p < 0.05$ was considered statistically significant.

RESULTS

Sociodemographic Characteristics

Of the total 1,500 survey respondents, 124 answered the questionnaires with inconsistent and incomplete responses. After eliminating the inconsistent and incomplete questionnaires, the remaining questionnaires were analyzed and the total response rate was 91.73%. A total of 1,376 participants were involved in the final analysis. **Table 1** represents the sociodemographic characteristic of the participants. The participants' mean age was 35.5 [Standard Deviation (SD) ±6.4] years. A total of 532 (38.7%) were males, and the remaining 844 (61.3%) were females. Among them, 1,316 (95.6%; males = 496, females = 820) were Christians. The proportions of ethnicity based on Akan, Ewe, Ga, and others were 71.8, 12.8, 7.3, and 8.1%, respectively. Five-point eight percent of the participants had been educated at the senior high school level and below. Sixteen-point, six percent of the respondents, were not on any insurance policy, and 46.8% had a monthly income of <2,000 Ghana cedis equivalent to $350. Fifty-one-point, 1% of the respondents, were working, and 39.5% were students. Majority of the respondents were single (86.9%) and 61.0% (males = 292, females = 548) had their first sexual intercourse at age 18 years old and above with 47.4% (males = 220, females = 432) having only "one sexual partner in the past 6 months." Statistical significance was noticed in most of the sociodemographic variables except medical insurance, marital status, and age.

TABLE 1 | Sociodemographic characteristics of participants.

Sociodemographic characteristics	Gender		Chi-square	*p*-value
	Male (*N* = 532)	Female (*N* = 844)		
Age				
<40	508 (95.5)	804 (95.3)		
40–60	20 (3.8)	32 (3.8)	0.142[a]	0.974[a]
Above 60	4 (0.8)	8 (0.9)		
Tribe				
Akan	396 (74.4)	592 (70.1)		
Ewe	64 (12.0)	112 (13.3)	11.127[b]	0.011[b]
Ga	24 (4.5)	76 (9.0)		
Others	48 (3.5)	64 (4.7)		
Religion				
Christian	496 (93.2)	820 (97.2)		
Muslim	28 (5.3)	20 (2.4)	14.472[a]	0.001[a]
Traditionalist	4 (0.8)	4 (0.5)		
Others	4 (0.8)	0 (0)		
Education				
Junior high school or below	4 (0.8)	4 (0.5)		
Senior high school	20 (3.8)	48 (5.7)	5.246[a]	0.133[a]
College/graduate and above	508 (95.5)	788 (93.4)		
Not applicable	0	4 (0.5)		
Occupation				
Student	232 (43.6)	312 (37.0)		
Working	252 (47.4)	452 (53.6)	11.215[b]	0.011[b]
Retired	12 (2.3)	8 (0.9)		
Unemployed	36 (6.8)	72 (8.5)		
Marital status				
Single/divorced/widow	468 (88.0)	728 (86.3)	0.843[b]	0.359[b]
Married	64 (12.0)	116 (13.7)		
Medical Insurance				
No insurance	104 (19.5)	124 (14.7)		
NHIS	364 (68.4)	612 (72.5)	5.716[b]	0.126[b]
Commercial Insurance	28 (5.3)	44 (5.2)		
Company Insurance	36 (6.8)	64 (7.6)		
Age at sex debut (year)				
<18	76 (14.3)	72 (8.5)		
>18	292 (54.9)	548 (64.9)	27.567[b]	< 0.001[b]
Don't know	48 (9.0)	36 (4.3)		
None	116 (21.8)	188 (22.3)		
Age at menarche (year)				
<12	0	144 (17.1)		
>12	0	660 (78.2)	1,813.155[a]	<0.001[a]
Unknown	0	40 (4.7)		
Not applicable for male	532	0		

[a]*Fisher's exact analysis was performed for tables which had at least one expected value <5 in the cells.* [b]*Pearson Chi-square test was performed for tables with 0 expectant cell count. The color values means Fisher's exact analysis was used.*

Knowledge About Cervical Cancer

Among the 1,376 participants involved in this survey, 1,240 participants (90.1%) representing 456 males (33.1%) and 784 females (57.0%) were aware of the terminology "cervical cancer." When stratified by gender, women had significantly greater knowledge, compared to men in terms of "cervical cancer being common in middle age (35–50) females" (75.5

vs. 67.5%, respectively, $p \leq 0.001$). These participants were examined further to test their knowledge on some risk factors and symptoms of cervical cancer, as presented in **Tables 2–4**. Majority of the respondents were aware of "bleeding after sexual intercourse (correctly identified by 51.8% of men and 70.4% of women, $p \leq 0.001$)," "lower abdominal pain (correctly identified by 59.6% of men and 71.9% of women, $p \leq 0.001$)" and "vaginal discharge with foul smell (correctly identified by 61.4% of men and 68.4% of women, $p \leq 0.001$)" as being the dominant cervical cancer symptoms. Likewise, a high proportion among the responses regarding the risk factors "Human papillomavirus infection (correctly identified by 47.4% of men and 55.6% of women, $p = 0.010$)" and "unprotected sexual practices (correctly identified by 50.9% of men and 63.8% of women, $p \leq 0.001$)" was noticed. Our survey respondents were ranked in each sub-category according to their level of knowledge in cervical cancer epidemiology, symptoms, and risk factors. In general, 75.3% of the respondents had good knowledge of cervical carcinoma epidemiology; however, it was accompanied by moderate knowledge in terms of cervical cancer risk factors (63.8%) and symptoms (61.6%) per the Bloom's cut-off point for accessing knowledge level. Respondents were asked regarding sources of information on cervical carcinoma and the main sources were social media/radio/television ($N = 851$, 68.8%), nurses/doctors ($N = 507$, 40.9%), newspapers/magazines ($N = 255$, 20.7%), and relatives/family ($N = 221$, 17.8%).

TABLE 2 | Knowledge on cervical cancer epidemiology.

Variable	Gender		Chi-square	*p*-value
	Male (*N* = 532)	Female (*N* = 844)		
Do you know about cervical cancer?				
Yes	456 (85.7)	784 (92.9)	18.87	<0.001
No	76 (14.3)	60 (7.1)		
Is cervical cancer a communicable disease (transmitted by skin contact, sneezing, and coughing)[a]				
Yes	16 (3.5)	16 (2.0)	39.221	<0.001
No	364 (79.8)	720 (91.8)		
Don't know	76 (16.7)	48 (6.1)		
Is cervical cancer more common in middle age females?[a]				
Yes	324 (71.1)	668 (85.2)	36.086	<0.001
No	125 (27.4)	106 (13.5)		
Don't know	7 (1.5)	10 (1.3)		
Are all women at risk of developing cervical cancer?[a]				
Yes	308 (67.5)	592 (75.5)	18.989	<0.001
No	68 (14.9)	120 (15.3)		
Don't know	80 (17.5)	72 (9.2)		
Is cervical cancer more common in middle age females?[a]				
Yes	264 (57.9)	584 (74.5)	39.221	<0.001
No	28 (6.1)	48 (6.1)		
Don't know	164 (36.0)	152 (19.4)		

Values are presented as number (%). The chi-square test was used and $p < 0.05$ was considered as statistically significant.

[a]*Only participants who have heard of cervical cancer answered these questions.*

Knowledge About HPV and Its Vaccine

As presented in **Table 5** of the participant who answered the questions, 50.3% ($N = 692$) have "heard of HPV," and only 36.9% have "heard of the HPV vaccine." When stratified by gender, women had significantly greater knowledge of human papillomavirus (54.5 vs. 43.6%, respectively, $p < 0.001$) and the human papillomavirus vaccine (39.3 vs. 33.1%, respectively, $p = 0.019$) compared to men.

Among the respondents who have heard of HPV, 59.8% ($N = 414$) of the respondents were aware that HPV infection is transmitted through sexual contact (correctly identified by 48.7% of men and 65.4% of women, $p < 0.001$) and 75.1% of the respondents were aware that "the persistent infection of high-risk HPV is the leading cause of cervical cancer and other HPV cancer types" with a significant $p < 0.001$. Furthermore, among respondents with knowledge of the HPV vaccine, only 55.9% ($N = 284$, $p < 0.001$) knew that cervical cancer could be prevented with the HPV vaccine. Additionally, only 21.7% ($N = 110$, $p = 0.001$) knew that "HPV vaccination is needed before first sexual intercourse."

TABLE 3 | Knowledge on cervical cancer symptoms answered by only participants who have heard of cervical cancer.

Symptoms	Gender		Chi-square	*p*-value
	Male (*N* = 456)	Female (*N* = 784)		
Asymptomatic (no symptoms)				
Yes	96 (21.1)	252 (32.1)	18.75	<0.001
No	136 (29.8)	220 (28.1)		
Don't know	224 (49.1)	312 (39.8)		
Post-menopausal bleeding				
Yes	200 (43.9)	424 (54.1)	15.315	<0.001
No	20 (4.4)	44 (5.6)		
Don't know	236 (51.8)	316 (40.3)		
Weight loss				
Yes	180 (39.5)	420 (53.6)	23.226	<0.001
No	56 (12.3)	80 (10.2)		
Don't know	220 (48.2)	284 (36.2)		
Vaginal discharge with foul smell				
Yes	280 (61.4)	536 (68.4)	6.739	<0.001
No	32 (7.0)	52 (6.6)		
Don't know	144 (31.6)	196 (25.0)		
Bleeding in between periods				
Yes	212 (46.5%)	484 (61.7)	29.448	<0.001
No	20 (4.4)	36 (4.6)		
Don't know	224 (49.1)	264 (33.7)		
Bleeding after sexual intercourse				
Yes	236 (51.8)	552 (70.4)	62.377	<0.001
No	20 (4.4)	52 (6.6)		
Don't know	200 (43.9)	180 (23.0)		
Lower abdominal pain				
Yes	272 (59.6)	564 (71.9)	19.905	<0.001
No	32 (7.0)	36 (4.6)		
Don't know	152 (33.3)	184 (23.5)		

TABLE 4 | Knowledge on cervical cancer risk factors answered by only participants who have heard of cervical cancer.

Risk factors	Gender		Chi-square	*p*-value
	Male (*N* = 456)	Female (*N* = 784)		
Early age at marriage				
Yes	88 (19.3)	168 (21.4)	3.544	0.170
No	204 (44.7)	308 (39.3)		
Don't know	164 (36.0)	308 (39.3)		
Immunocompromised/Human immunodeficiency virus/AIDS				
Yes	160 (35.1)	276 (35.2)	7.048	0.029
No	128 (28.1)	172 (21.9)		
Don't know	168 (36.8)	336 (42.9)		
Multiparity (giving birth to more than 3 children)				
Yes	48 (10.5)	112 (14.3)	8.511	0.015
No	220 (48.2)	316 (40.3)		
Don't know	188 (41.2)	356 (45.4)		
Unprotected sexual practices				
Yes	232 (50.9)	500 (63.8)	25.342	<0.001
No	52 (11.4)	92 (11.7)		
Don't know	172 (37.7)	192 (24.5)		
Smoking				
Yes	192 (42.1)	388 (49.5)	14.575	0.001
No	40 (8.8)	96 (12.2)		
Don't know	224 (49.1)	300 (38.3)		
Long term use of oral contraceptives pills				
Yes	208 (45.6)	396 (50.5)	14.216	0.001
No	28 (6.1)	84 (10.7)		
Don't know	220 (48.2)	304 (38.8)		
Human papillomavirus (HPV) infection				
Yes	216 (47.4)	436 (55.6)	9.117	0.010
No	16 (3.5)	32 (4.1)		
Don't know	224 (49.1)	316 (40.3)		

In general, 80.5% (N = 1,108) respondents were willing to have the HPV vaccination. Likewise, women had significantly greater willingness, compared to men (89.6 vs. 66.2%, respectively, $p < 0.001$). A total of 83.9% of women were willing to vaccinate their current and future children. Furthermore, the major reasons for respondents refusing to undertake the HPV vaccinations were "worrying about the safety of vaccine (30.2%)," "the HPV vaccine has not been widely accepted (13.1%)," "worrying about the price (13.1%)," and "worry about the effectiveness (12.7%)." Participants who said no to the payment for the HPV vaccine suggested that, WHO (70.4%) and the government (60.5%) should ensure the free supply of the HPV vaccine.

Willingness to Receive HPV Vaccine and Its Associated Factors

The bivariate regression analysis demonstrated a significant relationship exists between the willingness to be vaccinated and age, religion, economic status, education, age at first sex, participants with knowledge of cervical, HPV and its vaccine. This finding showed age 18–35 years (OR = 1.475; 95%CI = 1.142–1.591), respondents who are Christians (OR = 1.275; 95%CI = 0.729–1.459), college/graduate students (OR = 1.218; 95%CI = 1.054–1.878), respondents who had their first sex at age above18 years (OR = 1.670; 95%CI = 1.484–1.929), respondents with monthly income 2,000–3,999 Ghana cedis (OR = 1.686; 95%CI = 1.136–2.501), respondent with knowledge about CC (OR = 0.541; 95%CI = 0.364–0.803), respondent who have heard of HPV (OR = 0.760; 95%CI = 0.581–0.993) and heard of HPV vaccine (OR = 0.870; 95%CI = 0.657–1.150) were more willing to receive the HPV vaccinations. Hence, a strong association between these variables and a respondent willingness to be vaccinated. **Table 6** shows the outcome of the univariate and multivariate logistics analysis.

DISCUSSION

Advancement in understanding cervical carcinoma has been effective in acknowledging its preventive nature (33). It is firmly known that effective screening and HPV vaccination, to a large extent, will significantly decrease the prevalence of the disease (33, 34). For effective prophylaxis and screening, it is of paramount significance to understand the beliefs, perceptions, and knowledge of the general public. The assertion was that, females who were aware of cervical carcinoma are most likely to participate in vaccination and screening programs. Our findings confirmed the hypothesis when a participant responded that they know about cervical cancer, HPV, and its vaccine. This was evident in both women and men. Men who know of cervical carcinoma were most likely to offer encouragement to their partners to participate in vaccination and screening programs. The total awareness of study participants was poor, similar to past studies (35). These findings also complement the results of a systematic review, which reported lower knowledge levels of cervical cancer awareness but a higher willingness to receive vaccination in sub-Saharan Africa (36).

Our study findings showed that majority (90.1%) of the participants were knowledgeable of the term cervical cancer which is higher when compared with other similar studies in developing countries such as Pakistan, Ethiopia, and Zambia and where the percentage of participants knowledgeable of the term cervical cancer were 51.3, 76.8, and 36.8%, respectively (29, 37, 38). The variation may be attributable to the dissemination of information through various mass media and the availability of screening programs in Ghana. The knowledge of respondents on cervical cancer showed that 63.8% of participants know of cervical cancer risk factors.

Among these risk factors, "human papillomavirus infection" and "unprotected sexual practices" were correctly and highly identified as cervical cancer risk factors. This finding is lower when compared to past studies conducted in South Africa, Bhutan, Malaysia, and Ukraine (39–42). This lack of awareness normally leads to the higher death rate related with CC because women who are not enlightened about these risk factors will not undertake the appropriate preventive actions.

Regarding awareness of cervical cancer symptoms, respondents were aware of symptoms such as "vaginal discharge with a foul smell," "bleeding after sexual intercourse," and "bleeding in-between period" (67.8, 63.5, and 56.1%,

TABLE 5 | Attitude and awareness of HPV and the HPV vaccine among participants.

Items	Total (*N* = 1,376)	Male (*N* = 532)	Female (*N* = 844)	*p*-value
Have you heard of HPV?				
Yes	692 (50.3)	232 (43.6)	460 (54.5)	<0.001
No	684 (49.7)	300 (56.4)	384 (45.4)	
Is HPV infection a sexually transmitted infection?[a]				
Yes	414 (59.8)	113 (48.7)	301 (65.4)	<0.001
No	278 (40.2)	119 (51.3)	159 (34.6)	
Is persistent infection of high-risk HPV the leading cause of cervical cancer and other HPV cancer types?[a]				
Yes	520 (75.1)	155 (66.8)	365 (79.3)	<0.001
No	172 (24.9)	77 (33.2)	95 (20.7)	
Have you heard of the HPV vaccine?				
Yes	508 (36.9)	176 (33.1)	332 (39.3)	<0.001
No	868 (63.1)	356 (66.9)	512 (60.7)	
Can the HPV vaccine prevent cervical cancer and other HPV cancer types?[b]				
Yes	284 (55.9)	80 (45.5)	204 (61.4)	<0.001
No	224 (44.1)	96 (54.5)	128 (38.6)	
Must the HPV vaccination be received before the first sexual intercourse?[b]				
Yes	110 (21.7)	52 (29.5)	58 (17.5)	0.001
No	398 (78.3)	124 (70.5)	274 (82.5)	
Are you willing to vaccinate yourself?				
Yes	1,108 (80.5)	352 (66.2)	756 (89.6)	<0.001
No	268 (19.5)	180 (33.8)	88 (10.4)	
Are you willing to vaccinate your current or future children both male and female?[c]				
Yes	900 (81.2)	266 (75.6)	634 (83.9)	<0.001
No	208 (18.8)	86 (24.4)	122 (16.1)	
What are your reasons for unwillingness to take the HPV vaccine				
Worry about the safety	81 (30.2)	38 (21.1)	43 (48.9)	0.001
The HPV vaccine has not been widely accepted	35 (13.1)	23 (12.8)	12 (13.6)	
Worry about the price	35 (13.1)	15 (8.3)	20 (22.7)	
Worry about the effectiveness	34 (12.7)	28 (15.6)	6 (6.8)	
Not considering themselves at risk of cervical cancer	30 (11.2)	27 (15.0)	3 (3.4)	
The vaccine is not protective	28 (10.4)	26 (14.4)	2 (2.3)	
Other reasons	25 (8.6)	23 (12.8)	2 (2.3)	
Will you accept that you pay for the HPV vaccination by yourself?[d]				
Yes	331 (29.9)	145 (41.2)	186 (24.6)	<0.001
No	777 (70.1)	207 (58.8)	570 (75.4)	

Values are presented as number (%). The chi-square test was used and $p < 0.05$ was considered as statistically significant. [a]Participants who have heard of human papillomavirus (HPV) answered these questions, [b]Participants who have heard of the HPV vaccine answered these questions, [c]Participants who are not willing to take the HPV vaccine answered the question, [d]Participants who are willing to take the HPV vaccine answered the question.

respectively). Our results indicate that much effort is needed to educate the general public, especially women of cervical cancer symptoms, since failure to recognize these symptoms or late presentation can results in delaying medical care, resulting in poor prognosis and higher death rates.

There is no doubt that basic knowledge is essential in encouraging women to patronage preventive actions. This is in line with the intrapersonal level regression findings for SEM, where basic knowledge concerning cervical cancer was the key predictor of attitude (38). Communities based educational programs have been shown to be effective in increasing preventive practices, knowledge, and awareness (43). Social media /radio/television, nurses/doctors, and newspapers/magazines were found as reliable information sources and they could offer potential targets for performing interventional studies intended to improve awareness of cervical cancer in Ghana. It was presumed that females who receive backing from close relatives could influence the participation of women in cervical cancer screening. The findings back the assertion about women participating in screening. It was observed that women's recognized that the acceptance of spouses, families, and friends affected their screening practices. This result is consistent with another study conducted in Zambia that assessed that relatives and peers often spurred women's choice to screen (20). Ndejjo et al. (17) reported that Ugandan women who knew someone who had previously participated in the screening program would avail themselves to be screened. Furthermore, Anyebe et al. (18) and Cunningham et al. (19) found that the willingness of women participating in cervical cancer screening was influenced by their husbands' or partners'

TABLE 6 | Variables associated with the willingness to receive HPV.

Variables	Willingness to receive HPV vaccination Odd Ratio 95% Confidence Interval	Willingness to receive HPV vaccination Adjusted odd ratio 95%Confidence Interval
Gender		
Male	1	**1**
Female	0.228 (0.171–0.303)	0.423 (0.272–0.504)
Age		
18–35	1.475 (1.142–1.591)	1.527 (1.177–1.684)
36–60	0.600 (0.154–2.344)	0.547 (0.121–2.412)
Above 60	1	1
Tribe		
Akan	1.639 (0.945–2.843)	1.862 (1.218–2.945)
Ewe	0. 769 (0.380–1.557)	0.804 (0.430–1.752)
Ga	1.500 (0.729–3.085)	1.532 (0.932–3.148)
Others	1	1
Religion		
Christian	1.275 (0.729–1.459)	1.672 (1.129–1.814)
Muslim	1.151 (0.523–2.233)	1.407 (0.779–2.476)
Traditionalist	1	1
Education		
Junior high school or below	1	1
Senior high school	0.700 (0.700–3.037)	1.056 (1.032–3.193)
College/graduate and above	1. 218 (1.054–1.878)	1.431 (1.101–1.922)
Occupation		
Student	1.354 (0.801–2.287)	1.453 (0.843–2.356)
Working	0.832 (0.492–1.408)	0.946 (0.563–1.503)
Retired	2.933 (2.933–8.117)	2.965 (2.945–8.271)
Unemployed	1	1
Marital status		
Single/divorced/widow	1	1
Married	1.213 (0.830–1.774)	1.275 (0.992–1.806)
Medical Insurance		
No insurance	1	1
NHIS	0.676 (0.480–0.952)	0.772 (0.566–1.028)
Commercial Insurance	1.536 (0.864–2.730)	1.653 (0.941–2.804)
Company Insurance	0.419 (0.213–0.822)	0. 526 (0.391–0.987)
Monthly Income (GH Cedis)		
<2,000	1	1
2,000–3,999	1.686 (1.136–2.501)	1.719 (1.145–2.643)
4,000–5,999	2.205 (1.062–4.577)	2.259 (1.167–4.689)
6,000–9,999	1.102 (0.360–3.378)	1.174 (0.463–3.387)
Above 10,000	2.205 (0.649–7.485)	2.249 (0.754–7.584)
Age at first sex		
<18	1	1
>18	1.670 (1.484–1.929)	1.708 (1.526–1.981)
Number of sexual partners in the past 6 months		
1	1	1
>2	2.369 (1.549–3.625)	2.532 (1.671–3.732)
Do you know about cervical cancer?		
Yes	0.541 (0.364–0.803)	0.758 (0.495–1.205)
No	**1**	1
Have you heard of HPV?		
Yes	0.760 (0.581–0.993)	0.928 (0.685–1.112)
No	**1**	1
Have you heard of HPV vaccine?		
Yes	0.870 (0.657–1.150)	1.147 (0.973–1.342)
No	1	

The bold values are reference values.

decisions in helping or encouraging them. In addition, with the exception of men, women would be more prone to have their daughter's vaccinated if they gain approval from their spouses. It can be proposed that there exists an association between women needing support and participating in taking preventive programs. This suggests that the Ghanaian community is a patriarchal community in which men have a significant influence on the households—indicating that men must be included as a target group for the effectiveness of cervical cancer preventive programs.

Religious beliefs were believed to hinder screening and vaccine uptake. The findings contradict this hypothesis in that there was no influence of religion on screening choices, but rather religion had a good impact on the acceptability of vaccination. This is contradictory to other nations where religion was observed to hinder uptake of vaccination (24, 44). About 95.6% of the respondents acknowledged being Christians, and this improved the likelihood of having themselves vaccinated. This indicates that Ghanaian churches can play a part in enhancing vaccinations program in Ghana. The potential reason is that it is known that certain Christian denominations consciously enlighten their members on medical problems such as cervical cancer. These explanations are insufficient since the participant's Christian denominations were not evaluated. Again, beliefs vary from one Church to another. Likewise, the truthfulness of the information given by the churches requires further investigation.

Previous studies on HPV awareness with a large sample size reported that the percentage of women knowledgeable of HPV ranged from 15.0 to 44.9% (45–48). We noticed a greater percentage of 54.5% in only women, while the total percentage among all the respondents was 50.3%. This finding is lower when compared with results from developed nations since the percentage of women knowledgeable of the human papillomavirus was 71.8, 61.6, and 87.7% in Australia, United Kingdom, and the United States, which indicates that women in developed countries might be more knowledgeable of HPV (49). Furthermore, 36.9% of women were aware of the HPV vaccine. This outcome is slightly higher than what

was reported in Chinese women by Lin et al. (28) (21.0%). Nevertheless, women's knowledge of the human papillomavirus vaccine is still far from women in economically developed nations where governmentally funded HPV vaccination program has been implemented per WHO recommendation (49). The possible relationship between HPV awareness and its vaccine and socioeconomic characteristics requires further investigation because populations showed a variety of socioeconomic, ethnic, cultural, and other inequalities in published surveys.

Even though there was a higher willingness of participants to accept the vaccination, the primary complaints among participants not willing to accept the vaccination were how safe the vaccine is, in addition to the acceptability of it worldwide and the price of the vaccine. This finding is similar to a study by (50), where participants were willing to undertake the vaccination at a free cost or receives a subsidiary from the government (51). Endarti et al. further showed that the knowledge of vaccine effectiveness increased the willingness of people to undertake vaccination (51). Hence, the government may use the media houses to educate people on the efficacy of the vaccine. In addition, the economic status may play an important part in the acceptance of the vaccine because our study showed that the likelihood of respondents paying for the vaccination was relatively low in the general population sample, which corresponds with the assumption that the majority of the respondents had lower-income rates. However, it was also striking that higher income earners and participant with company insurance were less willing to pay for vaccination and this can be attributable to the fact that majority of the higher income earners have company or private insurance hence their unwillingness to pay for the vaccination but instead, wants the insurance companies to cover the cost for the vaccination. Again, a greater comprehension of how insurance coverage and certain variables influence HPV vaccination uptake is required to allow potential interventions to be planned, therefore our next research will concentrate on this.

In our survey, most Ghanaians claim to be affiliate with the Christian religion. Hence improvement in the vaccine coverage can be increased with clergy's the support since the majority of these churches are mostly arraigned of refusing western medication due to biblical and moral values. Again, regular visitation to Church must be taken to motivate church members to participate in vaccination programs and further ensure that accurate information on cervical cancer is disseminated during their health talk. There is a need for the general public to be educated to understand the significance of the vaccination in addition to risk factors, symptoms, and screening of cervical carcinoma due to low knowledge and awareness highlighted in our study. Furthermore, community-based programs and interventional strategies must be targeted at both women and men because men were an influential element in the acceptability of the HPV vaccination by women.

Some limitations need to be highlighted. First, study participation was voluntary. Hence, most of the participants may have been those who demonstrated greater interest in the subject. Secondly, the study was limited to three cities in Ghana; therefore, the entire population cannot be generalized to our findings. Thus, the participants in the study constitute a representative sample. Also, different results will be obtained from new studies targeting rural communities and different residential areas. Thirdly, our survey centered on acceptability instead of uptake of the HPV vaccine; thus, it is uncertain if the intentions of participants to be vaccinated would turn into actions. Lastly, due to different educational backgrounds, some participants may not have completely grasped the questions, contributing to possible bias.

Taking into account the findings of this survey, certain policies can be implemented. First of all, it is important to ensure that screening facilities for cervical carcinoma are accessible in all health centers. Because of low awareness, probably providing such services in all health centers will result in an effective outreach between women and healthcare providers who may visit the health center for certain health purposes. In regards to the HPV vaccines, the higher willingness among respondents to accept the vaccine was a good sign. Since reproductive age in Ghana starts very early, it is, however, essential to begin vaccinations early in adolescence. HPV vaccines can be added to the normal vaccination program as an effective-cost solution, or "community-based vaccine drives" can be launched via the Ministry of Health. In principle, advancement in acceptance of vaccines, along with changes in behavior, would have a huge influence on Cervical Cancer Prevention in Ghana.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Zhengzhou University and Henan Provincial People's Hospital. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

ED wrote and presented the original draft. LZ and QH were involved in data curation and visualization. CE, CA, GA, ES, and FF were involved in methodology, software, analysis, review and editing. KS was involved in supervision.

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10-Year Mortality Pattern Among Cancer Patients in Lagos State University Teaching Hospital, Ikeja, Lagos

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Background: Globally, cancer is a major leading health problem with an estimated 10 million incidences and 6 million cancer deaths annually. In Nigeria, an estimated 72,000 cancer deaths occur annually, and 102,000 new cases are diagnosed from its population of 200 million people. These are, however, estimates, it is necessary to document the yearly trends and patterns of cancer mortality with regards to the different regions in the country.

Methodology: we conducted this study at the Lagos State University Teaching hospital (LASUTH), Ikeja, Lagos to document mortality patterns from 2009 to 2018. Data extracted included those from the patient's case notes, admission and death registers, and death certificates. we also had records from the hospital records department and medical wards. We then documented cancer mortality over the study period.

Results: A total number of 6,592 deaths were recorded over ten years, and 1,133 cases were cancer-related deaths. This number puts the percentage of cancer-related deaths at 17.2%. Male patients accounted for 54.0%, and female patients are 46.0%. Breast cancer accounted for the highest mortality, followed by prostate cancer. The highest number of deaths were recorded in 2010 at 821, followed by 2011 at 799, 2015 at 780, and the least in 2017 at 513. There is also a significant general increase in odds of mortality with an increase in decades of life.

Conclusion: This study shows that about one in five deaths, over the last ten years, from this tertiary institution, is related to a cancer diagnosis. Even though a yearly decline in the number of cancer deaths was noticed, probably due to increased awareness and governmental intervention, the percentage still remains high.

Keywords: mortality, patterns, cancer patients, cancer related, Lagos state, cancer deaths

INTRODUCTION

Cancer is the world-leading cause of death, cancer mortality rates are more than deaths caused by HIV/AIDS, tuberculosis, and malaria put together. It is the second leading cause of death in developed regions and is among the three most causes of death for adults in developing regions (1–5). It estimates for 7.6 million deaths (about 13% of all deaths) in 2008 and is projected to continue increasing, with an account of 13.1 million deaths in 2030 (4). In 2002, there were 6.7 million world cancer deaths, with less than 5% of these in sub-Saharan Africa. Still, it has been accounted that, by 2020, cancer could lead to the death of 10.3 million people worldwide, with a 50 to 75% rise in cancer death in sub-Saharan Africa (4). Cancer is one of the most common non-communicable diseases and has become an essential contributor to the global burden of diseases. The burden of cancer is rising, and it is one of the most causes of death worldwide (6).

The cancer mortality pattern is quite different in Africa when compared to other parts of the developed world. In 2012, there were an estimated 626,400 new cases of cancer and 447,700 deaths from cancer in Sub-Saharan Africa. cancer incidence in Sub-Saharan Africa is projected to rise by 85% in the next fifteen years. Cancer in Africa is characterized by late diagnosis and presentation, low access to treatment, and poor treatment outcomes. Inadequate access to cancer treatment results in 80–90% of cases that are in an advanced stage to result in death.

Cancer is responsible for 72,000 deaths in Nigeria annually, with an accounted 102,000 (7) new cases of cancer annually. In Nigeria, with a population of nearly 200 million people, complex diseases such as cancer are currently emerging as critical health care priority for the future. The data available on cancer mortality is inadequate in Nigeria, especially with regards to yearly trends and patterns of cancer mortality with regards to the different regions and states in the country. This study was conducted to provide data on the patterns of cancer mortality in Lagos state university teaching hospital, LASUTH over ten years using the data obtained from the hospital death certificates and death registers.

METHODOLOGY

This study is a retrospective study in which the cancer deaths (outcomes) have already occurred. Data were extracted from patient's case notes, admission and death registers, and death certificates, retrieved from the hospital records department and medical wards. These were reviewed to document the cause of death. These data include patient demographic data age, sex, clinical information, and histopathological type of cancer, year of death, and data were analyzed according to sex and age distribution for all cases. Ethical clearance was obtained from the hospital's ethics committee before the commencement of study.

Analysis of the data was done using the Statistical Package for Social Sciences (SPSS version 22.0). Simple descriptive statistics were used. The data was analyzed statistically using simple figures, ratio, percentages, table, and graphs. Mean, and Standard deviation was applied for continuous variables. Inferential statistics included logistic regression to explain the relationship between variables, and P-value 0.05 was taken to be statistically significant.

RESULTS

This study aimed at providing data on the pattern of cancer mortality in Lagos State University Teaching Hospital, LASUTH. A total of 6592 deaths were recorded over ten years, with 1,133 being cancer-related deaths. This number puts the percentage of cancer-related deaths at 17.2% (**Figure 1**). It is observed that out of all deaths that occurred during the study, male patients accounted for 54.0%, and female patients are 46.0%. Mean age of cancer mortality for both ages was 51.3 ± 10.9 (**Table 1**). Based on age group as a variable, 50–59 and 60–69 as well as <10 at 15.5, 17.7, and 14.1% respectively have a high mortality pattern (**Table 1**). Most male and female deaths occurred between ages 60-69 and 50-59 respectively (**Table 2**). The highest number of deaths were recorded in 2010 at 821, followed by 2011 at 799, 2015 at 780, and the least in 2017 at 513 (**Table 3**). Mean age of cancer mortality for both ages was 51.3±10.9 (**Table 4**).

Of the total number of deaths recorded, male cancer patient's death was 14.9% while male non-cancer death was 85.1% and female cancer patient's death was 19.8% while female non-cancer patient's death was 80.2%. Cancer deaths were commoner in female compared to male ($p < 0.001$), as shown in **Table 4**. Among cancer-related deaths, male patients accounted for 46.9%, and female patients accounted for 53.1%. Cancer mortality was observed in different age groups as follows; 40–49 (21.6%), 50–59 (23.1%), 60–69 (21.6%), and 70–79 (18.7%) and the least was >90 (8.0%). **Table 4** compares yearly cancer and non-cancer mortality pattern; in 2011, cancer-related deaths recorded were 96 (12.0%) and non-cancer-related deaths were 703 (88.0%), also, in 2017, cancer-related deaths recorded were

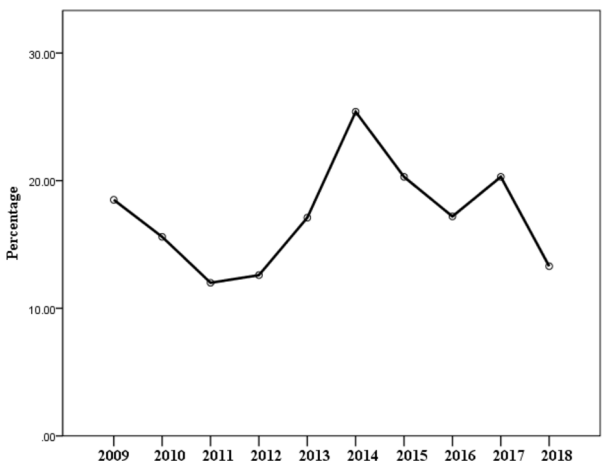

FIGURE 1 | A graph illustrating the yearly cancer mortality.

TABLE 1 | Showing gender and age distribution of mortality pattern over ten years.

Variable	Frequency (n = 6,592)	Percentage
Gender		
Male	3,559	54.0
Female	3,033	46.0
Age group (Years)		
<10	931	14.1
10–19	247	3.7
20–29	340	5.2
30–39	716	10.9
40–49	888	13.5
50–59	1,019	15.5
60–69	1,167	17.7
70–79	898	13.6
80–89	336	5.1
≥90	50	0.8

TABLE 2 | Showing age and gender distribution with regards to cancer mortality.

Variable	Male (n = 531)	Female (n = 602)	p-value
Age group (Years)			
<10	35(6.6)	17(2.8)	0.108
10–19	17(3.2)	17(2.8)	
20–29	20(3.8)	21(3.5)	
30–39	40(7.5)	68(11.3)	
40–49	74(13.9)	118(19.6)	
50–59	90(16.9)	145(24.1)	
60–69	125(23.5)	127(21.1)	
70–79	99(18.6)	69(11.5)	
80–89	20(5.6)	17(2.8)	
≥90	1(0.2)	3(0.5)	

TABLE 3 | Showing yearly mortality pattern.

Variable	Frequency (n = 6,592)	Percentage of Cancer cases
Year		
2009	541	8.2
2010	821	12.5
2011	799	12.1
2012	571	8.7
2013	604	9.2
2014	689	10.5
2015	780	11.8
2016	711	10.8
2017	513	7.8
2018	563	8.5

104 (20.3%) and non-cancer-related deaths were 409 (79.7%). The highest number of cancer deaths were recorded in 2014, 175 deaths, followed by 2015, 158 deaths, 2010, 128 deaths, 2015, 122 deaths and the least in 2012, 72 deaths. The yearly number of cancer-related deaths ranged between 12.0% (2011) to 25.4% (2014) (illustrated in **Figure 1** and **Table 5**), while yearly non-cancer-related deaths ranged between 74.6% (2014) to 88.0% (2011). The highest peak, as illustrated in **Figure 1**, depicts the highest number of cancer deaths recorded at 25.4%, 2014 and the lowest peak, the least number of deaths at 12.0% in 2011. Breast cancer was responsible for most of the deaths and accounted for

TABLE 4 | Showing the sex and age distribution for cancer and non-cancer mortality.

Gender	Cancer (n = 1,133)	Non-cancer (5,459)	p-value
			<0.001
Male	531(14.9)	3,028(85.1)	
Female	602(19.8)	2,431(80.2)	
Age group (Years)			<0.001
<10	52(5.6)	879(94.4)	
10–19	34(13.8)	213(86.2)	
20–29	41(12.1)	299(87.9)	
30–39	108(15.1)	608(84.9)	
40–49	192(21.6)	696(78.4)	
50–59	235(23.1)	784(76.9)	
60–69	252(21.6)	915(78.9)	
70–79	168(18.7)	730(81.3)	
80–89	47(14.0)	289(86.0)	
≥90	4(8.0)	46(92.0)	
Both sexes MeanAge ±SD	51.38 ± 10.9		
Mean Age for Males	51.73 ± 10.8		
Mean Age for Females	50.97 ± 11.1		

TABLE 5 | Showing yearly cancer and non-cancer mortality rate.

Year	Cancer (n = 1,133)	Non-cancer (5,459)	p-value
			<0.001
2009	100(18.5)	441(81.5)	
2010	128(15.6)	693(84.4)	
2011	96(12.0)	703(88.0)	
2012	72(12.6)	499(87.4)	
2013	103(17.1)	501(82.9)	
2014	175(25.4)	514(74.6)	
2015	158(20.3)	622(79.7)	
2016	122(17.2)	589(82.8)	
2017	104(20.3)	409(79.7)	
2018	75(13.3)	488(86.7)	

228 (20.1%) (**Table 6**), followed by prostate cancer which accounted for 102 deaths (9.0%). Colorectal cancer, hepatocellular, leukemia, and pancreatic cancer were responsible for 86 (7.6%), 84 (7.4%), and 86 (7.3%) respectively. Five commonest causes of cancer mortality are as depicted in **Figure 2**. In females, breast cancer was the commonest, followed by colorectal, hepatocellular, leukemia and pancreatic. For males, the commonest was Prostate cancer, followed by colorectal, hepatocellular, pancreatic and then gastric (**Figure 2**). Females had increase odd (1.447 95% CI = 1.270–1.648, $p < 0.001$) of dying when compared with males in this study. There is also a significant increase in odds of mortality with an increase in decades of life (**Table 7**). However, reduce odds was noted in terminal ages likely due to other factors associated with mortality in those age group ($p < 0.001$).

DISCUSSION

There is a rising trend in the incidence of cancer in Nigeria (8). Most patients present in a late-stage which leads to poor treatment outcomes, poor prognosis and increased cancer

TABLE 6 | Showing Organ-specific mortality in Males and Females over ten years.

	Male (n = 531)	Female (n = 602)	Total
Breast	5(0.9)	223(37.0)	228(20.1)
Prostate	102(19.1)	0(0.0)	102(9.0)
Colorectal cancer	47(8.9)	39(6.5)	86(7.6)
Hepatocellular	54(10.2)	30(5.0)	84(7.4)
Leukemia	44(8.3)	39(6.5)	86(7.3)
Pancreatic	40(19.1)	34(5.6)	74(6.5)
Gastric	25(10.2)	26(4.3)	51(4.5)
Lymphoma	29(5.5)	20(3.3)	49(4.3)
Renal	22(4.2)	9(1.5)	31(2.7)
Ovarian	0(0.0)	30(5.0)	29(2.6)
Intraabdominal	14(2.6)	14(2.3)	28(2.5)
Bile duct	13(2.5)	10(1.7)	23(2.0)
Myeloma	13(2.5)	7(1.2)	20(1.8)
Thyroid cancer	1(0.2)	4(0.7)	5(0.4)
Stomach	5(0.9)	3(0.5)	8(0.7)
Esophageal	8(1.5)	4(0.7)	12(1.1)
Skin	6(1.1)	3(0.5)	9(0.8)
Anorectal	5(0.9)	1(0.2)	6(0.5)
Bladder	10(1.9)	5(0.8)	15(1.3)
Rhabdomyosarcoma	11(2.1)	5(0.8)	16(1.4)
Sacrococcygeal teratoma	0(0.0)	2(0.3)	2(0.2)
Rectum	8(1.5)	8(1.3)	16(1.4)
Gall bladder	3(0.6)	9(1.5)	12(1.1)
Wilms tumor	1(0.2)	0(0.0)	1(0.1)
Endometrial	0(0.0)	11(1.8)	11(1.0)
Thymoma	2(0.4)	1(0.2)	3(0.3)
Lung	12(2.3)	7(1.2)	19(1.7)
Glioblastoma	2(0.4)	4(0.7)	6(0.5)
Nasopharyngeal	2(0.4)	1(0.2)	3(0.3)
Neuroblastoma	1(0.2)	1(0.2)	2(0.2)
Periampullary	1(0.2)	2(0.3)	3(0.3)
Neck	2(0.4)	0(0.0)	2(0.2)
Cervical cancer	1(0.2)	12(2.0)	13(1.1)
Laryngeal cancer	4(0.8)	1(0.2)	5(0.4)
Bronchogenic carcinoma	11(2.1)	5(0.8)	16(1.4)
Brain	3(0.6)	6(1.0)	10(0.9)
Others	24(4.5)	25(4.2)	50(4.4)

mortality. This study showed the pattern of cancer mortality in Lagos State University Teaching Hospital, LASUTH over ten years. Cancer deaths accounted for 17.2% of all the deaths in the hospital over the study period. The pattern is high when compared with other studies done in Africa, a study done in Tanzania showed that only 5.1% of deaths over ten years was cancer-related (9–11). The mortality pattern due to cancer in Africa is rising. In sub-Saharan Africa, cancer deaths have increased by 45 percent since 2000, with yearly mortality of more than half a million people (5, 12). Other attributable might include differences in climate, diet, genetic factors, development rate, and some other unknown factors (10, 11).

In 2011 and 2012, it was observed that the number of cancer deaths was low, but the number increased steadily in 2013 and 2014. From 2015 and into the following years, the number of deaths significantly decreased. This initially increases in cancer deaths in 2013 might be due to increased hospital presentations and reporting of cancer patients and cases, because of the commencement of Lagos state Ministry of Health cancer programs. More cancer patients presented to the LASUTH, which is a referral center for most state programs conducted at the Primary and secondary care levels and the only state-owned tertiary hospital offering tertiary cancer treatment in Lagos state. On the other hand, the reduction of deaths from 2015 onwards shows the impact of these screening programs conducted by the state government and some non-governmental organizations in Lagos State. As more patients presented with earlier disease, and fewer deaths were recorded, these numbers are however, still very high (13).

The mean age was 51.3 years, and the highest incidence of cancer deaths was seen in the age group 60–69. However, the age range of 50–59 and 40–49 also had very high incidence, and the pattern is similar to that reported by Akinde et al. (11), most mortality cases are seen in their study were between 51 and 60 years. The range of patients seen in this series falls within the stated life expectancy of Nigerians, which is 55 years and 56 years for males and females respectively, according to the WHO (14). Cancer mortality was observed to be higher in females at 53.1% compared to males, 46.9% giving a female to male ratio of 1.1:1. This is almost equal to the 1.2:1 female to male ratio in Kano Cancer Registry (KCR) (15). Reports from developed countries showed virtually identical or slightly increased M: F ratio as cancers take their toll in both sexes almost equally (16). The difference is noteworthy and can be proposed to be caused by the increased occurrence of breast cancer. In order of increasing frequency, organ-specific cancer mortality observed were, breast cancer, followed by prostate cancer, colorectal cancer, hepatocellular cancer and leukemia. This pattern is similar to the one observed in the University of Port Harcourt Teaching Hospital (UPTH) which was breast cancer, ranked first, followed by prostate cancer and hematolymphoid cancer while colorectal cancers ranked 4th (17). Among the least common were neck cancer, neuroblastoma, thymoma, nasopharyngeal and thyroid cancer.

Breast cancer is the commonest cause of cancer deaths recorded in this study, accounting for 228 deaths (20.1%). Global estimates for 2012 has revealed 1.67 million breast cancer cases worldwide ranking it as the second most common malignancy (18). Breast cancer is the most commonly diagnosed cancer in Africa and Sub–Saharan Africa and is also the leading cause of death from cancer (63,100 deaths in 2012) (18). Breast cancer carries a massive burden on the nation. Breast cancer mortality poses a severe public health threat in Nigeria and indeed, in most countries of the world (19). In our opinion, one key factor that, plays a crucial role in breast cancer mortality in our study is a late stage of presentation (20). often a consequence of poverty, ignorance, and inaccessible health care facilities.

Prostate cancer is the commonest cancer in males in Nigeria and Sub–Saharan Africa (2), it accounted for 9.0% deaths in this study, this is lower than the 13% in a South African study (21) and almost equal to the rate of 9.2% of mortality cases recorded in University of Port Harcourt Teaching Hospital (UPTH), Nigeria (17). According to GLOBOCAN 2012 estimates, prostate cancer ranked as the most common cancer in males worldwide with increasing survival rates due to screening programs available in most developed countries. The 5-year survival rates in the USA for men diagnosed with prostate

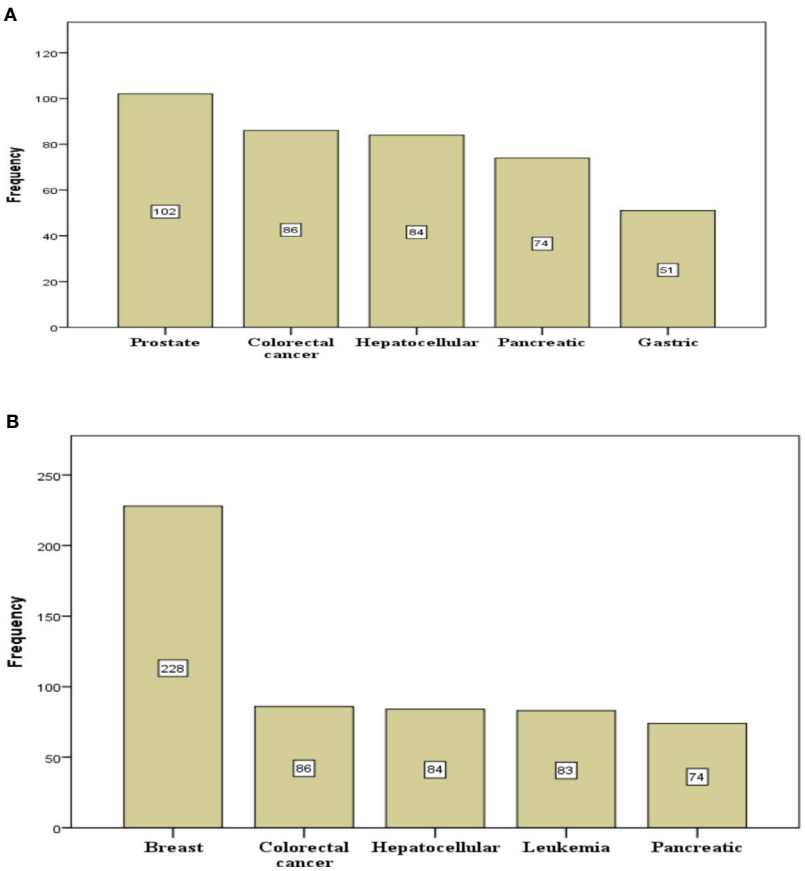

FIGURE 2 | Five commonest cancer-related mortality in male **(A)** and female **(B)** over ten years.

TABLE 7 | Logisticregression showing socio-demographic predictors of mortality.

	Odd ratio	95% CI	p-value
Gender			
Male	1	1.270–1.648	<0.001
Female	1.447		
Age group (Years)			
<10	1		
10–19	2.673	1.694–4.226	`<0.001
20–29	2.265	1.473–3.484	<0.001
30–39	2.929	2.070–4.144	<0.001
40–49	4.673	3.384–6.452	<0.001
50–59	5.090	3.711–6.981	<0.001
60–69	4.684	3.425–6.405	<0.001
70–79	3.915	2.824–5.427	<0.001
80–89	2.727	1.797–4.137	<0.001
≥90	1.394	0.482–4.030	<0.001

cancer is around 98% (22) while the data from Eurocare project (EUROCARE-5) from 2003 to 2007 showed that 5-year survival rate was 83% (23). More cases of prostate cancer will be diagnosed at an early stage if routine screening is available in Nigeria to screen men.

Colorectal cancer accounted for 7.6% deaths in this study; this result is like the 7.2% recorded in the Kano Cancer Registry (KCR) (15). Colorectal cancer is the third most common cancer in men and the second in women worldwide. The highest incidences are seen in the developed world while the lowest is noted in Africa, where it ranked as the fifth most common malignancy (2).

Carcinoma of the cervix accounted for 1.1% of cases in this study, this is low compared to global mortality and might indicate under-reporting of mortality cases from the disease, which is a significant challenge of documenting cancer patterns in Nigeria. Another attributable factor is that of a regional difference in the mortality of this disease in this state. Even though there is a policy on cancer control in Nigeria, the National Cancer Control Plan, (7) this policy has to be implemented effectively in other to reduce the burden of cancer on the nation. Currently, only 0.18% of the health care budget is allocated to cancer-related programs in Nigeria (24). The National budgetary allocation for health is also meager (25). The budgetary allocation for healthcare in 2020 was 4.3% of the total budget, as compared to other parts of the world, for instance, the US in 2020 dedicated about 21% (mandatory and discretionary spending's on health) (26) and the UK allocated about 19% of their total budget to healthcare. Also, there is an urgent need to increase funding and widen the coverage of these

programs directed towards cancer prevention, screening services for prompt diagnosis, and optimal treatment services.

Limitations

A major limitation of this study was the challenge of poor record keeping in the most centers in Nigeria, data collection is still paper based which is prone to loss of records. Also, the cancer registry is poorly funded and understaffed hence the need to include records from other sources like the death certificates and registers for this review.

CONCLUSION

Cancer mortality in Nigeria is still high. This study shows that about one in five deaths, over the last ten years, from this tertiary institution, is related to a cancer diagnosis. Breast cancer, which is more predominant in females, accounted for the highest mortality followed by prostate cancer which accounted for increased mortality in males. Even though a yearly decline in the number of cases was documented in this study, the percentage remains high. The decrease in the mortality pattern of cancer patients noticed in the last five years, shows the impact of the increased awareness, government intervention as well as regular screening for early detection and the practice of self-breast examination. However, there is still much that needs to be done, both locally and nationally, to reduce further the burden documented in this study.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

Ethical clearance was obtained from the Lagos State University Teaching Hospital ethics review committee, REF NO: LREC/06/10/1142.

AUTHOR CONTRIBUTIONS

OF, AS, and AP were involved in conceptualization and development of research idea. AO, SA, and OB were involved in data extraction and statistical analysis. AJ, AA, and OS were involved in final manuscript editing. All authors contributed to the article and approved the submitted version.

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Wilms Tumor in Sub-Saharan Africa: Molecular and Social Determinants of a Global Pediatric Health Disparity

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Wilms tumor (WT) is the most common renal malignancy of childhood. Global disparities in WT have been reported with the highest incidence and lowest overall survival occurring in sub-Saharan African nations. After a detailed search of PubMed, we reviewed available literature on WT in sub-Saharan Africa and summarized findings that explore biologic and social factors contributing to this alarming cancer health disparity. Access to care and treatment abandonment are the most frequently reported factors associated with decreased outcomes. Implementation of multidisciplinary teams, collaborative networks, and financial support has improved overall survival in some nations. However, treatment abandonment remains a challenge. In high-income countries globally, WT therapy now is risk-stratified according to biology and histology. To a significantly lesser extent, biologic features have been studied only recently in sub-Saharan African WT, yet unique molecular and genetic signatures, including congenital anomaly-associated syndromes and biomarkers associated with treatment-resistance and poor prognosis have been identified. Together, challenges with access to and delivery of health care in addition to adverse biologic features likely contribute to increased burden of disease in sub-Saharan African children having WT. Publications on biologic features of WT that inform treatment stratification and personalized therapy in resource-limited regions of sub-Saharan Africa have lagged in comparison to publications that discuss social determinants of health. Further efforts to understand both WT biology and social factors relevant to appropriate treatment delivery should be prioritized in order to reduce health disparities for children residing in resource-limited areas of sub-Saharan Africa battling this lethal childhood cancer.

Keywords: Wilms tumor, sub-Saharan Africa, health disparity, molecular features, social determinants of health

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INTRODUCTION

Wilms tumor (WT) is the most common renal malignancy of childhood. Black children of sub-Saharan African ancestry consistently show the highest incidence of WT worldwide at 11 cases per million (1). In sub-Saharan Africa, WT is reported as the second or third most common pediatric malignancy, which differs from its North American incidence (2). With the advent of cooperative

trials, multimodal treatment regimens, and multidisciplinary care models, overall survival at 5-years for patients with WT in developed nations is now greater than 90% (3). However, alarming disparities in outcomes persist for children with WT residing in sub-Saharan African nations, with overall survival at 5-years as low as 25% (4). Over the past 50 years, basic descriptions of WT prevalence, treatment challenges, and poor outcomes for children living in resource-limited settings of sub-Saharan Africa have been published, with the principal focus in more recent years on social determinants of health as contributing factors to this profound cancer disparity (2, 5–7). Only in the last decade has examination of WT biology as a molecular determinant of health in these austere contexts begun to gain momentum (8–11). The principal purposes of this review were to provide a comprehensive summary of existing literature on WT in sub-Saharan Africans and to describe the current epidemiology, biologic features, treatment strategies, and outcomes in these at-risk and vulnerable children. Further, we aimed to highlight areas of study where additional clinical and molecular research are needed.

METHODS

Publications related to WT and sub-Saharan Africa were included in this review. Using PubMed, the search terms "Wilms tumor" and "Africa" retrieved 192 results. Publications were reviewed for relevance and content by both authors and were included if WT in sub-Saharan African nations or Black populations was described. Key findings and results were abstracted from each paper and summarized. Publications were categorized as (1): biologic or molecular determinants of health, if content included description of clinical and molecular or genomic features of WT in a sub-Saharan African or Black population, or (2) social determinants of health, if content included description of access to care, treatment abandonment, cultural beliefs, or healthcare infrastructure. Date of publication and country of origin were also recorded.

RESULTS

Molecular Determinants of Health

Previous work has shown evidence of a biologic predisposition that may underlie an increased incidence of WT in children of Black sub-Saharan African descent (1). Specifically, a foundational study in 1984 showed that Black children living in the Greater Delaware Valley of the United States (i.e., Philadelphia, PA) were more likely to have congenital anomalies and syndromes associated with the development of WT. Specifically, a larger proportion of Black children had a WT-associated congenital anomaly, including aniridia, genito-urinary anomalies, Beckwith-Wiedemann Syndrome, and hemihypertrophy. Although not sequenced at the time of that seminal report, these developmental conditions associating with WT predisposition now have been attributed to alterations principally in two genes, *WT1* (11p13) and *WT2* (11p15.5) (12–14). Among younger patients, these authors reported a greater tendency for Black children to develop bilateral WT or to carry a tumor-associated anomaly. These features suggested a hereditary predisposition towards WT among Black children or less likely a greater susceptibility to toxins that induce germline mutations in these genes (15). After development and implementation of the National Wilms Tumor Study Group (NWTS) in 1969, which yielded 5 cooperative trials to optimize WT therapy, marked improvements in overall survival with reductions in treatment toxicity have since been realized (16–18). Moreover, a once significantly disparate survival gap for Black patients has now closed, at least in North America (3). However, Black populations globally continue to show greater frequencies to develop WT and to experience alarmingly poor survival in resource-constrained nations of sub-Saharan Africa. It was proposed in 1993 that, while global frequencies of WT were stable and not linked clearly or reproducibly with parental exposures to toxins, racial heredity and ancestry were greater determinants for development of WT than environmental exposures (1). To explore this concept of greater predisposition to develop WT among Black populations and potentially to harbor more treatment-resistant disease, both epidemiologic and somatic molecular differences between Black and White patients residing in Tennessee were explored. In Tennessee, Black children also appeared more susceptible than Whites to develop WT, and imaging mass spectrometry indeed identified peptide spectra from WT blastema and stroma that suggested race-specific molecular profiles (10).

Among sub-Saharan African populations, several initial studies described molecular features of WT that suggest a unique treatment-resistant and aggressive biology. These early studies aimed to quantify the frequency of p53 mutations that notoriously associate with diffuse anaplasia and more treatment-resistant disease. In one series of WT from Kenya, higher frequencies of p53 mutation were observed in comparison to White populations, and in accordance with previous literature, expression of p53 was associated with shorter survival period and unfavorable histology (19, 20). Additional molecular markers including E-cadherin, cadherin-11, alpha, beta and gamma-catenin were also studied within an African cohort. However, expression of these molecules did not show association with prognosis (21). Through multiple collaborations in Kenya and support from the Children's Oncology Group, disparate molecular profiles were explored between North American and Kenyan WT specimens (8, 9, 11). An unbiased proteomic screen revealed unique protein signatures between North American Black, White, and Kenyan Wilms tumor specimens with excellent and race-specific clustering. Interestingly, peptide signatures from the North American WT specimens of Black and White patients appeared more similar than those between Black North American and Kenyan patients, which suggested a unique biologic composition within this latter sub-Saharan African population and likely greater genetic admixture in the former (11). Furthermore, sequencing of the top 10 winner

peptides that associated with WT specimens from different race groups identified several interesting proteins and a novel association of Kenyan specimens with Fragile-X Related Protein – 1 (FXR1), which was subsequently characterized (22). FXR1 expression appeared to associate with undifferentiated cell types, specifically blastema, and may represent a pathway for cellular self-renewal hijacked from development (22). In Kenyan WT specimens, therefore, it is speculated that FXR1 emerged from the often blastemal-predominant cellular compartment in these cases that were analyzed commonly after neoadjuvant therapy and may represent a pathway for treatment resistance. Blastemal persistence after neoadjuvant therapy has been shown to be a poor prognostic feature, and indeed FXR1 has aligned with worse outcomes in several adult cancers (23).

Kenyan WT specimens have also been evaluated for histologic features and genomic alterations associated with somatic treatment resistance patterns. Specifically, Kenyan WT were analyzed for presence of diffuse anaplasia, which is an ominous harbinger of treatment resistance and failure, and in the majority of cases, is associated with alteration and mutation in *TP53*. While DAWT only comprises 5-8% of WT patients in high-income countries, anaplasia was present in 13% of Kenyan WT patients (9). Furthermore, an increased frequency of genetic and chromosomal alterations were uncovered in these specimens that have been associated with poor prognosis in high-income countries, including frequent mutations in p53, beta-catenin, and MYCN, loss of heterozygosity at 17p (which covers *TP53*) and 11q, and copy number gain at 1q (8, 9).

Social Determinants of Health

Differences in access to care, cultural attitudes and beliefs, infrastructure, and health care delivery mechanisms only exacerbate the dismal outcomes for children having biologic features of treatment-resistant WT and residing in sub-Saharan Africa. Loss to follow up and treatment abandonment remain the most commonly reported social challenges that contribute to treatment failure across the continent (24, 25). Studies from multiple countries have aimed to implement multidisciplinary treatment models and standardized therapy to improve outcomes. Risk factors and challenges for providing optimal treatments have been described by treatment center and country (24, 25).

In the Collaborative Wilms Tumor Project, an adapted WT treatment guideline was implemented in multiple centers across sub-Saharan Africa, including the countries of Malawi, Cameroon, Ethiopia, Uganda and Ghana. The principal aim was to decrease abandonment of treatment and to improve outcomes (26). Using this multi-center regional collaborative network, program implementation was associated with significantly higher survival without evidence of disease at the end of treatment compared to baseline evaluations (68.5% vs. 52%) (26–28). Financial support for medical treatment was highlighted as a key strategy to decrease abandonment of treatment (28). In the first multicenter prospective study in sub-Saharan Africa, seven units participated from Senegal, Madagascar, Cameroon, Cote d'Ivoire, Mali, Togo, and Burkina Faso. After protocolized treatment of unilateral, localized, standard-risk WT, a three-year overall survival rate of 73% was observed (29). However, fifteen percent of the patients did not receive optimal treatment, and principal barriers included limited access to care. Specifically, decreased availability of pathology reports, decreased availability of chemotherapeutic drugs, and lack of access to radiotherapy were described (29).

In Kenya, we reported recently a 2-year event-free survival from WT as 52.7%, which rose from 35% from prior publications. However, loss to follow up in our series was 50%, which tempered enthusiasm (24). Other studies have reported similar rates of loss to follow-up at 42%. Also reported, late presentations of WT with advanced stages of disease contribute to decreased overall survival (30, 31). For those who completed standard therapy, however, 2-year event free survival has been documented as high as 94%, in accordance with overall survival in high-income nations. Insurance status and enrollment in the Kenyan National Hospital Insurance Fund (NHIF) was associated with lower hazard of death, which suggests the importance of health insurance (24). Risk factors for treatment abandonment in Kenya include financial constraints, lack of education about WT and necessity to complete treatment, and lack of drug availability (24, 25).

In Nigeria, clinical characteristics and outcomes have also been evaluated, showing larger than average tumor size at presentation in comparison to Caucasian children in high-income nations. A high mortality rate due to late clinical presentation, poor availability of chemotherapeutic agents, and inadequate follow up and treatment completion have been documented (32). Later studies evaluated outcomes following introduction of multidisciplinary team management and patient treatment stratification according to tumor histology. In this population, one third of patients were lost to follow up. Among patients who completed chemotherapy treatment, 5-year overall survival was 73.7%, but overall 5-year survival (abandonment-sensitive survival) remained low at 35.6% Barriers to care included public health measures that allowed early diagnosis, improvement of facilities, and adequate healthcare funding to receive standard therapy (33). Additional studies advocate for the need for additional health information and collaboration with institutions in high-income countries (34).

In Rwanda, nephroblastoma, or WT, was reported as the most common childhood cancer. Significant challenges to survival include unaffordable treatment, late presentation, and lack of trained staff and multidisciplinary collaboration. Recommendations for improvement again highlight improvement in patient education, free health care for children with cancer, international partnerships with tertiary care centers (35).

In Malawi, presentation at advanced stage and high recurrence rates are reported even with completion of therapy at 15% (36). An adapted WT treatment guideline and strategies to enable children to complete treatment were introduced. Two- and five-year event-free survivals remained decreased at 46 and 42%, respectively, in comparison to high income countries, and causes of treatment failure included abandonment of care for 7%

of children, 15% with death during treatment, and 30% with disease-related deaths. Suggestions to optimize WT management in Malawi included strengthening social support programs, treatment compliance, nutrition, and modifications to reduce treatment-related deaths (37).

In South Africa, nutritional status was highlighted as a further prognostic feature impacting outcome from WT. Prevalence of malnutrition was as high as 66% using combined laboratory and anthropometric data. For this reason, early aggressive nutritional resuscitation for malnourished children in marginalized sub-Saharan African countries and populations was recommended (38). While presentation with advanced disease remained a challenge, treatment by multidisciplinary teams in Johannesburg showed improved survival outcomes relative to other sub-Saharan African nations (39). Furthermore, an additional study in South Africa showed that when treatment protocols employed in the United States were implemented in this African setting with robust surgical care, estimated 5-year overall survival was 94.4% (40).

The combined results of these publications from populations across sub-Saharan Africa highlight the need for improved access to care, availability of standard therapy for WT, supportive care, and patient education. These challenges remain significant and are cited as the primary determinant of decreased overall survival from WT in Africa in comparison to high-income nations (6, 7, 41, 42). Altogether, marginalized access to less than adequate therapies for malnourished children having advanced stage, treatment-resistant WT is exceedingly difficult to overcome, hence the horrific yet consistently poor survival in certain areas of sub-Saharan Africa.

Timeline and Categorization of Publications

A total of 26 papers were included in this review. Since the first included publication in 1981, a total of 19 papers described social determinants of health and the impact of various financial, cultural, and structural barriers to optimal treatment in African populations on survival from WT (**Table 1**). Significant improvements have been made to address these barriers, including collaborative clinical trials, implementation of treatment protocols, multidisciplinary teams, international partnerships, and unique strategies for increasing access to care. Since the first publication in 1984, a total of 7 papers described molecular and genomic features within WT of sub-Saharan Africa (**Table 1**). A timeline underscores the lag to investigate molecular features for more optimal risk stratification and treatment assignment (**Figure 1**).

DISCUSSION

WT disproportionately impacts Black children residing in sub-Saharan Africa and worldwide. This review illustrates significant progress in characterizing the clinical and molecular features of WT in sub-Saharan Africa and improving outcomes over the last 50 years, but clearly much work remains. Most sub-Saharan African nations categorized as low to middle income have seen improvement in survival outcomes since initial reports 4 decades ago, albeit not consistently near results from high income countries. Treatment abandonment remains a significant challenge reported by authors from multiple sub-Saharan African countries. The primary focus of research on WT in resource-limited regions of Africa is necessarily devoted to social determinants of health and decreasing barriers to care, of which there are many. Improving patient outcomes requires decreasing delayed presentation and diagnosis, increasing collaboration between interdisciplinary teams, improving access to pathology for treatment stratification, increasing availability of surgery, radiation, and chemotherapeutic agents, increasing adherence with follow up care, and comprehensive survivorship clinics. All of these factors are also likely impacted by finances, health literacy, and cultural beliefs. While these social determinants are certainly present in developed nations, it appears these inequities are exacerbated in low-resource settings of sub-Saharan Africa.

With the advent of targeted therapies, new frontiers of oncologic care focus on characterizing molecular signatures of disease with the goal of providing pathway- and cell-specific, personalized treatments. In high income nations, the focus of most WT research is optimizing therapy through further study of biomarkers associated with aggressive and treatment-resistance disease. This strategy to incorporate biologic features that assign risk of treatment failure within therapeutic regimens affords patients harboring a predictably sensitive WT to be exposed to less toxic therapy (4). The corollary of patients having a biologically high-risk WT will be assigned more appropriately intensive therapies. For example, specific genetic features of WT, including LOH for alleles spanning chromosomes 1p and 16q, are biomarkers that, when both present, associate with increased risk of relapse and death and have implications for more intensive management. Identifying additional prognostic biomarkers is an active area of study (43). Currently, the understanding of the genetic features of WT are based on specimens almost exclusively from patients in developed nations, which may not be generalizable to sub-Saharan African WT. Previous work has shown evidence of a predisposition among Black populations of sub-Saharan African ancestry to develop WT and that molecular markers associated with poor prognosis and treatment-resistant disease may well confound standard therapies. Further study and inclusion of African patients in molecular and genetic research is required to equitably advance treatment options for all patients with WT globally. Improved understanding of biologic features of WT in African populations will allow for risk stratification in parallel to the use of Children's Oncology Group (COG) and International Society of Pediatric Oncology (SIOP) treatment protocols. Advancement of personalized therapies for WT in Africa will require collaborative efforts to characterize molecular features, determine prognostic significance, and evaluate the efficacy of tailoring chemotherapy intensity accordingly.

Limitations of this review include the incorporation of only published work and lack of sub-Saharan collaborators. The data

TABLE 1 | Publications on Wilms tumor among sub-Saharan Africans.

Author	Journal/Date	Title	Country(ies)	Findings
Kyambi et al. (31)	EastAfrican Medical Journal/ 1981	The management of Wilms tumor in Kenya	Kenya	Late presentation and Loss to follow up (LTFU)
Kramer et al. (15)	Medical and Pediatric Oncology/1984	Racial Variation in incidence of Wilms tumor: relationship to congenital anomalies	United States	Genetic
Breslow et al. (1)	Medical and Pediatric Oncology/1993	Epidemiology of Wilms Tumor	Global	Genetic
Wessels et al. (38)	Pediatric Hematology Oncology/1999	Nutrition, morbidity, and survival in South African children with Wilms' tumor	South Africa	Malnutrition
Ekenze et al. (34)	Annals of Oncology/2006	The challenge of nephroblastoma in a developing country	Nigeria	Health education and collaboration
Davidson et al. (40)	Pediatric Blood Cancer/2006	Wilms tumor experience in a South African Centre	South Africa	Treatment protocols and collaboration
Uba and Chirdan (32)	West African Journal of Medicine/2007	Wilms tumor: prognostic features in North Central Nigeria	Nigeria	LTFU
Rogers et al. (39)	European Journal of Pediatric Surgery/2007	Experience and outcomes of nephroblastoma in Johannesburg 1998-2003	South Africa	Late presentation, collaboration
Israels et al. (37)	Pediatric Blood Cancer/2009	Acute malnutrition is common in Malawian patients with Wilms tumor: a role for peanut butter	Malawi	Late presentation, LTFU
Wilde et al. (36)	African Journal of Paediatric Surgery/2010	Challenges and outcome of Wilms' tumor management in a resource-constrained setting	Malawi	Malnutrition, late presentation, LTFU, drug availability
Axt et al. (10)	Journal of Surgical Research/ 2011	Race disparities in Wilms tumor incidence and biology	United States	Proteomic
Tenge et al. (30)	East African Medical Journal/ 2012	Management and outcome of patients with Wilms Tumor (nephroblastoma) at the MOI Teaching and Referral Hospital, Eldoret, Kenya	Kenya	Late presentation, LTFU, drug availability
Murphy et al. (9)	International Journal of Cancer/2012	Molecular characterization of Wilms' tumor from a resource-constrained region of sub-Saharan Africa	Kenya	Proteomic, Histologic
Axt et al. (24)	Journal of Pediatric Surgery/ 2013	Wilms tumor survival in Kenya	Kenya	LTFU, cost of treatment
Israels et al. (41)	Pediatric Hematology Oncology/2014	Management of children with Wilms tumor in Africa and Europe; thoughts about costs, priorities and collaboration	The Netherlands and Malawi	LTFU, malnutrition, cost of treatment, collaboration
Israels et al. (41)	Pediatric Hematology Oncology/2014	Clinical trials to improve childhood cancer care and survival in sub-Saharan Africa	Sub-Saharan Africa	LTFU, cost of treatment, collaboration
Libes et al. (11)	Journal of the American College of Surgeons/2014	Race disparities in peptide profiles of North American and Kenyan Wilms Tumor Specimens	United States and Kenya	Proteomic
Libes et al. (25)	Pediatric Blood Cancer/2015	Risk factors for abandonment of Wilms tumor therapy in Kenya	Kenya	Cost of treatment, education, drug availability
Paintsil et al. (27)	European Journal of Cancer/ 2015	The Collaborative Wilms Tumor Africa Project; baseline evaluation of Wilms tumor treatment and outcome in eight institutes in sub-Saharan Africa	Malawi, Cameroon, Ghana, Ethiopia, Uganda	LTFU and death during treatment
Kanyamuhunga et al. (35)	Pan African Medical Journal/ 2015	Treating childhood cancer in Rwanda: the nephroblastoma example	Rwanda	Late presentation, cost of treatment, education, health care personnel
Atanda et al. (19)	African Journal of Paediatric Surgery/2015	Wilms tumor: determinants of prognosis in an African setting	Kenya	Histologic
Lovvorn et al. (8)	Genes, Chromosomes, and Cancer/2015	Genetic and chromosomal alterations in Kenyan Wilms Tumor	Kenya	Genomic
Israels et al. (26)	Pediatric Blood & Cancer/ 2018	Improved outcome at end of treatment in the Collaborative Wilms tumor Africa Project	Malawi, Cameroon, Ghana, Ethiopia, Uganda	Collaboration, LTFU, cost of treatment
Yao et al. (29)	Journal of Global Oncology/ 2019	Treatment of Wilms Tumor in Sub-Saharan Africa: Results of the Second French African pediatric Oncology Group Study	Senegal, Madagascar, Cameroon, Cote D'Ivoire, Mali, Togo, Burkina Faso	Treatment availability
Ekenze et al. (33)	Pediatric Blood & Cancer/ 2019	Continuing barriers to care of Wilms tumor in a low-income country	Nigeria	Late presentation, cost of treatment, health care facilities
Chagaluka et al. (28)	Pediatric Blood & Cancer/ 2020	Improvement of overall survival in the Collaborative Wilms Tumor Africa Project	Malawi, Cameroon, Ghani, Ethiopia, Uganda	Collaboration, LTFU, cost of treatment

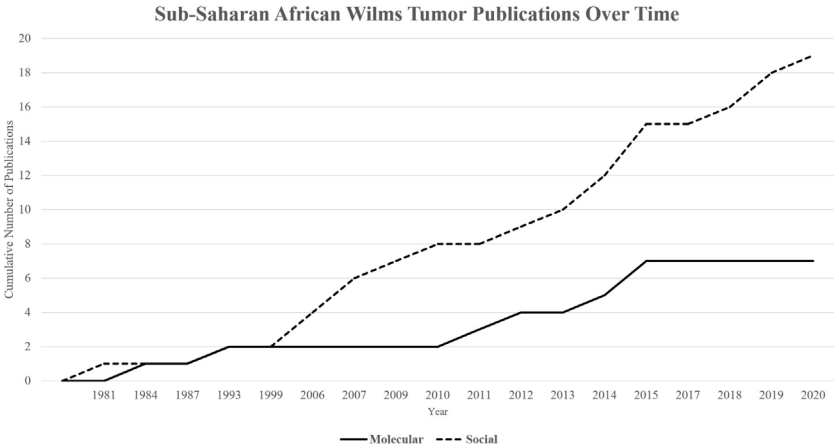

FIGURE 1 | Cumulative publications over time by category. Publications on molecular determinants of health (Total = 7) have lagged in comparison to publications on social determinants of health (Total = 19) over time.

reviewed may not reflect the entirety of research that has been conducted on Wilms Tumor in Africa, particularly studies that are ongoing or unpublished. Included publications were written by primarily sub-Saharan researchers and collaborators. However, the authors of this review do not practice in sub-Saharan Africa and therefore, may not capture additional perspectives or insights based on first-hand experience from within the region. Strengths of the review include the comprehensive summary of biologic and social factors relevant to understanding this pediatric health disparity, contemporary discussion of research trends over several decades, and suggestion of future directions to improve outcomes.

While social determinants are foundational and critical to improving outcomes for children with WT in sub-Saharan Africa, additional research is needed to better characterize disease at the genetic and molecular level. The results of this review show that publications on biologic and molecular features of disease in African WT are lagging in comparison to publications regarding social determinants of health. Sub-Saharan African children having WT are not only disproportionately impacted by structural and cultural barriers to care, but also may harbor a tumor biology that would benefit from additional risk stratification and personalized therapies. Indeed, even in high-income countries where access to appropriate care is assured, WT that acquire treatment-resistant molecular features are difficult enough to cure, let alone in resource-poor settings where social barriers abound, as described above. In order to address the persistent and widely reported health disparities in WT in Africa, efforts to address systems of care and decreasing treatment abandonment should remain a priority, in addition to improved understanding of Wilms tumorigenesis to advance personalized treatments.

AUTHOR CONTRIBUTIONS

HL contributed to the conception and design of the study. AA and HL contributed to the collection of the data, analysis, and manuscript development. All authors contributed to the article and approved the submitted version.

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REVIEW
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Cancer Omics in Africa: Present and Prospects

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During the last century, cancer biology has been arguably one of the most investigated research fields. To gain deeper insight into cancer mechanisms, scientists have been attempting to integrate multi omics data in cancer research. Cancer genomics, transcriptomics, metabolomics, proteomics, and metagenomics are the main multi omics strategies used currently in the diagnosis, prognosis, treatment, and biomarker discovery in cancer. In this review, we describe the use of different multi omics strategies in cancer research in the African continent and discuss the main challenges facing the implementation of these approaches in African countries such as the lack of training programs in bioinformatics in general and omics strategies in particular and suggest paths to address deficiencies. As a way forward, we advocate for the establishment of an "African Cancer Genomics Consortium" to promote intracontinental collaborative projects and enhance engagement in research activities that address indigenous aspects for cancer precision medicine.

Keywords: multi omics, cancer genomics, epigenomics, transcriptomics, metabolomics, proteomics, metagenomics, African continent

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INTRODUCTION

Cancer is essentially a multifactorial disease triggered by the interaction of multiple genes and numerous factors namely age, lifestyle, environmental toxins, and genetic syndromes (1). Cancer is also defined by a subset of abnormal cell clones that develop out of control and can infiltrate and metastasize towards distant organs beyond normal tissue borders (2). As cancer research has entered the precision medicine era, non-molecular characteristics have turned inadequate whilst the use of molecular characteristics is a progressively common research direction. Biomedical researchers aimed for implementing multi omics data in order to obtain new insight into cancer growth and development (3). "Omics" sciences including transcriptomics, genomics, metabolomics, proteomics, metagenomics, and epigenomics include several implementations and aim to significantly enhance our knowledge of cancer growth and progression processes (4). These omics approaches represent an essential part in influencing diagnosis, prognosis, and patients' treatment (4, 5). Additionally, they are naturally appropriate and very promising for the discovery of useful biomarkers (4). In the multi omics framework, the use of integrative methods became

important for gaining more insight into oncological phenomena and step towards the pattern of precision medicine (6).

Considering the enormous areas covered by developed-world advances in molecular and omics-based technologies, the adoption and implementation of these approaches in developed countries yet remain uncertain (7). Cancer is a widespread problem in African countries by dint of ageing and population growth, and increased prevalence of risk factors (8). Europe presents 23.4% of all cancer cases and 20.3% of cancer deaths, pursued by the Americas with 21% of cases and 14.4% of deaths worldwide. Unlike other regions, cancer mortality rates in Asia (57.3%) and Africa (7.3%) are higher than incidence rates (48.4 and 5.8%, respectively) due to the different distribution of cancer types and higher case mortality rates in these areas (9). In 2008, it is estimated that there were 715,000 new cancer cases and 542,000 deaths in Africa (10). The African population is expected to rise by 60 percent overall between 2010 and 2030 and by 90 percent for those 60 and older, the age at which cancer occurs most commonly, as per the United Nations population projections (8). However, facing this rising burden, cancer keeps receiving a relatively low public health priority in Africa, with few exceptions (8).

The International Cancer Genome Consortium (ICGC) was created to support large-scale genome studies regarding tumor cancer from 50 diverse forms and/or subtypes of cancer. It enables systematic studies at the genomic, epigenomic and transcriptomic levels of more than 25,000 cancer genomes (11). Many countries in America, Europe, and Asia are involved in this international project, but African countries shine by their absence. So as not to leave Africa behind in all these highly advantageous developments, there is an urgent need for creativity and maximization of existing infrastructure (7). In this study, we provide past and existing implementations of various multi omics strategies in the African continent's cancer research sector and address the key challenges regarding the development of these approaches in Africa such as the lack of training programs in bioinformatics in general and omics strategies in particular. Paths forward to address deficiencies will be suggested.

CANCER GENOMICS

Valuable new pieces of information about genomic drivers of cancer onset and progression across several anatomical locations have been highlighted thanks to the application of next-generation sequencing (NGS) techniques to discovery projects on large-scale cancer genomics (12). Unlike traditional Sanger sequencing, the NGS has the ability to sequence, very efficiently and at high throughput, gigabases of DNA (13). The majority of NGS approaches rely on DNA template preparation, sequencing and imaging, and data analysis. To prepare the template, current techniques involve randomly splitting the genomic DNA into smaller sizes. The generated template is then attached or immobilized to a rigid support or surface. Thousands to billions of sequencing reactions can occur concurrently due to the immobilization of spatially detached template sites. Because the majority of imaging systems are unable to reveal the fluorescent events, the amplified templates are needed to boost the intensity of sequencing signals (2). NGS can be used to detect small deletions and insertions, loss of heterozygosity in tumor DNA samples, sequence mutations, structural rearrangements, and copy number alterations (12). Due to NGS, beyond the genomic sequencing, which was the initial development objective and application, emerging applications and fields in medicine and biology are becoming a reality. The NGS provides new and fast methods for genome-wide characterization and profiling of transcription factor regions, small RNAs, mRNAs, DNA methylation patterns and structure of chromatin, microbiology, and metagenomics (14).

Given that cancer is a genetic disease, sequencing the patient's genome will allow detecting recurring alterations. Up to now, sequencing of more than 80 forms of cancer worldwide has been achieved. Most prominent actors are the Cancer Genome Atlas (TCGA) project and the International Cancer Genome Consortium (ICGC). They not only broadened cancer list genes but further identified novel dysregulated cellular processes, namely those engaged in chromatin regulation and epigenomic control and those involved in RNA splicing, metabolism, lineage maturation, and protein homeostasis (15).

It is well known that the genetic diversity among African populations is the most high and, therefore, its study requires a greater number of variants in order to determine the same amount of variation as in European ancestry groups, to do this, a larger sample size is required (16). TCGA project, which aims to uncover the main genomic alterations that cause cancer and construct a complete "atlas" of cancer genomic profiles (17), is targeting a large cohort of 11 122 patients involving 33 cancer types from 27 primary sites (18). TCGA, due to its cohort size, is considered to be one of the greatest projects with numerous samples, multidimensional genomic profiles, and thorough clinical information which are essential to detect the impact of genetic ancestry on genomic alterations. Despite these advantages, for *de novo* identification of genomic alterations specific to a racial group at a level specific to the type of cancer (18) and to capture even relatively common somatic mutations that are specific to those groups, the absolute number of samples of racial minorities like African ancestry groups in TCGA is still relatively small (19). Therefore, to better understand the genomic basis of the differences among all racial/ethnic groups, there is an increasing need to augment the number of underrepresented patients samples (18).

A large number of genomic variants were reported to be causally linked to or associated with a higher risk for various types of cancer. For example, in 11 members of two families of Greek origin, Karageorgos et al. introduced a NGS method for classifying all genetic variants with the propensity for family members to be predisposed to cancer. A total of 571 variants were reported in cross-comparison with data from the Human Gene Mutation Database, 47 percent of which were disease-related polymorphisms, whilst 26 percent were disease-related polymorphisms with further functional data, and 19% were

functional polymorphisms. However, with some residual confusion as to their pathological importance, 4% were mutations causing putative diseases and 3% were mutations causing disease (20).

Laryngeal cancer is known to affect African-Americans more than European-Americans. In order to distinguish between environmental and ancestrally-inherited factors, Ramakodi et al. studied the genome-wide somatic point mutations from the tumors of a cohort including 57 European Americans and African Americans patients from TCGA. Differences between the two population in the distributions of the number of somatic point mutations per sample (the number of mutations varied from 29 to 313 with a mean of 151.31 for African-Americans and the number of mutations ranged from 46 to 1,026 with a mean of 277.63 for European-Americans) and the prevalence of context nucleotide signatures for somatic point mutations (C >G and C >A) were found. These nucleotide signatures in parallel with other factors may contribute to the variations observed in the mutation landscape between the two races. These findings suggest that the race, at the molecular level, play a significant role in the progression of laryngeal cancer with ancestral genomic signatures and explain the origin of the differences observed between the two studies races (21).

Similarly, for the sake of determining the role of ethnic differences in clear cell renal cell carcinoma (ccRCC) somatic mutation rate and gene expression, a cohort of 419 white and 19 African American patients identified through TCGA clear cell kidney (KIRC) dataset was examined. The GSE25540 dataset comprising 125 white and 10 African American patients was utilized for validation. The results showed that African American compared to white patients were enriched in the clear cell type B (ccB) molecular subtype that has worse prognosis and were significantly less susceptible to have Von Hippel-Lindau (VHL) mutations. Equally, in African American, the RNA expression disclose relative down-regulation of hypoxia-inducible factor and vascular endothelial growth factor -associated pathways. The outcomes of this work suggest that the genomic differences observed between African American and white ccRCC patients could be involved in the worse survival of African American patients (22).

The second most frequent malignancy in men worldwide is prostate cancer with 1,276,106 new cases and 358,989 deaths in 2018. When compared to white men, the incidence rates of prostate cancer in African American are higher with 158.3 new cases per 100,000 men and their death rate twice that of white men (23). The higher incidence and mortality of prostate cancer (CaP) observed in men of African Ancestry (AA) compared to men of predominantly European Ancestry (EA), may be due to genomic factors. To investigate this theory, the authors evaluated genomic profiles from the TCGA CaP cohort (n = 498) and analyzed the data from only 61 AA and 414 EA cases. Considerable differences were spotted by ancestry in the frequency of Transmembrane Serine Protease 2- ETS related gene (TMPRSS2-ERG) fusions (29.3% AA vs. 39.6% EA), speckle-type POZ protein (SPOP) mutations (20.3% AA vs. 10.0% EA), and Phosphatase and Tensin (PTEN) deletions/losses (11.5% AA vs. 30.2% EA). Differentially expressed genes (DEGs) between AAs and EAs demonstrated significant enrichment for prostate eQTL target genes. Enrichment of highly expressed DEGs for immune pathways has been observed in AA and for PTEN/Phosphatidylinositol 3-kinase (PI3K) signaling in EA. These results, through both genomic and transcriptomic analysis, indicated that the differences found may be biological contributors to racial discrepancies in the incidence and consequences of CaP (24).

Likewise, in order to highlight the genomic alterations linked to race, Koga et al. compared the frequencies of somatic alterations in a cohort comprising AA and AE prostate cancer patients. Mutations in Zinc finger homeobox 3 (ZFHX3), focal deletions in ETS Variant Transcription Factor 3 (ETV3), c-myc (MYC) amplifications in metastatic PCa, Histone-lysine N-methyltransferase 2D (KMT2D) truncations and Cyclin D1 (CCND1) amplifications in primary PCa were more frequent in tumors from AA patients. While rearrangements in Transmembrane protease, serine 2 (TMPRSS2-ERG) and deletions in PTEN were less frequent in AA compared with EA patients. In contrast, tumor mutation burden, microsatellite instability (MSI) status, and genomic alterations in select DNA repair genes, Cyclin Dependent Kinase 12 (CDK12), and in Androgen receptor (AR), which are the genomic features that could influence the clinical decisions, were found not to differ significantly between the two groups studied. Despite the results indicating genomic disparities amongst AA and EA, the similarities found in the frequencies of genomic alterations in PCa therapeutic targets, suggest that precision medicine strategies could be evenly useful if applied fairly (25).

In another study, in order to perform deep sequencing of complete mitochondrial genomes in prostate cancer, McCrow et al. analyzed 87 tissue samples extracted from South African men with matched blood and prostate (77 with an African origin). Clinical presentation was skewed towards severe illness and contrasted either with or without benign prostatic hyperplasia to men without prostate cancer. One hundred forty-four somatic mitochondrial DNA (mtDNA) single nucleotide variants (SNVs) were identified, of these, 80 were found in 39 men with severe illnesses. Higher pathological stages were correlated with the number of somatic mtDNA SNVs and their frequency. Similarly, in men of African descent, the authors equate mutational load with the aggressive status of prostate cancer (26).

Abbad et al. indicated that the majority of genetic studies regarding African Breast Cancer (BC) remain restricted to studying BRCA1 and BRCA2 genes and their mutation spectrum variations. Thus, by collecting pertinent data from 43 studies in Africa depending on the following features: case control research, and the association of genetic variants with BC risk. Data on mutations and BC-related polymorphisms were given without setting a particular time. This research had omitted case-only studies and clinical trials. Therefore, to guide precise and more appropriate treatment interventions for the people of Africa, African scientists should be encouraged to identify more genes associated with BC employing high throughput methods such as NGS (27).

For Africa, Jaratlerdsiri et al. conducted the first tumor-normal paired genome sequencing. They registered for 15 cases a 1.8-fold rise in minor somatic variants in tumors of African and European origin, except one single hyper-mutated tumor with 55 mutations per mega base. In addition, they found a rise in oncogenic driver mutations in African tumors; approximately 30 percent of the affected genes were described for the first time in prostate cancer, and 79 percent of reoccurring tumorigenesis driver mutations emerged early. In African prostate tumors, complex genomic rearrangements were less frequent. Despite the fact that this research is preliminary, the findings indicate that further confirmation and analysis of the possible implications of increased mutational tumor load and tumor initiating gene alterations in clinically inauspicious prostate cancer will boost clinical outcomes in Africa (28).

It is important to point out that Sub-Saharan Africa's (SSA) genomics research potential is relatively low and may hinder full benefits from genomics applications in medicine and clinical practice. About one-tenth of papers published on this genomics topic was related to non-communicable diseases where cancer present 6.1%. There are currently significant differences in genomics research ability among SSA countries and South Africa has the highest research performance in genomics, expressed in the investments made in its genomics and biotechnology activities (29). Challenges related to scarce resources affecting the implementation of genomics research in Africa include ill-equipped laboratories, lack of expertise, and enabling climate for local hospital research activities and inadequate connectivity to research centers. The research study challenges include comprehensive procedures, delayed funding, delays in building research units and inadequate human resource instruction, language difficulties and underestimation of cultural rules (30).

Several new major ventures, including the Human Heredity and Health in Africa (H3Africa) initiative, resolve a couple of the aforementioned barriers towards the establishment of precision and personalized medicine in African countries (31). The H3Africa project was built up to drive new genetic and environmental aspects forward on an African-relevant human diseases basis, as well as create resources for genomic research on the continent. For more than 70,000 members across the continent, this consortium jointly collects samples and data, followed by detailed clinical data on a range of communicable and non-communicable diseases. The consortium also invested substantial resources in the establishment of advanced African biorepositories, a bioinformatics network together with a prominent educational and training programs that drew up genomic data analysis skills and interpretation among bioinformaticians, health-care professionals and wet-lab researchers (32, 33).

CANCER EPIGENOMICS

In addition to genetic modifications, mutations, and polymorphisms, environmental factors also influence carcinogenesis through epigenetic changes. Epigenetics are heritable gene expression modifications that happen without altering the DNA sequences (34). Chemical elements are added to nucleotides and can regulate the expression of the surrounding gene(s). The epigenome concerns all of the chemical elements that have been attached to the entirety of an individual genome as a strategy to control the activity of all that genome components including genes. These epigenetic modifications cover two primary categories: methylation of DNA and modifications of histones. DNA methylation at the cytosine site of the 5th carbon typically occurs on CpG (CpG dinucleotide rich regions) islands present at the promoter and the proximal first exon of genes (35). Abnormal epigenetic pathways lead to the development of various diseases, including cancer. The aberrations found in the DNA methylation of human cancer could be assumed to fit into either of two types: transcriptional repression of tumor suppressor genes through the CpG Island promoter of hypermethylation and an extensive genome wide hypomethylation. In nearly every human malignancy, global DNA hypomethylation has been recorded (36).

One of the most prevalent kidney cancers is Renal Cell Carcinoma (RCC) (90% cases) with clear cell RCC (ccRCC) being the most common histological form (70% RCC cases). For unknown and unclear reasons, the incidence rates of ccRCC are higher amongst African American than European American. To reveal the causes of these differences, the authors performed a comparative integrative genomic and transcriptomic analysis on 50 AA and 266 EA. The findings of the differential methylation analysis showed 2,048 genes significantly varied by race. These genes have been found to be implicated in biologic processes, various molecular functions, and cellular component localization. Additionally, through the analysis of differential gene expression, 3,296 genes were found to be altered in AA compared with EA race. This work indicates that DNA methylation and mRNA expression are involved in tumor biology dissimilarities observed between AA and EA with kidney cancer (37).

Rubicz et al. carried out a study on a cohort of 76 African American men patients with prostate cancer to investigate if clinical manifestations of a more aggressive disease at diagnosis and prostate cancer recurrence are related to differential DNA methylation. Long-term monitoring detected recurrence of prostate cancer in 19 patients. Additionally, patients with cancer recurrence compared to patients without recurrence, were characterized by 23 differentially methylated CpGs. Methylation differences were also highlighted between regional vs. local pathological stage, men with metastatic-lethal prostate cancer vs. no recurrence, and higher vs. lower tumor aggressiveness. These findings show that prostate cancer aggressiveness observed in tumor tissues of African American patients, may be due to differentially methylated CpG sites (38).

Nieminen et al. characterized 69 sporadic Egyptian colorectal cancers for promoter methylation at 24 tumor suppressor genes, microsatellite instability, expression of mismatch repair, p53, and beta catenin proteins. Data were compared with 80 sporadic and familial Finnish colorectal cancers. The results indicated that

Egyptian colorectal carcinoma significantly marked by elevated methylation of the microsatellite stable tumors as reflected by the average number of methylated genes per case and by the tumor suppressor gene methylator phenotype which was defined as methylation of 5 or more genes. Compared with these Egyptian samples, sporadic western, namely Finnish, cancers were characterized by a lower rate of methylation. Four genes are distinctly methylated between Egyptian and Western cases, wherein the relation in cyclin-dependent kinase inhibitor 2B (CDKN2B)/p15 to Egyptian roots was noteworthy. These results illustrate the potential impact of environmental exposures through DNA methylation in carcinogenesis (39). Another Abdulkareem et al. research showed different patterns of DNA methylation between Africans and European patients with colorectal cancer. Genome wide DNA methylation of 480,000 CpG sites revealed 4,103 of distinctively methylated sites between the two races, with 92% of CpGs (over 1,986 genes) being mainly methylated in Africans contrasted with 8% (246 genes) in European (40). As with all aspects of cancer omics, epigenetics in sub-Saharan Africa is poorly explored in cancer as in other non-communicable diseases (34).

CANCER TRANSCRIPTOMICS

Transcriptomics is the analysis of RNA molecules on a wide scope, using high-throughput techniques, namely microarrays or RNA-seq. It explores the abundance and composition of a cell transcriptome (41). Transcriptomics helps us to view the genome's functional elements and expose the global gene expression profiles associated with the disease (42). Transcriptome research is widely supported for the identification of biomarkers, precision medicine and investigation of biological and functional processes involved in health condition as well as in disease state such cancer (43). In a study conducted by Bernard et al. single cell transcriptomes analysis indicated the possibility of achieving high-resolution profiling of transcriptomic fluctuation occurring during multiphase progression of cystic pancreatic ductal adenocarcinoma precursors to pancreatic cancer (44). In addition to metabolomics, using a transcriptomic approach in another cervical cancer research, the authors assessed genes in 7 substantially enriched pathways, of which 117 differentially articulated genes appeared to be essentially involved in catalytic action. These findings suggested that both transcriptomic and metabolomic variables were associated with cervical cancer (45). In a study interested in non-small cell lung cancer (NSCLC), researchers performed a transcriptomic study of 1,027 NSCLC patients and 108 neighboring peritumoral tissues obtained from TCGA resource. This work revealed 2,202 genes presenting significantly diverse expressions in cancer cells in contrast with healthy controls (42).

To investigate the influence of racial variance in gene and miRNA expression on the biology of lung tumors with clinical relevance in African Americans (AA) and European Americans (EA), Mitchell et al. performed a comparative molecular profile on normal tissue and lung tumor samples, from AA and EA, using mRNA (n = 22 AA and 19 EA) and miRNA (n = 42 AA and 55 EA) expression arrays. The results of this study demonstrated that differential gene expression in EA lung tumors has been mostly affecting cell proliferation pathways. Whereas, the differential gene expression enriched in AA concerned stem cell and invasion pathways. Population-specific gene expression was in part determined by population-specific miRNA expression profiles. This comparative transcriptomic profiling highlighted intelligible distinctions between AA and EA in lung tumor biology (46).

Furthermore, Paredes et al. conduct a study to investigate the contribution of tumor immunology in the disparities observed between AA and Caucasian Americans (CA) populations. The authors performed a whole transcriptome sequencing to inspect the tumor and non-tumor adjacent tissues gene expression of AA and CA colon cancer patients. Additionally, as a validation cohort, they used the TCGA database from AA and CA. AA tumor samples present significant fold-change elevation in gene expression compared with CA for Interleukin 8 (IL8), forkhead box P3 (FOXP3), and Interleukin 1 beta (IL1B) genes. On the other hand, excessive gene expression of markers related to antitumor activity such as Interferon Gamma (INFG), Granzyme B (GZMB), and the immunotherapy targets Cytotoxic T-lymphocyte associated protein 4 (CTLA4) and Programmed death-ligand-1 (PDL1) proteins was observed in CA patients. Regarding the study of immune cell populations, the results showed that AA when compared to CA has an elevated number of mast cells, exhausted CD8+ cells and augmented T regulatory cells. Moreover, the differences between the two groups studied were also evident in the patterns of cytokine production in plasma. This work indicated the dissimilarities in colon cancer immune characteristics between AA and CA that may be implicated in insufficiency of proper immune defense mechanisms (47).

Esophageal cancer (EC), which is the seventh leading cause of cancer-related deaths, is a malignant tumor in the epithelial cells filling the esophagus. EC is accountable for over 400,000 deaths each year (48). Of all the cases of EC diagnosed globally, Esophageal Squamous Cell Carcinoma (ESCC) represents about 90% of the 456,000 incident esophageal cancers each year (49), and among them, around 80% take place in low-income regions of Asia and Africa (50). In sub-Saharan Africa (SSA) regions, ESCC is widely spread and considered as the third leading cancer. In Malawi, 59 patients with ESCC were reported by Liu et al. as a whole-exome tumor/normal sequencing and RNA transcriptome analysis. Based on the study of the genome transcription, ESCC may be divided into three different subgroups, which were distinguished by their cell cycle expression and the neuronal transcripts. The findings of the study revealed distinctive subtypes of ESCC in SSA and concluded that the endemic existence of this disease reflects exposure to carcinogens different from oncogenic viruses and tobacco (51).

In addition, the most prevalent pediatric cancer in equatorial Africa with endemic malaria is the Endemic Burkitt lymphoma (eBL) which almost constantly comprises the Epstein-Barrvirus

(EBV), different from sporadic Burkitt lymphoma (sBL) characterized by decreased incidence in developed countries. For the purpose of understanding pathogenesis, Kaymaz et al. performed transcriptomic analysis using RNA sequencing from several primary eBL tumors versus Burkitt lymphoma (BL) tumors. Based on EBV genome type, in-hospital survival rates, anatomical presentation site, and suggesting that eBL tumors are homogeneous without marked subtypes, low expression distinctions were found within eBL tumors. The remarkably reduced expression of key genes in the immunoproteasome complex in eBL tumors carrying type 2 EBV compared with type 1 EBV is the salient difference revealed using surrogate variable analysis. In this study, the main part of pathway and expression differences was associated with PTEN/phosphoinositide 3-kinase (PI3K)/mechanistic target of rapamycin (mTOR) signaling pathway and was robustly compatible with EBV status rather than geographic specification. Moreover, a group of novel genes mutated in BL, including the coding gene for MutS Homolog 6 (MSH6), phospholipase C gamma 2 (PLCG2), Protein Kinase, DNA-Activated Catalytic Subunit (PRKDC), Regulation of Nuclear Pre-MRNA Domain Containing 2 (RPRD2), DNA repair protein (RAD50), Transcription factor activating enhancer binding protein 4 (TFAP4), BAF Chromatin Remodeling Complex Subunit (BCL7A), Proline Rich Coiled-Coil 2C (PRRC2C), and Forkhead box protein O1 (FOXO1) have been distinguished. Generally speaking, the data of this work demonstrated that EBV, in particular type 1, catalyzes BL tumor formation, reducing the requirement for certain specific mutations from the human genome (52).

Increasing transcriptomic innovations are nowadays recurring in order to diagnose cancer faster and more reliably, giving better prediction and prognostic value to cancer medical specialists and patients. Modern technologies like sequencing of RNA may replace existing imaging techniques to furnish further precise analysis of the transcriptoma and the aberrant expression that induces oncogenesis. Transcriptomics is used for the diagnosis of different cancer types for instance breast cancer, colorectal cancer, lung cancer, prostate cancer, and other tumors of unknown origin (53). Nevertheless, cancer transcriptomics and postgenomic medicine demand bioinformatics innovation and a critical review of the existing algorithm's performance. Even so, due to interdependencies within gene entries, this analysis frequently faces considerable difficulty (43). Despite the importance currently given to cancer transcriptomics, the application of this approach in the African continent is still very poor compared to developed countries.

CANCER PROTEOMICS

The proteomics domain deals with the detection of the complete peptide and protein complement produced in an organism, tissue, or a cell and can be, in theory, more specifically linked to phenotypic modifications related to the pathogenesis of a certain disease. Proteomic studies may describe the functional situation of protein activities, protein-protein as well as protein-ligand interactions (54). Unlike transcriptomics, proteomics methods take the post-transcription, translation, and post-translational changes of polypeptides into account (55). In cancer, proteomic analysis can be used to follow disease development, to potentially distinguish markers for cancer diagnosis, and to characterize therapeutic targets on a body wide scale (56).

Urine and blood are both very promising sources of preclinical biomarkers for prostate cancer (PCa) in Africa. Contrary to African American populations, there is a lack of PCa proteomics research on indigenous peoples of African descent. Although several potential preclinical biomarkers of PCa were disclosed in Western studies, a limited number of studies in Africa have discovered and validated new possible PCa biomarkers (57). The study carried out by Adeola et al. on multi-ethnic cohorts of South African patients discovered novel candidate urinary protein biomarkers for prostate cancer. Throughout this study, proteomic analysis was performed based on mass spectrometry of pooled individual PCa samples, benign prostatic gland enlargement, normal healthy prostate samples, as well as patients carrying other uropathies to classify proteomic profile spectrum. A total of 1,102 and 5,595 protein groups and non-redundant peptides, respectively, were found in the pooling experiments. Twenty possible biomarkers in PCa were revealed and fold differences were spotted in 17 proteins. The analysis of 45 individual samples generated 1,545 and 9,991 protein groups, and non-redundant peptides, respectively. Seventy-three protein groups were identified as potential PCa biomarkers along with some known putative PCa biomarkers and demonstrated ethnic patterns within the PCa cohort. The identification of useful biomarkers tailored to several races and the good understanding of interethnic distinctions in this studied cohort, has been achieved thanks to the distinct proteins with ethnic orientation. The revealed candidate biomarkers, in addition to the demonstration of ethnic trend, regularly differentiated between PCa, benign prostatic hyperplasia, patients with other uropathies, and normal healthy individuals (58)

Ovarian cancer is the seventh most common cancer among women. In 2018, 295,414 cases and 184,799 deaths due to ovarian cancer have been identified. The lack of access to suitable treatment may be the cause of the elevated mortality-to-incidence ratio among African women (59). Ovarian cancer is characterized by the uppermost rate of mortality of all gynecological cancers because of its tardy detection and ambiguous symptoms. Hence, promising new potential tools for ovarian cancer diagnosis are needed. Rizk et al. intended to find a characteristic pattern of plasma proteomes that could be used to detect epithelial ovarian cancer in Egyptian females, compared to benign ovarian masses and normal controls. They further aimed to distinguish amongst early and advanced ovarian cancer profiling of plasma proteins, and between extremely serious and non-serious histopathological forms. The findings showed a 21-peak plasma proteome profile differentiating patients with epithelial ovarian cancer from healthy

individuals, whereas a 5-peak profile distinguished patients with epithelial ovarian cancer from those with benign ovarian masses. With a recognition capability of 88.3% and an overall cross validation of 70%, the profile of 20 peaks was developed to differentiate between early and late disease stages. Of these 20 peaks, 14 were overexpressed in early stage ovarian cancer patients (stages I and II), but not significantly. Whereas, 6 peaks were over-expressed in late stage ovarian cancer (stages III and IV) (60).

The proteomics field has developed tremendously over the past 10 years especially in Europe, North America, and Asia, whilst it comparatively remains quite poor in Africa. In South Africa, the introduction of proteomics research is recent and a small number of scientists use it as a routine approach. The main challenges facing the large application of proteomics are associated with the rarity of scientists, and technical support in biotechnology in general. The handful of proteomics-trained researchers prefer to move on to other unconnected occupations upon accomplishment of training, often even before their research is publishable or published (55).

CANCER METABOLOMICS

Metabolomics is the new omics technique used for the investigation of the presence and the abundance of metabolites (low weight biomolecules) in body fluids and cells (54). Urine, tissue, and serum are the most common specimens compatible with metabolomics analysis. Through genomics and proteomics, the metabolome changes according to the individual's physiological and pathological condition and the detection of particular metabolites provide a potentially useful insight towards pathogenetic disease mechanisms (54). Metabolomic research is currently the prevailing approach for early detection and precise medicine and it may also provide information from a metabolic point of view regarding the development of cancers (42). Therefore, the comparison of the metabolic profile alterations of cancer cells with those of normal cells can contribute to the discovery of metabolites that would trigger carcinogenesis (61). Yang et al. published a detailed metabolomics and transcriptomics study on the possible diagnostic implications of cervical cancer and its metabolic character profile. 62 metabolites varied between cervical cancer (CC) and standard controls, five of which were selected as candidate biomarkers for CC, and were able to pave the way for diagnosis and screening (45). The Combination of transcriptomics and metabolomics approaches has elevated the effective recognition of both important functional genes and metabolic pathways in lung cancer patients. In a study in which the authors made an untargeted metabolomics assessment of 142 patients with non-small cell lung cancer (NSCLC) and 159 safe controls; 35 reported metabolites significantly differentiated between NSCLC patients and healthy controls, of which 6 metabolites were selected as possible combination biomarkers for NSCLC. Like in the previous one, the findings of this study confirm that the discriminating metabolic biomarkers detected can be used for screening and diagnosis of NSCLC (42). Researchers combined transcriptomics and metabolomics in another study on human prostate cancer to compare 25 paired tumor and adjacent non-cancerous tissues. Further confirmation of the results has been performed in an expanded cohort of 51 PCa patients and 16 patients with benign prostatic hyperplasia. The findings showed many abnormally expressed pathways at both the metabolic and transcription levels, including metabolism of methionine and cysteine, metabolism of nicotinamide adenine dinucleotide, and hexosamine biosynthesis. The sphingosine metabolite has also shown capacity to distinguish prostate cancer from benign prostatic hyperplasia with high sensitivity and specificity (62).

Breast cancer is the most common cancer among women. In 2012, 1.67 million new cases and 324,000 deaths of breast cancer were identified worldwide. The incidence rate of breast cancer varies considerably among different regions of the world (27 per 100,000 in Middle Africa and East Asia and 92 per 100,000 in Northern America) with the knowledge that the highest age-standardized mortality rate around the world was recorded in Africa (63). Triple negative breast cancer (TNBC), which is more common in African Americans, is a cancer in which the expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor 2 receptor (HER2) is missing. In the study conducted by Kanaan et al. the authors, through a comprehensive gas chromatography (GC)-mass spectrometry (MS) and liquid chromatography (LC)/MS/MS-based and unbiased metabolomic analysis, addressed a molecular understanding to detect the differences between TNBC and ER(+) breast cancer. The analysis was carried out on a series of breast carcinomas from African-American patients. The results of the global metabolomic profiling of tumor tissues determined a total of 418 featured metabolites, out of which 133 were found to be different between ER (+) and TNBC tumors. In the TNBC when compared to ER(+) tumors, the distinct biochemical pathways affected included those reflecting general augmentations in energy metabolism and transmethylation. Moreover, high levels of biochemicals linked with increased proliferation, redox balance, and the recently proposed oncometabolites, sarcosine and 2-hydroxyglutarate were found in TNBC compared to ER(+) tumors. The outcomes of this study highlighted the possibility of discovering new treatments based on the distinctive metabolic characteristics of these tumors (64).

In a study conducted on an Egyptian cohort, researchers aimed to compare the levels of metabolites in sera of 49 patients with cirrhosis and 40 individuals with hepatocellular carcinoma (HCC). The use of ultraperformance liquid chromatography coupled with quadrupole time-of-flight mass spectrometer (UPLC-QTOF MS)-based metabolomics, supplies helpful insight into suitable computational methods and experimental design for the discovery of serum biomarkers. The findings allowed candidate cirrhotic controls. It is important to recall that this is the first MS-based metabolomics study conducted on Egyptian cohort in order to discover candidate metabolites that could be used to detect HCC early in cirrhosis patients (65).

Despite all these studies carried out on cancer patients and these interesting results, the oncology community not only in Africa but worldwide still lacks knowledge of metabolomics and is uncertain about its methodological methods, technological problems, and clinical applications (66).

CANCER METAGENOMICS

Metagenomics has widened the potential in targeting the microbes responsible for causing different kinds of cancer (67). Metagenomics is a valuable strategy to characterize and classify microorganisms in their home environment. The identification, analysis, and targeting of microbial diversity in tissue samples from cancer patients have been revolutionized with the implementation of metagenomic approaches (67).

Colorectal cancer (CRC) is the third most deadly type of cancer in the United States. The estimates of 2016 showed 134,490 new colorectal cancer cases (70,820 in males and 63,670 in females) and 49,190 deaths (68). In sub-Saharan Africa, colorectal cancer has been estimated to be the fifth most prevalent malignancy, according to the International Agency for Research on Cancer and the American Cancer Society (69). GLOBOCAN's estimates for several countries in sub-Saharan Africa vary considerably (9). In Gambia and Mozambique, the estimated age-standardized incidence rate (ASR) per 100,000 was identified to be the lowest (1.5 in men and 1.0 in women for Gambia; 1.5 and 1.0 for Mozambique). In contrast, the highest ASR was reported in South Africa (15.6 and 9.5), due to racial and ethnic diversity (70). Given that the third leading cause of death in Morocco is colorectal cancer, Allali et al. contrasted the stool microbiome of Moroccan healthy individuals with the one of CRC patients. They follow a 16S rRNA amplicon sequencing approach to characterize the microbiome diversity and richness of samples from 11 CRC patients and 12 healthy individuals. Results revealed that cancer samples had higher amounts of Firmicutes, explicitly Clostridia, and *Fusobacteria*, notably *Fusobacteria.* Whilst Bacteroidetes were enriched in healthy samples, especially the *Bacteroidia* class. In diseased patients, *Porphyromonas*, Clostridium, *Ruminococcus, Selenomonas*, and *Fusobacterium* were substantially overrepresented. Outcomes of this study have enabled the identification of bacterial taxa pertinent to the Moroccan population and call for broader research to raise population-driven therapeutic methods (71).

African men are exposed to increased risk of prostate disease and infection. Feng et al. assume that the high-risk manifestation of PCa in Africa and the observed ethnic difference in turn, at least in part, may be due to pathogenic microbes. In this study, the authors reveal the microbial composition within prostate tumor tissue from 22 patients by means of metagenomic analysis of host-derived whole-genome sequencing results. What is interesting about this study is that it divided patients by race. The research revealed 23 common genera of bacteria amongst African, Australian, and Chinese prostate tumor samples. In the African vs Australian samples, the authors have found a substantial increase in the diversity of bacterial species. With an excess of *Eubacterium* linked to host tumor hypermutation, prostate tissue samples from African patients seem enriched for *Escherichia* and *Acidovorax*, considering core human gut microbiota. The high tumor mutation load in African vs. non-African specimens together with the increasing bacterial composition and abundance, suggests that bacterially-driven carcinogenesis in the prostate microenvironment may lead to aggressive manifestation of the disease in Africa (72).

Micro-organisms cause a large percentage of cancers, so metagenomics studies may promote cancer research by recognizing the microbes that are involved in cancer genesis and progression. Therefore, coming studies are promoted to scout the microbes roles in other different forms of cancer (67). Once again, this is noteworthy the very few studies on the microbiome relationships with most cancer types in the continent.

CHALLENGES AND RECOMMENDATIONS FOR CANCER OMICS DEVELOPMENT IN AFRICA

Given the enormous encumbrance of cancer in Africa, healthcare strategies need to catch the most cost-effective and precise approaches to test and diagnose the disease at an early stage. Even though up to 80% of the cancer incidence is in low- and middle-income countries, it only benefits from about 5% of global cancer spending (7). This low investment in the field of cancer is reflected in the limited number of studies carried out in the continent, in particular, those using developed methods such as the omics strategies. **Table 1** summarizes African studies on cancer omics.

Precision medicine, also known as personalized medicine and individualized medicine (73), is a modern healthcare approach that aims to produce the accurate treatment at the proper dose and time based on the individual's health, diet, lifestyle, family history of disease, and ethnicity (74). We must emphasize that the majority of studies aimed at revealing the molecular profile of cancer have been carried out in patients from high-income countries. As cancer has become a global burden and cancer medicine is progressively guided by molecular alterations in high-income settings, low-income settings can be left behind. Therefore, researchers, funders, and policymakers must increase their efforts internationally to allow cancer research to cover the entire world (75). The main challenges facing the precision medicine implementation in Africa are manifested in the deficiency of infrastructure, equipment, transport, funding, trained personnel in laboratory medicine and data sciences, and evidence to support the applicability of the clinical response (75). In order to overcome this shortage and make precision medicine a reality in Africa, further genomics research and data collection relevant to indigenous populations are needed (76). Additionally, there is an increased need to transform genomics knowledge into genetic tests, diagnostics, or improved dosing algorithm (76).

Data such as medical histories and genetic test data are the basis of sizable cohort studies and personalized medicine (77). In the case of big data, it is hard for an organization to analyze,

TABLE 1 | List of African studies on cancer omics.

Type of cancer	Omics method	Year of publication	Country	Reference
Prostate Cancer	Genomics	2015	South Africa	(26)
Prostate Cancer	Genomics	2018	South Africa	(28)
Colorectal Cancer	Epigenomics	2016	Nigeria	(39)
Esophageal Squamous Cell Carcinoma	Transcriptomics	2017	Malawi	(51)
Endemic Burkitt lymphoma	Transcriptomics	2017	Kenya	(52)
Prostate Cancer	Proteomics	2015	South Africa	(58)
Ovarian Cancer	Proteomics	2019	Egypt	(60)
Hepatocellular Carcinoma	Metabolomics	2012	Egypt	(65)
Colorectal Cancer	Metagenomics	2018	Morocco	(70)
Prostate Cancer	Metagenomics	2019	South Africa	(71)

manage, and extract value from it through traditional methods and systems due to its big volume, velocity, and diversity (78). With the huge advance of sequencing techniques in biomedical domain, tons of molecular sequencing and genome profiling data were generated. Extensive projects such as TCGA gathered large scale genomics data which are publicly accessible. These data sets supply criteria for method development and raising of big data analysis performance (77). In low and middle income countries (LMICs), such as African countries, the main challenge facing the use of big data in precision medicine is learning how to start generating and harnessing the value of sharing big data. Additionally, because of the restricted availability of patients' data, scientists and epidemiologists carrying out research in these regions, may find limited use of big data. One of the significant challenges in using health big data is managing the transition from using paper to using electronic documents, especially with the fact that the clinicians still prefer to utilize paper and are less affected by the capabilities that the infrastructure provides for sharing data through information system exchange. We must point out that the infrastructure needed to implement big data initiatives is generally sophisticated because it encompasses many technology platforms, data types, and stakeholders (78). For these reasons, big data initiatives should be encouraged by the ministries of health and research. In order to ensure greater benefits, the ministries must also construct efforts in an open and public framework and include public-private partnerships. Moreover, to link data with practice, ministries should establish relationships with physicians and data scientists. In this context, in order to provide an extensive infrastructure that can lead, in the health sector, to the production and use of big data, as example to follow, the Rwandan government has proposed the Rwanda Health Information Exchange (RHIE) initiative. RHIE strives to continuously collect and assemble health data and encourage service providers, organizational decision makers, and patients to reuse it (78). Generally, LMICs are rapidly beginning to generate data that has become "big" in nature, especially with the widespread and growing prevalence of cloud infrastructure, web-based technologies, mobile devices, and other technologies (78).

The emergence of high-performance omics-based technology has illustrated the demand for computational biology, and also, state-of-the-art experimental biospecimen banking. Inappropriate biological specimen documentation and storage can lead to distorted biochemical inferences, histopathological examination, and expected therapy. A suitable biorepository specimen, associated with pertinent data for research purposes and following rules of relevant ethics, policies and processes, is, therefore, essential infrastructure component for the development of personalized medicine based on high-throughput omics in Africa. Many revolutionary genomics projects including the Human Genome Project (HGP), the Human Proteome Project (HPP), and TCGA have gained greatly from biorepositories of specimens (79). In Africa, however, it is evident that there is restricted scope for biobanking, and that processes such as fresh snap-frozen tissue sampling are not easy to be conducted in the majority of the parts of the continent because liquid nitrogen is mainly out of control (57).

Regarding cancer genomics research capacity in Africa and mainly in East Africa, only Kenya and Sudan have the maximum capacity to carry out research into cancer genomics. Both countries have academic facilities fitted with state-of-the-art labs. Biosciences Eastern and Central Africa Hub Genomics and Bioinformatics Platform in Kenya is equipped with capillary and second-generation sequencing facilities that will enable East African researchers to conduct genotyping activities and sequencing for genomes and metagenomes (80).

African authorities should concentrate on financing facilities, researchers, and support for scientific training and with favorable improvements in health policy, molecular methods based on omics should be incorporated into routine clinical practice (7). Furthermore, to exploit the advantages of bioinformatics and data science in cancer omics research in Africa, the first step is to overcome the issue of limited skills in bioinformatics and genomics all over the continent. Adequate computational infrastructure, teaching laboratories, availability of training spaces, server systems, and social and political stability are some of the factors influencing the organization of sustainable training programs. Across Africa, several bioinformatic training initiatives have been launched such as the doctoral training in bioinformatics provided in Uganda and Botswana through the Collaborative African Genomics Network (CAfGEN) (81), the Eastern Africa Network of Bioinformatics Training (EANBiT) (81) which supplies bioinformatics training in Kenya as part of a M.Sc. program in bioinformatics, and the African Genomic

Center (TAGC) launched in Cape Town, South Africa in 2018 which comprises a powerful bioinformatics training component (81), not to mention the H3 African Bioinformatics Network (h3ABioNet) (82) that, in different countries, organize training programs aimed at enhancing the computational skills of biology and health scientists in Africa (81) (83). In addition, African scientists, regardless of their location, can be trained through online training programs, workshops on bioinformatics organized by world leading scientific organizations, short courses, and complete online degree programs established by some African universities (83). The current efforts in Africa to improve training opportunities in Bioinformatics and Genomics are expected to generate scientific experts to drive the prosperity of genetic and genomic research in Africa (83). In this regard, to improve skills in medical genetics and genomics, key healthcare personnel must be involved. For this purpose, training of the healthcare staff and clinical researchers in genomic medicine, through professional development courses, is the foundation of efficacious adoption of genetic and genomic evidence into clinical cancer application. Generally speaking, training initiatives in genomic medicine domain are in their infancy, but the African continent confront further challenges at the institutional and logistical levels. To achieve the objective of developing knowledge and capacity in genomic medicine, during a common conference of the African Society of Human Genetics and the US National Health Institutes (NIH)-funded H3Africa Consortium in 2016, Senegal, the participants launched The African Genomic Medicine Training (AGMT) Initiative (84). Healthcare staff like doctors, pharmacists, nurses, who are not geneticists, are the main beneficiaries from this durable genomic medicine training initiative. This approach provided graduate and postgraduate programs, short courses as well as public engagement activities. The AGMT initiative also gives the opportunity to patients who wish to be advocates in the fields of genetics and genomics to participate in the courses. We must point out that, across Africa, AGMT was the first extensive community training initiative in genomic medicine (84).

Regardless of the fact that bioinformatics typically needs much less infrastructure investment compared to science-intensive disciplines, basic equipment like robust computer systems, access to basic databases and software, dependable source of electricity, high-speed Internet are essential. Fortunately, the challenge of the lack of internet and computers is gradually disappearing in Africa allowing research to progress. Moreover, building research centers well-equipped with bioinformatics resources and integrating specific departments in bioinformatics within the existing institutes is improving training conditions (83). Furthermore, to help Africa mitigate some of the infrastructure hurdles, the H3ABioNet project has participated in the renovation of several training laboratories and the provision of servers and computers within the network in Africa. In order to allow geographically different classrooms to take part in a live and interactive training workshop and to deal with the wide geographical distances, in the continent, live video streaming services such as Vidyo are used (85).

THE WAY FORWARD IN OMICS FOR AFRICA

There is an intensified optimism about the role omics may help in addressing general health disparities, particularly after the accomplishment of the human genome project and the ongoing African genome sequencing. From this, we can say that there is an increased need for genomics research alliances in Africa to carry out omics studies and to reveal means in which the results of these research could be practically incorporated into health care for the interest of African populations (86). For all these reasons we propose the immediate establishment of an "African Cancer Genomics Consortium". Our call to this all-omics initiative aims to raise the efforts in order to minimize the massive negative effect of this fatal disease on an already brittle continent and to encourage others to become concerned. For example, studying African genomic variation constitutes the next frontier of genetic medicine, it will therefore be necessary to develop an African genomics workforce to implement such broad research in cancer. These efforts can build on the foundation of many successful initiatives such as H3Africa (87).

CONCLUSION

As mentioned earlier, compared to non-African populations, the genetic diversity of the African race is the highest. Therefore, comparative studies conducted on ethnically diverse populations, mainly in Africa, are crucial to investigate the genetic basis of complex disease and phenotypic adaptation. In Addition to phenotypic details about various traits such as disease likelihood and response to drug, the comprehension of levels and patterns of difference in African genomes, will be pivotal to highlight the genetic basis of environmental adaptation and to discover new and effectual therapeutic treatments for disease (88). In the case of cancer, racial dissimilarities observed in the mortality and the morbidity of the illness, could be minimized through an understanding of contributing genetic factors. This objective can be attained by studies conducted, for example, on African American participants (89). In the field of cancer omics, the USA is the country with the most efforts to apply these approaches to African immigrant populations (**Table 2**).

The world is expecting massive data/information generation at most cancer levels in the genomics, transcriptomics, proteomics, metabolomics, epigenomics, metagenomics, etc. The translation of these data into practical clinical ways (i.e., identifying pathways/pathophysiology) will require considerable work in the coming years. To do this, we need integration of system biology approaches, emerging technology, and new computational and mathematical methods for in-depth research into cancer.

As technologies and strategies in Omics are continually evolving, the emerging technique single cell sequencing offers a valuable tool to enhance our knowledge of tumor cell heterogeneity in order to guide tailored cancer treatments.

TABLE 2 | List of studies on cancer omics carried out on migrant African.

Type of cancer	Omics method	Year of publication	Country	Reference
Laryngeal cancer	Genomics	2016	USA	(21)
Clear cell renal cell carcinoma	Genomics	2016	USA	(22)
Prostate cancer	Genomics/Transcriptomics	2020	USA	(24)
Prostate cancer	Genomics	2020	USA	(25)
Clear cell renal cell carcinoma	Epigenomics/Transcriptomics	2020	USA	(37)
Prostate cancer	Epigenomics	2019	USA	(38)
Non-small cell lung cancer	Transcriptomics	2017	USA	(46)
Colon cancer	Transcriptomics	2020	USA	(47)
Triple negative breast cancer	Metabolomics	2014	USA	(64)

Moreover, this new technique is a way of distinguishing subpopulations of cancer cells in a single patient. Single cell sequence analysis can tend to be crucial to comprehend the etiology, development, and drug resistance of cancer countries (90). Although the single cell genomics is its infancy, African cancer research is called to engage in this very promising trends for research in cancer.

Being a continent predominantly populated by low and middle-income countries and highly impacted by cancer, various obstacles have been identified working against the common implementation of these strategies in Africa. There is an urgent need to expand country- or regional-based cancer research initiatives and collaborate with partners inside and outside the continent to overcome these limitations. African governments should also be involved in the implementation of cancer omics strategies by providing a useful and sustainable research environment in local government-owned institutions that will provide researchers with many opportunities to build their capacity in bioinformatics and omics through training programs. In this context, it is reassuring that many initiatives and projects have been put in place in different African countries (81), but additional efforts should be made to generalize these approaches on the continent. In addition, the hurdle of the limited number of studies conducted on African populations must be overcome, and research should be encouraged and pushed towards the detection of omics (genomics, transcriptomics, proteomics, metabolomics, epigenomics, and metagenomics) alterations in the case of cancer in a highly genetically diverse population such as the African one. Collaborative research geared towards the investigation of these cancer omics in African patients must also be motivated, both at continental level and with international partners. The prospected "African Cancer Genomics Consortium" would be mandated to promote such collaborative projects and engage in research activities for cancer precision medicine.

AUTHOR CONTRIBUTIONS

IE wrote the manuscript. IA conceived the study and wrote the manuscript. SS wrote the manuscript. KO conceived and designed the study, and reviewed the writing of the manuscript. SH and NA reviewed the writing of the manuscript. CN and SA conceived and designed the study. YB conceived the study and reviewed the writing of the manuscript. HG conceived and designed the study and and wrote and reviewed the writing. All authors contributed to the article and approved the submitted version.

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REVIEW
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Cancer in Africa: Is It a Genetic or Environmental Health Problem?

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Patients of African ancestry have the poorest outcome and the shortest survival rates from cancer globally. This could be attributed to many variables including racial, biological, socioeconomic and sociocultural factors (either single, multiple or combined), which may be responsible for this major health problem. We sought to assess the most common types of cancer that endanger the health of the African people, and tried to investigate the real differences between African and other Non-African patients regarding incidence, prevalence and mortality rates of different cancers. Therefore, identifying the underlying aetiological causes responsible for the increased incidence and mortality rates of African patients will allow for changing the current plans, to make optimized modalities for proper screening, diagnosis and treatment for those African patients, in order to improve their survival and outcomes.

Keywords: Africa, cancer, incidence, survival, mortality

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INTRODUCTION

Cancer is a major public health problem worldwide. It is one of the most leading causes of death in several regions depending upon disparities among different people (1). These disparities include socioeconomic, ethnic, racial and cultural factors that differ between low and high-income countries. According to the records obtained from the GLOBOCAN 2018 database of the International Agency for Research on Cancer (IARC) (2), the estimated results of 36 cancer types available from 47 countries of the African region of WHO (AFRO) revealed that there are 811,200 new cancer cases (4.5% of the total world) and 534,000 cancer deaths (7.3% of the total world) reported in the AFRO countries in 2018 (**Figure 1**).

The estimated cancer burden in the AFRO countries is mainly attributed to breast cancer which represents 27.7% of the total cancer cases, followed by cervical cancer which represented 19.6% of the total cases. Taken together, this represents the most common in African females. Meanwhile, prostate cancer (18.1% of total cases), followed by liver cancer (9.7% of total cases) and colorectal cancers (6.9% of total cases) were the most common in African males (**Figure 2**). Concerning survival rate of childhood malignancies, the survival rate is as low as 20% in African children and 80% in high income countries (4).

In an interesting study, Pinheiro and his colleagues (5), analysed the cancer mortality data obtained from South Florida for white, Hispanic, and black populations with disaggregation for Cuban, Puerto Rican, South American, African American, and Afro-Caribbean groups, during the period 2012–2016.

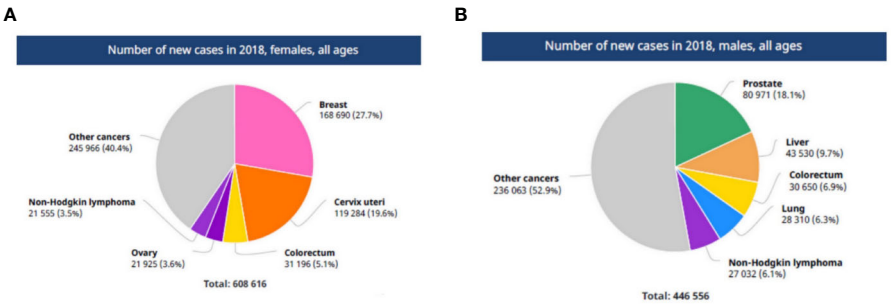

FIGURE 1 | The number of new cancer cases in AFRO region. **(A)** In African females, **(B)** in African males. Reproduced from "The Global Cancer Observatory, Africa Globocan 2018" (3).

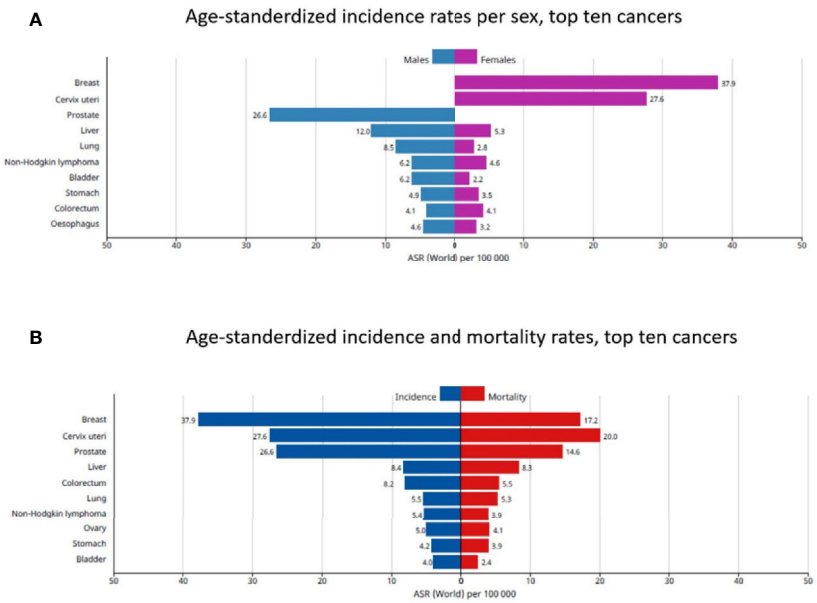

FIGURE 2 | The top 10 cancers in African patients. **(A)** Age-standardized incidence rates per sex, **(B)** Age-standardized incidence and mortality rates. Reproduced from "The Global Cancer Observatory, Africa Globocan 2018" (3).

Pinheiro et al., provided an evidence that, the African American males and females had the highest all sites-combined cancer mortality rates among all groups. As well as the highest mortality rates for many cancers including breast, prostate, lung, stomach, colorectal carcinoma, liver and multiple myeloma. According to their data, the Afro-Caribbean patients had significantly higher mortality rates compared to the white populations especially for stomach, prostate, multiple myeloma, premenopausal breast and endometrial carcinomas. In contrast, lower rates were reported for the other cancer types, particularly the lung cancer. These data are similar to other previous studies reported higher race-specific rates among both Afro-Caribbean and African American populations for endometrial, premenopausal breast, prostate, and multiple myeloma cancers in South Florida's black population (6, 7). They also reported that lung cancer was the first leading cause of cancer-related death in African American men, followed by Prostate and colorectal cancers. While, for the Afro-Caribbean's and other Hispanics, prostate cancer was the leading cause of cancer-related death followed by lung and colorectal carcinoma. On the other hand, breast and lung cancers were the first and the second leading causes of cancer- related death in African American females, followed by colorectal cancer, while lung cancer preceded breast and colorectal cancers in the Afro-Caribbeans (5).

It is a well-known fact that, cancer outcome is not equal in all people, and there are many factors that can affect its behaviour and its impact on the patients' survival or response to treatment. Here, we review the most common types of cancer that endanger the health of the African people or those with African ancestry, and

investigate the differences between African and non-African patients regarding incidence, prevalence, and mortality rates of different types of cancer. This will pave the way to produce an appropriate screening method or targeted therapy for such patients.

PROSTATE CANCER

Prostate cancer is the first leading cause of cancer deaths in African males, and the second leading cause of cancer deaths in the united states (1, 2). It was obviously noted that, racial disparity plays a crucial role in its incidence and mortality rates among American patients (8). In 2007, It has been reported that prostate cancer incidence among black men in the US was 60% higher and its mortality was more than double the estimated rates in white men respectively (9). Later on, Siegel et al. (8), reported in 2014 that African-American men were 2.4 and 5.0 times more likely to die from prostate cancer compared to Americans of European or Asian ancestries, respectively. Various studies tried to investigate this racial disparity in prostate cancer regarding its incidence, prevalence, aggressive behaviour, and mortality rates. Some of these studies proposed that, the poor outcome in black men may be attributed partially to the inaccessible medical care and/or inadequate screening and treatment facilities (10, 11). Meanwhile, other studies mentioned the differences in germline and genetic background between black and white men as a reason (9, 12–14). Moreover, the socioeconomic status and lifestyle variation had also been suggested, however after adjusting for these factors, African ancestry remains a significant risk factor for prostate cancer (15). Supporting these data, Moul et al. and Faisal et al. (16, 17), concluded that the black race has to be considered an independent prognostic factor for disease recurrence, allowing for a more biologically aggressive phenotype. Though, the explanation of this disparity is still unknown and require more in depth studies (18). Tsodikov and his colleagues have also investigated this issue through establishing three predictive models of prostate-specific antigen (PSA) screening patterns in the USA, to compare the prostate cancer natural history in black men compared to the general population using an updated reconstruction of PSA screening. The obtained data were collected through the National Health Interview Survey in 2005, and the prostate cancer incidence from the Surveillance, Epidemiology, and End Results program (SEER) in 1975–2000 (18). They found that 30–43% of black men developed preclinical prostate cancer by the age of 85 years, which was relatively 28–56% higher than in the general population. Also, black men showed a similar risk of diagnosis (35–49%) compared to the general population (32–44%), but their risk of progression to a metastatic disease by the time of diagnosis was 44–75% higher than in the general population. Taken together, these results are consistent with those published by Powell et al. (19), which based on autopsy and surgical pathology data. They observed that black men have an increased risk of transformation to clinically significant cancer compared to white men.

Blackburn and his colleagues (20) tried to investigate the association between the underlying genetic differences for prostate cancer with the racial variations among peoples. They reported a lower frequency for *TMPRSS2-ERG* fusion which is inversely associated with aggressive prostate cancer in black South Africans males compared to those from European ancestry. Similarly, Zhou et al. (21), performed a meta-analysis study and reported that the highest incidence of *TMPRSS2-ERG* fusion was recorded in 49% in men of European ancestry. While, lower incidence rates were found in Asian (27%) and African (25%) male ancestries. Moreover, Magi-Galluzzi et al. (22) reported a racial discordance in the mechanism(s) of *TMPRSS2-ERG* fusion occurrence, since the African Americans more commonly had *TMPRSS2-ERG* fusion through deletion, whereas the European and Asian Americans had *TMPRSS2-ERG* fusion through translocation.

For more confirmation of these data, an interesting study was done by Jaratlerdsiri et al. (23), who performed deep whole-genome sequencing for paired tumor-normal tissues obtained from African patients compared to non-African patients. The results of the study revealed a 1.8-fold increase in the small somatic variants, and also elevated oncogenic driver mutations in the African- derived tumors in comparison to the European counterpart. The *ERG* fusions and *PIK3CA* mutations were absent, *PTEN* loss was less frequent, whereas *CCND1* and *MYC* were frequently gained. In addition, out of the commonly affected prostate cancer gene pathways, genes regulating the calcium ion-ATPase signal transduction were disrupted in the African tumors. Therefore, it is quite clear that, a special screening program for the black men of African ancestry is highly required, and this should be done depending upon their own genetic makeup.

BREAST CANCER

Breast cancer (BC) is the most commonly diagnosed cancer in the African females, and it also represents the second leading cause of cancer- related deaths following cancer cervix in sub-Saharan Africa (SSA) (2). Its incidence had been increased in the last six years by more than 23% (from 1.7 million new patients in 2012 to 2.1 million in 2018) (24, 25). In addition, its five-year survival rate is less than 40% in SSA, compared to 86% in the United States (26). In their observational study on BC patients from USA, Iqbal et al. (27), reported that black women with small-sized tumors had 9.0% increase in the risk of death compared to the non-Hispanic white women who had only 4.6% increased risk of death. These data are in accordance with many previously published studies which showed that black women usually have higher risk of BC recurrence regardless of the age, tumor size or tumor grade. Based on these data, the African ancestry, by itself, should be considered an independent predictor factor for poor survival rates (28, 29).

Although BC mortality rate is now decreasing in the developed countries due to the implementation of screening programs including mammography, which is the gold standard for early detection and successful management of BC. The screening of BC in Africa is still a great challenge (30). This is attributed mainly to the lack of financial and technical support, in addition to decreased numbers of well-trained radiologists and technicians (31, 32). It was reported in some previous studies that, the age of peak incidence of BC is lower in SSA, with most of the women had advanced-stage disease at the time of diagnosis (33). At the same time, mammography is less effective for detecting tumors at advanced stages, as well as in younger women due to changes in breast tissue density according to the hormonal profile

of the patients (34). Moreover, mammography is not available in most countries of SSA, and it is only available in urban centers, that made it rater costy for women living in semi-urban or rural areas to compensate for the travel and accommodation (35, 36). Another major obstacle which could also be responsible for the poor outcomes of the BC patients in Africa, is the failure to deliver the proper treatment to the patients. This is because that the treatment options for advanced stages of breast cancer are limited and restricting mainly to mastectomy, in addition to lacking other modalities including chemotherapy and/or radiotherapy facilities (37, 38). Taken together, these factors prevent many women from getting their proper medical treatment(s) for their disease. They seek for other non- medical and non- effective options such as prayer camps and herbs, and accordingly, they usually present with advanced high grade and advanced stage tumors (37). Based on the previous data we can conclude that, breast cancer is the major health problem threatening African women, owing to poverty, social and cultural barriers, as well as limited diagnostic and treatment facilities. Black et al. (30), suggested in their study that increasing public awareness for breast self-examination and clinical breast examination (CBE) could help, at least partially, in down staging of BC in the African females. This has also been supported by the relatively recent study of Dos Santos et al. (39), who reported in their study, which was done in Sudan and Tanzania, that training health workers for CBE together with awareness campaigns can effectively improve the patients' outcomes.

UTERINE CERVICAL CANCER

carcinoma of the cervix uteri is among the most preventable malignancies worldwide (40), however it remains the first leading cause of cancer deaths in African women [(2), **Figure 3**]. Human papillomavirus (HPV) types 16 and 18 are the most common etiological factors for the pathogenesis of cervical cancer in Africa (42). The reported prevalence rate of HPV was 97.0% in Malawi (43), 92.1% in South Africa (44), 90.7% in Ibadan Nigeria (45), and 69.8% in Maiduguri and Nigeria (42). In fact, the HPV infection is usually cleared in the immunocompetent women (46). However, in women with underlying human immuno-deficiency virus (HIV) infection; as a common situation in Africa, there is an increasing risk of developing cancer cervix rather than in women without HIV infection, with the annual detection rates are 1.4 *versus* 0.4 per 100 persons per year; respectively (47–49). It was reported by de Martel et al. (50), that SSA had the highest age standardized incidence rate (ASIR) of HPV attributable cancer all over the world (ASIR 19.3 cases per 100,000 person/years). A recent meta-analysis study performed by Drolet et al. (51), including midline studies published between February 1, 2014, and October 11, 2018, reported that nearly two thirds of all cervical cancers cases caused by HPV16 and HPV18 could be prevented with the currently available HPV vaccines. At the same time, Cervical screening programs either with cytology, HPV testing, or both could prevent most of the remainder of cases especially in developed countries. However, in Africa it is rather challenged by many factors including limited resources, lack of knowledge about the cervical cancer and unavailability of screening centers (52, 53). It was estimated that the overall cervical cancer screening in Ethiopia was 0.8% according to the ICO Information Centre on HPV and Cancer 2017 (54). Similarly, it was reported to be 1% in another study done by Getachew et al. (52). All these factors contributed to inefficient early detection and consequently later diagnosis and poorer survival rates.

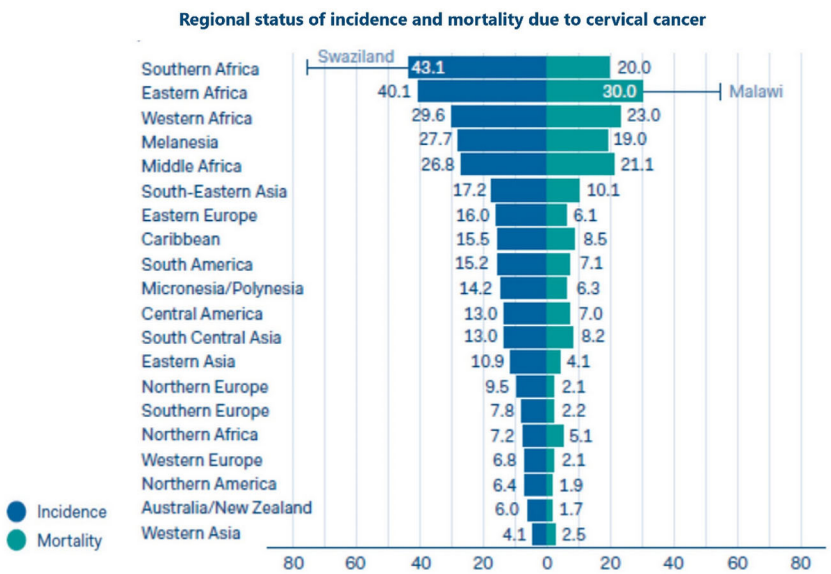

FIGURE 3 | Regional status of incidence and mortality due to uterine cervical cancer (41).

HEPATOCELLULAR CARCINOMA

Hepatocellular carcinoma (HCC) is the third leading cause of cancer related death in Africa, and a major health problem all over the world (1). It was recorded that 80% of HCC cases occurred in the SSA and eastern Asia according to Cancer Today, which is an international agency for research and cancer (24). The prevalence of HCC is heterogeneous because it has variable risk factors, since hepatitis B (HBV) and aflatoxin exposure are the major risk factors for HCC in SSA, whereas hepatitis C (HCV) is the major risk factor for HCC in USA, Europe, and Japan (55). In a large, retrospective observational study done by Yang et al. (56), which included 2,566 patients who were treated in 21 tertiary referral centers from different countries in Africa, they observed that the African patients presented with HCC were at a younger age (median of 45 years), with advanced disease stage, severe liver dysfunction and poor performance status. Additionally, Mak et al. (57), reported that the mortality rate of HCC black African patients is higher than that in white patients. Many studies had addressed this disparity between the black and the white population, among those are Ladep et al. (58), who concluded that this disparity might be due to different biological and etiological risk factors that should be urgently identified, as those patients represent high-risk group patients who need a prompt effective treatment. Other studies attributed this poor outcome to the absence of comprehensive surveillance programs for HCC, inaccessible expert medical care, socioeconomic and sociocultural factors that affect treatment decision making (59, 60). In addition to the previously mentioned etiological factors, it is clear that the HIV epidemic has had a major demographic and health impact on the black African population, which also should be assessed (57).

LUNG CANCER

Lung cancer remains the first leading cause of cancer- related deaths in the United States (1), with the highest lung cancer mortality rate being detected in the African-American population (61, 62). Indeed, there were a conflicting data regarding the racial disparity of the prevalence and outcome of patients with lung cancer. Many studies reported a significantly lower frequencies of *EGFR* mutations in black compared white patients (63, 64). However, other groups failed to find any significant association between *EGFR* mutations and patients' races (65, 66). An important study done by Campbell et al. (67), who performed genomic sequencing for a panel of 504 cancer genes in lung cancer tissue specimens obtained from 245 black patients compared to 264 white patients. Based on the data of their study, they concluded that there was no significant difference regarding mutational frequencies and copy number changes between the black and white patients. Also, there was no significant difference in the genetic alterations of the receptor *tyrosine kinase/Ras/Raf* pathway including *EGFR* and *KRAS*. Additionally, Mitchell et al. (68), reported no significant association between lung cancer survival and ethnic variations especially in West African ancestry. These data were confirmed by previous studies suggested that genetic ancestry did not adversely contribute to lung cancer risk or survival (69, 70). Therefore, some investigators suggested that other factors including socioeconomic, environmental or cultural variables could explain these disparities (68, 69).

Consistent with these results, Murphy et al. (71), concluded that African Americans consumed greater amounts of nicotine per cigarette compared to other American ancestry groups. This was measured by the urinary total nicotine equivalents (TNE), which is a more objective measure of smoking intensity than the number of cigarettes per day (CPD). Accordingly, TNE is correlated with the uptake of the well-known tobacco carcinogens such as *nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol* (NNAL) and polycyclic aromatic hydrocarbons (72). Therefore, it seems that, exomic mutations does not contribute to the observed racial disparities between black and white populations regarding lung cancer development and outcome. However, further investigations are suggested into other genomic variations such as mutations in noncoding regions and epigenetic changes, or assessment of other socioeconomic factors including smoking behavior and access to health care facilities (67).

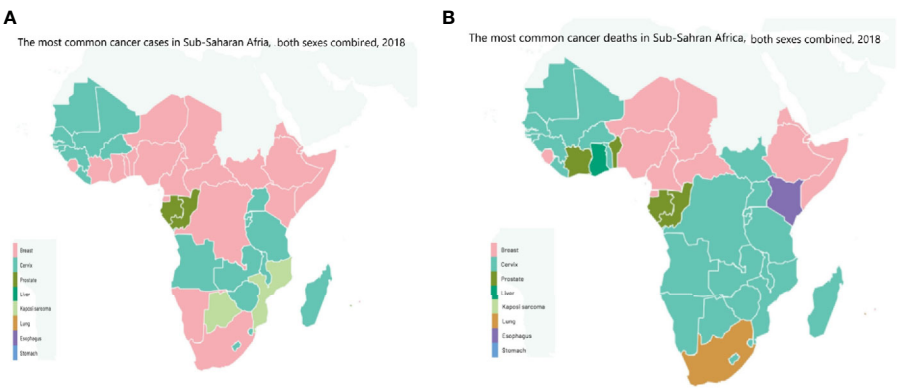

FIGURE 4 | The most common **(A)** cancer cases, **(B)** cancer deaths in Sub-Saharan Africa, both sexes combined, 2018 (3). Reproduced from "The Cancer Atlas," canceratlas.cancer.org (73).

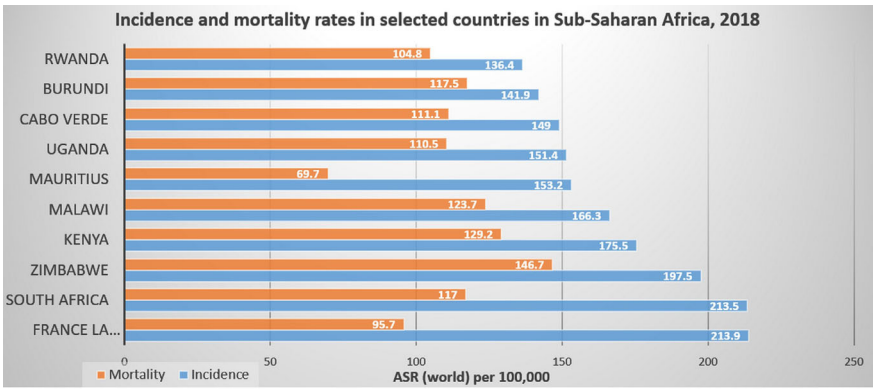

FIGURE 5 | Incidence and mortality rates in selected countries in Sub-Saharan Africa, 2018 (3). Reproduced from "The Cancer Atlas," canceratlas.cancer.org (73).

Based on our previous discussion, we can conclude that cancer is a major public health problem in Africa, with increased incidence, financial toxicities and mortality (**Figures 4**, **5**). Racial disparities seem to played an important role for the increasing incidence and prevalence of many cancers including prostate and breast cancers which are genetically more common in black patients rather than in white population. However, the increased incidence of other cancer types including lung, hepatocellular and uterine cervical cancers could be attributed to many factors other than racial disparities. Actually, Africa is challenged by many problems including mainly the prevalence of oncogenic viruses such as *HIV* for non-Hodgkin lymphoma, *HHV-8* for Kaposi sarcoma, *HPV* for cervical cancer, *HBV* and *HCV* for HCC. Other factors including limited screening programs as PAS, TURS for prostate cancer, and mammography for breast cancer. Also included is poor implementation of *HPV* vaccines as for uterine cervical cancer and *HBV* for HCC. Moreover, African patients were challenged by poor economic circumstances, low life standard, inaccessible medical care and poor medical services. All these factors together with the racial disparities contributed to increased cancer incidence and mortality among African patients. Therefore, identifying the underlying aetiological causes for increased cancer death in Africans will contribute to better modalities for screening, diagnosis, treatment and prevention.

AUTHOR CONTRIBUTIONS

AB: revised the manuscript. MA: collecting data and writing the manuscript. A-RZ: directing the work and revised the manuscript. All authors contributed to the article and approved the submitted version.

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Globally Rare *BRCA2* Variants With Founder Haplotypes in the South African Population: Implications for Point-of-Care Testing Based on a Single-Institution *BRCA1/2* Next-Generation Sequencing Study

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Breast cancer patients historically benefitted from population-based genetic research performed in South Africa, which led to the development of founder-based *BRCA1/2* diagnostic tests. With the advent of next-generation sequencing (NGS) technologies, the clinical utility of limited, targeted genetic assays were questioned. The study focused on mining NGS data obtained from an extensive single-institution NGS series (n=763). The aims were to determine (i) the prevalence of the most common recurrent/founder variants in patients referred for NGS directly; and (ii) to explore the data for inferred haplotypes associated with previous and potential new recurrent/founder variants. The identification of additional founder variants was essential for promoting and potentially advancing to rapid founder-based *BRCA1/2* point-of-care (POC) technology as a time- and cost-effective alternative. NGS revealed actionable *BRCA1/2* variants in 11.1% of patients tested (*BRCA1* – 4.7%; *BRCA2* – 6.4%), of which 22.4% represented variants currently screened for using first-tier targeted genetic testing. A retrospective investigation into the overall mutation-positive rate for an extended cohort (n=1906), which included first-tier test results, revealed that targeted genetic testing identified 74% of all pathogenic variants. This percentage justified the use of targeted genetic testing as a first-tier assay. Inferred haplotype analysis confirmed the founder status of *BRCA2* c.5771_5774del (rs80359535) and c.7934del (rs80359688) and revealed an additional African founder variant (*BRCA2* c.582G>A – rs80358810). A risk-benefit analysis using a questionnaire-based survey was performed in parallel to determine genetic professionals' views regarding POC testing. This was done to bridge the clinical implementation gap between haplotype analysis and POC testing as a first-tier screen during risk stratification of breast and ovarian cancer

patients. The results reflected high acceptance (94%) of *BRCA1/2* POC testing when accompanied by genetic counselling. Establishing the founder status for several recurrent *BRCA2* variants across ethnic groups supports unselected use of the BRCA POC assay in all SA breast/ovarian cancer patients by recent local and international public health recommendations. Incorporating POC genotyping into the planned NGS screening algorithm of the Department of Health will ensure optimal use of the country's recourses to adhere to the set standards for optimal care and management for all breast cancer patients.

Keywords: *BRCA2*, founder variants, South Africa, breast cancer, next-generation sequencing, point-of-care assay

INTRODUCTION

The development of hereditary breast cancer (BC) results in most cases, from highly penetrant pathogenic variants in several genes, of which the most frequently studied are *BRCA1* and *BRCA2*. Pathogenic variants present in these genes predispose to hereditary breast and ovarian cancer (HBOC) syndrome, with related cancers often described as being more aggressive compared to sporadic cancers. *BRCA1*-related tumors are more frequently negative for hormone receptors and of high grade, with *BRCA2*-related disease on average being of a higher histological grade than sporadic cases (1–5). *BRCA1/2* pathogenic variants predispose women to breast and ovarian cancer (OVC) (6, 7). The cumulative risk for *BRCA1/2* mutation carriers of developing BC to the age of 80 years has been approximated at 72% (95% CI 65–79%) and 69% (95% CI 61–77%), respectively. The risk for developing OVC is lower, at around 44% (95% CI 36–53%) for *BRCA1* and 17% (95% CI 11–25%) for *BRCA2* heterozygotes (8). Current management strategies for pathogenic mutation carriers range from intensified surveillance from a younger age to risk reduction surgery of the breasts and/or ovaries and include risk-reducing medications (9). Detection of inherited pathogenic variants in asymptomatic carriers allows for the development of appropriate management strategies to reduce cancer incidence and enable early detection, thus reducing mortality and improving quality of life.

The interesting history of sub-Saharan Africa has highlighted the populations of South Africa (SA) concerning the field of medical and population genetics. Due to various migration events, including European colonialism from predominantly north-western Europe, the indigenous expansion to the south, and admixture introduced mainly by slaves and laborers from southern Asia, various unique genetic signatures have been imprinted on its peoples. With genetic drift and natural selection, these major events have created uniquely admixed populations residing at Africa's southern-most region. Their composition and heritage have incited various population studies that attempted to identify each group's genetic architecture (10–14).

Over the past two decades, HBOC families in SA have derived great benefit from similar studies, which resulted in the development of a diagnostic, cost-effective first-tier genotyping assay based on a limited number of population-specific pathogenic *BRCA1/2* founder or recurrent variants. With the advent of low-cost next-generation sequencing (NGS) technologies, this assay's clinical utility was questioned based on the SA populations' collective genetic diversity. It caused a divergence from founder/recurrent variant testing to comprehensive *BRCA1/2* screening, which resulted in increased strain on the financially challenged health sector. Concerns were also raised that medical professionals and patients may misinterpret the exclusion of population-specific pathogenic *BRCA1/2* variants as a negative test result.

This study focused on exploring the potential of a new genetic counselling model that incorporates rapid point-of-care (POC) *BRCA1/2* founder-based genotyping as a cost-effective alternative to SA's current practices. Such a POC assay will allow for rapid clinical decision-making in mutation-positive patients and indicate extended NGS testing for deserving uninformative cases. Furthermore, this investigation relied on knowledge obtained regarding the incidence of founder variants in patients diagnosed with BC or OVC and the distribution of population-specific variants, including those not previously described. Thus, haplotypes associated with founder and recurrent *BRCA2* variants identified in the most extensive national, single-institution NGS series performed to date, were reconstructed. The confirmed founder/recurrent SA *BRCA2* pathogenic variants are suitable for inclusion in a customized DNA test kit developed under the South Africa-United Kingdom Newton Collaborative Research Development Program in Precision Medicine (https://gtr.ukri.org/projects?ref=103993). A current version of this kit was recently evaluated in a pilot study performed by Mampunye (15), highlighting the novel BRCA POC 1.0 Research Assay's cost-saving potential. A qualitative survey was used as a first step towards assessing the thresholds that need to be overcome to bridge the clinical implementation gap between newly obtained research results and their incorporation into a POC assay.

MATERIALS AND METHODS

Samples of a total of 763 BC and/or OVC patients who attended various genetic clinics between 2017 and 2020 were received at the National Health Laboratory Service (NHLS) Human Genetics laboratory in Bloemfontein for comprehensive screening of *BRCA1/2* using NGS. Genomic DNA was isolated

from peripheral blood (5–10 ml) using the salting-out method (16). The initial DNA quality was assessed with the NanoDrop® ND-1000 Spectrophotometer (NanoDrop® Technologies Inc., Wilmington, DE, USA), whereas the Qubit dsDNA High Sensitivity Assay kit was used to quantify DNA with the Qubit® Fluorometer (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) for NGS. Reference sequences used for *BRCA1* and *BRCA2* analyses were GenBank NM_007294.3 (*BRCA1*) and NM_000059.3 (*BRCA2*).

NGS was performed using the Oncomine™ BRCA Research Assay (Life Technologies, Carlsbad, CA, USA). The primer pools targeted the entire coding region together with intronic flanking sequences for both genes. The amplicon library was constructed using multiplexed primer pools during PCR-based targeted amplification. Sequencing was performed on the Ion Proton Platform (Life Technologies, Carlsbad, CA, USA) and the Ion Reporter™ Software (Life Technologies, Carlsbad, CA, USA) used to filter out possible artifacts. Raw signal data were analyzed using the Torrent Suite™ versions 5.2 to 5.14.

Genotyping for the most common SA pathogenic variants (17) was performed as a first-tier test (n=1906) for all breast and OVC patients. It was performed on the LightCycler® 480 II instrument (Roche Diagnostics Applied Science, Mannheim, Germany) using hybridization probe technology for six of the variants (*BRCA1* c.68_69delAG, p.Glu23ValfsX17; *BRCA1* c.1374delC, p.Asp458Glufs; *BRCA1* c.2641G>T, p.Glu881Ter; *BRCA1* c.5266dupC, p.Gln1756Profs; *BRCA2* c.7934delG, p.Arg2645Asnfs) and two simple probe assays for *BRCA2* c.5771_5774del, p.Ile1924Argfs and *BRCA2* c.6448_6449dup, p.Lys2150fs. The primer and probe sequences have been listed by Oosthuizen (18). Each qPCR reaction contained 50 ng genomic DNA, 3 μM of each primer (TIB MolBiol, Berlin, Germany), 2 μM of each probe (TIB MolBiol), 4 μl LightCycler® 5X Genotyping Master Mix (Roche Diagnostics GmbH, Mannheim, Germany), together with 12.6 μl molecular grade H_2O. A standard qPCR regime was utilized, followed by a melt curve acquiring fluorescence data at a frequency of 5 readings per °C to determine the melting point (T_m) (18). Each variant was tested for individually, together with a positive, negative and no template control to ensure sensitivity and specificity. The genotyping reports generated over the years were retrospectively analyzed to evaluate the assay's success as a first-tier test (**Table 1**) (19).

All patients included in the NGS cohort (n = 763) were born in the country and represented the SA population. They were selected for comprehensive screening by their healthcare professionals based on their diagnosis with either breast or OVC at an early age (<40 years) or the presence of a personal and family history of the disease. All the patients underwent pre- and post-test counselling at the respective referring national hospitals. Information regarding a personal and/or family history of the disease, together with written informed consent for testing, was provided. The cohort included medium- (two affected family members) to high-risk families (>3 affected family members), with most representing low-risk patients who had no family history of either condition but was diagnosed at an early age of onset (<40 years). Population group was determined by patient self-identification and represented all main SA ethnic groups. The Ethics Committee of the Faculty of Health Sciences at the University of the Free State, together with the Health Research Ethics Committee of Stellenbosch University, approved all study procedures (UFS-HSD2019/1835/2910, UFS-HSD2020/0194/3006, US-N09/08/224) and the NHLS permitted use of the data.

Inferred haplotype analysis was performed for the four most prevalent *BRCA2* pathogenic variants to determine the presence

TABLE 1 | Detection rates for the most common SA pathogenic variants included in the first-tier genotyping assay, according to ethnicity and clinical diagnosis.

First-tier pathogenic variants	Ethnicity[b]	Number of patients tested	Number of negative results	Number of positive results	Detection rate
NM_007294.3(*BRCA1*):c.1374delC p.Asp458Glufs	Afrikaner	758	749	9	1.2%
		Affected: 436	433	3	0.7%
		Pre-symptomatic: 322	316	6	1.9%
NM_007294.3(*BRCA1*):c.2641G>T p.Glu881Ter	Afrikaner	758	733	25	3.3%
		Affected: 436	424	12	2.8%
		Pre-symptomatic: 322	309	13	4.0%
	Coloured[a]	600	597	3	0.5%
		Affected: 537	534	3	2.8%
		Pre-symptomatic: 63	63	0	0.0%
NM_000059.3(*BRCA2*):c.7934delG p.Arg2645Asnfs	Afrikaner	758	623	135	17.8%
		Affected: 436	351	85	19.5%
		Pre-symptomatic: 322	272	50	15.5%
	Coloured[a]	600	597	3	0.5%
		Affected: 537	584	16	2.7%
		Pre-symptomatic: 63	61	2	3.2%
NM_000059.3(*BRCA2*):c.5771_5774del p.Ile1924Argfs	Coloured[a]	600	587	13	2.2%
		Affected: 537	527	10	1.9%
		Pre-symptomatic: 63	60	3	4.8%
	Black	548	508	40	7.3%
		Affected: 521	487	34	6.5%
		Pre-symptomatic: 27	21	6	22.2%
Total		**1906**	**1665**	**241**	**12.6%**

[a]Mixed ethnicity; [b]Only the ethnic groups in which the respective variants were detected are listed, as the variants are population-specific.

of possible founder effects (**Table 2**). Haplotypes that were positively associated with these internationally rare *BRCA2* pathogenic variants would support a potential founder effect. Genotypes based on multiple SNPs retrieved from the NGS data were compared among patients carrying a specific pathogenic variant (n≥5) and checked against a reference haplotype constructed by using mutation-negative individuals.

Linkage disequilibrium (LD) analysis was performed to construct reference haplotypes for *BRCA2*, using the NGS data. The process commenced with the identification of SNPs and their associated minor allele frequencies (MAF). This step was necessary to eliminate rare variants unique to individuals that could weaken the LD analysis and prevent the reconstruction of haplotype blocks assorting independently as determined by contingency $\chi 2$. A total of 36 SNPs were selected for LD analysis based on a MAF > 0.001 in the SA population. The SNP identification codes and genomic positions based on the GRCh37/h19 human genome build are listed in **Table 3**. The selected SNPs were distributed across 82 kb and were situated mainly in the exons and exon/intron boundaries.

Haplotype blocks were constructed using Haploview 4.2 (https://www.broadinstitute.org/haploview/haploview) (21). The program created an LD plot (**Figure 1**) using the logarithm of the odds (LOD score) and average obsolete value (D') between two SNPs. Built-in quality checks of the software resulted in the exclusion of 18 SNPs based on a MAF < 0.01 and deviation from the Hardy Weinberg equilibrium. Haplotype blocks were constructed according to the algorithm and block definitions

TABLE 2 | Details of the most common *BRCA2* pathogenic variants observed and their recurrence internationally.

***BRCA2* variant**		**rs ID**	**HGVS[a]**	**ClinVar**	**PAGE study**	**Current SA study[b]**
DNA level	**Protein level**					
c.582G>A	p.Trp194Ter	rs80358810	NC_000013.10:g.32900701G>A	5	3	11
c.5771_5774del	p.Ile1924fs	rs80359535	NC_000013.10:g.32914263_32914266del	5	0	45
c.6447_6448dup	p.Lys2150fs	rs397507858	NC_000013.10:g.32914939_32914940dup	6	0	7
c.7934del	p.Arg2645Asnfs	rs80359688	NC_000013.10:g.32936788delG	10	0	161

[a]The variants are defined according to the Human Genome Variation Society guidelines; [b]The numbers indicated include both pre-symptomatic carriers and affected individuals. Genomic positions are according to the GRCh37/h19 human genome build.

TABLE 3 | Complete list of *BRCA2* SNPs detected by means of NGS amongst the mutation carrier and control cohorts.

SNP number	**rs ID**	**Variant name**	**Chromosome Position**	**Global MAF ALFA Global: 183,188 chromosomes**	**MAF in SA population**	**MAF of variants included in haplotype (MAF>0.01) African: 6656 chromosomes European: 159208 chromosomes South Asian: 4904 chromosomes**
SNP1	rs1799943	c.-26G>A	chr13:32890572	0.256	0.121	African: 0.110 European: 0.265 South Asian: 0.291
SNP2	rs76874770	c.-11C>TA	chr13:32890587	0.004	0.02	African: 0.017 European: 0.000 South Asian: 0.000
SNP3	rs81002794	c.317-22C>T	chr13:32899191	0	0.009	Excluded
SNP4	rs81002804	c.517-4C>G	chr13:32900632	0	0.028	African: 0.000 European: 0.000 South Asian: 0.000
SNP5	rs80358810[a]	c.582G>A	chr13:32900701	0	0.005	Excluded
SNP6	rs2126042	c.681+56C>T	chr13:32903685	0.186	0.226	African: 0.243 European: 0.185 South Asian: 0.124
SNP7	rs144848	10: c.1114A>C	chr13:32906729	0.279	0.216	African: 0.149 European: 0.283 South Asian: 0.339
SNP8	rs750755676	11: c.2299A>C	chr13:32910791	0	0.001	Excluded
SNP9	rs1801406	11: c.3396A>G	chr13:32911888	0.311	0.178	African: 0.248 European: 0.314 South Asian: 0.305

(Continued)

TABLE 3 | Continued

SNP10	rs543304	11: c.3807T>C	chr13:32912299	0.182	0.169	African: 0.188 European: 0.184 South Asian: 0.114
SNP11	rs80359406	11: c.3858_3860delAAA	chr13:32912345	0	0.004	Excluded
SNP12	rs41293485	11: c.3869G>A	chr13:32912361	0	0.01	African: 0.013 European: 0.000 South Asian: 0.000
SNP13	rs56248502	11: c.4090A>C	chr13:32912582	0	0.024	African: 0.015 European: 0.000 South Asian: 0.000
SNP14	rs545444016	11: c.4502A>G	chr13:32912994	0	0.005	Excluded
SNP15	rs206075	11: c.4563A>G	chr13:32913055	0.988	1	African: 0.929 European: 0.998 South Asian: 1.000
SNP16	rs55639415	11: c.5198C>T	chr13:32913690	0	0.009	African: 0.001 European: 0.000 South Asian: 0.000
SNP17	rs80358765	11: c.5414A>G	chr13:32913906	0	0.027	African: 0.000 European: 0.000 South Asian: 0.000
SNP18	rs80359535[a]	11: c.5771_5774del	chr13:32914260	0	0.003	Excluded
SNP19	rs11571659	11: c.6412G>T	chr13:32914904	0	0.009	African: 0.002 European: 0.000 South Asian: 0.000
SNP20	rs397507858[a]	11: c.6447_6448dup	chr13:32914939	0	0.003	Excluded
SNP21	rs206076	11: c.6513G>C	chr13:32915005	0.996	0.998	African: 0.956 European: 0.999 South Asian: 1.000
SNP22	rs1799955	14: c.7242A>G	chr13:32929232	0.213	0.149	African: 0.229 European: 0.219 South Asian: 0.170
SNP23	rs169547	14: c.7397T>C	chr13:32929387	0.997	0.978	African: 0.941 European: 0.999 South Asian: 1.000
SNP24	rs56070345	15: c.7505G>A	chr13:32930634	0	0.001	Excluded
SNP25	rs9534262	17: c.7806-14T>C	chr13:32936646	0.514	0.484	African: 0.554 European: 0.515 South Asian: 0.484
SNP26	rs80359688[a]	17: c.7934delG	chr13:32936787	0	0.009	Excluded
SNP27	rs81002827	17: c.7976+12G>A	chr13:32936842	0	0.005	Excluded
SNP28	rs146430937	18: c.8010G>A	chr13:32937349	0	0.005	Excluded
SNP29	rs80359052	18: c.8092G>A	chr13:32937431	0	0.009	African: 0.000 European: 0.000 South Asian: 0.000
SNP30	rs28897747	18: c.8149G>T	chr13:32937488	0.001	0.002	African: 0.000 European: 0.001 South Asian: 0.000
SNP31	rs81002808	19: c.8332-66T>C	chr13:32944473	0	0.009	African: 0.010 European: 0.000 South Asian: 0.000
SNP32	rs11571744	21: c.8487+47C>T	chr13:32944741	0	0.032	African: 0.010 European: 0.000

(Continued)

TABLE 3 | Continued

SNP33	rs4942486	22: c.8755-66T>C	chr13:32953388	0.512	0.429	South Asian: 0.000 African: 0.501 European: 0.514 South Asian: 0.484
SNP34	rs4987047	22: c.8830A>T	chr13:32953529	0.001	0.036	African: 0.030 European: 0.000 South Asian: 0.000
SNP35	rs56121817	27: c.9875C>T	chr13:32972525	0	0.008	African: 0.000 European: 0.000 South Asian: 0.000
SNP36	rs1801426	27: c.10234A>G	chr13:32972885	0.007	0.06	African: 0.088 European: 0.001 South Asian: 0.000

[a]*Pathogenic variants not included in the haplotype illustrated in* ***Figure 1****; Global and continental MAF reference according to NCBI and ALFA Release Version: 20200227123210 (20).*

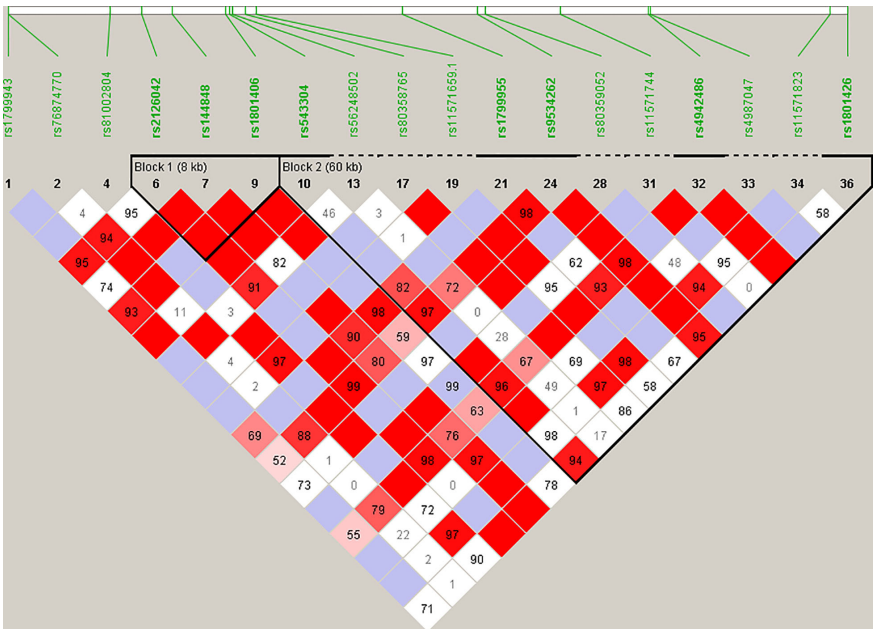


FIGURE 1 | Linkage disequilibrium (LD) plot constructed for *BRCA2* with Haploview 4.2, using a total of 18 SNPs. Schematic diagram of the gene on chromosome 13. Marker variants and their relative locations are represented by vertical lines or boxes (SNP number is indicated above each box together with the rs ID). The LD plot is based on the logarithm of the odds (LOD score) and average obsolete value (D') to characterize the LD between two SNPs in the population data. The diamond color where two SNPs intersect reflected the LD's level, with bright red indicating very strong LD (LOD = 2, D' = 1), a white color for no LD (LOD < 2, D' < 1), with pink-red (LOD = 2, D' < 1) and blue (LOD < 2, D' = 1) for an intermediate LD.

stipulated by Gabriel et al. (22). Using Haploview 4.2, the diamond color where two SNPs intersect reflected the LD's level, with bright red indicating very strong LD (LOD = 2, D' = 1), white color for no LD (LOD < 2, D' < 1), with pink-red (LOD = 2, D' < 1) and blue (LOD < 2, D' = 1) for an intermediate LD. The *BRCA2* pathogenic variants and their observed genotypes were assigned to predicted haplotypes through association frequency after LD analysis and haplotype block construction.

Before incorporating newly obtained results into a POC BRCA assay, the expressed demand for it to be used as a first-tier assay was assessed as part of a risk-benefit analysis through a survey published on the Open Genome Project website (https://www.gknowmix.org/opengenome/survey/). The survey distributed among SA genetics healthcare professionals also explored the most appropriate clinical setting within which such an assay should be performed. Responses from these professionals who attended the South African Society for Human Genetics conference held in Cape Town in 2019 were evaluated after excluding six questions and answering data sets that were considered irrelevant to the current study. The remaining questions were divided into two groups, relating to perceived benefits and risks.

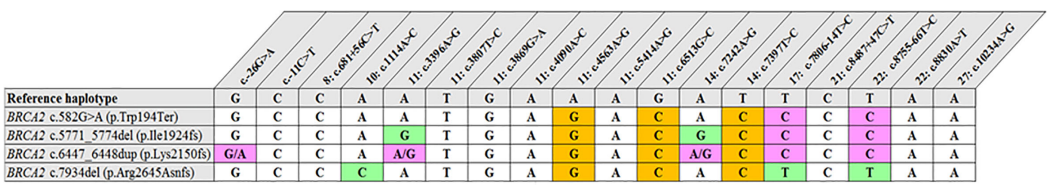
RESULTS

Of the 763 patients screened using NGS, 85 (11.1%) carried a likely-to pathogenic *BRCA1/2* SNV (*BRCA1* 36/763, 4.7% and *BRCA2* 49/763, 6.4%). The mutation rates differed among the ethnic groups, with 13 variants detected for the SA Indian (13/142, 9.1%; 7 in *BRCA1* and 6 in *BRCA2*), 13 Coloured individuals of mixed ancestry (13/120, 10.8%; 4 *BRCA1* and 9 *BRCA2*), 22 White Afrikaners (22/124, 17.7%; 11 *BRCA1* and 11 *BRCA2*), 35 Black patients (35/379, 9.2%; 13 *BRCA1* and 22 *BRCA2*) and two *BRCA1* variants in the non-Afrikaner White population (2/30, 6.7%). The rates detected for the Afrikaner and the Black populations included 11 and six patients, respectively, carrying previously described founder variants, generally excluded using the first-tier genotyping assay. The mutation rates for copy number variants were reported elsewhere (23). Of the 85 pathogenic variants detected, 19 (22.4%) represented variants included in the first-tier genotyping assay (*BRCA1* c.68_69delAG – rs80357783, 1.2%; *BRCA1* c.2641G>T – rs397508988, 1.2%; *BRCA2* c.5771_5774delTTCA – rs80359535, 7.1%; *BRCA2* c.7934delG – rs80359688, 12.9%).

Statistical reconstruction of reference haplotypes was performed using 18 SNPs, with MAF > 0.01. Haplotype analysis showed that the SNPs segregated in two LD blocks (>95% probability), encompassing eight SNPs in strong LD (**Figure 2**). The blocks consisted of an 8 kb segment (block 1: rs2126042–rs1801406) and a 60 kb segment (block 2: rs543304–rs1801426) (**Figure 2**). The blocks encompass eight SNPs in strong LD (LOD ≥ 2, D' = 1), with three indicated in block 1 and five in block 2 (**Figure 2**). Block 1 consisted of four alleles, whereas block 2 indicated six alleles (multi-locus D' = 0.77; **Figure 3**). Block 1A was involved in the most recombination events and was, therefore, the least conserved. This was in stark contrast to block 1B, which exhibited no recombination upon a well-conserved haplotype. Recombination between block 1B and 2B represented the most common haplotype (0.22). The lowest level of recombination was observed between block 1C and 2B (**Figure 3**). All the observed associations accounted for 96% of the haplotypes observed, indicating several unknown events present in the SA population, possibly involving rare SNPs (MAF < 0.01).

Most of the SNPs observed among mutation carriers representing the four pathogenic variants listed in **Table 2** were rare, with MAF < 0.01 (ALFA: Allele Frequency Aggregator) (20) (**Table 3**). Only eight SNPs had a MAF > 0.01 (**Table 3**). From the low frequencies indicated on ALFA, it is clear that most variants excluded proved to be unique to the African continent or SA individuals (**Table 3**). These differences in MAFs reflected the diversity of the SA population. Despite the low MAF scores for the majority of SNPs, a segregating haplotype was associated with three of the four pathogenic variants, namely *BRCA2* c.582G>A (based on seven affected mutation carriers compared to controls, haplotype 1A2B), *BRCA2* c.5771_5774delTTCA (n=8, haplotype 1D2D), and *BRCA2* c.7934delG (n=11, haplotype 1C2C) (**Figure 3**). These haplotypes were based on the allelic combinations observed at 18 markers (**Figure 3**). This confirmed the previous founder status classification of *BRCA2* c.7934delG and *BRCA2* c.5771_5774delTTCA based on genealogy (>10 generations) and phased microsatellite markers (24). For *BRCA2* c.6447_6448dup, the alleles observed at three distinct loci (*BRCA2* c.-26G>A, *BRCA2* c.3396A>G and *BRCA2* c.7242A>G; **Figure 3**) were not common amongst carriers of this variant. This finding resulted in the variant being classified as recurrent rather than a founder variant. Therefore, haplotype analysis confirmed a single additional founder variant in the Black SA population, namely *BRCA2* c.582G>A (rs80358810). This variant has not yet been included in the BRCA 1.0 POC Research Assay.

Table 4 shows an extract from the qualitative survey results obtained from genetic professionals regarding the appropriateness for performing a first-tier genetic test in the form of the novel BRCA 1.0 POC Research Assay. This newly-developed assay currently includes all eight common SA variants screened for by the diagnostic laboratories of the National Health Laboratory Service and several private laboratories in SA (19), but has the potential to be more cost-effective and less time consuming when compared. The vast majority (94%) of survey participants indicated that it would be very convenient to have a rapid, affordable POC test available that can alter patient care with regard to clinical intervention and genetic counselling support. While 75% of participants argued that founder mutation analyses might be used widely in government hospitals as a first-tier test, only 9% reported that it would be used in private practice as gene panel testing is more often requested. However, 91% of stakeholders agreed that when patients (setting unspecified) cannot afford HBOC panel testing, targeted genetic testing will be better than no testing, keeping in mind the limitations of population-based testing. With regards to diagnostic and predictive *BRCA1/2* testing, 81 and 84% of participants, respectively, expressed concerns about the associated psychosocial impact of results made available within the hour or on the same day.



Reference haplotype	G	C	C	A	A	T	G	A	A	A	G	A	T	T	C	T	A	A
BRCA2 c.582G>A (p.Trp194Ter)	G	C	C	A	A	T	G	A	G	A	C	A	C	C	C	C	A	A
BRCA2 c.5771_5774del (p.Ile1924fs)	G	C	C	A	G	T	G	A	G	A	C	G	C	C	C	C	A	A
BRCA2 c.6447_6448dup (p.Lys2150fs)	G/A	C	C	A	A/G	T	G	A	G	A	C	A/G	C	C	C	C	A	A
BRCA2 c.7934del (p.Arg2645Asnfs)	G	C	C	C	A	T	G	A	G	A	C	A	C	T	C	T	A	A

FIGURE 2 | Shared haplotype of recurrent pathogenic variants. The core haplotype associated with each variant is represented by 18 SNPs spread throughout *BRCA2*. A core haplotype was observed for three of the four pathogenic mutation carriers representing a specific, actionable variant but was inconsistent in the control chromosomes. The pink blocks represent an alternative allele to that of the reference, whereas the blocks highlighted in green indicate unique genotypes associated with the specific variant.

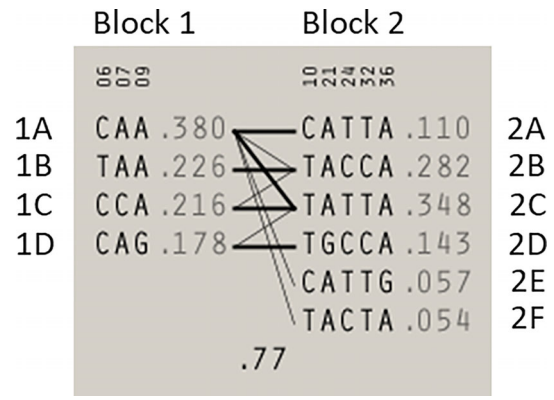


FIGURE 3 | Haplotype blocks and their associated allele frequencies constructed for *BRCA2* with Haploview 4.2 using eight SNPs with a MAF > 0.01 with a strong LD. Schematic diagram of the two blocks. The multi-locus D', which measures the LD between two blocks, is indicated below the crossing lines. Thick connection lines represent haplotype block recombinations observed >10%, whereas a thin connection line represents haplotype block recombination >1%.

DISCUSSION

The results obtained from this single-institution NGS series delivered a positive mutation rate of 11.1%. Of the 85 mutation-positive patients, 17 patients (20%) carried one of the eight most common SA founder or recurrent pathogenic variants. Compared to the overall mutation-positive rate for the extended cohort (n=1906), targeted genetic testing identified 74% of all the pathogenic variants detected (241 of 326, including those detected by NGS, **Table 1**). These results indicate that performing targeted genetic testing as a first-tier assay remains extremely valuable for the country's financially depleted healthcare system. By performing it for all affected breast and OVC patients, irrespective of cancer in the family or ethnic group, most familial variants will still be identified at a fraction of the costs involved with comprehensive screening. This observation corresponds to the findings of various international studies performed on founder populations such as French-Canadian (25) and Ashkenazi Jewish (26) groups. The data obtained from these studies justified a place for cost-effective targeted genetic testing for founder variants and even future population-based screening for cancer predisposition.

Our results mimic the recommendations of the NCCN in the United States, which state that standard care for all Ashkenazi Jewish individuals starts with screening for founder variants first (27). Using a similar approach will improve the results obtained with risk prediction tools such as the Manchester scoring system in the SA population. The inclusion of founder variant status will enhance the predictive score of a *BRCA1/2* variant being present. This is in line with our risk-benefit analysis based on 12 issues addressed in the needs-assessment survey, which provided useful information for paving the way forward (**Table 4**).

The two recurrent and two SA founder variants investigated are internationally rare (**Table 3**). *BRCA2* c.582G>A in exon 7 is located in an area of the gene (c.517 to c.587) that has global

TABLE 4 | Survey results indicating the responses of 32 workshop participants to statements relating to BC management including diagnostic and treatment-related *BRCA1/2* POC genetic testing.

B/R	Questions related to benefits (B) and risks (R)	Yes	N/A	No
B	It will be very convenient to have a rapid, affordable POC test available that can alter patient care with access to clinical intervention and genetic counselling support.	30 (94%)	0	2 (6%)
B	POC screening for founder mutations is important in the context of ancestry and family history.	25 (78%)	3	4 (13%)
R	The detection rate of *BRCA1/2* founder mutations is reducing due population diversification, therefore a population specific POC test will not be useful.	14 (44%)	4	14 (44%)
B	Often the report is provided when the patient has already started therapy or had surgery and that defeats the purpose of genetic testing.	11 (34%	0	21 (66%)
B	When patients cannot afford panel testing founder mutation testing will be better than nothing, knowing the limitations related to population-based testing.	29 (91%)	1	2 (6%)
R	From a private practice perspective, a *BRCA1/2* POC test will not be widely used as founder mutation testing is hardly ever requested anymore.	21 (66%)	8	3 (9%)
B	Founder mutation analyses are still first line testing for many conditions in the state sector and may therefore be used widely in government hospitals.	24 (75%)	0	8 (25%)
R	When a patient is going to pay for a genetic test out of pocket, most of the patients would prefer more comprehensive cancer gene panels the first time around rather than having to do more than one test later.	28 (88%)	2	2 (6%)
R	With regards to *BRCA1/2* predictive testing, the waiting period for results is helpful in giving the patient's time to mentally prepare for the results.	27 (84%)	4	1 (3%)
R	Same day delivery of *BRCA1/2* results might be a bit daunting as these results have major implications with regards to the patients themselves, their reproductive choices and their children.	26 (81%)	0	6 (19%)
B	A missed genetic diagnosis of HBOC* is unlikely with the use of a combination of tests ranging from a rapid POC diagnostic assay for known *BRCA1/2* pathogenic mutations to MinION/whole genome sequencing using an integrated service and research approach for return of results.	25 (78%)	5	2 (6%)
R	Genetic counselling is essential for POC genetic testing that may require extension to clinical sequencing when the results are uninformative.	30 (94%)	1	(3%)

**HBOC, hereditary breast and ovarian cancer.*

splicing enhancer properties (28). This area is known for harboring both the highest density of exonic splicing enhancers and the lowest density of exonic splicing silencers. This exon is therefore very sensitive to nucleotide variants affecting potential exonic splicing regulatory elements (29). The variant was initially reported by Francies et al. (30) in a single SA patient and later by Chen (31). This variant also represented one of the causative variants reported for a SA Black Fanconi anemia infant reported by Feben et al. (24). The variant was confirmed as a new SA founder variant based on the SNP haplotype analysis results.

The recurrent variant *BRCA2* c.6447_6448dup in exon 11 (historically known as *BRCA2* 6676insTA, rs397507858) entailed the duplication of two base pairs and was first described by Meindl in 2002 (32). The variant is globally rare and results in a null variant, directly affecting the associated protein. It was detected in eight self-identified Coloured patients (17, 18, 33). The age at onset/diagnosis in these patients varied from 27 to 63 years, with an average age of 49.2 years. Different genotypes were observed in mutation carriers at three loci, namely c.-26G>A, c.3396A>G and c.7242A>G (**Figure 1**), which resulted in its proposed classification as a recurrent variant. This finding is noteworthy given its current restriction to a single SA population group despite apparent uncertainty of the exact insertion/deletion position at a potential *BRCA2* mutational hotspot. This variant was initially listed by Agenbag (33) as *BRCA2* c.6449_6450insTA, and as c.6448_6449dupTA by Van der Merwe et al. (17) and Oosthuizen (18), despite corresponding electropherograms. Based on the new Human Genome Variation Society guidelines (http://www.HGVS.org/varnomen), this variant is currently officially known as *BRCA2* c.6447_6448dup. This entails noting the nucleotide number of the two base pairs involved in the duplication (nt 6447 and nt 6448) and not the location where the repeat was inserted.

BRCA2 c.5771_5774del, historically known as *BRCA2* 5999del4, represents the most common pathogenic variant observed in both the Black and Coloured populations (**Table 1**). This variant is absent in the Afrikaner and SA Indian population and was identified seven times during the NGS study (7/763, 0.92%). The majority of the patients (n=5) were Black, with two patients who self-identified as Coloured. All the mutation carriers were diagnosed with BC, with most diagnosed ≤40 years (range 35–53 years). The deletion is predicted to cause loss of normal protein function through either protein truncation or nonsense-mediated mRNA decay. The variant occurred in the BRC domain (aa1009–2083) that facilitates the binding of RAD51 (34). This variant currently forms part of the first-tier genotyping assay that precedes comprehensive NGS analysis. The variant was detected collectively 53 times (53/1906, 2.8%), mostly in patients from the Western Cape (**Table 1**). The age at onset for mutation carriers ranged from 25 to 71 years, with many patients not reporting a family history of cancer. The seven BC patients included for the haplotype analysis represented both the Black and Coloured ethnic groups. Although a unique haplotype was observed, there was no distinction between patients representing each of these groups. The single-base deletion in exon 17 (*BRCA2* c.7934delG – rs80359688, historically known as *BRCA2* 8162delG) represents the most common Afrikaner founder variant, also included in the current first-tier genotyping assay (17, 35). Founder status was previously proven by genealogical and haplotype analysis using flanking and intragenic microsatellite markers (17, 35). The genealogical study involved 12 independent families linked to the variant, mapped over a minimum of 10 generations (data not shown). A total of 151 mutation carriers were identified, with 99 of them being affected with cancer (151/1906, 7.9%). It represents the country's most common recurrent variant. It accounts for most BC and/or OVC patients in two populations, namely the Afrikaner and Coloured populations (**Table 1**) (19). The variant is located in the gene's helical domain (oligonucleotide/oligosaccharide-binding fold OB1) responsible for the binding single- and double-stranded DNA (36, 37). The age at onset varied from 21 to 73 years (average 42.9 years) and included uni- and bilateral female and male BC, OVC, six men affected with prostate cancer, and a single case with pancreatic cancer. The founder haplotype did not differ between the self-identified patients representing the Afrikaner and Coloured groups.

Female *BRCA1/2* mutation carriers are at significantly increased risk for BC, OVC, and pancreatic cancer. In contrast, male mutation carriers are at increased risk for breast, prostate, and pancreatic cancer, among other types (38, 39). The benefit of targeted genetic testing of affected patients is encompassed in identifying healthy at-risk related family members early in life. By knowing their mutation status, individuals can take advantage of the options available in terms of screening and medical therapies and benefit from risk-reducing strategies to manage their risks (27). Over the past 20 years, our experience indicates a low uptake of carrier testing, which varies considerably between ethnic groups (**Table 1**). Individuals with an Afrikaner heritage are most inclined to opt for susceptibility testing (n=322, **Table 1**). Varied perceptions of the benefits related to cancer risk management (40) have a significant impact on the responsiveness and openness to cancer prevention using cascade testing in families. Many patients or individuals may be unaware of a family history of cancer and, therefore, do not consider genetic testing.

The SA Department of Health has recently recognized that health and the country's development are integrally linked. The department has pledged to reform this sector, which is firmly embedded in its National Development Plan for 2030 (Our Future – make it work) (41). The department has since released clinical guidelines for BC control and management in which they set standards for optimal care and management to improve survival. This standard includes, among others, referral of all patients with BC (diagnosed <40 years) and/or OVC (<60 years) for comprehensive genetic testing of at least *BRCA1*, *BRCA2* and *Tp53* by means of NGS, and decreasing the time to presentation, diagnosis and treatment. The national implementation of these guidelines will dramatically increase the demand for genetic testing and exponentially contribute to this sector's financial burden. By implementing more cost-effective targeted genetic testing as a first-tier screen, full advantage will be taken of the budget available.

These obstacles were recently addressed by the development of a novel rapid *BRCA1/2* POC assay aimed at improving the

clinical management of patients with BC and associated co-morbidities (https://gtr.ukri.org/projects?ref=103993). As a more cost-effective alternative than the current assay, the ParaDNA BRCA 1.0 Research Kit using HyBeacon probes was designed. The new assay can simultaneously detect all eight recurrent SA variants in four multiplexed reactions. This assay proved to be both time- and cost-effective, although careful consideration is required before its implementation in clinical practice. The value of this innovative approach has been recognized as a future focus area when addressing personalized medicine for SA patients in both the public and private sectors.

South Africa's extensive population diversity originated due to its geographical location with respect to historical trade routes between the east and the west, and a multi-faceted colonization history (10–14). It contributed to a unique composition, incorporating genetic signatures from Europe, Asia and Africa into SA. This diversity creates diagnostic challenges, as certain pathogenic variants are restricted to specific ethnic groups (**Table 1**). The development of an appropriate population-directed POC assay based on the results presented will help achieve the Department of Health goals to ensure optimal and standard care to all citizens. This pathology-supported genetic testing strategy was piloted by Mampunye (15) in BC patients previously referred for gene expression profiling to reduce the risk of chemotherapy overtreatment (42, 43), as well as the risk of tamoxifen resistance (44, 45).

The survey results used in the risk-assessment analysis provided valuable information and gave direction to where the ParaDNA BRCA POC assay should ideally be placed. Timeous receipt of a patients' genotyping results may dramatically affect surgical decision-making. Receipt of a predictive, mutation-positive *BRCA1/2* POC result within an hour or on the same day was perceived as a risk by 84% of health professionals, as they thought it might be overwhelming for at-risk family members. Most healthcare professionals' sentiment was also reflected in relation to a diagnostic test result that has implications for reproductive health and recurrence risk to offspring (81%, **Table 4**). However, this perception could be drastically influenced depending on the setting in which the test is being offered. Individual clinicians/surgeons were consulted to obtain their opinion (data not shown) before and after the results of the pilot study performed by Mampunye (15) became available. It was clear that the reaction to a positive test result on-site will differ based on the motivation for testing, namely whether it was intended for surgical decision-making or to determine familial risk.

Historically, *BRCA1/2* pathogenic variants are suspected in families with multiple women with BC and/or OVC, early ages of cancer onset, bilateral or male breast cancer. In 2001, the NCCN recommended genetic testing for patients diagnosed with breast cancer at age ≤40 (46). In 2009, however, the upper limit for age was increased in the guidelines to age 45 years (47). This evolution in guidelines demonstrates how practices change over time as new knowledge becomes available, reflecting the importance of an integrated service and multidisciplinary research model as described by Kotze et al. (48). The risk-benefit analysis supports recent suggestions to preclude relying solely on family history and pursuing the idea of testing all women diagnosed with BC or OVC for pathogenic *BRCA1/2* variants (49). The argument here is three-fold: even though these variants are relatively rare, they engender 1) high cancer risks (predictive), 2) actionable treatment targets (therapy selection), and 3) uncover inherited predisposition that may be hidden by the family structure (differential diagnosis). Some families are very small, making it difficult to recognize a strong inheritance pattern versus environmentally-induced or lifestyle-triggered genetic risk. Furthermore, in families with a male predominance, pathogenic variants may be passed through generations of men and become evident only later in female carriers. Schoeman et al. (50) reported that even in women who meet the current guidelines for genetic testing (based on family history), as few as 17.3% have been tested at a Western Cape Academic Hospital.

While clinical implementation of useful research findings may take many years, direct to the consumer applications with limited clinical utility and support have become widely available. We propose rapid founder testing supported by genetic counselling to address the associated psychosocial concerns. The survey was ideally positioned to explore some of the barriers to translation of research findings, which needs to be addressed if genomics research is to fulfil on the promises of personalized medicine (51). Barriers to *BRCA1/2* testing and extended NGS analysis include clinicians not discussing or offering testing due to a potential lack of training or knowledge, cost and insurance coverage, as well as long turn-around time of laboratory-based tests involving sample collection and transport, which all adds to the cost. Other concerns include the use of race as a proxy for risk stratification in genetics studies. This constituted an important discussion point at the SASHG conference and pre-conference workshop during which this question was addressed. Oncology specialists who expressed interest in incorporating POC *BRCA1/2* genetic testing in their cancer care pathway confirmed that they would screen all SA patients with this assay, regardless of ethnic group or language. The proposed model, which incorporates targeted genetic testing at the POC in a genomic counseling or laboratory-based near-patient setting, may overcome these barriers regardless of which of the three indications the test may be performed under, as per the clinician's discretion.

The genetic diversity of *BRCA2* in the SA population unveiled during this investigation could potentially aid in the etiology of BC in SA, once explored, similar to the work performed by Lilyquist et al. (52). The large size of the haplotype blocks observed justifies future investigation by including polymorphic variants situated further up and downstream of the gene, together with deep intronic variants. This approach corresponds to the standard STR profiling approach. Comparing the STR (1.7 Mb) and SNP (82 kb) haplotypes for *BRCA2* c.5771_5774del and c.7934delG, showed that not all haplotypes could be distinguished when focusing on a locus spanning a relatively small genomic distance which is limited to relative conserved sequences. This was evident from the exclusion of rare minor allele variants, which could have been family or population-specific (53). The inclusion of SNPs further away from *BRCA2* might assist refining SNP haplotyping in the SA population.

This study's significance in future investigations can be improved once a minimum of 1000 samples have been screened comprehensively. It will result in the inclusion of SNPs at a MAF > 0.001. A larger cohort will increase the p-value for variants that deviated from the Hardy-Weinberg equilibrium and justify their future inclusion in the haplotype inference. Furthermore, dividing the cohort into sub-populations before LD analysis might increase the statistical significance of the LD between SNPs, which have a low MAF in the combined population. This might increase the sensitivity of the data set for the prediction of haplotypes with a very low MAF. The continuous addition of SNP data of patients harboring these founder variants will also increase the sensitivity and accuracy of the haplotype associations. The complex diversity of the SA population observed in this study shows the need for population-based analysis performed in parallel with NGS. This will drive more appropriate population-based first-tier genotyping assays in third world countries with limited resources for pathology.

Furthermore, it could be of diagnostic significance to perform pathological association studies for each *BRCA2* haplotype with enough variation to be classified as a different *BRCA2* isoform due to the number of missense variants. As the study identified *BRCA2* SNPs in LD, together with their associated distances from each other, it could represent valuable markers for *de novo* assembly during long-range sequencing to confirm segregation patterns of novel or rare VUS. Finally, it would be important to evaluate the incidence of these variants and their impact on management in a prospective cohort of newly diagnosed breast and/or ovarian cancer patients, while comparing the results to testing strategies using local and international guidelines for founder and panel-based testing.

DATA AVAILABILITY STATEMENT

The full survey results, together with the haplotype analysis, are available at the website of the Open Genome Project (https://www.gknowmix.org/opengenome/survey/), with restricted access to supplementary data sets and analyses generated during the current study.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The Ethics Committee of the Faculty of Health Sciences at the University of the Free State, together with The Health Research Ethics Committee of Stellenbosch University approved all study procedures (UFS-HSD2019/1835/2910, UFS-HSD2020/0194/3006, US-N09/08/224) and the NHLS permitted use of the data. Written informed consent to participate in this study was provided by each participant.

AUTHOR CONTRIBUTIONS

NCM authored the original draft of this publication and obtained ethics approval. JO, PB, and NCM collated the NGS data and performed the haplotype analysis. JO performed the statistical analyses. NM and MK assisted with the risk-benefit analysis. MK, NM, JO, and EM provided critical feedback and assisted with shaping the final version of the manuscript. NCM, JO, and MK contributed significantly to the conception of the idea on which this manuscript is based. All authors contributed to the article and approved the submitted version.

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Survival Status of Esophageal Cancer Patients and its Determinants in Ethiopia: A Facility Based Retrospective Cohort Study

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Background: Globally, the incidence and mortality due to esophageal cancer are increasing, particularly in low- and middle-income countries. Cancer of the esophagus is the eighth in incidence and seventh in cancer mortality in Ethiopia. A few studies have shown an increasing burden, however, little is known about the survival pattern and its determinants among esophageal cancer patients in Ethiopia. Therefore, we assessed the survival pattern and its determinants among esophageal cancer patients.

Methods: We conducted a retrospective cohort study among 349 esophageal cancer patients who were diagnosed at or referred to Tikur Anbessa Specialized Hospital, Ethiopia from January 2010 to May 2017. Using an abstraction form, nurses who were working at the oncology department extracted the data from patient charts. To estimate and compare the probability of survival among covariate categories, we performed a Kaplan–Meier survival analysis with the log-rank test. To identify the prognostic determinants of survival, we performed a multivariable Cox proportional regression analysis.

Results: The median follow-up time was 32 months with interquartile range of 15 to 42. Overall, the median survival time after diagnosis with esophageal cancer was 4 months with one-, two- and three-year survival of 14.4, 6.3, and 2.4% respectively. In the multivariable Cox proportional hazards model, receiving chemotherapy [Adjusted Hazard Ratio (AHR)=0.36, 95%CI: 0.27–0.49], radiotherapy [AHR=0.38, 95%CI: 0.23–0.63] and surgery [AHR=0.70, 95%CI: 0.54–0.89] were statistically significant.

Conclusions: In Ethiopia, esophageal cancer patients have a very low one-, two- and three-year survival. Despite a very low overall survival, patients who received either chemotherapy, radiotherapy or surgery showed a better survival compared with those who did not receive any treatment. Hence, it is essential to improve the survival of patients with esophageal cancer through early detection and timely initiation of the available treatment options.

Keywords: survival, esophageal cancer, prognostic determinants, Ethiopia, cohort

INTRODUCTION

Cancer is the second leading cause of morbidity and premature mortality globally, with an estimated 24.5 million new cancer cases and 9.6 million deaths in 2017 (1). Esophageal cancer is ranked ninth in incidence and sixth in cause of cancer deaths in both sexes worldwide, with over a million new cases and 508,585 new deaths in 2018 alone (2).

In the past, non-communicable diseases (NCDs), particularly cancer, were considered as a disease of high-income countries, but recent evidence indicates that it is an important public health issue in low- and middle-income countries (LMICs). A change in lifestyle including sedentary behavior and unhealthy dietary habit, urbanization, cultural transition, and an increase in life expectancy in LMICs might be the possible reason for an increasing incidence (3–5). The largest increase in the incidence of cancer from 2007 to 2017 was observed in middle-income countries (1). By 2030, the cancer burden in sub-Saharan Africa is expected to increase by 85% (6). Similarly, in Ethiopia, the burden of NCDs including cancer is rising. In 2018, with 1,752 estimated new cases, cancer of the esophagus was the eighth most incident and the seventh leading cause of mortality (7, 8). Areas in the African rift valley, particularly Arsi and Bale regions of Ethiopia, Western Kenya, Northern Tanzania and Malawi, are the known hot spots of esophageal cancer (9).

The availability of advanced diagnostic services and early treatment options improve the survival rate in high-income countries. In contrast, in LMICs including Ethiopia the cancer prognosis is very poor, which could be attributable to lack of diagnostic equipment, limited treatment options, and patients visit healthcare at advanced stages (10–13). Hence, the mortality due to cancer, principally cancer of the esophagus is disproportionately higher in LMICs than in high-income countries (14). More than two-third of all cancer deaths happen in LMICs (15).

Esophageal cancer is often associated with an unfavorable prognosis worldwide, with five-year survival ranging from 4 to 40% (14). It is essential to estimate the average survival rate to evaluate and monitor the quality and effectiveness of care provided to cancer patients. However, little is known about the care and management given as well as the survival of patients with cancer in Ethiopia. Although a few studies have been conducted describing the disease burden, little is known on the prognosis of esophageal cancer. Assessment of survival has practical implications for healthcare providers and patients to understand the prognosis over time and for decision making on better treatment options. Thus, this study assessed the overall survival rate and identified its determinants among esophageal cancer patients in Ethiopia.

METHODS AND MATERIALS

Study Setting and Period

This study was conducted at one of the tertiary level hospitals with a cancer diagnostic and treatment facility in Ethiopia named Tikur Anbessa Specialized Hospital (TASH). Under the TASH, the Addis Ababa Population-Based Cancer Registry (AAPBCR) was established in 2011, which serves a catchment population of more than four million inhabitants. The main sources of cases for the registry are pathology centers, hospitals, and higher diagnostic clinics. The time of diagnosis with esophageal cancer was taken as the starting point for follow-up, while the date of death, loss to follow-up, last contact or the end of follow-up time (May 31, 2017) was the end point of the study.

Study Design and Participants

We conducted a retrospective cohort study among all esophageal cancer patients registered in TASH who were diagnosed or referred from January 1, 2010 to May 31, 2017. The inclusion criteria were all clinically and pathologically confirmed esophageal cancer cases by oncologist. We excluded patient charts with missing information on both histopathology and cancer stage reports. Using the medical record number obtained from the registry, the charts of all esophageal cancer patients were retrieved. Out of 367 charts retrieved, 18 (4.9%) were excluded due to unavailability of neither histopathology nor cancer stage report. Then, we extracted information from 349 patient charts and included them in the analysis.

Data Collection Procedures

After reviewing literature and consulting experts on important variables, we prepared a data abstraction form considering the availability of information on patient charts and feasibility to get *via* a phone interview. Initially, we identified the charts of all esophageal cancer patients and retrieved using the medical registration number. Then, data collectors reviewed baseline and follow-up patient characteristics including sign and symptoms, laboratory and imaging results, and pathology report.

To ascertain the main outcome, death, the death certificate was identified from the TASH cancer registry. When the death certificate was not available, we did a phone interview with patients or their attendants. Information that was not available from the patient chart or medical register was also collected during the phone interview. In this study, an event was defined as the death of a patient due to esophageal cancer. Patients who were lost to follow-up before developing the event, have incomplete information on the date of death, who died due to other known causes unrelated to esophageal cancer, who do not have registered phone numbers and their current status is unknown, were censored to the last follow-up date. Patients who survived until the last follow-up date were censored to May 31, 2017. Data collection and facilitation of phone interview was conducted by trained oncologic nurses who were working at the oncology center. To improve the data quality, training was given for data collectors on the aim, materials and methods, and data collection procedure for two days.

Data Processing and Analysis

After checking for completeness, data were coded and entered into EpiInfo version 7.1 and exported to R programming version 3.6.1 for further processing and analysis. For categorical variables, descriptive statistics were computed using frequencies with percentages and rates, whereas continuous variables were

summarized using mean with standard deviation (SD) or median with interquartile range (IQR). We calculated the overall death rate from diagnosis to end of follow-up. The variation in overall survival pattern across covariate categories was presented using the Kaplan–Meier curve and tested using the log-rank test. A reverse Kaplan–Meier estimator was used to estimate the median follow-up time (16). We performed a bivariate Cox proportional hazards regression model to identify the crude association of covariates with time to death. Finally, we performed a multivariable Cox regression for ten variables upon checking for the assumptions. Significant multicollinearity was detected between distant metastasis and organ metastasis, then, we excluded the latter from the final model. P-values less than 0.05 in the multivariable Cox proportional hazards model were considered as statistically significant. We presented the results using crude and adjusted Hazard Ratio (HR) with 95% confidence interval (95%CI).

There were 31 (8.9%), five (1.4%), 80 (22.9%), 12 (3.4%), and seven (2.0%) missing values for histology type, tumor location, cancer stage, tobacco use, and family history of cancer respectively. Under missing data at random (MAR) assumption, we managed using a multivariate imputation technique of the '**mice**' package in R (17). We imputed 100 datasets using variables included to the model and additional auxiliary variables. The hazard ratios were estimated in each imputed dataset separately, and combined using Rubin's rules (18). Missing observations were imputed for the predictor variables used in the multivariable Cox regression model. The outcome variable, death, was not imputed as we analyzed only participants for whom the outcome was ascertained. We performed a sensitivity analysis to assess whether the MAR assumption is valid, and the results were reasonably comparable (**Supplementary Material**).

Participant Consent and Ethical Approval

The protocol of this study was approved by the institutional review board of Addis Ababa University, College of Health Sciences. Before starting the phone interview, informed consent was obtained from patients or caretakers. This study is in compliance with the principles of the declaration of Helsinki. The confidentiality of patients' data was kept at each step of data collection and processing.

RESULTS

Sociodemographic and Behavioral Characteristics of Patients

The sociodemographic and behavioral characteristics of patients are summarized in **Table 1**. The mean age of patients was 51.4 years (SD: 11.9), and 206 (59.0%) were females. More than half (56.7%) were from the Oromia region and 319 (91.4%) were married. Sixty-two (17.8%), 18 (5.3%), and 92 (26.4%) had a history of alcohol intake, smoking and Khat (*Catha edulis*) chewing respectively. The prevalence of alcohol consumption significantly varied across gender, in which 9.2% of females and 30.1% of males had a history of alcohol intake ($p < 0.001$). Similarly, none of female participants reported tobacco use, whereas 15.4% of males use tobacco and the difference was statistically significant ($p < 0.001$). Moreover, the prevalence of Khat chewing was significantly higher among males (35.0%) than females (20.4%) ($p = 0.004$).

TABLE 1 | Sociodemographic and behavioral characteristics of patients with esophageal cancer in TASH, Addis Ababa, Ethiopia, 2010–2017 (n = 349).

Variables	Frequency	Percent
Age (years) (mean/SD)	51.4	11.9
Sex		
Male	143	41.0
Female	206	59.0
Marital status		
Single	10	2.9
Married	319	91.4
Widowed	14	4.0
Divorced	6	1.7
Residence region		
Addis Ababa	54	15.5
Amhara	26	7.4
Oromia	198	56.7
SNNPR	54	15.5
Others[¥]	17	4.9
Consume alcohol	62	17.8
Chew Khat	92	26.4
Tobacco use (n = 337)	18	5.3
Has family history of cancer (n = 342)	2	0.6

[¥]*Dire Dawa, Harari, Gambella, Somali, Afar, Tigray.*
TASH, Tikur Anbesa Specialized Hospital.

Histologic Types, Anatomic Site, and Stage of Esophageal Cancer

Out of 349 cases registered, 318 (91.1%) and 57 (16.3%) of patient charts had histological type of cancer and histologic grade report, respectively. Among those with histopathology test results, 287 (90.3%) were squamous cell carcinoma, whereas 31 (9.7%) were adenocarcinoma type. Over half (54.1%) of the cases had lesions at the lower third of the esophagus, whereas 105 (30.5%) at the middle third. Two hundred sixty nine (77.1%) of charts had reports of cancer stage at diagnosis, of which 188 (69.9%) and 51 (19.0%), respectively, were stages IV and III at diagnosis (**Table 2**).

Treatment Options Given to Patients

Out of 349 patients, 183 (52.1%) were treated with trans-hiatal esophagectomy surgical procedure, while 112 (31.9%) transthoracic esophagectomy, and 112 (31.9%) were managed using feeding gastrostomy. Above one-fourth (25.8%) of them received chemotherapy, whereas only 26 (7.7%) were treated with radiotherapy (**Table 3**).

Survival Time From Diagnosis to Death

The median follow-up time was 32 months with IQR of 15 to 42 months. Three-hundred ten (88.8%) patients died during the 1,932 person-month follow-up period, resulting in an overall event rate of 160.5 per 1,000 person–months [95%CI: 119.2–

TABLE 2 | Distribution of histologic types and histologic grades of patients with esophageal cancer in TASH, Addis Ababa, Ethiopia, 2010–2017 (n = 349).

Variables	Frequency	Percent
Histological type (n = 318)		
Squamous-cell carcinoma	287	90.3
Adenocarcinoma	31	9.7
Tumor location (n = 344)		
Upper third	53	15.4
Middle third	105	30.5
Lower third	186	54.1
Histological grade (n = 57)		
Well differentiated	38	66.7
Moderately differentiated	10	17.5
Poorly differentiated	6	10.5
Undifferentiated	3	5.3
Stage at diagnosis (n = 269)		
Stage I	3	1.1
Stage II	27	10.0
Stage III	51	19.0
Stage IV	188	69.9

TASH, Tikur Anbesa Specialized Hospital.

TABLE 3 | Treatment options given to esophageal cancer patients at TASH, Addis Ababa, Ethiopia, 2010–2017 (n = 349).

Treatment received	Frequency	Percent
Surgery	183	52.4
Type of surgery (n = 183)		
Trans-hiatal esophagectomy	145	79.2
Trans-thoracic esophagectomy	29	15.8
Feeding gastrostomy	112	61.2
Laparotomy	71	38.8
Chemotherapy	89	25.5
Type of chemotherapy (n = 90)		
Neoadjuvant	3	3.4
Adjuvant	70	78.6
Palliative	17	19.1
Radiotherapy	26	7.4
Type of radiotherapy (n = 26)		
Adjuvant	2	7.7
Radical	1	3.8
Palliative	23	88.5

TASH, Tikur Anbesa Specialized Hospital.

320.6]. The overall survival rate was very low with one-, two- and three-year survival rates of 14.4% [95%CI: 11.0–18.9], 6.3% [3.9%–10.2], and 2.4% [0.9%–6.0%] respectively, and the median survival time was 4 months [95%CI: 2–8] (**Figure 1**).

Variation in Survival Rates Among Groups of Esophageal Cancer Patients

The rate of survival varied across categories of covariates such as the stage of cancer, chemotherapy, and radiotherapy treatment status. The survival varied along with the cancer stage, with lower stages at diagnosis showing a better survival (log-rank test, $p < 0.03$). Similarly, patients who received chemotherapy showed a better overall survival compared with those who did not (log-rank test $p < 0.001$). Moreover, the overall survival was significantly different among patients based on their radiotherapy treatment status, in which those who received showed a better survival (log-rank test, $p < 0.001$). No significant variation was observed on overall survival according to sex and location of the tumor ($p = 0.057$). The variation in survival pattern among covariate categories is presented in **Figures 2–4**.

Prognostic Determinants of Survival Among Esophageal Cancer Patients

In the multivariable Cox proportional hazards model, receiving chemotherapy, radiotherapy, and surgery independently determine the survival from esophageal cancer. The death rate decreased by 64% for those patients who received chemotherapy compared with those who did not (AHR = 0.36, 95%CI: 0.27–0.49). Similarly, those who were treated with radiotherapy had 62% lower rate of death than those who did not (AHR = 0.38, 95%CI: 0.23–0.63). The death rate was also 30% lower for patients who were treated with any type of surgery in comparison with those who did not (AHR = 0.70, 95%CI: 0.54–0.89). However, there was no statistically significant interaction between any of the predictors. Age, baseline hemoglobin, sex, and histology type were not statistically significant in the multivariable Cox regression (**Table 4**).

DISCUSSION

In this study, we highlighted the survival pattern and prognostic determinants of esophageal cancer among patients who were diagnosed at or referred to Tikur Anbessa Specialized Hospital. Such a study has not been reported from Ethiopia to date. We found that the overall survival after diagnosed with esophageal cancer was very low. Despite very low overall survival, those who received either chemotherapy, radiotherapy, or surgery showed a better survival compared with those who did not.

In Ethiopia, the survival from all types of cancer is relatively low in comparison with high-income countries. A study by Beksisa and his colleagues showed a three-year survival of prostate cancer was estimated to be 38.9% (19). Similarly, a study from other parts of the country showed a two-year survival of breast cancer to be 53% (20). Several studies in other parts of the world indicated esophageal cancer has a poor prognosis in comparison with other types of cancers (21, 22). Hence, the survival from esophageal cancer is expected to be worse in LMICs including Ethiopia.

In our study, the overall one-, two- and three-year survival for all stages combined was below 15%. This finding is lower than the rate reported by a study done in Brazil, which showed a 22.8 and 20.2% five-year survival for squamous and adenocarcinoma, respectively (23). Moreover, in our study the median survival time after diagnosis was 4 months, which is in line with a study from Mozambique which reported 3.5 months (24). On the other hand, a study in Cameroon and Tanzania reported a relatively higher median survival of 6.7 and 6.9 months respectively (25, 26). In our study, patients visited healthcare at later stages of the disease, majorities (89%) were diagnosed either at stage III or IV. The lower survival could also be attributed to the lower socio-development index (SDI) of the country (27). A study by Wong

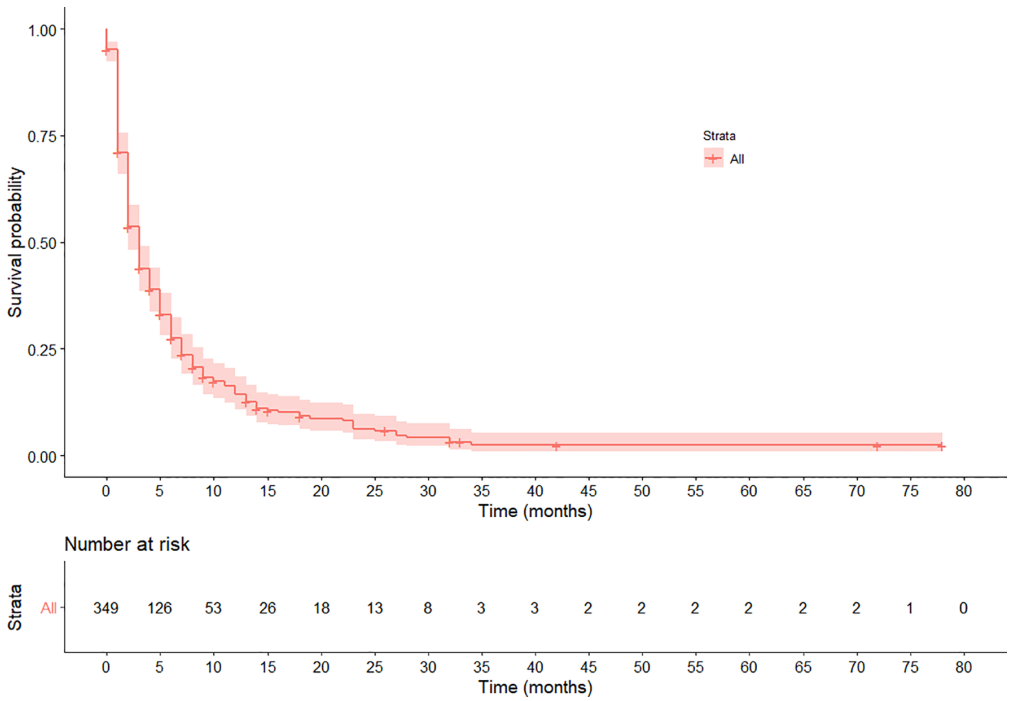

FIGURE 1 | Kaplan–Meier survival curve of the overall survival pattern among esophageal cancer patients registered in Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia, 2010–2017. The curve shows the median survival is 4 months.

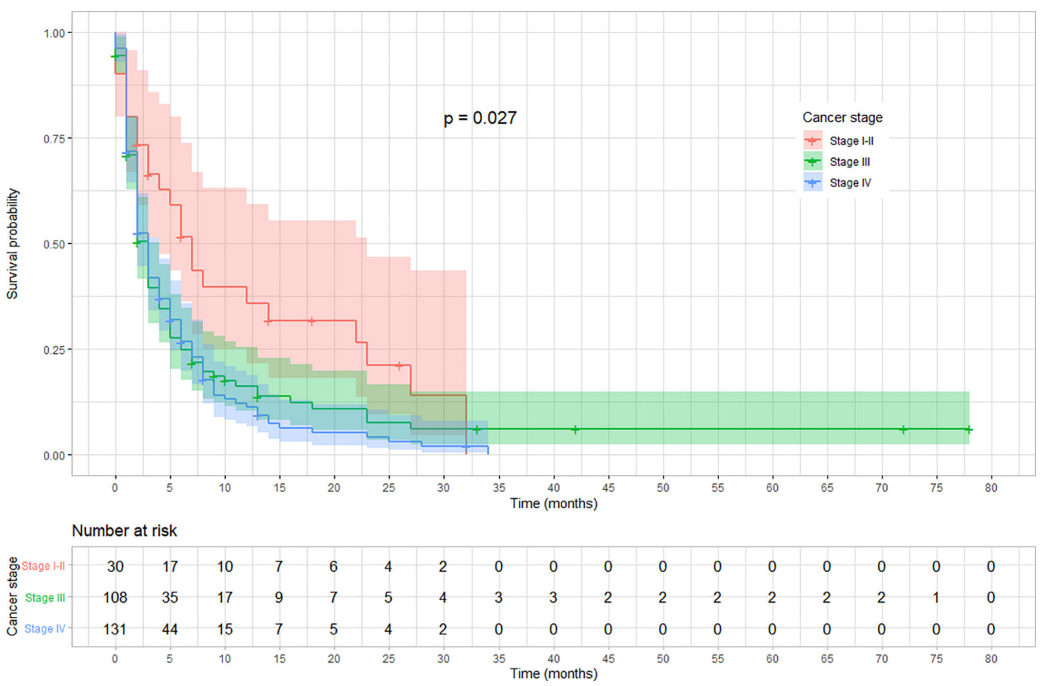

FIGURE 2 | Kaplan–Meier survival curve showing the variation in overall survival based on cancer stage at diagnosis among esophageal cancer patients in Ethiopia, 2010–2017 (Log-rank test, $p = 0.027$).

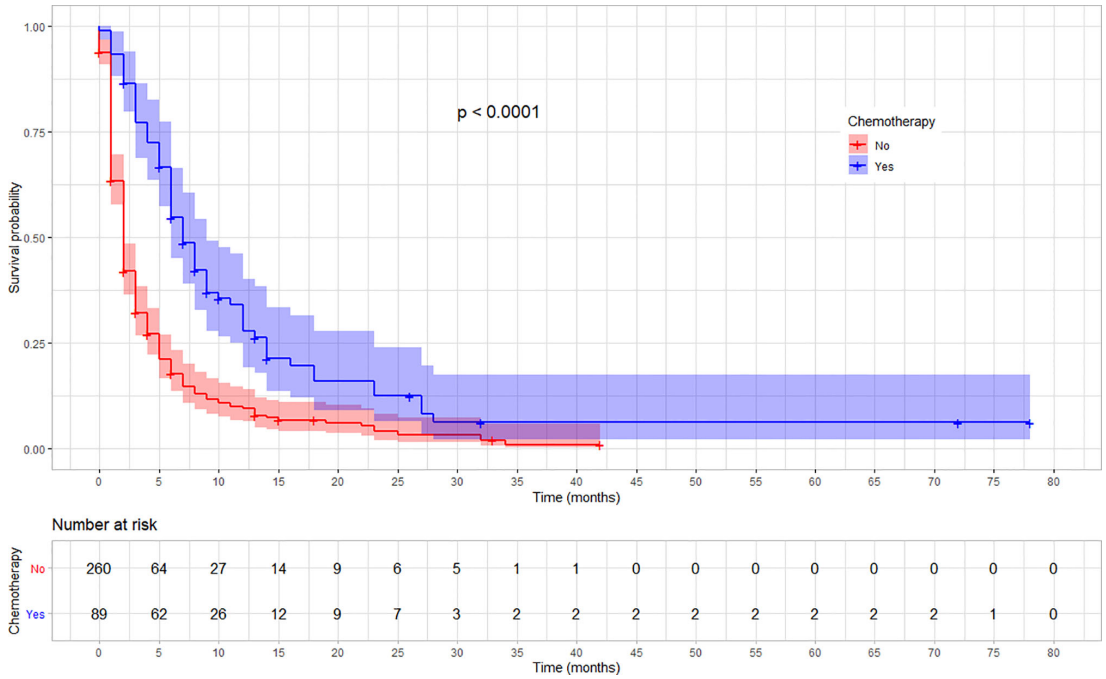

FIGURE 3 | Kaplan–Meier survival curve showing the difference in overall survival based on the chemotherapy treatment among esophageal cancer patients in Ethiopia, 2010–2017 (Log-rank, $p < 0.01$).

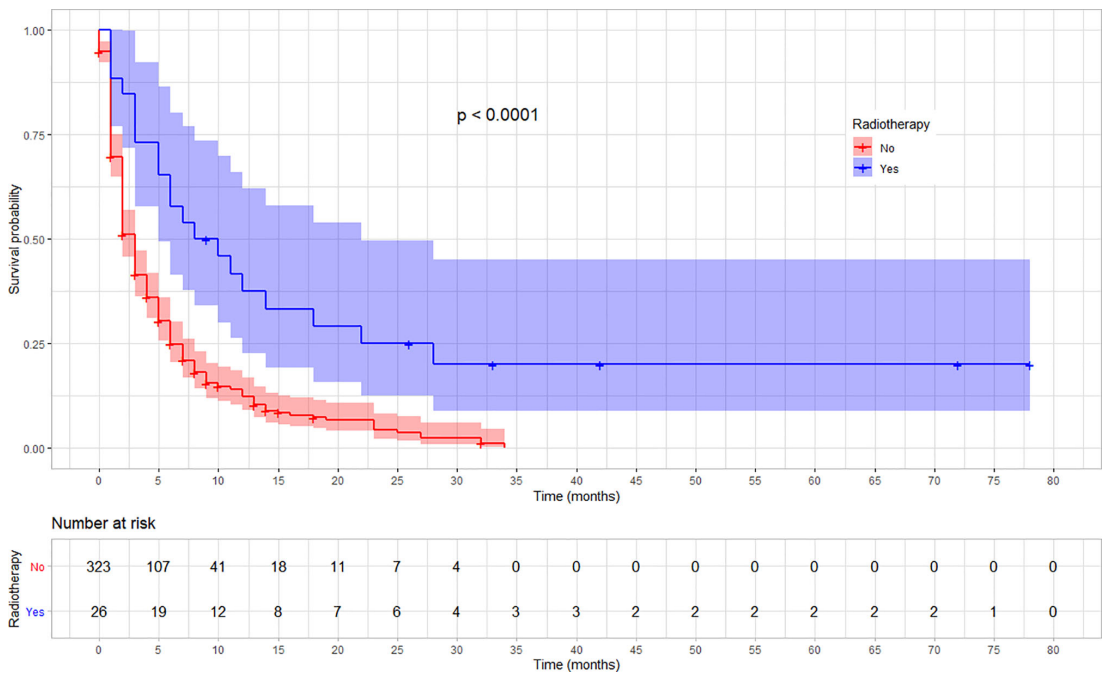

FIGURE 4 | Kaplan–Meier survival curve showing the difference in overall survival by radiotherapy treatment status among esophageal cancer patients in Ethiopia, 2010–2017. (Log-rank test, $p < 0.001$).

TABLE 4 | Cox proportional hazards model of the determinants of survival among esophageal cancer patients registered at TASH, Addis Ababa, Ethiopia, 2010–2017.

Determinants	CHR (95%CI)	AHR (95%CI)
Age	1.01 (0.99–1.02)	1.01 (0.99–1.02)
Sex *(female)*	1.17 (0.93–1.48)	1.06 (0.84–1.36)
Distant metastasis *(yes)*	1.30 (0.98–1.71)	1.16 (0.85–1.59)
Histology type		
Squamous-cell carcinoma	1	1
Adenocarcinoma	0.93 (0.64–1.36)	0.79 (0.52–1.18)
Cancer stage		
I and II	1	1
III	1.37 (0.92–2.02)	1.16 (0.76–1.75)
IV	1.48 (1.02–2.15)*	1.28 (0.84–1.94)
Tumor location		
Upper	1	1
Middle	1.40 (0.99–1.99)	0.99 (0.68–1.44)
Lower	1.07 (0.78–1.48)	0.74 (0.52–1.07)
Chemotherapy *(yes)*	0.43 (0.33–0.56)**	0.36 (0.27–0.49)**
Baseline hemoglobin	1.03 (0.98–1.09)	1.02 (0.97–1.08)
Surgery *(yes)*	0.87 (0.69–1.09)	0.70 (0.54–0.89)*
Radiotherapy *(yes)*	0.39 (0.24–0.62)**	0.38 (0.23–0.63)**

*P < 0.05; ** P < 0.01.

AHR, Adjusted Hazard Ratio; CHR, Crude Hazard Ratio; CI, Confidence Interval; *TASH, Tikur Anbesa Specialized Hospital.*

Multivariate multiple imputations were performed (n = 349).

and his associates indicated the incidence and mortality of esophageal cancer is highly correlated with SDI of countries (28). The economic development of a country determines the patient's health seeking behavior and lifestyle, access to screening and management options, which in turn impact the survival from esophageal cancer (29, 30).

In our study, patients who received chemotherapy have a 64% lower probability of death, supporting the hypothesis that chemotherapy is an efficacious treatment option for advanced esophageal cancer. Coherently, a study in China showed esophagectomy and chemo-radiotherapy were associated with a better survival (31). A systematic review in Africa also reported consistent results (32).

Furthermore, the rate of death is 62% lower among patients who were treated using radiotherapy than those who did not. Similar studies indicated radiotherapy improves survival from esophageal cancer (33, 34). Hence, expansion of radiotherapy centers and training of skilled professionals could help to reduce mortality from esophageal as well as other types of cancer.

This study also showed that patients treated with surgery had a 30% lower rate of death than their counterparts, which is supported by a systematic review that showed the best treatment options to be esophagectomy with a 3-year survival rate of 76.6% (32). Consistently, a study in Kenya indicated patients treated with esophagectomy had a better survival compared to intubations (35). A study in Japan also showed the 5-year survival rates for patients who undertook surgery and those who did not were 17 and 13%, respectively, indicating the importance of surgery (36).

As part of the strength of this study, we used a multivariable Cox regression, which allowed estimating survival patterns of patients with an unequal follow-up period and also took account of censoring. Furthermore, due to the inclusion of all the patients who fulfilled the eligibility criteria, sampling error was avoided or minimized. In addition, in our study more than three hundred patients experienced the event, which made our Kaplan–Meier and Cox regression estimates more precise. Harrel et al. (37) suggest that the Cox regression model needs a minimum of 10 events per each covariate in the model, indicating our analysis is sufficiently powered to identify determinants of survival. However, the following limitations need to be considered in interpretation of findings. First, since we used existing patient charts, data were missed for some variables, particularly histological grade. Nevertheless, we managed missing data using multiple imputation, which provided more precise estimates. Second, confirmation of death and its cause for some of the patients used verbal autopsy through phone interview, which may not be as accurate as hospital death records or vital event registrations. As a result deaths due to esophageal cancer might be overestimated, leading to outcome ascertainment bias. Nevertheless, since the misclassification is independent of the prognostic factors, the effect on the hazard ratios is negligible. At last, since the majority of patients were diagnosed at an advanced stage of cancer, the overall median survival was too small, leading to the estimation of time specific survival rates being less precise. Further determinants of survival could be identified using studies which recruit larger sample size.

CONCLUSIONS

This study identified, in Ethiopia, patients diagnosed with esophageal cancer have a very low survival rate. The death rate due to esophageal cancer is significantly different according to the stage of cancer at diagnosis and treatment modalities they received, radiotherapy, chemotherapy, and/or surgery. Patients diagnosed at an advanced cancer stage and those who did not receive either of the treatment options showed lower survival rate. These indicate early diagnosis and timely initiation of the available treatment options are essential to improve survival of patients with esophageal cancer. Hence, improvements in cancer control programs, including screening, prevention, timely initiation of available treatment, and establishment of comprehensive cancer registry are recommended. Moreover, public health experts should collaborate with clinicians and community leaders to increase awareness on prevention strategies and early symptoms of esophageal cancer to assist early visit to healthcare. To improve utility of data for further research and policy, healthcare providers working at oncology units need to give more attention to document all relevant patient information on the medical record and the cancer registry. We recommend future studies employing prospective design and larger samples.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available upon request from the authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the institutional review board of Addis Ababa University, College of Health Sciences. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

HH: Conceptualization, methodology, formal analysis, software, validation, investigation, data curation, visualization, supervision, writing—original draft, writing—reviewing and editing. MT: Conceptualization, methodology, validation, investigation, resources, data curation, visualization, supervision, project administration, funding acquisition, writing—reviewing and editing. AA: Methodology, supervision, project administration, validation, resources, writing—reviewing and editing. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2020.594342/full#supplementary-material

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REVIEW
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A Review of Cancer Genetics and Genomics Studies in Africa

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Cancer is the second leading cause of death globally and is projected to overtake infectious disease as the leading cause of mortality in Africa within the next two decades. Cancer is a group of genomic diseases that presents with intra- and inter-population unique phenotypes, with Black populations having the burden of morbidity and mortality for most types. At large, the prevention and treatment of cancers have been propelled by the understanding of the genetic make-up of the disease of mostly non-African populations. By the same token, there is a wide knowledge gap in understanding the underlying genetic causes of, and genomic alterations associated with, cancer among black Africans. Accordingly, we performed a review of the literature to survey existing studies on cancer genetics/genomics and curated findings pertaining to publications across multiple cancer types conducted on African populations. We used PubMed MeSH terms to retrieve the relevant publications from 1990 to December 2019. The metadata of these publications were extracted using R text mining packages: RISmed and Pubmed.mineR. The data showed that only 0.329% of cancer publications globally were on Africa, and only 0.016% were on cancer genetics/genomics from Africa. Although the most prevalent cancers in Africa are cancers of the breast, cervix, uterus, and prostate, publications representing breast, colorectal, liver, and blood cancers were the most frequent in our review. The most frequently reported cancer genes were *BRCA1*, *BRCA2*, and *TP53*. Next, the genes reported in the reviewed publications' abstracts were extracted and annotated into three gene ontology classes. Genes in the *cellular component* class were mostly associated with *cell part and organelle part*, while those in *biological process* and *molecular function* classes were mainly associated with cell process, biological regulation, and binding, and catalytic activity, respectively. Overall, this review highlights the paucity of research on cancer genomics on African populations, identified gaps, and discussed the need for concerted efforts to encourage more research on cancer genomics in Africa.

Keywords: cancer, genetics, genomics, Africa, molecular biology

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INTRODUCTION

Cancer is the second leading cause of death globally (1). In Africa, cancer incidence and mortality continue to grow rapidly. According to the 2018 Globocan data, new cancer cases and cancer deaths in Africa were estimated at 1,049,800 and 700,800, respectively (2). In 2018, women in East Africa had the highest cumulative risk of dying from cancer globally. The burden of cancer in Africa is increasing, and this burden is expected to increase by 60% by the year 2030. To lower this projected increase in cancer burden, population-relevant biological studies and the identification of innate risk factors among African populations are needed (3–5).

As cancer is a genetic disease, scientific studies investigating its causes, diagnosis, and treatment in developing countries need to focus more on genetics and genomics. The African or Black population is not a homogenous group and, as such, necessitates the need for genomic/genetic studies to reflect the diverse African populations. The population history of Africa shows that the people of Africa are the most genetically and phenotypically diverse population (6, 7). The peopling history of Africa has been described by Campbell et al. and Tucci & Akey (8, 9), and their reviews showed that African ethnic groups and tribes are genetically heterogeneous. Hence, there is likely a critical contribution of the underlying within-group genetic differences to the disparity in cancer prognosis seen among Blacks (10). Therefore, cancer genetics/genomics studies are expected to significantly impact the understanding of the risk, susceptibility, diagnosis, and treatment of this disease.

The genomic heterogeneity of human populations was driven by ancient migration and heterogeneous adaptive pressures on the human genome, particularly on the African Continent (11, 12). These evolutionary events resulted in the split of human populations into five distinct groups: southern Khoe-San, northern Khoe-San, central African hunter-gatherers, West Africans, and East Africans, out of which a subset migrated out of Africa and is now recognized as the out-of-Africa population (11, 12). Therefore, the African continent could be considered to harbor the repository of human genomic diversity and serves as the resource reference for understanding the role of genomics in human health equity. This repository is further deepened by the present-day North African populations enriched with the genetic pool of the out-of-Africa's Euro-Asian populations. Still, Africa's contribution to global genetic and genomics information is grossly disproportionate to its population's diversity and size. For example, very few African populations were included in the HapMap and 1000Genome projects (13). This is a serious shortcoming for a group of people that represent over 90% of human genomic diversity. A recent review of genome-wide association studies (GWAS) showed that Africans (including African Americans) only represent 2.4% of individuals included in all GWAS studies (14).

The proper understanding of genetics and genomics among African populations will expectantly improve prevention, diagnosis, and treatment outcomes of cancer. Although recent evidence shows that the burden of cancer is in Africa, there remains a huge deficit in requisite skills and infrastructure required to carry out the necessary research studies to alleviate this knowledge gap, requiring still non-African nations to fill this gap (15).

Accordingly, in this review, we discuss both genetics and genomics study findings across multiple cancer types in African populations. The goal here is to demonstrate the existing knowledge and to crucially identify the gaps that should be filled in order to address the cancer burden across Africa.

METHODS

The peer-reviewed publications included in this review were extracted from PubMed and covered the period between January 1990 and December 2019, as shown in the flow chart in **Figure 1**. Since PubMed Medical Subject Heading (MeSH) terms involve synonym control, it yields more precise and inclusive search results (16). Our literature search approach, therefore, utilized an integration of MeSH terms that incorporated "the disease" (neoplasm), 54 African countries, and combinations of study parameters ('gene or protein or molecular biology or mutation or genetics or genomics'). After extracting African cancer papers, we next filtered those to include only papers pertaining to cancer molecular biology (protein or nucleic acid). Cancer molecular biology papers were then further filtered using "genetic* OR genomic* OR mutation*[MeSH Terms]." The final criteria were that the studies must utilize biospecimens of African origin. Two authors (SOR and OAR) manually verified these publications to ensure the accuracy of terms.

For the purpose of data extraction, the metadata and abstract of each publication returned from our search were collected in a single corpus and subjected to text-mining using the R packages RISmed (17) and Pubmed.mineR (18). The publications returned were analyzed in R to identify the cancer types/sites associated with each publication, as described by Acharya et al. (19). Furthermore, the R package "PubmedmineR" was used for obtaining the names and frequency of occurrence of genes denoted in "Human Genome Nomenclature Committee" (HGNC) symbols (20). For this purpose, we considered the genes reported in the abstract as the genes associated with the most prominent findings of the publications. Next, these genes were pulled and subjected to gene ontology functional profiling for three gene ontology classes ("molecular function", "biological process", and "cellular component") using "goProfiles" (21).

RESULTS

The total numbers of publications returned by our search on the topics of cancer globally, as well as cancer, cancer molecular biology, and cancer genetics/genomics within Africa between 1990 and December 2019, are shown in **Figure 1**. Out of nearly two and half million publications on cancer globally, only 7,697 (0.329%) papers were returned by our search on cancer in Africa, with only 1,456 (0.061%) related to molecular biology (protein or nucleic acid). Of these publications, only 375 articles were found using the search terms "genetic, genomics, mutations".

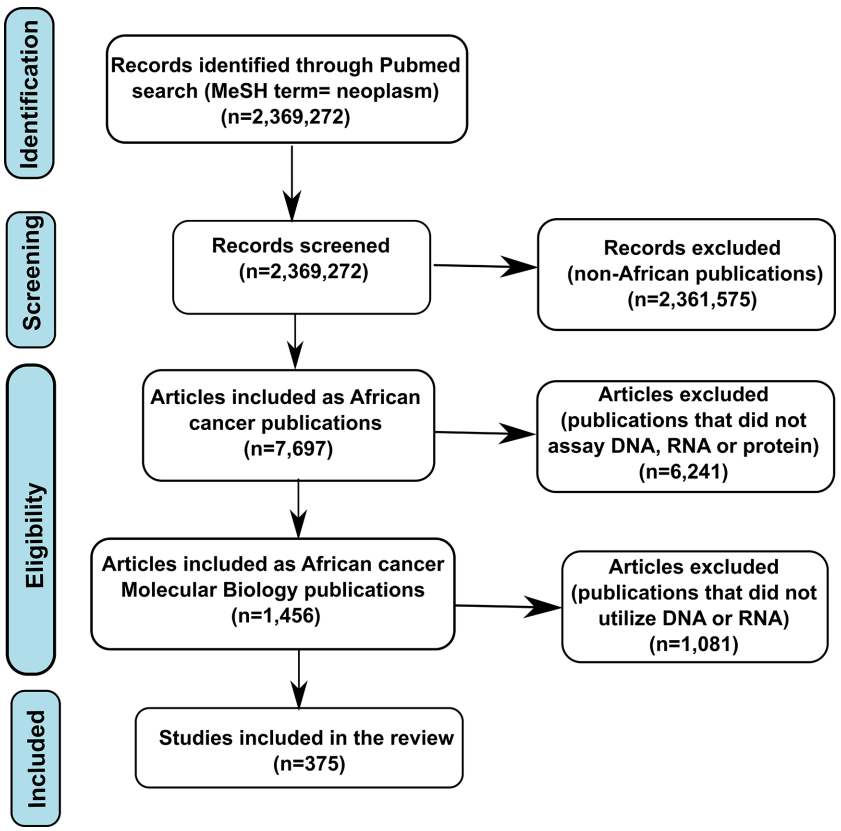

FIGURE 1 | Flow diagram of the literature search strategy.

Among all cancer publications pertaining to Africa, the cancer sites with the highest number of published studies represented cancers of the cervix, breast, liver, head/neck, and colorectal while, lung, brain, bladder, ovarian, and uterine cancers were the least frequently reported on (**Figure 2A**). For publications related to cancer molecular biology in Africa, breast, liver, colorectal, blood, and prostate cancer were the most frequent. In contrast, cancers of the brain, stomach, lung, skin, and uterine cancer had the fewest publications (**Figure 2B**). Most papers reporting cancer genetics or genomics reported on breast, colorectal, liver, blood, and ovarian cancer, with the fewest cancer genetics or genomics studies on the brain, stomach, lung, skin, and uterine cancers (**Figure 2C**).

There were also disparities in the publications by country, as illustrated in **Figures 3A–C**. Nigeria had the most papers on cancer overall, followed by South Africa, Egypt, Tunisia, Morocco, and Kenya (**Figure 3A**). For cancer molecular biology papers, Egypt took the lead, followed by Tunisia, South Africa, and then Nigeria (**Figure 3B**). Tunisia, however, returned the most search results for cancer genetics/genomics papers followed by Egypt, South Africa, and Morocco (**Figure 3C**). Overall, only seven African countries contributed at least 10 cancer genetics/genomics publications, while 22 African countries returned no search results on cancer genetics/genomics studies. The search results show clear evidence of regional differences in publishing capacity, with North Africa and South Africa leading in cancer research.

Next, we focused specifically on the list of 375 genetics/genomics publications for gene curation and review. We did this to identify the functional contributions of these studies to the understanding of biological processes associated with carcinogenesis, using functional correlations comparison (22). A total of 152 genes in the abstracts of 375 publications on cancer genomics were extracted and further annotated into the following gene ontology classes: *cellular component, biological process, and molecular function* (**Figures 4A–C**). In the *cellular component* class, the genes studied were mostly associated with cell part, organelle, organelle part, and cell membrane. In contrast, the genes in the biological process were mainly associated with cell process, biological regulation, response to stimulus, and positive regulation of the biological process. The *molecular function* ontology genes were mostly associated with binding, catalytic activity, molecular function regulator, molecular function transducer activity, and transcription regulation in the molecular function class, which are dysregulated in cancer. The most studied genes in the publications were *BRCA1*, *BRCA2*, *TP53*, *EGFR*, and *MLH1* (**Table 1**), indicating a dearth of data on the plethora of other

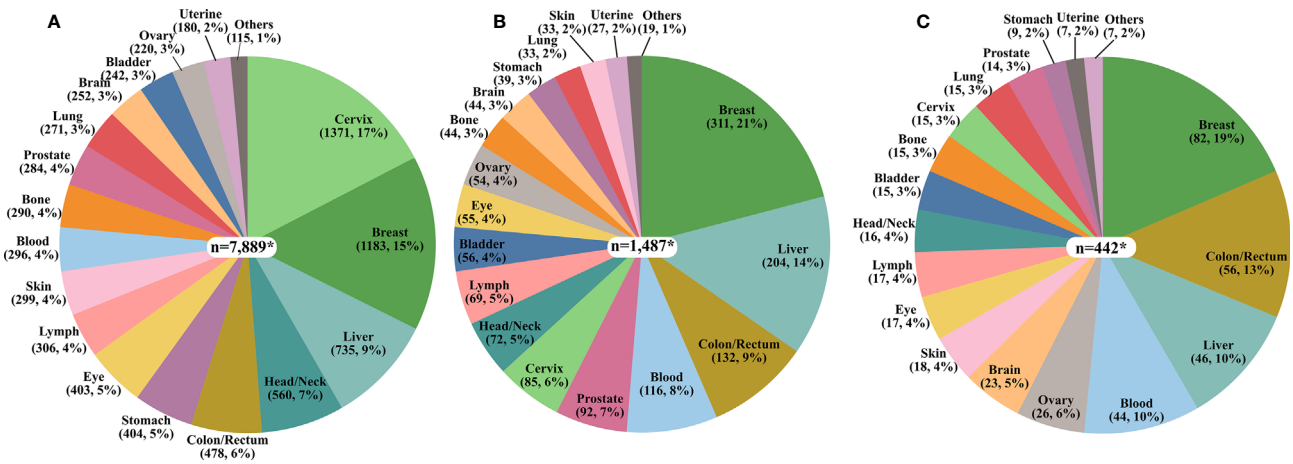

FIGURE 2 | The proportion of the number of publications on each cancer type. **(A)** Cancer in Africa (n=7,697) **(B)** Cancer Molecular Biology in Africa (n=1,456), and **(C)** Cancer Genetics/Genomics in Africa (n=375). *The total values presented in the pie charts are greater than the sum of publications in each category due to the multiplicity of cancer sites for some publications as exemplified by studies on breast/ovary and blood/lymph.

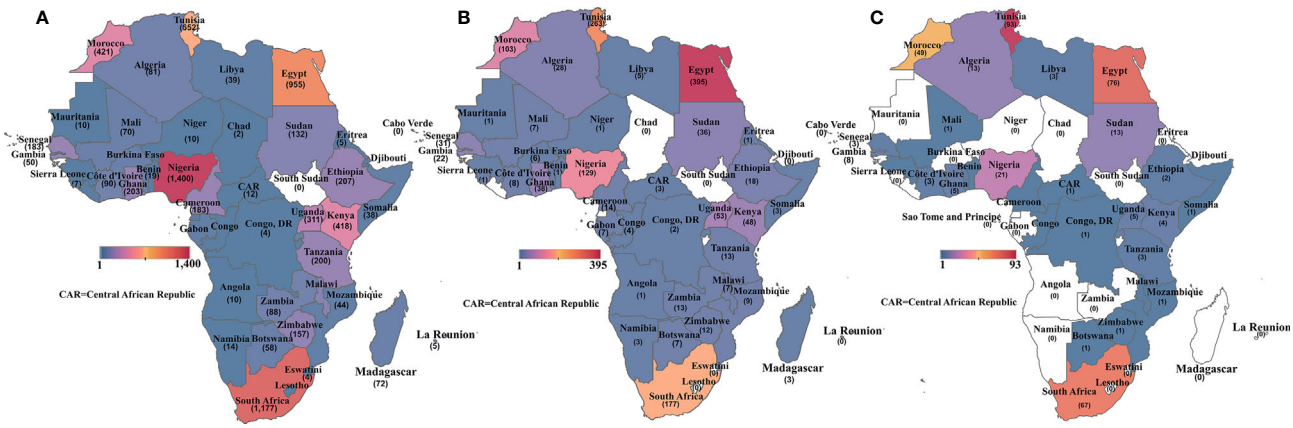

FIGURE 3 | Heat map showing the number of publications retrieved for **(A)** all cancer publications per African country; **(B)** cancer molecular biology publications per African country and **(C)** cancer genetics/genomics publications per African country. Countries without any publication in each category are shaded in white.

critical cancer-associated genes. Next, we reviewed some of the key findings reported across the 375 genomics papers for each of the major and most frequently published cancer types below.

Breast/Ovarian Cancer

Breast cancer has continued to be the leading cause of cancer morbidity and mortality in Africa, with an incidence and mortality rate of 37.9 and 17.2 per 100000, respectively, according to GLOBOCAN 2018 data (2). Breast cancer's prominence in Africa dates back to around 3000BC in the ancient Egyptian medical text - the Edwin Smith Papyrus, the oldest cancer record (23, 24). Not surprisingly, breast cancer had the highest number (n=82, 19%) of peer-reviewed cancer genetics/genomics publications in Africa. With the current understanding of cancer as a genomic disease and the unique phenotype that breast cancer presents in the people of African ancestry, attempts to address its burden require rigorous genomics investigations.

Together with cancer of the ovary, breast cancer risk is greatly increased in women with inherited mutation(s) in tumor suppressor genes (25). Not surprisingly, the earliest publications on breast and ovarian cancers in African populations focused on understanding the contribution of variations in the tumor suppressor genes *BRCA1/2* and *TP53*, particularly in North African populations of Morocco, Tunisia, Egypt, and Sudan (26–40). While these findings hold immense benefits for those populations, their *BRCA* variants are not dissimilar to those present in the out-of-Africa populations.

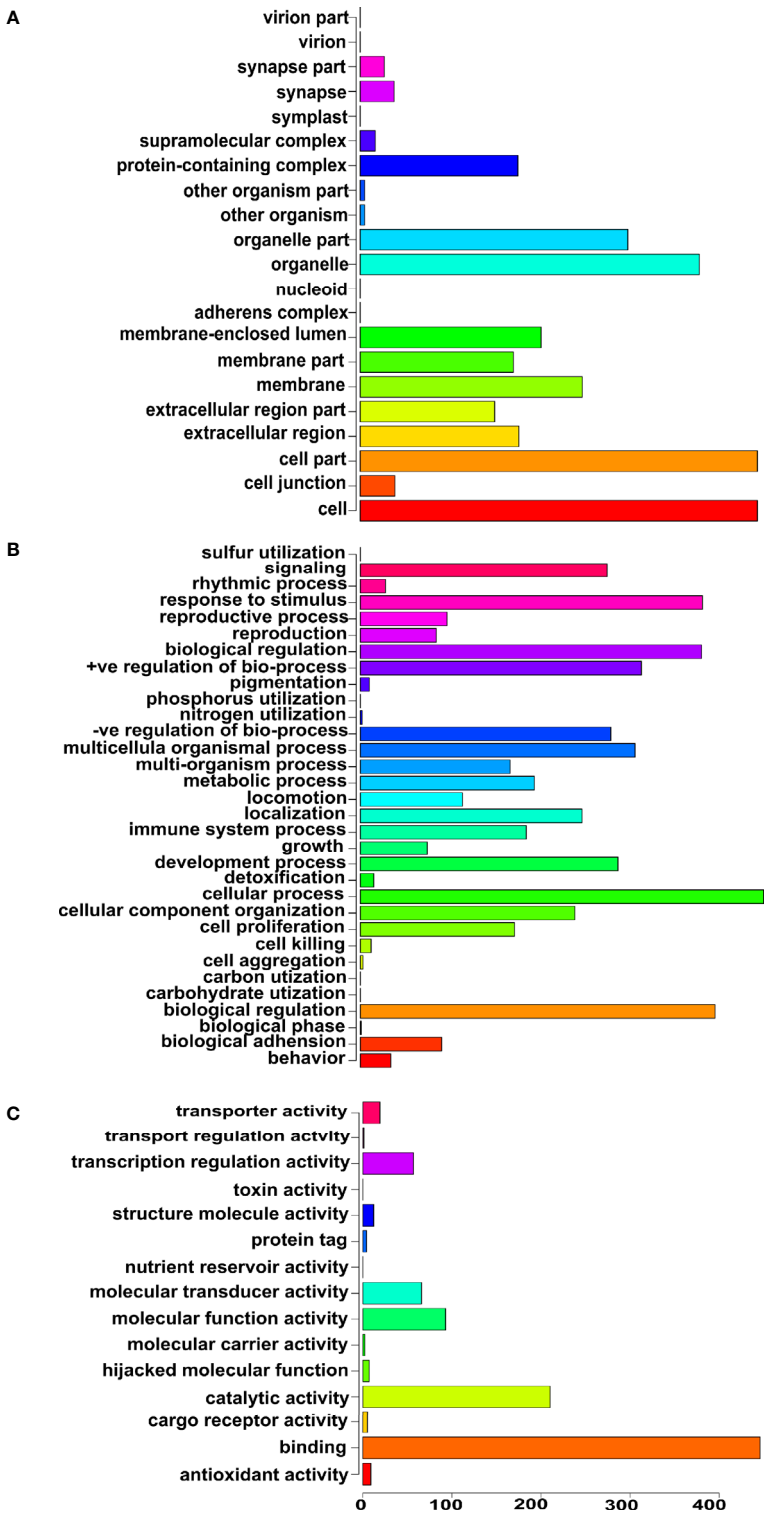

FIGURE 4 | Gene ontology of the genes reported in the abstracts of publications on cancer genomics in Africa. **(A)** Cellular component ontology, **(B)** Biological process ontology, and **(C)** Molecular function ontology.

TABLE 1 | List of top 20 genes reported in the abstracts of publications on cancer genetics and genomics in Africa.

	Gene symbol	Genes	Frequency
1	*BRCA1*	breast cancer 1, early onset	164
2	*BRCA2*	breast cancer 2, early onset	108
3	*TP53*	tumor protein p53	73
4	*EGFR*	epidermal growth factor receptor	53
5	*MLH1*	mutL homolog 1, colon cancer, nonpolyposis type 2 (E. coli)	41
6	*KRAS*	v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog	39
7	*BRAF*	v-raf murine sarcoma viral oncogene homolog B1	30
8	*XPA*	xeroderma pigmentosum, complementation group A	29
9	*RET*	ret proto-oncogene	22
10	*NPM1*	nucleophosmin (nucleolar phosphoprotein B23, numatrin)	21
11	*NLRP7*	NLR family, pyrin domain containing 7	20
12	*APC*	adenomatous polyposis coli	19
13	*JAK2*	Janus kinase 2	19
14	*MSH2*	mutS homolog 2, colon cancer, nonpolyposis type 1 (E. coli)	19
15	*ABCB1*	ATP-binding cassette, sub-family B (MDR/TAP), member 1	16
16	*GSTT1*	glutathione S-transferase theta 1	16
17	*MGMT*	O-6-methylguanine-DNA methyltransferase	15
18	*RB1*	retinoblastoma 1	15
19	*WT1*	Wilms tumor 1	15
20	*MDM2*	MDM2 oncogene, E3 ubiquitin protein ligase	14

This, therefore, limits the translational impact of such findings to controlling breast/ovarian cancer in the Sub-Saharan African populations.

Furthermore, the major epidemiological implication of *BRCA* mutations lies in identifying specific founder mutation(s) within each population, with the view of using it as a predictive molecular risk marker and treatment recommendation. For instance, advances in understanding the role of *BRCA* proteins in tumorigenesis have now led to improved therapeutic choices with the availability of PARP inhibitors for breast cancer patients with germline mutations (41). Also, the identification of founder *BRCA* gene mutations in populations like Ashkenazi-Jewish (Hungarian and Russian), Polish, Norwegian and Icelandic people has resulted in improved low-cost genetic testing and the determination of high-risk individuals for breast and ovarian cancers (42, 43). Therefore, these have made it imperative for the founder mutations of the *BRCA* gene within Africa populations to be identified and included in breast cancer screening, diagnosis, and treatment.

In an attempt to consider *BRCA* contributions to breast cancer in Africa, Rebbeck et al. (44) published a global distribution of *BRCA1* and *BRCA2* germline mutations by including women from Nigeria and South Africa. However, the extent to which their subjects represent the ethnic and genetic diversity in these countries is unclear. They did note that the mutations observed in African American families were of African origin because they are unlike the mutations seen in out-of-African ethnic groups (44–46). This study of Rebbeck et al. (44) was part of the Consortium of Investigators of Modifiers of *BRCA1/2* investigations, which only included the nation of South Africa (http://cimba.ccge.medschl.cam.ac.uk/cimba-groups/study-groups/). A more detailed study of Zheng et al. (47) on Nigerian women established that up to 20% of inherited invasive breast cancer cases in Nigeria are associated with inherited mutations in *BRCA1*, *BRCA2*, *PALB2*, or *TP53*. Their findings on *BRCA1* and *BRCA2* built on the earlier report of Fackenthal et al. (48) that Nigerian breast cancer patients have a very high frequency of *BRCA1* and *BRCA2* mutations. These mutations were reported by Pitt et al. (49) to be associated with greater structural variation and aggressive biology in Nigerian women with HR + /HER2 − tumors. Similar findings were reported by Pegoraro et al. (50) in Black South Africans with ovarian epithelial malignancies.

Recently, Mahfoudh et al. (51) showed that the 5382insC *BRCA1* mutation contributes to the development of triple-negative breast cancer (TNBC) in Tunisia. The higher mortality of breast cancer in women of West African ancestry is due in part to higher levels of TNBC (compared to whites), which is associated with the poorest prognosis of all breast cancer subtypes. Hence, *BRCA* screening in Africa could help identify women who can benefit from *PARP* inhibitors leading to improved clinical outcomes. In South Africa, Reeves et al. (52) characterized *BRCA1* mutations in breast and/or ovarian cancer to identify founder mutations in Afrikaner families. However, this population is also of European ancestry, and the mutations that were identified were similar to those reported in the Netherlands and in Ashkenazi Jews (53). They also reported that variants of *PALB2*, a partner and localizer of *BRCA2* was also associated with the early onset of breast cancer in some South African patients (53). *PALB2* functions as a scaffold between *BRCA1* and *BRCA2*. Similar *PALB2* mutations have previously been identified in women of European ancestry but not in women with Nigerian ancestry, as reported by Sluiter et al. (54).

The first publication on *BRCA* mutations in the indigenous Sub-Saharan African population was by Zhang et al. (55), who identified an ancient *BRCA1* mutation (Y101X) in Yoruba (Nigeria, West Africa) breast cancer patients. The team further reported a non-pathogenic novel exon 21 deletion of *BRCA1* (c. 5277 + 480_5332+672del) in Nigeria in addition to a novel deleterious *BRCA1* mutation (c. 1949_1950delTA) in a woman from Senegal (West Africa) (56). Another novel founder, *BRCA2*

mutation, was identified by var der Merwe et al. (53) in the Bantu-speaking Xhosa population (South Africa). Other studies have identified new *BRCA* mutations and their contribution to early-onset and sporadic breast and/or ovarian cancer in Arabic speaking countries (57) of Egypt (58), Tunisia (51, 59–66), Algeria (67–69), Morocco (70–72), and Sudan (73, 74), in addition to Senegal (75), Mauritius (76) and South Africa (50, 77–79) in the Sub-Saharan region.

Additional studies on *BRCA* genes have expanded to identifying the population-based mutation frequency and screening/genetic testing in the Democratic Republic of the Congo (80), Morocco (81), Tunisia (82, 83), Algeria (84, 85), familial studies in Morocco (86, 87), and large genomic rearrangement in Egypt (88, 89). Of these, the contribution of *BRCA* mutations to male breast cancer was reported only in the Moroccan study by Guaoua et al. (86). A mutation in the *TP53* gene often accompanies *BRCA* mutations in breast and ovarian cancers, making the mutations in these DNA repair genes relevant in therapeutic interventions (90, 91). The publications on *TP53* mutation have focused on its expression in breast cancer and the contribution of its polymorphism, particularly codon 72 to breast cancer (28, 31, 33, 36, 92–94), as well as to its interaction with *MDM2* 344T>A polymorphism in response to chemotherapy of breast cancer in Tunisia (95). Other DNA repair genes that have been studied in Africa include *XRCC1* and XPD in Egypt (96, 97). Overall, even though it is one of the most studied genes in African cancer research, there remains a very small number of publications on *BRCA* mutations in the indigenous African population, clearly showing a knowledge gap on a hereditary gene critical in managing incidence and clinical outcomes in breast cancer.

Exogenous factors that drive DNA damage include viruses and xenobiotics. The presence of these agents and genetic alterations that mediate the ensuing host-response can promote carcinogenesis. The first reports of virus-associated breast cancer in Africa were by Levine et al. (98) and Hachana et al. (99), who reported the presence of a human breast carcinoma virus (a virus similar to mouse mammary tumor virus) in 74% of tumors in Tunisia. These were the only two studies that reported this virus in Africa. Studies have also shown an association of the hepatitis C virus in Egypt (100) and Human papillomavirus (HPV) in Rwanda (101) to breast cancer progression. However, the most reported virus linked to breast cancer in Africa is the Epstein-Barr virus (EBV), with studies published in Algeria (102), Eritrea (103), Egypt (104), and Sudan (105). EBV was the first identified human oncogenic virus that was detected in Uganda in 1964 by Denis Parsons Burkitt (106–108) and its molecular pathogenesis has been reviewed by Lawson et al. (109). The virus is responsible for many cancers across the continent, and the host genomic factors that facilitate tumorigenesis are described below.

The detoxification of carcinogenic chemical entities is primarily catalyzed by cytochrome P450 (phase I) and a host of phase II xenobiotic-metabolizing enzymes. Polymorphisms in these genes dictate, in large part, the effect of xenobiotics on the biological system. Such polymorphisms have been reported in *CYP1A1* and *CYP1B1* in Nigeria and Egypt (110, 111), *CYP2D6* in South Africa (112), and *CYP1A2* in Tunisia (113). Furthermore, as hormone-responsive cancers, these cytochrome P450 genes play critical roles in estrogen metabolism and the response of the tumor to endocrine therapy. For genes coding for phase II xenobiotics metabolizing enzymes, the deletion of *GSTT1* and *GSTM1* were reported by Khedhaier (114) to predict the early onset and prognosis of breast cancer among Tunisian women. The number of TA repeats in the promoter of low activity *UGT1A1* was reported to be protective against breast cancer in pre-menopausal Nigerian women (115, 116). Similarly, the association of polymorphisms in paraoxonase, cyclooxygenase, glyoxalase, and glutathione peroxidase genes with breast cancer were reported in Egypt and Rwanda (117–120).

Inflammation is a major hallmark of cancer, and it is known to contribute to aggressive tumor biology. This makes understanding the variations in immuno-oncogenic genes important in understanding the population biology of cancer in Africa. Mestiri et al. (121, 122) reported that polymorphisms in *TNF-α* and *TNFRII* increase the susceptibility to breast cancer in Tunisian women, with *TNFRII* -196R prevalent in premenopausal women. Conversely, *FASL* (rs763110) was associated with a good prognosis in the same population (123). However, *HLA-DQB1* and *HLA-G* +3142C>G (rs1063320) polymorphisms were related to increased breast cancer susceptibility (124, 125). Pathogenic polymorphisms of other inflammatory genes like *NRF2*, *IL1α*, *IL1β*, *IL6*, *IL8*, and *CXCR2* have also been identified in Tunisian and Egyptian breast cancer patients (126–130).

Recent evidence suggests that inflammation-driven cancer in Blacks is influenced by vitamin D levels (131, 132). To establish the association of vitamin D variants and related genes with breast cancer, El-Shorbagy et al. (133), Abd-Elsala et al. (134) and Shaker & Senousy (135) showed that polymorphisms in the vitamin D receptor (*VDR*) increases the risk of breast cancer in Egyptian women who carry the ATT haplotype. The risk of developing breast cancer due to these mutations was elevated in women who also carry *RANKL* (rs9533156), *OPG* (rs2073617), and *CHI3L1* (rs4950928) (135). Similar studies have also reported the risk allele in Ethiopian women as *VDR* rs2228570 (FokI) (136) but the study of Wang et al. (137) did not identify variants in vitamin D related genes as risk factors for breast cancer in Nigerian women that were used as the ancestral population for African American women. This genome-wide association study, however, identified *TYRP1* (rs41302073), a melanin synthesis regulatory gene, as a significant risk allele for breast cancer in their dataset that included African American and Barbadian women. Furthermore, the authors also used the same dataset to identify *WWCI* as an important susceptibility locus in the Hippo pathway for breast cancer (138).

Polymorphisms in the angiogenesis-associated genes have also been identified in breast cancer in African populations and include the *LEP*, *LEPR*, *VEGF*, and *MMP2*. Leptin and *LEPR* Q223R (rs1137101) were identified as risk factors for breast cancer in Egyptian and Nigerian women (139–141)

while leptin alone was notably reported as a key driver of breast cancer progression through the induction of *JAK/STAT3*, *ERK1/2*, and estrogen pathways in obese Egyptian women (142). Furthermore, variants of *VEGF* and *MMPs*, which induce the upregulation of these proteins, were reported as risk factors in North African countries of Morocco, Egypt, and Tunisia (143–148). Other overexpressed angiogenic proteins reported are *EGFR* in Tunisia (149) and *IGFBP2* and *IGFBP5* in Nigerian women (150). The authors proposed these angiogenic proteins as druggable targets in breast cancer treatment. Another therapeutic pathway that has been studied is the *PIK3/AKT* pathway. Jouali et al. (151) reported *PIK3CA* hotspot mutations in 13% of triple-negative breast cancer cases in Morocco. They suggested that this pathway could be of therapeutic importance for triple-negative breast cancer in Morocco.

Cancer is a polygenic disease, and scientific investigation to understand breast cancer's population biology, therefore, cannot be simplified to a single genetic variant. Hence, techniques to investigate multiple genes at a time such as with next generation sequencing are now being utilized to understand the genetic risk factors of breast cancer in Africa. To that effect, genome-wide studies (GWAS) published primarily on breast cancer in African populations include GWAS in Tunisia and South Africa (152–154) and whole-exome sequencing in Tunisia and Egypt (155–157). In the Tunisian population, Shan et al. (154) and Hamdi et al. (152) identified rs1219648, rs2981582, rs8051542, rs889312, and rs889312 as breast cancer susceptibility single nucleotide polymorphisms (SNPs), with rs9911630 as the SNP with the strongest effect on the expression of *BRCA1* and two long non-coding RNAs (NBR2 and LINC008854). The genome-wide copy number alteration analysis of breast cancer in South African women (153) identified the amplification in Xp22.3 and 6p21-p25, and other regions that affect known cancer genes like *CCND1*, *CDKN1A*, *MDM2*, *TP53*, and *SMAD2*. Meanwhile, the whole-exome sequencing study by Hamdi et al. (152) and Riahi et al. (156) linked breast cancer in Tunisian women to alterations in *MMS19*, *DNAH3*, *POLK*, *KATβ6*, and *RCC1* in *BRCA1/2* mutation-negative patients with familial breast cancer. A similar study in Egypt also found other novel genetic variants responsible for familial breast cancer. These genetic variants are different from those linked to DNA damage repair (like *BRCA1* and *BRCA2*) but are linked to other functional genes like *NBPF10*, *ZNF750*, *CHTI5*, *NPIPB11*, and *PHIP*, that are involved in RNA binding, transcriptional regulation, extracellular matrix, a structural protein, and signal transduction, respectively.

The contribution of epigenetic factors to risk and prognosis of breast cancer reported in Africa included the roles of tissue microRNA, circulating free mRNA, circulating long non-coding RNA (158–163) as well as DNA methylation status of breast cancer susceptibility genes like *APC*, *ERα*, *RASSFIA*, *UCHL1*, *COX-2*, and *FHIT* (161, 164–167) in breast tumor across Africa.

Prostate Cancer

Prostate cancer continues to be the leading cause of cancer morbidity and mortality among African men (168, 169). Although genetics is a major risk factor for this disease, there are only a few publications on prostate cancer genomics in Africa. In this subsection, we review 14 papers that were relevant to prostate cancer out of the list of 375 papers extracted. Prostate cancer presents with an aggressive phenotype among men of African descent, and like breast cancer, it is a hormone-responsive tumor. Consequently, early studies on this disease identified androgen's influence in the control of normal prostate growth and, in its transformation into adenocarcinoma, a phenomenon called the "androgen hypothesis" (170, 171). Therefore, peer-reviewed publications on prostate cancer genetics in African populations have reported genetic variants that contribute to elevated circulating androgens, including androgen reduced clearance and upregulated activity of androgen receptor. These include the polymorphisms in cytochrome P450 genes like *CYP3A4*, *CYP3A5*, *CYP1A1*, *CYP17* in Morocco, Tunisia, Nigeria, South Africa, and Senegal (172–176). Besides, alterations in CAG and GGN repeats in the androgen receptor gene have been reported as risk factors in North Africa, Ivory Coast, and Nigeria (177, 178). Unlike the North African populations, prostate cancer in Sub-Saharan African populations and North African Berbers were associated with high frequencies of low size alleles (CAG under 18 repeats, and GGC under 15 repeats) (178). Other reported genetic variations that increase African populations' susceptibility to prostate cancer include *GSTM1*, *GSTT1*, UDP-glucuronosyltransferase, and sulfotransferase in Tunisia and Algeria (179–182).

A deeper understanding of the disease's polygenic risk was elucidated by four studies that have investigated the genome-wide genetic variations in prostate cancer across Africa. These included GWAS of prostate cancer in Tunisia, Ghana, and Uganda (183–185), as well as a whole-genome sequencing of six individuals in South Africa (186). It is interesting to note that these four studies did not identify any common high-risk prostate cancer variants. The Tunisian study identified three regions (on chromosomes 9, 17, and 22) containing 14 significant SNPs, three of which are shared with Caucasian populations (185). The Ghanaian study of Cook et al. (184) identified 30 most significant SNPs distributed across chromosomes 1, 2, 3, 5, 6, 7, 8, 9, 10, 13, and 20.

Meanwhile, the Ugandan study identified risk alleles on chromosomes 1, 6, 11, 13, 14, and 17 (183). Although the Ugandan and Ghanaian populations shared cytoband 6p21.32 in common, the nucleotide positions and risk alleles were still different. This chromosome position codes for *HLA-DQB1*, which has been reported to be important for the adaptation of African ancestral populations to the African rainforest environment. These studies further add to the existing evidence of the heterogeneity of African populations (12) and that cancers in these populations may have a different biology. These findings provide further evidence for the need to disaggregate the Black population by genetic lineage in studying the contributions of genomics to racial disparities of diseases like prostate cancer. Importantly, it is yet to be revealed whether these differences influence the disease phenotype and disparity in outcome.

The most commonly reported genomic alteration that drives prostate tumorigenesis is *TMRPSS2-ERG* fusion, and this androgen-upregulating fusion is known to correlate with higher grades of the disease. Although men of African ancestry are known to present with higher disease grade, only three studies have examined the *TMRPSS2-ERG* fusion on the Continent (187–189). This fusion often results from either a chromosomal translocation or an interstitial deletion, and these studies reported rates that were less than 20% in Ghanaian and Black South African patients (187–189).

Liver Cancer

According to the 2018 GLOBOCAN data, hepatocellular carcinoma accounted for 8.4 cases per 100,000 and 8.3 deaths per 100,000 globally (2) and it is the 4th most common cancer in Africa. We retrieved 46 publications that studied liver cancer genetics/genomics in Africa. Several of these studies investigated the contribution of the hepatitis virus and mycotoxins to this malignancy. These biotic and abiotic agents represent the major causes of this disease on the continent (190, 191). Hence, a preponderance of publications on liver cancer in Africa focused on understanding the contribution of mutation and expression of *TP53*, and other tumor suppressors like *TP73*, *RB*, *KLF6*, and *CTNNB1*, to liver carcinogenesis (190, 192–207), particularly in Senegal, Gambia, Nigeria, South Africa, Egypt, and Morocco. These studies identified the mutation in codon 249 of *TP53* as a genetic risk factor for developing hepatocellular carcinoma following exposure to either the hepatitis virus or mycotoxins (see Lin et al. (208) for detailed mechanism). In Morocco, *MDM2* 309 T>G was associated with liver cancer (209, 210). These mutations are known to upregulate this oncogene's expression, which in turn binds p53 and prevents its tumor suppression function (209) resulting in increased genomic instability as demonstrated by loss of heterozygosity in chromosome 4-q13 in Black South Africans (211).

The development of hepatocellular carcinoma is often preceded by chronic inflammation of the liver. In Africa, hepatic inflammation is exacerbated by high-prevent co-morbid conditions like non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), and liver cirrhosiss. For instance, the prevalence of NAFLD in Nigeria, Ethiopia, and South Africa has been reported to be 68.8%, 73%, and 87%, respectively (212).

Despite the pervasiveness of liver cancer across Africa, only the Egyptian and Tunisian populations have been studied for the contribution of variation in inflammation-related genes to this disease. These studies reported mutations in *IL3R*, *IL17A*, *IL8*, *IL1*, *IL16*, *IL12*, *IL27*, and *TNF-*α as risk factors for hepatitis and hepatocellular carcinoma (213–219).

Other authors have focused on the development of biomarkers for liver cancer, using epigenetic factors like microRNAs. These include serum Mir-224, Mir-215, Mir-143, Mir-122, Mir-199a, and Mir-16 (220, 221). Specifically, Mir-122 and Mir-222 levels were reported by Motawi et al. (222) as a discriminating biomarker for distinguishing liver injury from liver cancer. This group further reported that LncRNA *HULC* rs7763881 and *MALAT* rs619586 were associated with decreased susceptibility of Egyptian hepatitis virus-persistent carriers to liver cancer (223).

Mycotoxicosis, with concomitant early-life protein malnutrition, is an important driver of liver cancer in Africa (224–226). One group of enzymes that are involved in detoxifying mycotoxins are the glutathione-S-transferases ($\mu, \theta, \pi, \alpha, \sigma$). Hence, individuals who do not express all the enzymes due to homozygous deletion are more susceptible to myco-carcinogens (227). Overall, two studies have identified the deletion of *GSTM1* and *GSTT1* haplotypes as risk factors for aflatoxin-associated hepatocellular carcinoma (228, 229) in Africa.

The last group of genes that have been studied on hepatocellular carcinoma in Africa are those involved in angiogenesis, including *VEGF*, *MMP*, *RASSF1A*, and *RECK*. In Egypt, Samamoudy et al. (230) reported that patients with *MMP9* (rs3918242) are at high risk of developing liver cancer while *RECK* (rs12814325) (231) could account for the disease progression and metastasis.

Cervical Cancer

Cervical cancer continues to be responsible for the highest cancer mortality in Africa, accounting for 2,000,000 deaths in 2018 (2), and its incidence rates continue to increase in most Sub-Saharan African countries (232). However, studies on cervical cancer genetics/genomics only represented 3% of the publications we retrieved. Similar to liver cancer, cervical cancer is viral-related and primarily caused by Human Papillomavirus (HPV). Several reviews have discussed the burden, distribution, and contribution of HPV serotypes to cervical cancer in Africa (233–235). Despite the burden of HPV in Africa, only a small proportion of women that are infected develop cervical cancer (236, 237). It is, therefore, essential to understand the genetic factors that contribute to the risk of progression from HPV infection to cervical cancer across Africa.

One of such genetic factors that increase susceptibility to HPV-associated cervical carcinogenesis is the *TP53* R72P mutation (238), which was reported in Gabon, Senegal, Sudan, Morocco, and South Africa; and this risk increases when combined with the chromosomal allelic loss of *RB* or with aberrant methylation of *DAPK1*, *RARB*, *TWIST1*, and *CDH13* (79, 239–244). Furthermore, aberrant methylation of these genes was proposed by Feng et al. (245) to be useful in Senegal for the screening of cervical cancer, either alone or in combination with cytology. The importance of this homozygous arginine polymorphism at codon 72 of *TP53* in determining genetic susceptibility of a population has been shown in Israeli Jewish women who have been reported to have reduced susceptibility to HPV-associated cervical cancer (246).

The variations in genes involved in inflammatory and apoptotic response pathways have also been reported to increase African women's susceptibility to cervical cancer (247). The reported polymorphisms in Africa include those of *TLR 2/3/4/9* and *IL1/10/15* genes in Tunisia and -308 promoter polymorphism of *TNF-*α in South Africa (248–250). Meanwhile, polymorphisms in *FASR*-670A and *CASP8*-652 were associated with a reduced risk of developing cervical cancer in South African women (251).

Colorectal Cancer

Colorectal cancer is the 5th most common cancer in Africa and accounted for 550,000 deaths in 2018 (2). We retrieved 56 publications on colorectal cancer genetics/genomics from Nigeria, Ghana, South Africa, Algeria, Tunisia, Morocco, and Egypt. The findings in these publications included: (1) the identification of I130K *APC* polymorphism in the indigenous Black population in South Africa and Tunisia to development of familial adenomatous polyposis coli (252–255), (2) the presence of mutations in the *MUTYH*, *MLH1*, and *MSH2* gene in patients with colorectal cancer and attenuated polyposis in Algeria, Egypt, Morocco, Tunisia, and South Africa (256–266), (3) the burden of *KRAS* and *BRAF* mutations in colorectal cases in Morroco, Nigeria, Ghana, Egypt and Tunisia (267–275) and (4) the level of microsatellite instability in South African, Nigerian, Ghanian, Tunisian, and Moroccan colorectal cancer patients (259, 271, 274, 276–281). Other studies have also explored the contribution of epigenetic changes to colorectal cancer carcinogenesis in Africa (278, 282–285). For example, the methylation of *UCH1* and *p14ARF* genes were reported to drive colorectal cancer in the presence of *TP53* mutation in Tunisia (282, 283, 286, 287). Other studies on the North African populations reported the influence of polymorphisms in telomere and mitochondrial D-loop region on the clinicopathological characteristics of the colorectal cancers among their patients (288, 289). Hence, the dearth of data on the genomics of this disease makes it difficult to explain the increase in the level of sporadic colorectal cancers reported in African countries, despite the difference in lifestyle and dietary habits. Profiling of these genes, including the use of targeted next-generation sequencing, in the screening and clinical management of this disease is essential in reducing its burden (255, 290).

Lung Cancer

Across Africa, lung cancer ranks 6th, with about 550,000 cases in 2018 (2). However, the burden of this disease is on the North African countries and South Africa (2). This burden reflects the pattern of tobacco smoking reported through national surveys (291). Lung cancer genetics/genomics studies have also largely been conducted on the North African populations of Tunisia and Egypt. These studies investigated the role of angiogenic pathway genes like *EGFR* and *MMP-3* in lung carcinogenesis (292–297). The expression of *EGFR* was associated with poor prognosis, and the frequency of the mutations observed in Tunisian and Moroccan patients was similar to those of Europeans (294, 296, 298). However, Dhieh et al. (292) found that abnormal p53 expression in these patient populations was more frequent than in Europeans. Similarly, a nonsense mutation (Arg-196-Term) in exon 6 of *TP53* was identified in the small cell lung cancer from gold miners in South Africa (299).

Cigarette and air pollution are major sources of lung carcinogens; hence, studies have reported polymorphisms in *CYP1A1*, *CYP1A2*, *CYP2F1*, *CYP2A6*2*, and *CYP2A6*9* (300–305) in lung cancer patients in North Africa. These polymorphisms alter the detoxification rate of toxicants, and individuals who carry the slow metabolizer variants have an increased risk of lung cancer (300). For example, Hussein et al. (302) concluded that Egyptian smokers with *CYP1A1* m1 (rs4646903) and *CYP1A1* m2 (rs1048943) are more likely to develop squamous cell carcinoma. Furthermore, lung carcinogens are highly inflammatory and studies in Tunisia, for example, identified alterations in inflammatory genes-*TNF-α*, *IL8*, *IL17A*, *IL17F*, *CCR2*, and VDR *Fok1* (rs2228570) and *ApaI* (rs7975232) that predispose to lung cancer (306–310).

There were additional studies that used epigenetic techniques to develop diagnostic or prognostic markers for non-small cell lung cancer in Egypt. These included the study of Haroun et al. (311) that identified *FHIT* methylation and that of Hetta et al. (312) which reported circulating microRNA-17 and microRNA-22 as potential biomarkers for early detection of lung cancer.

Bladder Cancer

Chronic inflammation with attendant oxidative stress induced by *Schistosomia haematobium* infection remains a major cause of bladder cancer in Africa (313–315), with squamous cell carcinoma being the most common (316, 317). Schistosomiasis (or bilharzia) is a neglected tropical disease that is widespread across Africa (318). This cancer is the 10th most prevalent cancer in Africa and accounted for 240,000 death in 2018. Studies on its genetics/genomics represented about 3% of the publications that we reviewed.

Its pathogenesis involves the bladder infection by *S. haemotobium*, which induces the formation of carcinogenic N-nitrosamine that contributes to squamous cell carcinogenesis (319), particularly in individuals with *TP53* mutation (320). In addition, mutations in genes associated with inflammation and detoxification of carcinogenesis are critical risk factors. One of which is the polymorphisms in *CYP2D6* and *CYP1A1* that have been studied in Egypt and Tunisia (321–323) and that of *CYP2D*1A*, which was found to increase the risk and clinicopathological outcome of both transitional and squamous cell carcinomas in Egypt (322). Similar findings were reported in the same North African countries for individuals with *GST* null genotypes and *NAT*5* (341T>C) (324–331).

The neoplastic transformation and progression of bladder cancer are enhanced through oxidative stress-induced genomic instability and chromosomal aberrations, which particularly involve the loss of heterozygosity on chromosomes 8 and 9 (332–338). These aberrations, coupled with p53 and p16 loss, have been reported in both bilharzial and non-bilharzial bladder cancer in Egypt and Tunisia (36, 332, 339–343).

The pattern of CpG island hypermethylation was studied by Gustierrez et al. (344) and they showed that the Schistosoma-associated tumors in Egyptian patients had higher hypermethylation of genes like E-cadherin, DAP-kinase, *TP14*, *TP15*, *TP16*, *APC*, *GSTP1*, and *TP73*. Other authors have further proposed using these unique epigenetic modifications for the early diagnosis of bladder cancer by utilizing plasma circulating microRNA and urinary DNA methylation profile (345, 346).

It is important to note that pesticides have also been implicated in bladder tumorigenesis (347, 348) through

oxidative stress and *KRAS* mutation in Egyptian occupationally-exposed individuals (347).

Other Solid Tumors

Studies in South Africa, Egypt, Sudan, and Tunisia identified the EBV as the major cause of head and neck cancer (349–354). The genetic risk factors that have been reported include *TP53* mutations in Sudan and Egypt (355–357), *XRCC1*, *TNF-α*, *IL10* promoter, *CYP1A1*, *CYP2D6*, and *NAT2* polymorphisms in Tunisia (358–361) as well as genome-wide aberrations associated with chromosomes 2p, 3p, 5q, and 18q and microsatelite instabilities (362–364) and mutations in the mitochondrial D-Loop region and Cytochrome b gene (365).

The genomic studies on the cancer of the brain, kidney, pancreas, and other organs are still emerging with very limited publications (366–378). The emphasis of these publications on the polymorphisms of genes associated with inflammatory response is an indication of the importance of this biological process to the neoplastic transformation of normal tissue and the progression of the malignancy. In addition, studies on retinoblastoma concentrated on identifying the constitutional mutations in *RB* within the North African populations (379–382) while publications on esophageal and gastric cancers focused on identifying the role of *RAS* genes mutations as drivers of genomic instability (383–386).

Lymph and Hematological Malignancies

The most prevalent lymphoma in Africa is Burkitt lymphoma. Its pattern and geographical spread are similar to that of malaria and ancient human migration on the continent (387–392). This aggressive pediatric B-cell non-Hodgkin lymphoma is caused by the EBV, which induces genomic instability in the B-cell that results in hyperproliferation (393, 394) and it is associated with unique *TP53* mutations that are clustered between codons 213 to 248 (395–397).

Other studies on lymphoma include: (1) the role of *TP73* and *FOXP3* in the pathogenesis of reactive lymphoid hyperplasia and diffuse B-cell lymphoma, as well as the contribution of *HLA-G* polymorphism to non-Hodgkin lymphoma in Egypt (398–400), (2) susceptibility of individuals with A/A genotype of *TNF* promoter (-308A/G) to non-Hodgkins lymphoma in Tunisia (401) and Egypt (402) and the identification of *HLA-B*18*, *DRB1*03*, *DRB1*07*, and *DQB1*02* as lymphoma susceptibility loci in Algerian children (403).

Studies from Egypt, Tunisia, and Morocco have identified the susceptibility or prognostic implications of mutations in *FLT3-ITD*, *NPM-1*, *KIT*, *NPM1*, *HFE*, *DNMT3A*, *TERT*, and *NRAS* in hematological malignancies (404–410). *NRAS* G12D and *NRAS* G13C mutations were reported in Nigerian leukemia patients Anyanwu et al. (411).

DISCUSSION

In order to provide an overview of research progress in African cancer genomics with the view of identifying the critical gaps, we searched and reviewed publications on cancer genetics and genomics in Africa. The 375 publications on cancer genetics/genomics retrieved on PubMed represented only 0.016% of total publications on cancer globally.

According to the 2018 GLOBOCAN data on cancer in Africa, the most frequently diagnosed cancers were breast, cervix, prostate, liver, and colorectum, while the leading causes of cancer deaths were from cancers of the cervix, breast, prostate, liver, and colorectum (2). However, of the top ten frequently diagnosed cancers and the leading cause of cancer deaths in Africa, only breast, colorectal, liver, and ovarian cancers were proportionately represented in cancer genetics/genomics studies returned from search terms.

Overall, Africans are grossly underrepresented in cancer genomics and molecular biology research globally. For example, research on prostate cancer in African men or breast cancer in African women, both leading causes of death in Africa, are still understudied compared to cancers in their non-Black and white counterparts (412).

Although Africa seems to be on the right track in terms of focusing on some of the top cancers, researchers and funding agencies, need to elevate and prioritize genetics and genomics research on cancers that remain hugely underrepresented or unrepresented in the literature for which there is a significant burden in Africa. These include cancers of the lung, ovary, stomach, bladder, prostate, and non-Hodgkin lymphoma, which are among the leading ten causes of death but remain understudied in the literature. Filling this research gap is essential to improving awareness, prevention, diagnosis, and treatment outcomes for people affected by cancer across the continent.

It is also worth noting that most studies on cancer in Africa are clustered to a few regions, mainly North Africa, Nigeria, Ghana, and South Africa. Most of the continent lacks any appreciable data, is often excluded from research efforts, and is devoid of the infrastructure and resources needed to contribute to cancer genomics/genetics discoveries.

It is important to reiterate that this review was based on publications that were indexed in Pubmed only. This is because Pubmed is considered as the most reputable index for biomedical publications, and the data we have retrieved are a good representation of the spectrum and scope of this review. It is also possible that our search did not retrieve some studies that included African populations, and this could be because those studies were not focused on African countries or groups but have used them for comparative purposes, thereby making the data obscure and less prominent in their findings. The use of MeSH terms ensured that relevant publications were extracted from Pubmed.

CONCLUSION AND FUTURE DIRECTIONS

As presented in this review, the preponderance of the peer-reviewed publications on cancer genomics in Africa was on the North Africa populations. Hence, there is a need for a concerted effort to address the gaps in the contribution of genomic variance and alterations to cancer in Sub-Saharan African populations.

Recently, Durvasula and Sankararaman (413) reported the presence of ghost archaic introgression into the genome of Sub-Saharan Africa populations, and some of this introgression included regions involved in carcinogenesis. This and the details presented in this review lay credence to the inadequacy of the use of predominantly Caucasian genomics data for cancer control in Africa. The use of personalized medicine and targeted therapy in cancer management rely on understanding the genomics of the population. Hence, there is a need to step up cancer genomics studies for Africa to benefit from medical advances. Also, because Africa is the root of humanity, understanding the genetic basis of this disease in Africans will contribute to improving cancer health equity globally.

In addition, scientific investigations on cancer racial disparity have largely considered the Black race as a homogenous group. However, the evidence is now emerging that there are within-group differences in cancer risk among Blacks (414). This review also clearly demonstrated the need to disaggregate Africa in cancer studies. To reduce cancer disparity and achieve equity in treatment outcomes, cancer genetics and genomics studies in African should endeavor to stratify populations by their ancestry roots, tribes, or languages rather than countries. This is imperative to identifying population-relevant genetic variants since African countries are geopolitical constructs that bear no relationship with the biological relatedness of the people that are clustered together in those countries.

Furthermore, every genomic study requires a reference to make an appropriate inference, but African populations are presently inadequately represented in the current reference genomes. To address this unmet need, Shermanet et al. (415) recently published a pan-African reference genome. The African Pan Genome sequences they assembled revealed that up to 10% of the genome will be missed by any efforts relying only on GRCh38 to study human variation. Yet, it is important to note that their study only included representative samples (5%) from Ibadan, Nigeria, and may not be a true "Pan African Genome" and may best represent the West African human population, which the Yoruba people belong to. Further research efforts are, therefore, needed to assemble more African reference genomes, which should be based on the genetic divergence of human populations in Africa.

AUTHOR CONTRIBUTIONS

BS conceived, designed, and supervised the review. SR and OR collected and analyzed the data. BS, SR, and OR wrote the manuscript. All authors contributed to the article and approved the submitted version.

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Identification of Eleven Novel *BRCA* Mutations in Tunisia: Impact on the Clinical Management of *BRCA* Related Cancers

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Background: Breast cancer is the world's most common cancer among women. It is becoming an increasingly urgent problem in low- and middle-income countries (LMICs) where a large fraction of women is diagnosed with advanced-stage disease and have no access to treatment or basic palliative care. About 5-10% of all breast cancers can be attributed to hereditary genetic components and up to 25% of familial cases are due to mutations in *BRCA1*/2 genes. Since their discovery in 1994 and 1995, as few as 18 mutations have been identified in *BRCA* genes in the Tunisian population. The aim of this study is to identify additional *BRCA* mutations, to estimate their contribution to the hereditary breast and ovarian cancers in Tunisia and to investigate the clinicopathological signatures associated with *BRCA* mutations.

Methods: A total of 354 patients diagnosed with breast and ovarian cancers, including 5 male breast cancer cases, have been investigated for *BRCA1/2* mutations using traditional and/or next generation sequencing technologies. Clinicopathological signatures associated with *BRCA* mutations have also been investigated.

Results: In the current study, 16 distinct mutations were detected: 10 in *BRCA1* and 6 in *BRCA2*, of which 11 are described for the first time in Tunisia including 3 variations that have not been reported previously in public databases namely *BRCA1*_c.915T>A; *BRCA2*_c.-227-?_7805+? and *BRCA2*_c.249delG. Early age at onset, family history of ovarian cancer and high tumor grade were significantly associated with *BRCA* status. *BRCA1* carriers were more likely to be triple negative breast cancer compared to *BRCA2* carriers. A relatively high frequency of contralateral breast cancer and ovarian cancer

occurrence was observed among *BRCA* carriers and was more frequent in patients carrying *BRCA1* mutations.

Conclusion: Our study provides new insights into breast and ovarian cancer genetic landscape in the under-represented North African populations. The prevalence assessment of novel and recurrent *BRCA1/2* pathogenic mutations will enhance the use of personalized treatment and precise screening strategies by both affected and unaffected North African cancer cases.

Keywords: *BRCA* cancers, genetic testing, novel *BRCA* mutations, clinicopathological signatures, precision medicine

INTRODUCTION

Breast cancer is the most common malignancy among women worldwide (1). Incidence and mortality rates of breast cancer differ between populations (1). In Tunisia, it remains the most common cancer among females and represents the first leading cause of cancer mortality among women. The mean age at diagnosis of Tunisian breast cancer cases is around 50 years old, a decade younger than Western countries (2, 3).

BRCA1 and *BRCA2* are the most prominent breast cancer susceptibility genes that convey high risk of breast and ovarian cancers (4). Since their discovery, a wide range of mutational spectrum have been described for both genes. So far, more than 1800 distinct *BRCA1* and 2000 *BRCA2* mutations have been reported in the Breast Cancer Information Core (BIC) database. These mutations explain around 20-30% of breast cancer genetic component and seem to be associated with different other cancers such as prostate, pancreatic, endometrial and melanoma (5). The identification of novel *BRCA1/2* mutations has important clinical implications. Indeed, unaffected *BRCA* mutation carriers have various preventive options including extensive and regular surveillance, chemoprevention, and risk-reducing surgery (6–8), while, affected cases carrying *BRCA* mutations could benefit from personalized therapeutic options such as platinum-based chemotherapy and poly (ADP-ribose) polymerase (PARP) inhibitors (9, 10). However, a full *BRCA1* and *BRCA2* gene screening remains a labor and time-consuming challenge due to the large gene size, diverse mutations, or variants of unknown significance (VUS) and complexity of large genomic rearrangements (LRs) and copy number variations (CNVs) requiring special technical approaches. Recent advances in high throughput sequencing technologies including Target panels and whole exome sequencing (WES) allowed rapid, sensitive, and cost-effective screening of the large *BRCA* genes. In addition, the decreased cost of genotyping and sequencing offered affordable targeted testing options.

Sequencing thousands of cancer samples showed that the frequency of germline mutations in *BRCA* genes varies widely among populations. Some mutations are shared between different populations and others are ethnic specific (11, 12). Indeed, in certain countries and ethnic communities, the *BRCA* mutation spectrum is limited to a few founder mutations (13, 14). This is mainly observed in geographically, culturally, or religiously isolated populations and in countries with high rates of consanguinity and endogamy that undergo rapid expansion from a limited number of ancestors. Consequently, some alleles become more frequent which explain the high frequency of some founder mutations in these populations. The founder effect may, therefore, influence mutation prevalence and gene penetrance. Since cancer risk is a function of mutation prevalence and penetrance that seems to vary by ethnicity, investigating the prevalence, the frequency, and the penetrance of novel *BRCA* mutation in different populations will bring new insights on cancer risk and etiology.

In Tunisia, previous studies on *BRCA* genes have focused only on breast cancer patients. In some studies, the genetic investigation concerned all the coding regions of *BRCA1* and/or *BRCA2* genes and in others only hotspot exons have been investigated. These reports have revealed a total of only 18 distinct mutations of which 12 are localized within *BRCA1* gene including 2 large rearrangements encompassing exons 5 and 20. Among the identified mutations c.211dupA, c.5266dupC in *BRCA1* and c.1310_1313delAAGA in *BRCA2* were the most recurrent mutations encountered among the hereditary breast cancer cases (11, 15–23). Despite these efforts, the mutational spectrum of *BRCA1/2* genes is still not well established. The main goal of the present study was to identify additional novel *BRCA* mutations and to investigate the contribution of these mutations to the missing heredity of breast and ovarian cancers. We also aimed to compare breast cancer clinicopathological characteristics in BRCA+ vs *BRCA*- Tunisian breast cancer cases.

MATERIALS AND METHODS

Patients

A total of 354 breast and ovarian cancer patients (335 breast cancer patients and 19 ovarian cancer patients) were included in this study referred from different medical oncology departments in Tunisia including those of Abderrahman Mami Hospital, Military Hospital of Tunis, Salah Azaiez Institute of Cancer and Farhat Hachad University Hospital University of Sousse.

Abbreviations: BCCRs, Breast Cancer Cluster Regions; ER, Estrogen receptor; gDNA, Genomic DNA; GxE, Genetic and Environmental factors; IDC, Invasive Ductal Carcinoma; LMICs, Low- and middle-income countries; OCCRs, Ovarian cancer cluster regions; PR, Progesterone receptor; SBR, Scarff-Bloom-Richardson; TNBC, Triple Negative Breast Cancer; VUS, Variants of Unknown Significance; WES, Whole Exome Sequencing.

Written informed consents were obtained from all participants. The study has been conducted according to the Declaration of Helsinki Principles and ethical approval was obtained from the biomedical ethics committee of Institut Pasteur de Tunis (2017/16/E/Hôpital A-M). Clinico-pathological characteristics and follow-up data were collected from patients' medical records. Probands were selected based on the following selection criteria (1): Presence of at least 3 related first or second-degree breast cancer cases at any age (2), Young cancer patients aged less than 35 years (3), Presence of at least two cases of breast or ovarian cancer, regardless of age, and at least one case of pancreatic cancer or prostate cancer in a related first- or second-degree patient (4), one case with triple negative breast cancer (TNBC) at an age ≤40 years (5), one breast cancer and one ovarian cancer cases diagnosed at first or second degree relatives at any age. A study flowchart is illustrated in **Figure 1**.

DNA Isolation

Total genomic DNA was isolated from peripheral blood using DNeasy blood DNA extraction Kit (Qiagen) according to the manufacturer's instructions. DNA purity and concentration were measured using a NanoDrop™ spectrophotometer.

Screening for Recurrent Mutations in *BRCA1* and *BRCA2* Genes Using Sanger Sequencing

Before performing Next Generation Sequencing (NGS) analysis, the studied cohort was screened for at least one of the recurrent *BRCA1/2* mutations previously reported in the Tunisian population, namely exon5-c.211dupA (rs397508938), exon11-c.798_799delTT (rs80357724), exon11-c.2551delG (rs397508977), exon11-c.3331_3334delCAAG (rs80357701) and exon20-c.5266dupC (rs80357906) of the *BRCA1* gene and exon10-c.1310_1313 delAAGA (rs80359277), exon16-c.7654dupA (rs879255463) in *BRCA2* gene respectively. The reference sequences used were NM_007294.3 for *BRCA1* and NM_000059.3 for *BRCA2*.

PCR reactions were performed on genomic DNA (gDNA), following standard protocols. Sanger sequencing has been performed using an automated sequencer (ABI 3500; Applied Biosystems, Foster City, CA) and a cycle sequencing reaction kit (Bigdye Terminator v3.1 kit, Applied Biosystems). The data were analyzed using BioEdit software version 7.2.5.

Sanger sequencing technique was then used to validate the identified mutations resulting from NGS.

NGS was performed on 75 breast and ovarian cancer cases. Targeted *BRCA1/2* sequencing and whole exome sequencing were performed on 60 and 15 patients respectively.

Targeted Gene Sequencing

Targeted gene sequencing was performed on *BRCA1/2* for 60 breast and ovarian cancer patients with strong family history. All targeted coding exons and exon–intron boundaries of *BRCA1/2* genes were amplified with 253 pooled primer pairs. After the targeted amplification and construction of a library through QIAGEN Library Kit v2.0, the libraries were pooled prior to emulsion PCR and bead enrichment steps that were carried out

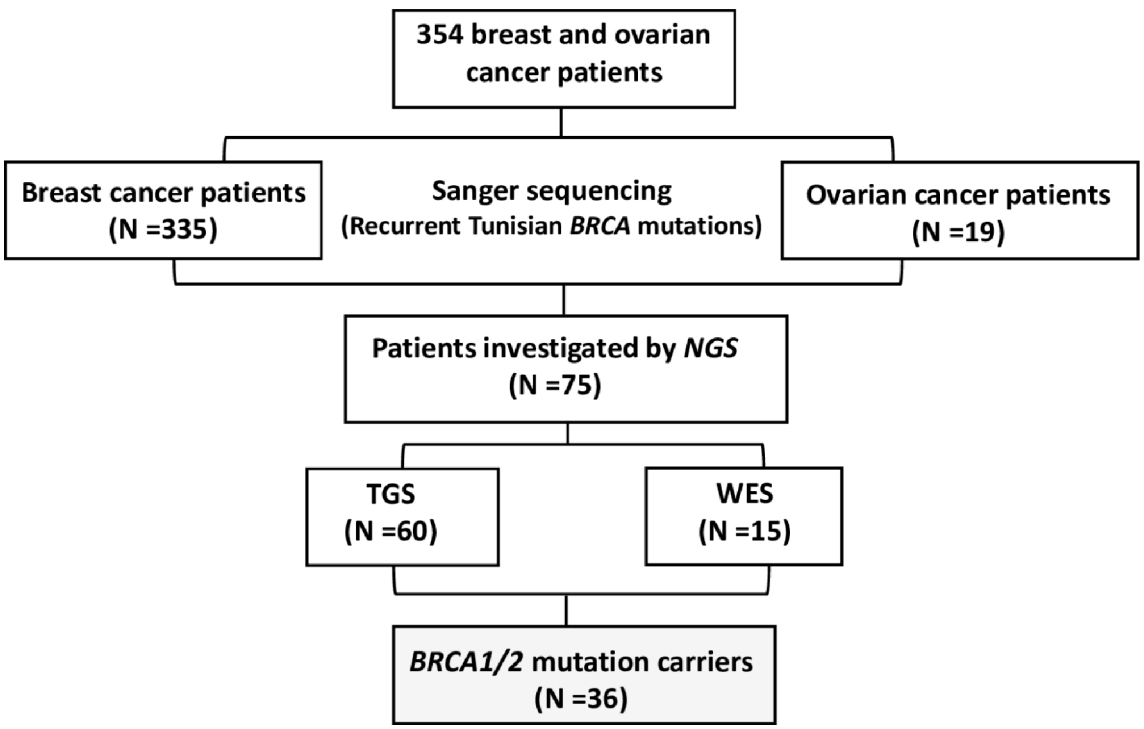

FIGURE 1 | Study flowchart.

using an automated protocol on the GeneRead QIAcube (QIAGEN, Hilden, Germany) using the GeneRead Clonal Amp Q Kit (QIAGEN, Hilden, Germany), according to the manufacturer's protocol. Following bead enrichment, the pooled libraries were sequenced using the GeneReader platform (QIAGEN, Hilden, Germany).

Whole Exome Sequencing

WES was performed for 15 breast cancer Tunisian patients. Samples were prepared according to Agilent's SureSelect Protocol Version 1.2 and enrichment was carried out according to Agilent SureSelect protocols. Enriched samples were sequenced on the Illumina HiSeq2000 platform using TruSeq v3 chemistry with paired-end (2 × 100). Exome DNA sequences were mapped to their location in the build of the human genome (hg19/b37) using the Burrows–Wheeler Aligner (BWA) package. The subsequent SAM files were converted to BAM files using Samtools. Duplicate reads were removed using Picard. GATK was then used to recalibrate the base quality scores as well as for SNP and short INDEL calling. Annotation and prioritization of potential disease-causing variants were performed using VarAFT (Variant Annotation and Filtering Tool) (http://varaf t.eu). To annotate variants, VarAFT uses ANNOVAR, a command line tool. INDELs and SNPs annotated were filtered according to several criteria (1): considering breast cancer as autosomal dominant disease and removing variants that were found in a homozygous state (2), variants identified as intronic, intergenic, and non-coding or synonymous were discarded (3), assuming that causal variants are rare, we removed all variants with an allele frequency > 1% either in ExAC (24), 1000 genomes (25) or ESP6500 (http://evs.gs.washington.edu/EVS/) (4), Using different *in silico* prediction tools, the functional impact of all identified variants has been assessed. Based on this assessment, Benign and tolerated variants were removed. Finally, significant candidate variants were obtained after filtering against their phenotypic relevance.

Clinico-Pathological Features of *BRCA1* and *BRCA2* Carriers

Clinical and pathological features of *BRCA*+ vs *BRCA*- patients as well as *BRCA1* vs *BRCA2* carriers were compared and evaluated. Statistical analysis was performed using SPSS software (version 23). Quantitative variables with normal distribution were analyzed by Student's t test. Comparison of qualitative data was performed using Chi-square test. Fisher's exact test was used for the study of small sample size. Correlation is considered statistically significant between two variables if the *P* value is less than or equal to 0.05.

RESULTS

Epidemiological and Clinico-Pathological Features of Investigated Breast and Ovarian Cancer Patients

A family history of breast and ovarian cancer was present in 35.24% and 11.14% of patients respectively. In addition, 2.68% of patients presented both breast and ovarian cancers. Consanguineous families represent 35.31% of the studied patients. Mean age at menarche was 12.81 years. Mean age at first pregnancy was 26.62 years. Oral contraception was reported by 47.31% of patients, 25.99% of patients have never breastfed and 31.85% were premenopausal.

The mean age at diagnosis of breast cancer was 43.10 years and 31.94% of patients were ≤35 years. Among investigated patients1.49% were male breast cancer (MBC) cases. Inflammatory breast cancer (IBC) (T4d) was seen in 8.65% of patients. Invasive ductal carcinoma (IDC) was the most frequent (90.04%) while infiltrating lobular carcinoma (ILC) was observed in only 3.94% of cases. Scarff-Bloom-Richardson (SBR) grade III was the most common (47.71%). Mean Tumor size was 33.62mm. Patients with positive lymph node disease represented 53.37% of our cohort, 88.67% of patients had Ki-67>14%. Luminal B tumors were the most common (56.88%) followed by triple negative breast cancer (TNBC) (23.85%), Her2+ (11.93%) and luminal A (7.34%). Distant metastases were observed in 26.34% of patients.

For ovarian cancer cases, the mean age at diagnosis was 52.62 years and the majority with serous ovarian carcinoma.

Genetic Analysis

Genetic analysis results showed that 36 out of 354 tested breast and ovarian cancer patients were *BRCA1/2* mutation carriers (31 breast cancer cases and 5 ovarian cancer patients), including 21 patients with *BRCA1* mutation and 15 patients carrying *BRCA2* mutation. A total of 16 mutations have been identified including 11 short indels, 4 single nucleotide variations (3 nonsense & 1 splicing) and 1 large rearrangement.

Identified *BRCA1/2* Pathogenic Mutations in Breast Cancer Cases

Within the studied breast cancer cohort, 13 pathogenic mutations have been identified: 8 in *BRCA1* and 5 in *BRCA2* genes. Among the identified mutations, 9 are described for the first time in Tunisian population (6 in *BRCA1* and 3 in *BRCA2*) **(Table 1).**

Considering the *BRCA1* gene, 6 patients belonging to 5 unrelated families were carriers of the recurrent c.211dupA mutation. Three patients belonging to 2 unrelated families were positive for c.5266dupC mutation. The missense c.1612C>T mutation has been identified in 2 related patients. c.19_47del, c.668dupA, c.2418dupA and c.5030_5033delCTAA mutations have been identified each in one patient. c.2433delC has been identified among 2 related patients. Except c.211dupA and c.5266dupC mutations, all remaining *BRCA1* mutations are reported for the first time in the Tunisian population.

In the *BRCA2* gene, 3 frameshift mutations as well as 1 splicing and 1 large rearrangement mutation were detected. Our results revealed 6 patients belonging to 5 unrelated families that are double heterozygous for *BRCA2* gene. Indeed, these families were carrying two mutations classified as pathogenic in the ClinVar database namely c.632-1G>A and c.1310_1313delAAGA. Four additional patients carrying only the c.1310_1313delAAGA mutation have been identified

TABLE 1 | Mutations in the *BRCA1/2* genes identified in breast cancer and ovarian cancer patients by Sanger and next generation sequencing technologies.

Gene	Exon	Coding change	Protein variation	dbSNP rs ID	Number of families carrying mutations	Number of patients carrying mutations	Screening method
BRCA1	2	c.19_47del	p.Arg7fs*24	rs80359871	1	1 (BC)	NGS
	5	c.211dupA	p.Arg71fs*10	rs397508938	5	6 (BC)	Sanger sequencing
	10	c.668dupA	p.Ala224Glyfs*4	rs80357537	1	1 (BC)	NGS
	11	c.915T>A	Cys305*	–	1	1 (OC)	Sanger Sequencing
	11	c.1612C>T	p.Gln538*	rs80356893	3	4 (2 BC, 2 OC)	NGS
	11	c.2418dupA	p.Ala807Serfs*3	rs886040036	1	1 (BC)	NGS
	11	c.2433delC	p.Lys812fs*3	rs80357524	1	2 (BC)	NGS
	11	c.3049G>T	Glu1017*	rs80357004	1	1 (OC)	NGS
	17	c.5030_5033delCTAA	p.Thr1677fs*2	rs80357580	1	1 (BC)	NGS
	20	c.5266dupC	p.Gln1756Profs*74	rs80357906	2	3 (BC)	Sanger sequencing
	3	c.249delG	p.Glu83Aspfs	–	1	1 (OC)	NGS
BRCA2	8	c.632-1G>A	–	rs81002820	5	6 (BC)	NGS
	10	c.1310_1313delAAGA	p.Lys437fs*22	rs80359277	9	10 (9 BC, 1 MBC)	NGS/Sanger sequencing
	10	c.1389_1390delAG	p.Val464fs*3	rs80359283	1	1 (MBC)	NGS
	16	c.7654dupA	p.Ile2552Asnfs*2	rs879255463	1	2 (BC)	Sanger sequencing
	1-16	c.-227-?_7805+?	–	–	1	1 (BC)	NGS

BC, breast cancer; MBC, Male Breast Cancer; OC, ovarian cancer; NGS, Next Generation Sequencing.
*The * symbol design the codon stop/frameshift mutation (fs).*

including one male breast cancer. The c.7654dupA mutation was identified in 2 related patients with a strong family history of hereditary breast and ovarian cancer. The c.1389_1390delAG mutation has been identified in 1 additional male breast cancer case. Similarly, a large rearrangement mutation of *BRCA2* gene (Del exons 1-16) has been identified in one patient. Among the identified *BRCA2* mutations, *BRCA2*-Del exons 1-16 mutation is novel and was not described in public databases. c.632-1G>A and c.1389_1390delAG are described for the first time in the Tunisian population.

BRCA1/2 Pathogenic Mutations Identified in Ovarian Cancer Cases

A total of 19 ovarian cancer patients were screened for *BRCA* pathogenic mutations using Sanger and/or NGS. Four distinct deleterious mutations were identified: 3 mutations in *BRCA1* gene (c.915T>A, c.1612C>T and c.3049G>T) and one *BRCA2* mutation (c.249delG).

BRCA1-c.915T>A and *BRCA2*-c.249delG mutations are novel and not described in public databases. The other identified mutations are described for the first time in the Tunisian population. c.1612C>T mutation was identified among 2 patients. This same mutation was also identified in 2 related breast cancer patients. Screening for additional carriers of the identified mutations, based on their geographic origin, was performed using Sanger sequencing. Consequently, the geographic origin of the identified *BRCA1/2* mutations has been clearly established (**Figure 2**). We have also illustrated the distribution of *BRCA* mutations identified in hereditary breast and ovarian cases on *BRCA1* and *BRCA2* genes (**Figure 3**). Breast cancer cluster regions (BCCRs) and ovarian cancer cluster regions (OCCRs) were assigned in **Figure 3** according to the study of Rebbeck et al., 2015 (26). Among *BRCA* mutations identified in breast cancer patients, *BRCA1_c.*211dupA, *BRCA1*_c.5266dupC, *BRCA2*_c.-227-?_7805+?, *BRCA2*_1310_1313delAAGA, *BRCA2*_c.1389_1390delAG and *BRCA2*_c.7654dupA occurred in BCCRs. Considering *BRCA* mutations identified in ovarian cancer patients *BRCA1*_c.1612C>T and *BRCA1*_3049G>T arose in OCCRs.

Polymorphisms and Variant of Unknown Significance Identified in *BRCA1* and *BRCA2* Genes

In addition to the pathogenic mutations that have been identified in *BRCA* genes, several SNPs and variants of unknown significance (VUS) have been observed (**Supplementary Table 1**). Among the 101 identified variants, 45.54% were coding, 50.49% were intronic and 3.96% were localized within regulatory regions. The majority of variations were classified as benign or likely benign in the ClinVar database (91.08%) and five intronic variations were not reported. One patient carried a VUS rs397507308 in *BRCA2* and 3 other patients carried 2 intronic variations (rs276174878 and rs276174816) that have conflicting interpretations of pathogenicity. Another patient diagnosed with early onset bilateral breast cancer had an in-frame variant with conflicting interpretations of pathogenicity rs80358343 (c.5017_5019delCAC) in the *BRCA1* gene.

Clinico-Pathological Features of *BRCA* Carriers Among Breast Cancer Cohort

Clinico-pathological characteristics of breast cancer cases carrying *BRCA1* and *BRCA2* mutations are described in **Tables 2**, **3** respectively. We investigated these clinico-pathological features in *BRCA1/2* mutation carriers vs *BRCA negative patients* (**Table 4**) and between *BRCA1* and *BRCA2* mutation carriers (**Table 5**), as well.

BRCA+ vs BRCA-

Family history of ovarian cancer was significantly associated with *BRCA* positive status (p=0.004). Regarding the mean age at

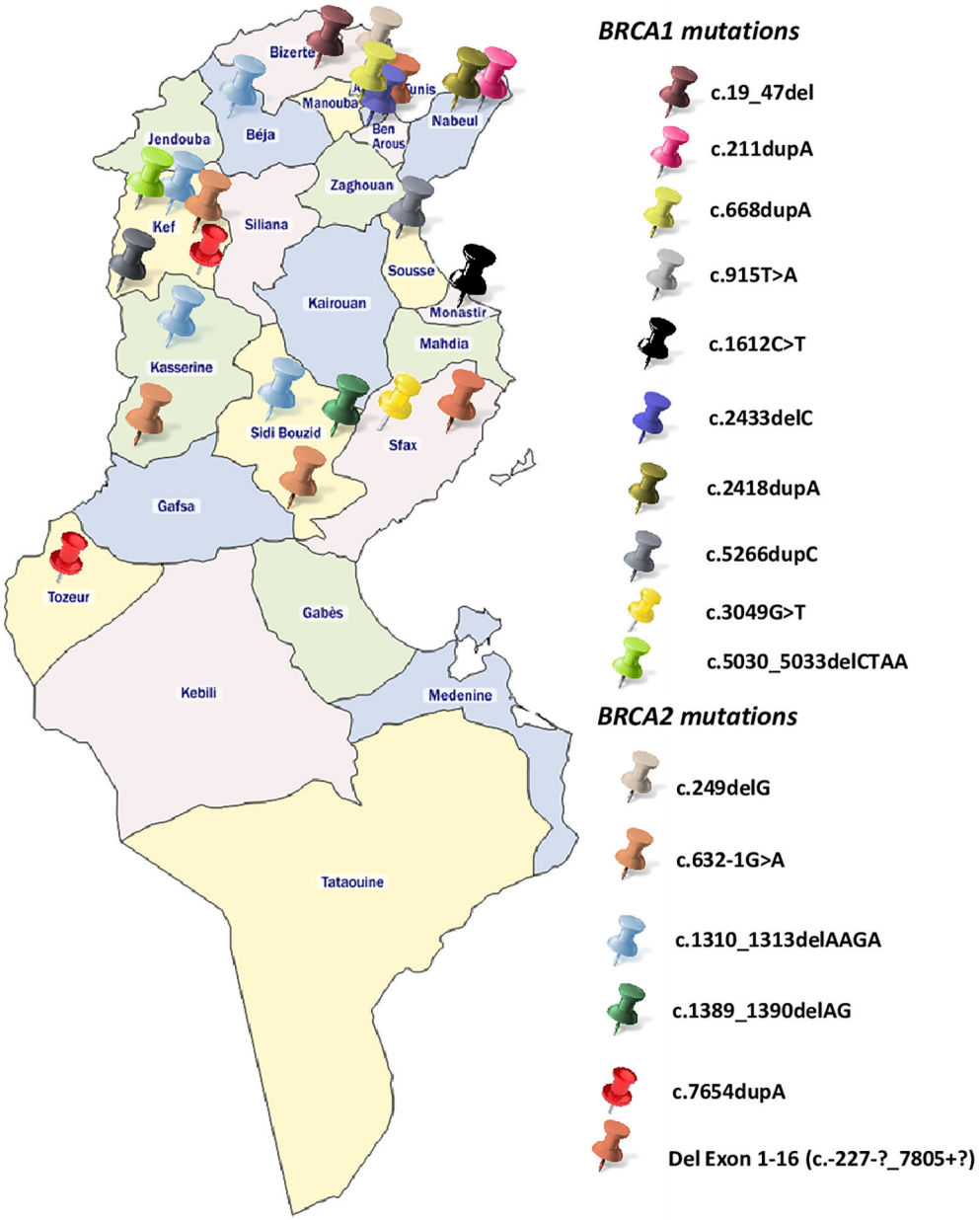

FIGURE 2 | Geographical distribution of the identified *BRCA1* and *BRCA2* mutations.

diagnosis *BRCA* carriers seem to be younger than *BRCA*- patients (38.37 vs 43.14) (p= 0.049). However, no significant difference has been observed between both groups regarding family history of breast cancer, personal history of cancer and consanguinity. Similarly, no significant differences have been observed between the 2 groups in histological subtype, nodal involvement, tumor stage, hormonal receptors status, HER2 status, molecular subtypes, Ki-67 index, and metastases (**Table 4**). Nevertheless, SBR grade III was found in 65.38% of patients with *BRCA1/2* mutations against a frequency of 28.57% among non-carriers, this difference appears to be statistically significant (p= 0.027).

BRCA1 vs BRCA2

Association between clinico-pathological features and *BRCA* status (*BRCA1+*, *BRCA2+* and *BRCAx*) was shown in **Figures 4**, **5**. Our results showed that there were no significant differences between *BRCA1* and *BRCA2* mutated groups regarding the mean age at diagnosis, the family history of personal cancers, of breast cancer and ovarian cancer (**Table 5**).

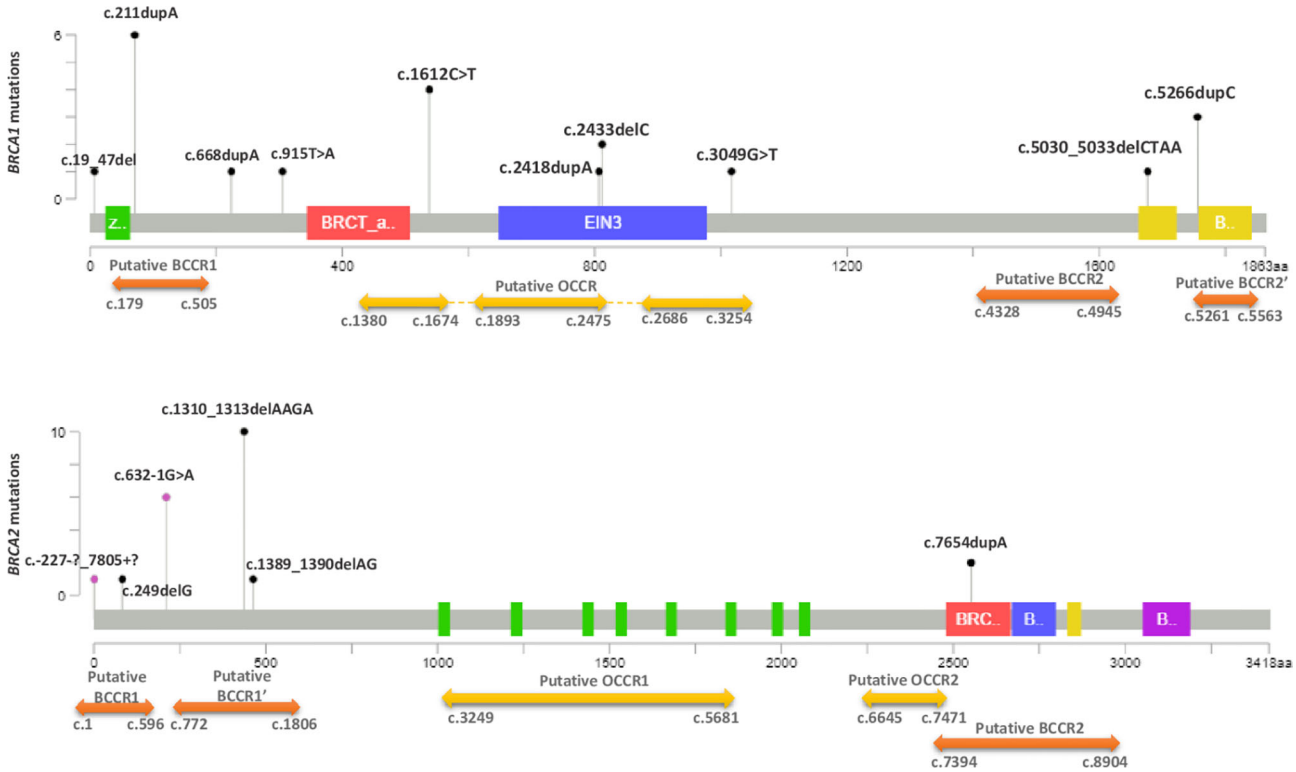

FIGURE 3 | Distribution of *BRCA1* and *BRCA2* mutations identified in hereditary breast and ovarian cancer cases. The Length of mutation indicator reflects the number of observed carriers. The diagrams linearly represent BRCA1/2 protein domains (x-axis). BRCA1 domains: Zinc/Ring finger (green); BRCT_assoc: serine-rich domain associated with BRCT (red); Ethylene insensitive 3 (blue); BRCA1 C terminus domain (yellow). BRCA2 domains: BRC repeats (green); BRCA-2_helical (red); oligonucleotide/oligosaccharide-binding, domain 1 (blue); tower domain (yellow) and oligonucleotide/oligosaccharide-binding, domain 3 (purple). Black mutation indicators depict truncating mutations and purple indicators represent the other types of mutations (splicing, LR). Cluster regions (breast cancer cluster regions (BCCRs) (orange) and ovarian cancer cluster regions (OCCRs) (yellow) were assigned according to the study of Rebbeck et al. (26).

Pathology showed that the infiltrating ductal carcinoma was the most common histological type in both groups (100% and 92.86%). HER2 status, lymph node involvement, SBR grade, tumor size, Ki-67 index and metastatic status showed no statistically significant difference between both studied groups. However, *BRCA1* carriers were more likely to have triple negative breast cancer (p=0.002) and *BRCA2* carriers were more likely to have luminal B breast cancer tumors (p=0.000078). In addition, positive estrogen receptor (ER) status and positive progesterone receptor (PR) status studied separately were both associated with *BRCA2* mutated tumors (p=0.000056 and p=0.000084), respectively.

Follow Up of *BRCA1* and *BRCA2* Carriers

Among *BRCA* carriers, contralateral breast cancer and ovarian cancer co-occurrence were observed respectively in 22.58% and 16.12% of cases. One patient diagnosed with early onset breast cancer has undergone a contralateral prophylactic mastectomy and is currently under regular surveillance. Both contralateral breast cancer and ovarian cancer occurrence were more frequent in *BRCA1* than *BRCA2* carriers. Also, 22.58% of the carriers have developed distant metastases and 5 cases died due to disease progression.

DISCUSSION

Detection of mutations in hereditary breast and ovarian cancer related *BRCA1* and *BRCA2* genes is an effective method of cancer prevention, early detection, and treatment. Mutations in the highly penetrant *BRCA* genes explain around a quarter of these cases (27). The frequency of germline mutations identified on both genes varies depending on the geographic and ethnic distributions. In some populations, a wide spectrum of different mutations is present, whereas in other groups specific recurrent *BRCA* mutations have been reported that may be due to the founder mutation effect (28–32).

Our previous studies, investigating breast cancer loci and Nucleotide Excision Repair pathway, have shown that the Tunisian population is an admixed and intermediate population between Sub-Saharan Africans and Europeans (33, 34). This genetic diversity reflects the inter-ethnic variability in the frequency distribution of the studied polymorphisms. Indeed, allele frequencies of several variants were found to be statistically different between Tunisian and other populations including rs2046210 and rs941764 that site in breast cancer susceptibility loci (33). These findings are in favor of the

TABLE 2 | Clinicopathological features of *BRCA1* carriers.

Mutation	Carrier ID	Pathology	Age at diagnosis (years)	Family History BC/OC	Family history of other cancers	Histological subtype	SBR grade	ER status	PR status	HER2 status	Ki67-index (%)	Nodal status	Tumor size (mm)	Follow-up
c.19_47del	BC320-1	BC	35	3 BC	1 gastric, 2 lung, 1 esophageal	IDC	III	ER+	PR-	HER2+	70%	N+	25	Bone and lung metastases at 37 years old.
	BC9-1	BC/OC/CBC	42	1 BC/OC 1 OC	1 Cervical cancer	IDC	NA	NA	NA	NA	NA	NA	NA	OC at 57 years old CBC at 63 years old Died at 67 years old
c.211dupA	BC49-1	BC/CBC	29	2 BC	Leukemia, Prostate, Colon, Gynecological cancer, Larynx	IDC	III	ER-	PR-	HER2-	NA	N+	35	Spontaneous pregnancy 6 months after the end of CT CBC at 32 years old (ER+, PR-, HER2-)
	BC49-2	BC	37	2 BC		IDC	III	ER +	PR +	HER2-	40	N+	15	Patient in complete remission, under regular surveillance.
	BC199-1	BC	58	1 BC 1 OC	1 Endometrium	IDC	III	ER -	PR -	HER2-	2	N+	40	Bone and Lung metastases at initial diagnosis Disease progression, cerebral metastases Died at 59 years old
	BC204	BC/OC	28	2 BC 2 OC	1 Thyroid	IDC	NA	ER -	PR -	HER2-	20	N-	30	OC at 41 years old. Patient in complete remission, under regular surveillance.
	PEC50-1	BC	38	2 BC	2 Lung, 1 Pancreatic	IDC	II	ER -	PR-	HER2-	NA	NA	30	NA
c.668dupA	BC420	BC	64	1 BC	1 Colorectal,1 tongue cancer	NA	NA	NA	NA	NA	30	NA	NA	NA
c.1612C>T	BC276-1	BC	25	4 BC 1 BOC 1 OC	1 Lung, 1 head and neck	IDC	II	ER -	PR-	HER2-	NA	N+	22	Disease progression, multiple metastases
	BC276-3	BC/OC	ND	1 BC 1 OC	1 Lung	NA	NA	NA	NA	NA	80	NA	NA	Discovery of ovarian involvement during a preoperative examination for a prophylactic oophorectomy
c.2418dupA	BC93	IBC	34	None	1 Lung, 1 pancreatic	IDC	II	ER -	PR -	HER2-	NA	NA	NA	Bone metastases Died at 36 years old due to disease progression
c.2433delC	BC178-1	BC/CBC/Endometrial cancer	42	2 BC 1 OC	2 Lung, 1 colorectal, 1 bladder	IDC	III	NA	NA	NA	30	N+	25	CBC at 45 years old; Endometrial cancer at 55 years old with peritoneal metastases
	BC178-2	BC/CBC	45			IDC	II	ER -	PR -	HER2-	NA	N-	13	CBC at 52 years old
c.5030_5033delCTAA	BC70	BC/OC	47	3 BC 1 OC	1 Lung	Polymorphic IDC	III	ER -	PR -	HER2-	65	N-	30	Complete remission Died at 58 years old
c.5266dupC	BC81-1	BC/(CBC: PABC)	27	4 BC	1 Pancreatic 1 colorectal	IDC	III	ER -	PR -	HER2-	NA	N-	24	PABC at 32 years
	BC81-6	BC	36		cancer	IDC	III	ER -	PR -	HER2+	NA	NA	20	NA
	BC314	BC	29	6 BC	1 Prostate	IDC	III	ER +	PR +	HER2-	40	NA	NA	NA

BC, breast cancer; OC, ovarian cancer; CBC, contralateral breast cancer; IDC, invasive ductal carcinoma; PABC, Pregnancy associated breast cancer; NA, non available; PR, Progesterone receptor; ER, Estrogen receptor.

TABLE 3 | Clinico-pathological features of *BRCA2* carriers.

Mutation	Carrier ID	Pathology	Age at diagnosis (years)	Family History BC/OC	Family history of other cancers	Histological subtype	SBR grade	ER status	PR status	HER2 status	Nodal status	Tumor size (mm)	Ki67-index (%)	Follow-up
DH (c.632-1G>A, c.1310_1313delAAGA	BC6-1	BC	40	4 BC	1 Throat cancer	IDC	NA	NA	NA	NA	NA	NA	NA	Died at 44 years old
	BC17-1	BBC	36	9 BC	1 Gastric cancer 1 Kidney cancer	IDC	III	ER+	PR+	NA	N+	25	NA	Esophageal Carcinoma at 48 years old. Disease progression, laterocervical, bone and liver metastases. Died at 50 years old
	BC17-2	BC (PABC)/ CBC	25	9 BC	1 Gastric cancer 1 Kidney cancer	IDC	II	ER +	PR +	HER2-	N+	55	20	CBC Bone metastases, Unplanned pregnancy during BC treatment Lung metastases at 27 years old
	BC39	BC	27	None	None	IDC	III	ER +	PR +	HER2-	NA	NA	NA	Bone and liver metastases at initial diagnosis Disease progression, patient died at 35 years old
	BC95	BC	32	None	1 Colorectal cancer	IDC	I	ER+	PR+	HER2-	N-	7	50	Patient in complete remission, under regular surveillance
	BC225-1	BC	50	5 BC	1 cerebral cancer 2 esophageal	IDC	III	ER+	PR+	HER2-	N+	20	60	Under regular surveillance
c.1310_1313delAAGA	BC245	IBC (PABC) CBC	36	2 BC	None	IDC	II	ER+	PR+	HER2-	N+	NA	80	CBC at 37 years old
	PEC009	BC	33	1 BC	None	IDC	II	ER+	PR+	HER2-	N+	NA	25	Under regular surveillance
	PEC0035	MBC	43	2 MBC 1 BC	None	IDC	III	ER+	PR+	HER2-	N+	7	30	Under regular surveillance
	BC354-1	BC	37	2 BC 1 IBC 1 MBC	1 pancreatic 1 Lung	ILC	I	ER+	PR+	HER2-	N+	25	15	NA
c.1389_1390delAG	PEC0056	MBC	59	1 BC	1 bladder	IDC	III	ER+	PR -	HER2-	N+	54	30	Under regular surveillance
c.7654dupA	BC231-1	BC/OC	47	8 BC 1 BOC	2 Gastric, 1 prostate,	IDC	III	ER+	PR +	HER2-	N-	55	NA	OC at 51 years old
	BC231-2	BC	34		1 hepatic cancers	IDC	III	ER+	PR +	HER2-	N+	16	20	Under regular surveillance
c.-227-?_7805+?	BC287	IBC (PABC)	36	3 BC 1 BBC 1 BOC 1MBC/ prostate cancer 1 IBC	1 Larynx	IDC	III	ER+	PR +	HER2-	NA	NA	40	Contralateral prophylactic mastectomy Under regular surveillance

BC, breast cancer; OC, ovarian cancer; CBC, contralateral breast cancer; IDC, invasive ductal carcinoma; PABC, Pregnancy associated breast cancer; NA, non available; PR, Progesterone receptor; ER, Estrogen receptor.

genetic heterogeneity to breast cancer predisposition in the Tunisian population. So far, only 18 deleterious *BRCA* mutations have been reported. In the current study, 16 *BRCA* mutations, including 11 novel variations, have been identified in a cohort of 354 Tunisian breast and ovarian cancer patients. For breast cancer cases, high fractions of young patients (31.94%), cases with family history of breast cancer (35.24%), Triple negative breast cancer (24.31%) and high tumor grade (47.41%) have been observed. As reported in previous studies, the high fractions of early onset, triple negative cases and also the presence of family history of breast cancer may be associated with germline *BRCA* mutations (35, 36). Indeed, it is now well documented that breast cancer patients in North Africa are almost 10 years younger than patients from western countries (37). In Tunisia, around 11% of breast cancer cases are under 35 years old (38). In fact, at a young age, the human organism usually functions as well as it ever will. However, interactions between some genetic and environmental factors (GxE) may cause a physiological decline of some organism systems leading to early disease presentation. Therefore, the influence of specific genetic background, differences in variant penetrance and frequency between populations along with environmental factors may explain this early onset of the disease. Large cohorts of young breast cancer patients should be studied to elucidate these GxE factors.

For ovarian cancer cases, the mean age at diagnosis was 52.62 years and the majority presented with serous ovarian carcinoma. Previous studies have shown that among all patients diagnosed with serous ovarian carcinoma, which is the most common subtype, over 15% will have germline *BRCA* mutations (39).

Among the 16 distinct deleterious mutations that have been observed c.19_47del, c.668dupA, c.915T>A, c.1612C>T, c.2418dupA, c.2433delC, c.3049G>T and c.5030_5033delCTAA in *BRCA1* and c.-227-?_7805+? (Del exons 1-16), c.249delG, c.632-1G>A, c.1389_1390delAG in *BRCA2*, are reported for the first time in the Tunisian population. We have also identified an inframe deletion reported to have a conflicting interpretation of pathogenicity effect in early onset bilateral breast cancer patient *BRCA1*_c.5017_5019delCAC. This variation has been described in multiple breast and ovarian cancer cases, with some families showing incomplete co-segregation of the variation (40–42).

Among *BRCA* mutations identified in breast cancer patients *BRCA1_c*.211dupA, *BRCA1*_c.5266dupC,*BRCA2*_c.-227-?_7805+?,*BRCA2*_1310_1313delAAGA, *BRCA2*_c.1389_1390delAG and *BRCA2*_c.7654dupA occurred in BCCRs that are considered to be associated with an increased likelihood of breast cancer compared to ovarian cancer. Considering *BRCA* mutations identified in ovarian cancer patients *BRCA1*_c.1612C>T and *BRCA1*_3049G>T arose in OCCRs. Other mutations, namely c.19_47del, c.668dupA, c.915T>A, c.2418dupA, c.2433delC, c.5030_5033delCTAA in *BRCA1* and c.249delG, c.632-1G>A in *BRCA2* do not overlap with previously reported breast or ovarian cancer cluster regions. This could be explained by ethnic differences in *BRCA* mutation spectrum or it may indicate shared cluster regions for both breast and ovarian cancer.

In the *BRCA1* gene, the c.19_47del mutation was identified in one breast cancer patient. This mutation was previously described only in the Algerian population (43). The c.2433delC mutation was described in Korean breast and ovarian patients (44, 45), and in Mexican patients (46, 47). The pathogenic c.1612C>T mutation was identified in 4 breast and ovarian cancer patients. This mutation has been identified in Brazilian population (48), in ovarian cancer patients from Israeli population (49) and in Macedonian population (50). We also detected the c.668dupA mutation in one patient. This latter has not been reported in previous studies neither in Tunisia nor in other populations. Nevertheless, it is already listed and classified as pathogenic in ClinVar and predicted to result in the substitution of Alanine to Glycine (p.Ala224Glyfs) which leads to BRCA1 protein truncation. Another new mutation was identified in *BRCA1* gene, c.2418dupA, that was reported by our group for the first time in the Tunisian population and was not reported previously in other populations (51). c.3049G>T has been identified in one ovarian cancer patient. This mutation has been reported in Thai patients with non-mucinous epithelial ovarian cancer (52). The c.5030_5033delCTAA mutation was identified among one patient with breast and ovarian cancers and it is reported in Brazilian population (48). The c.915T>A mutation is novel and not described in public databases.

In addition to the identification of rare and novel *BRCA1* mutations, other mutations seem to be recurrent and/or were described in previous Tunisian reports. The c.211dupA mutation was shared by 6 patients belonging to the same geographical origin. This mutation has so far been reported only in hereditary breast/ovarian cancer families of Tunisian origin, particularly in the North-East region, suggesting a founder effect. In order to unravel the genetic specificities of this mutation and to trace its origin a haplotype analysis has been conducted by our group on the North Eastern region (51). Results have determined the founder haplotype segregating with this mutation and have revealed that it arose in the period of colonization approximately 130 years ago.

The c.5266dupC mutation has been identified among two families. This mutation was previously described in 8 Tunisian breast cancer families (11, 16, 17, 20). It was originally described as an Ashkenazi founder mutation. Haplotype analysis has shown that this mutation arose approximately 1800 years ago in Northern Europe (53). Then, it has been reported in several other populations such as, Italian, Russian Slovenian and Greek (54).

Interestingly for *BRCA2* gene, 6 breast cancer patients were double heterozygous carrying the two deleterious mutations c.632-1G>A and c.1310_1313delAAGA, and 4 other unrelated patients carried only the c.1310_1313delAAGA mutation including one male breast cancer (MBC). c.632-1G>A mutation appears to be rare in other populations since it was only reported in one patient with prostate cancer in the UK (55). However, c.1310_1313delAAGA seems to be a founder mutation in Maghrebin countries (16, 17, 56, 57). It has been also identified in patients with Lebanese (58), European (59–62), African (63), Asian (64) and Latino ancestry (65) as well as in Caribbean

TABLE 4 | Epidemiological and clinico-pathological characteristics of patients carrying or not *BRCA1/2* mutations.

Variables	*BRCA1/2*+N=31	*BRCAx*N=52	*P* value
Mean age at diagnosis (years)	38.37	43.14	0.049
Early age at onset (≤35 years)			
Yes	12/30 (40.0%)	11/50 (22%)	0.085
No	18/30 (60.0%)	39/50 (78%)	
Family history of breast cancer			
Yes	28/31 (90.32%)	42/52 (80.77%)	0.353
No	3/31 (9.68%)	10/52 (19.23%)	
Family history of ovarian cancer			
Yes	11/31 (35.48%)	5/51 (9.80%)	0.004
No	20/31 (64.52%)	46/51 (90.20%)	
Personal history of cancers			
Yes	5/31 (16.13%)	4/51 (7.84%)	0.288
No	26/31 (83.87%)	47/51 (92.16%)	
Consanguinity			
Yes	7/30 (23.23%)	13/50 (26%)	0.790
No	23/30 (76.77%)	37/50 (74%)	
Histological type			
IDC	28/29 (96.55%)	26/30 (86.67%)	0.353
Other	1/29 (3.45%)	4/30 (13.33%)	
SBR Grade			
Grade I	2/26 (7.69%)	5/28 (17.86%)	0.027
Grade II	7/26 (26.92%)	15/28 (53.57%)	
Grade III	17/26 (65.38%)	8/28 (28.57%)	
Mean tumor size (mm)	27.15	36.15	0.201
T stage			
T1-T2	9/15 (60.00%)	9/14 (64.29%)	0.750
T3	1/15 (6.67%)	2/14 (14.29%)	
T4	5/15 (33.33%)	3/14 (21.42%)	
Nodes involvement			
N+	15/21 (71.43%)	14/26 (53.85%)	0.218
N-	6/21 (28.57%)	12/26 (46.15%)	
Mean Ki-67 (%)	38.79	34.95	0.598
Ki-67 index status			
Ki-67 ≤14%	1/19 (5%)	2/21 (9.52%)	1
Ki-67>14%	18/19 (95%)	19/21 (90.48%)	
Molecular subtypes			
Luminal A	1/23 (4.35%)	2/29 (6.90%)	0.926
Luminal B	12/23 (52.17%)	14/29 (48.28%)	
Her2+	1/23 (4.35%)	3/29 (10.34%)	
TNBC	9/23 (39.13%)	10/29 (34.48%)	
ER receptor status			
RE+	16/26 (61.54%)	23/37 (62.16%)	0.960
RE-	10/26 (385.46%)	14/37 (37.84%)	
PR receptor status			
PR+	14/26 (53.85%)	22/37 (59.46%)	0.658
PR-	12/26 (46.15%)	15/37 (40.54%)	
HER2 receptor status			
HER2+	2/25 (8.00%)	8/33 (24.24%)	0.163
HER2-	23/25 (92.00%)	25/33 (75.76%)	
TNBC			
TNBC	9/23 (39.13%)	10/29 (34.48%)	0.778
Non-TNBC	14/23 (60.87%)	19/29 (65.52%)	
Metastatic status			
M0	18/24 (75.00%)	22/32 (68.75%)	0.608
M1	6/24 (25.00%)	10/32 (31.25%)	

cohorts (66, 67). These results show the genetic heterogeneity of breast and ovarian cancers in Tunisian patients and the admixed origins of *BRCA* mutations in Tunisia.

In addition, 5 male breast cancer cases were investigated among which 2 carried *BRCA2* mutations (c.1310_1313delAAGA and c.1389_1390delAG). Male breast cancer is a rare disease accounting for less than 1% of all breast cancer cases and it was previously shown that nearly 90% of MBC arising in *BRCA* mutation carriers are found to harbor a *BRCA2* mutation (68). Unfortunately, being a man with "a women's disease" makes MBC a disease surrounded by social taboo and lack of awareness especially in underdeveloped countries. Indeed, the treatment of MBC has been extrapolated from the knowledge of female breast cancer, despite the multiple differences in the pathogenesis, biology and genetics of

TABLE 5 | Epidemiological and clinico-pathological characteristics of patients carrying *BRCA1* and *BRCA2* mutations.

Variables	*BRCA1*+N=17	*BRCA2*+N=14	*P* value
Mean age at diagnosis (years)	38.50	38.21	0.939
Early age at onset (≤35 years)			
Yes	7/16 (43.75%)	5/14 (35.71%)	0.654
No	9/16 (56.25%)	9/14 (64.29%)	
Family history of breast cancer			
Yes	16/17 (94.12%)	12/14 (85.71%)	0.576
No	1/17 (5.88%)	2/14 (14.29%)	
Family history of ovarian cancer			
Yes	8/17 (47.06%)	3/14 (21.43%)	0.258
No	9/17 (52.94%)	11/14 (78.57%)	
Personal history of cancers			
Yes	4/17 (23.53%)	1/14 (5.88%)	0.344
No	13/17 (76.47%)	13/14 (94.12%)	
Consanguinity			
Yes	2/17 (11.76%)	5/13 (38.46%)	0.190
No	15/17 (88.24%)	8/13(61.54%)	
Histological type			
IDC	15/15 (100%)	13/14 (92.86%)	0.483
Other	0/15	1/14 (7.14%)	
SBR grade			
Grade I	0/13	2/13 (15.38%)	0.673
Grade II	4/13 (30.77%)	3/13 (23.08%)	
Grade III	9/13 (69.23%)	8/13 (61.54%)	
Mean tumor size (mm)	25.36	29.33	0.555
T stage			
T1-T2	3/5 (60%)	6/10 (60%)	0.045
T3	1/5 (20%)	0/10	
T4	1/5 (20%)	4/10 (40%)	
Nodes involvement			
N+	6/10 (60%)	9/11 (81.82%)	0.361
N-	4/10 (40%)	2/11 (18.18%)	
Mean Ki-67 (%)	40.78	37.00	0.727
Ki-67 index status			
Ki-67 ≤14%	1/9 (11.11%)	0/10	0.474
Ki-67>14%	8/9 (88.89%)	10/10 (100%)	
Molecular subtypes			
Luminal A	1/13 (7.69%)	0/10	0.000078
Luminal B	2/13 (15.39%)	10/10 (100%)	
Her2+	1/13 (7.69%)	0/10	
TNBC	9/13 (69.23%)	0/10	
ER receptor status			
RE+	3/13 (23.08%)	13/13 (100%)	0.000056
RE-	10/13 (76.92%)	0/15	
PR receptor status			
PR+	2/13 (15.38%)	12/13 (92.31%)	0.000084
PR-	11/13 (84.62%)	1/13 (7.69%)	
HER2 receptor status			
HER2+	2/13 (15.38%)	0/12	0.480
HER2-	11/13 (84.62%)	12/12 (100%)	
TNBC			
TNBC	9/13 (69.23%)	0/10	0.002
Non-TNBC	4/13 (30.77%)	10/10 (100%)	
Metastatic status			
M0	8/11 (72.73%)	10/12 (83.33%)	0.640
M1	3/11 (27.27%)	2/12 (16.67%)	

these two disease entities. These evidence make MBC a gender issue that requires more attention from the scientific community.

The introduction of the c.1310_1313delAAGA mutation, that have been encountered in diverse populations, in the Tunisian population could be explained by the immigration of Andalusians in Tunisia which has been intensified after the fall of Granada in 1492 and lasted for two centuries before the total expulsion of all Andalusian Moriscos from the Iberian Peninsula in 1610. The diverse geographical distribution of this mutation may further suggest independent origins as shown for the 4184del4 *BRCA1* mutation reported to have at least three independent origins in the study of Neuhausen et al. (69). The c.7654dupA *BRCA2* gene mutation which was identified in a unique family with a strong family history of breast and ovarian cancer is reported previously

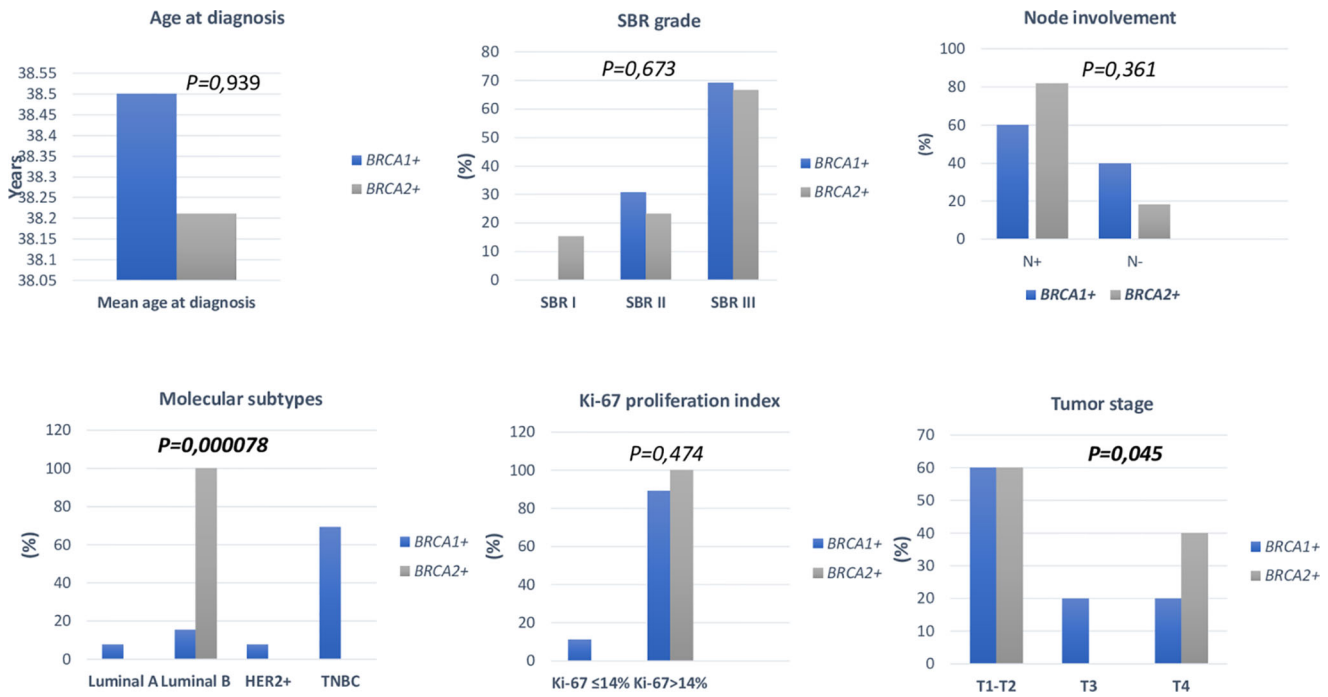

FIGURE 4 | Distribution of clinico-pathological features of breast cancer in *BRCA1+* and *BRCA2+* patients.

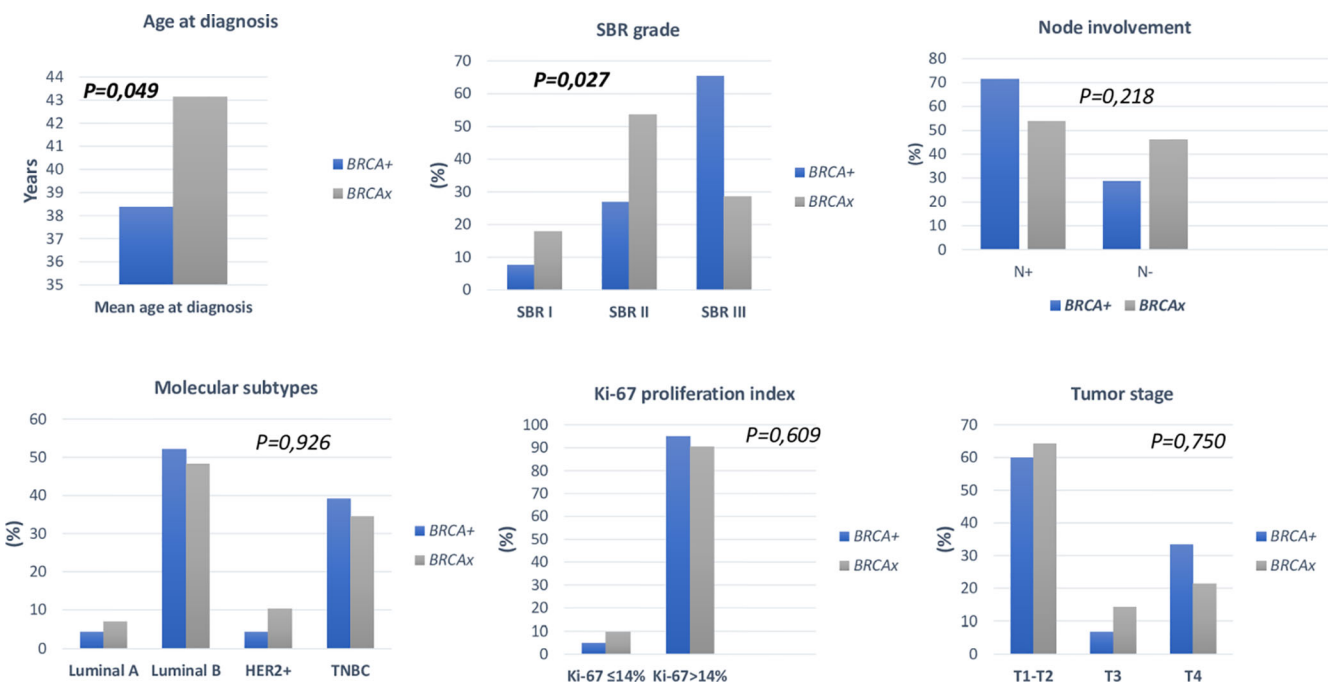

FIGURE 5 | Distribution of clinico-pathological features of breast cancer in *BRCA+* and *BRCAx* patients.

and exclusively in Algerian population (70) and could be therefore specific to North African countries.

Through this report and despite the identification of novel mutations in Tunisian population, it is clear that the genetic susceptibility to breast cancer is explained in a vast majority of cases by recurrent mutations. Indeed, more than 44.44% of carriers harbor *BRCA1*-c.211dupA or *BRCA2*-1310_1313deAAGA mutations which highlights the importance of screening these mutations in the treatment workflow of cases with early onset or strong family history of breast cancer. In fact, identifying germline *BRCA1* and *BRCA2* pathogenic mutations is a crucial component in the medical management of affected patients. Regular surveillance and/or prophylactic mastectomy of the second breast or prophylactic salpingo oophorectomies, which have been shown to reduce the risk of developing cancer, are recommended to these carriers. Moreover, relatives who test positive for a germline *BRCA* pathogenic mutation may take appropriate action to prevent cancer or have cancer diagnosed as early as possible for better treatment options (59).

In addition, mutations in the *BRCA* genes and their associations with clinico-pathological features were reported in several studies (71–74). However, in Tunisia this aspect was not previously investigated. This point was raised in the present study and our results showed that patients with *BRCA1* and *BRCA2* mutations were similar with regard to several epidemiological and clinico-pathological parameters. Nevertheless, *BRCA1* carriers were more likely to be triple negative breast cancer compared to *BRCA2* carriers (p=0.002) and *BRCA2* carriers were more likely to be luminal B breast cancer tumors (p=0.000078). Consistent with our findings, various previous studies reported that there is a much higher rate of TNBC among *BRCA1* mutation carriers (75, 76) and *BRCA2*-related breast cancer is often luminal (77). Additionally, positive ER was significantly associated with *BRCA2*+ tumors (p=0.000056). PR status was significantly different between *BRCA1* and *BRCA2* mutation carriers; *BRCA2* carriers are more likely to develop progesterone receptor (PR) positive tumors and PR-negative breast cancer are associated with *BRCA1* mutation carriers (p=0.000084). It was reported that the ER positivity was predominantly seen in *BRCA2* mutation carriers, which is consistent with our findings (71, 78). Furthermore, a previous report has found that *BRCA2*-associated cancers are mainly PR positive (79). Other studies have raised some pathological differences between *BRCA1/2* mutation carriers and *BRCAx* patients. In our study, *BRCA* carriers seem to be younger than *BRCA-negative* patients (p=0.049). Furthermore, patients with a positive family history of ovarian cancer are more likely to be *BRCA* positive (p=0.004). We also observed a significant predominance of SBR grade III tumors among *BRCA1/2* mutations carriers (p= 0.027). These findings are in line with previous literature (35, 80, 81).

Furthermore, we have assessed disease outcomes in *BRCA* carriers, and we have observed a relatively high proportion of contralateral breast cancer and ovarian cancer occurrence that were more frequently observed in *BRCA1* carriers. Previous reports have demonstrated that women carrying a pathogenic mutation in the *BRCA1* or *BRCA2* genes have an increased risk of developing a second primary cancer in the contralateral breast. The cumulative risk 20 years after breast cancer diagnosis was estimated to be 40% for *BRCA1* carriers and about 26% for *BRCA2* carriers (82). In accordance with our findings, it was shown also that the occurrence of both breast and ovarian cancer in a woman is associated with a high likelihood of a germline *BRCA1* mutation (83).

Besides the *BRCA* genetic mutations that have been identified in our study, mutations on other high to moderate breast cancer genes such as *TP53*, *ATM, BLM* and *CHEK2* have been also identified for the first time in North African populations (data not shown). All these findings reflect the genetic heterogeneity of cancer predisposition in Tunisia and highlights the importance of the use of NGS to identify clinically actionable genetic variants that have a crucial role in disease management. Therefore, technological advances in terms of array and DNA sequencing technologies made the route towards the examination of genetic risk largely clear. However, practical challenges related to marked population-specific differences still exist. In this context, Manolio and colleagues conveniently classified *LRRK2* as a high penetrant gene associated with Parkinson disease (84) with G2019S mutation being the main cause of Parkinson familial cases. Recently the international LRRK2 consortium reported a worldwide frequency of 1% of *LRRK2* G2019S, 30–40% in Arab patients from North Africa and 10–30% in Ashkenazi Jews, but is very rare in Asians (85, 86). As a variant´s frequency has a direct impact on its penetrance, this example shows the ethnic-dependent penetrance of some important variants involved in complex diseases and the role of consanguinity and endogamy in shaping the genetic susceptibility to these diseases. Therefore, the same reflection can be applied on high and low penetrant breast cancer variants in order to review their penetrance in underrepresented populations such as North Africans. A disproportionate distribution of the identified mutations is observed between the Northern and Southern regions of Tunisia (**Figure 2**), with the vast majority found in the North. This can be explained by a selection bias because most of the recruited participants come from Northern governorates, but it can also be explained by the very high consanguinity rates in the South that reaches 98% in some cities and that may have an impact on *BRCA* mutations frequency and prevalence.

Additional limitations of our study have been observed. Indeed, to our best knowledge, this work represents the largest BRCA1/2 study in Africa. However, we believe that the sample size is still small and larger cohorts are needed to trace a clear and complete BRCA1/2 mutational spectrum in Tunisia. In addition, because of the limited resources dedicated to this work, we were not able to perform a complete sequencing of both genes for the whole cohort. Therefore, the frequency and prevalence of the identified mutations need to be assessed in larger studies. Clearly, the prevalence assessment of *BRCA1* and *BRCA2* mutations also rely on the quality of both cohort selection criteria and mutation ascertainment methods. The identification of novel BRCA mutations and the assessment of their penetrance in a specific population will help to implement more affordable and cost effective targeted genetic testing strategies.

Finally, up until now, most data on *BRCA1*/2 mutations associated with high risk for hereditary breast and ovarian cancer

do not cover the North African populations. Accordingly, the novel mutations identified in this study will help to improve knowledge on the genetic component of hereditary breast and ovarian cancer in the North African region and will lead to a better clinical management of cancer patients. In addition, we are aiming to share genetic and phenotypic data with larger multi-ethnic Consortia of *BRCA1/2* mutation carriers such as the Consortium of Investigators of Modifiers of BRCA1/2 (CIMBA) (87). This will make our findings more broadly useful and will give us a global overview of the similarities and differences that the Tunisian population has compared to other ethnicities.

CONCLUSION

In conclusion we have identified 16 distinct *BRCA* mutations in breast and ovarian cancer patients including 11 novel mutations in the Tunisian population. The recognition of the *BRCA* mutational spectrum and its geographical distribution in Tunisia is of keen interest for the scientific and medical communities as it helps to develop precise risk assessment tools, accurate genetic testing, cost-effective approaches for prevention and early detection of the disease as well as personalized treatments of *BRCA* related cancers for both affected and unaffected cancer cases.

DATA AVAILABILITY STATEMENT

The minimal dataset that would be necessary to interpret, replicate and build upon the findings reported in this study are included in this article and in its supplementary files. All identified mutations with their related details have been shared in the public database ClinVar under the following link "https://www.ncbi.nlm.nih.gov/clinvar/submitters/507986/". Any additional datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The Biomedical Ethics Committee of Institut Pasteur de Tunis (2017/16/E/Hôpital A-M). The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from participants for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

YH prepared the study concept and design, supervised the study, did data analysis, data interpretation, drafted, and critically revised the manuscript. NMi and MB did the experiments, participated in participant recruitment, and participated in drafting and reviewing the manuscript. NMe contributed to clinical data analysis and reviewed the manuscript. SN contributed to participants recruitment and reviewed the manuscript. MR and OM contributed to data collection and reviewed the manuscript. HanB, YB, HR, OJ, ND, AZ, JA, HEB, SL and JBH contributed to the clinical investigation and recruitment of patients. AH, KR, FB, RM, SBA and HamB critically revised the clinicopathological section and the whole manuscript. SB and SA contributed to the study concept, design and supervision and critically revised the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fonc.2021.674965/full#supplementary-material

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Pioneering *BRCA1/2* Point-Of-Care Testing for Integration of Germline and Tumor Genetics in Breast Cancer Risk Management: A Vision for the Future of Translational Pharmacogenomics

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Research performed in South African (SA) breast, ovarian and prostate cancer patients resulted in the development of a rapid BRCA point-of-care (POC) assay designed as a time- and cost-effective alternative to laboratory-based technologies currently used for first-tier germline DNA testing. In this study the performance of the new assay was evaluated for use on a portable screening device (ParaDNA), with the long-term goal to enable rollout at POC as an inventive step to meet the World Health Organization's sustainable development goals for Africa. DNA samples for germline testing were obtained retrospectively from 50 patients with early-stage hormone receptor-positive breast cancer referred for genomic tumor profiling (MammaPrint). Currently, SA patients with the luminal-type breast cancer are not routinely selected for *BRCA1/2* testing as is the case for triple-negative disease. An initial evaluation involved the use of multiple control samples representing each of the pathogenic founder/recurrent variants included in the BRCA 1.0 POC Research Assay. Comparison with a validated laboratory-based first-tier real-time polymerase chain reaction (PCR) assay demonstrated 100% concordance. Clinical utility was evident in five patients with the founder *BRCA2* c.7934delG variant, identified at the 10% (5/50) threshold considered cost-effective for *BRCA1/2* testing. *BRCA2* c.7934delG carrier status was associated with a significantly younger age (p=0.03) at diagnosis of breast cancer compared to non-carriers. In three of the *BRCA2* c.7934delG carriers a high-risk MammaPrint 70-gene profile was noted, indicating a significantly

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increased risk for both secondary cancers and breast cancer recurrence. Initiating germline DNA testing at the POC for clinical interpretation early in the treatment planning process, will increase access to the most common pathogenic *BRCA1/2* variants identified in SA and reduce loss to follow-up for timely gene-targeted risk reduction intervention. The ease of using cheek swabs/saliva in future for result generation within approximately one hour assay time, coupled with low cost and a high *BRCA1/2* founder variant detection rate, will improve access to genomic medicine in Africa. Application of translational pharmacogenomics across ethnic groups, irrespective of age, family history, tumor subtype or recurrence risk profile, is imperative to sustainably implement preventative healthcare and improve clinical outcome in resource-constrained clinical settings.

Keywords: breast cancer, *BRCA1*, *BRCA2*, Africa, point-of-care, first-tier genetic testing, pathology, pharmacogenomics

INTRODUCTION

Breast cancer (BC) is a leading cause of cancer among women globally, with poor survival and higher mortality rates reported in Africa. These are generally ascribed to late-stage presentation and a delay in diagnosis, partly due to sub-optimal healthcare systems (1–3). From studies conducted in Sub-Saharan Africa, more advanced breast disease is seen in patients living in rural areas than those in urban centers (2, 4). This is also the case for South Africa (SA), where the stage of cancer and age at diagnosis differs according to geographic location as well as psychosocial and personal financial status (5, 6). Fear of dying from cancer or refusal of recommended medical treatment methods due to cultural beliefs are all factors affecting overall survival (7). Conversely, should patients agree to undergo therapy, the costs related to follow-up visits may be unsustainable. Lack of community awareness relating to genetic testing and the benefits of presymptomatic diagnosis of BC contribute to the increased mortality (3).

Epidemiological studies have indicated multiple risk factors associated with the development of BC, both modifiable and non-modifiable. The influence of modifiable factors on BC risk can be controlled and is associated with lifestyle and the environment, for example, obesity and alcohol consumption (8, 9). Non-modifiable risk factors include sex, age, and age at menarche (10, 11). Menarche before the age of 12 and menopause after age 55 prolong the time that breast tissue is exposed to hormonal influence and increase the risk of BC. Genetic risk factors for cancer development or recurrence play a prominent role, especially in the presence of a family history of the disease in first-degree or multiple relatives, and a personal history of atypical hyperplasia or carcinoma *in situ* of the breast. Radiation therapy to the chest area for other malignancies before the age of 30, especially if the patient is left with intact ovarian function for ≥20 years post-treatment, may also increase the risk of BC (12).

Translational research performed in SA involving the highly penetrant *BRCA1* and *BRCA2* cancer susceptibility genes has identified various recurrent and founder variants as targets for both pharmacogenetic and cascade testing across population groups (13–17). The present article is the second in a series initiated by Oosthuizen et al. (18), aimed at the development of practical solutions for the challenges currently experienced with implementation of genomic medicine in Africa. The authors provided an historical view on *BRCA1/2* testing performed in nearly 2000 breast/ovarian cancer patients extending from a first-tier *BRCA1/2* population-based assay to next-generation sequencing (NGS) in a subset of patients. Detection of founder/recurrent variants in the majority (74%) of SA patients justified the use of a first-tier assay to select patients eligible for NGS of the *BRCA1/2* or other cancer susceptibility genes. However, uptake of laboratory-based *BRCA1/2* testing in affected families was relatively low, despite the knowledge that gene-targeted therapy and surgical intervention could be life-saving. These findings provided a strong incentive for development of a novel point-of-care (POC) test kit (https://gtr.ukri.org/projects?ref=103993) including eight of the pathogenic founder/recurrent variants previously identified in SA (17): *BRCA1* c.68_69delAG (rs80357914), c.1374delC (rs397508862), c.2641G>T (rs397508988), c.5266dupC (rs80357906)] and *BRCA2* c.5771_5774delTTCA (rs80359535), c.5946delT (rs80359550), c.6447_6448dupTA (rs397507858), c.7934delG (rs80359688). A risk-benefit analysis showed strong support (94%) for clinical implementation of a BRCA POC assay as a rapid first-tier test combined with genetic counseling (18). Implementation of our pathology-supported genetic testing (PSGT) strategy will enable *BRCA1/2* screening in BC patients unselected by age or family history through integration of germline DNA testing with tumor gene profiling, as envisaged for future application of pharmacogenomics in Africa. The cost-saving PSGT approach was first implemented in SA to reduce chemotherapy overtreatment as informed by multi-gene expression profiling (MammaPrint) (19) and to facilitate reclassification of early-stage BC into treatment groups by combining immunohistochemistry (IHC) assessment at the protein level with molecular subtyping (20), using formalin fixed paraffin embedded (FFPE) tumor biopsies. Germline DNA testing and tumor genetics based on RNA analysis are not routinely integrated to facilitate differential diagnosis and recurrence risk assessment in the same patient.

As a targeted genetic testing approach proved valuable as a first-tier test in the age of low-cost NGS (18), this study aimed to evaluate the BRCA 1.0 POC Research Assay as a robust, cost-effective alternative to currently-used laboratory-based testing protocols in BC patients unselected by family history. To our knowledge, *BRCA1/2* POC testing is not currently available internationally either as a stand-alone test or incorporated into the PSGT framework (17–20). Once assessed in relevant clinical settings, this cost- and time-effective genetic testing approach using DNA obtained from crude saliva, mouth swabs, or blood samples in conjunction with parallel genetic counseling, may be presented as a model to the policymakers at the SA Department of Health for rollout in primary health clinics. The benefits of transferring a laboratory-based assay (requiring sample transport and batching) to a rapid assay performed at POC would be three-fold: (i) to alleviate the financial burden of genetic testing in the country by identifying the most common founder/recurrent *BRCA1/2* variant carriers early using cost-effective rapid POC technology; (ii) increase healthcare accessibility of all citizens, and (iii) contribute to community awareness and education by simultaneously explaining the value of pharmacogenomics and presymptomatic diagnosis in high-risk families.

MATERIALS AND METHODS

This study included 50 patients and ten control individuals. DNA samples for germline DNA testing were obtained with written informed consent from a subset of SA patients previously referred for transcriptional gene profiling (MammaPrint/BluePrint) using FFPE tumor biopsies (19, 20). The study cohort was selected retrospectively based on a personal history of BC and IHC assessment of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor-2 (HER2) status incorporated into the PSGT framework. The specific selection criteria for germline *BRCA1/2* POC DNA testing were different from conventional germline *BRCA1/2* testing, as it was not based on the age at onset, the presence of a family history of cancer and/or triple-negative disease (17, 21). Ethics approval was obtained from both the Health and Wellness Sciences Research Ethics Committee of the Cape Peninsula University of Technology in Cape Town (CPUT/HW-REC 2018/H10), and the Ethics Committee of the Faculty of Health Sciences, University of the Free State (UFS-HSD2019/1835/291001). The research was also approved as a sub-study under reference number N09/06/166 by the Health and Research Ethics Review Committee of Stellenbosch University, SA.

The BRCA 1.0 POC Research Assay and instrumentation were provided by the LGC Limited (Teddington, UK), using HyBeacon probes synthesized by LGC, Biosearch Technologies (Petaluma, USA). Kit development by LGC was based on the ParaDNA polymerase chain reaction (PCR) amplification principles as previously described (22, 23). The reaction plate kits (BRCA 1.0) were stored at -20°C and thawed at room temperature for 15–20 min before use. DNA samples were diluted to a final concentration of 1 ng/ul, and 2 µl of each sample transferred into each well of the ParaDNA reaction plate (**Figure 1**). The ParaDNA assay comprised all the reagents required for multiplex melt curve analysis of eight *BRCA1/2* targets in a four-tube format (**Table 1**) using the fluorescent dyes FAM, CAL Fluor Orange 560 (CAL560), and CAL Fluor Red 610 (CAL610).

Prior to the analysis of the 50 patient samples using DNA extracted from whole blood and/or saliva, a no template control and two *BRCA1/2* variant-negative controls, as well as eight variant-positive samples of known genotype were tested. The genotypes of the DNA samples used as positive controls were previously determined using a combination of validated hybridization and simple probe technologies (18). Negative controls were previously screened using NGS based on standard selection criteria for *BRCA*/other high-moderate penetrance cancer susceptibility genes (17). The accuracy of the genotyping calls was assessed by adding different DNA samples representing the known SA founder/recurrent variants

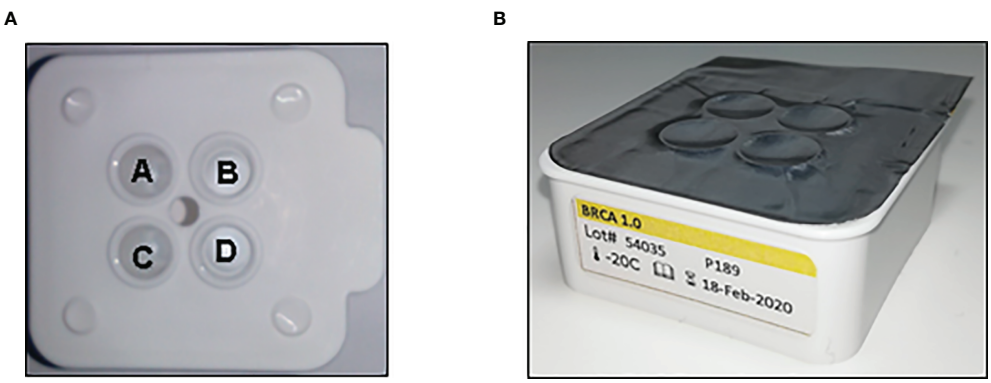

FIGURE 1 | The ParaDNA reaction plate with positions of wells A–D as indicated **(A)**. The probe mixes for *BRCA1* c.1374delC (rs397508862) and *BRCA2* c.7934delG (rs80359688) are multiplexed in well A, with mixes for *BRCA1* c.2641G>T (rs39750888) and *BRCA2* c.5771_5774del (rs80359535) in well B, *BRCA1* c.5266dupC (rs80357906) and c.68_69delAG (rs80357914) in well C and *BRCA2* c.6447_6448dupTA (rs397507858) and c.5946delT (rs80359550) loaded in well D. **(B)** The ParaDNA reaction plates are provided foil sealed, ready for use.

TABLE 1 | Multiplex analysis of eight *BRCA1/2* founder/recurrent variants in a four-tube ParaDNA closed system format.

Well	Gene	Founder/recurrent variant[a]	Variant	Probe label
A	*BRCA1*	c.1374delC	rs397508862	FAM
	BRCA2	c.7934delG	rs80359688	CAL560
B	*BRCA1*	c.2641G>T	rs397508988	FAM
	BRCA2	c.5771_5774del TTCA	rs80359535	CAL560
C	*BRCA1*	c.5266dupC	rs80357906	FAM
	BRCA1	c.68_69delAG	rs80357914	CAL610
D	*BRCA2*	c.6447_6448dupTA	rs397507858	FAM
	BRCA2	c.5946delT	rs80359550	CAL560

[a]*Reference sequences used for BRCA1 and BRCA2 analyses were GenBank NM_007294.4 (BRCA1) and NM_000059.3 (BRCA2).*

to each well of the ParaDNA plates. The plates were inserted into the ParaDNA instrument for rapid thermal cycling. Following an initial denaturation step (98°C for 1 min), the targets were amplified using 50 PCR cycles of 99°C for 7 sec, 62°C for 12 sec and 72°C for 12 sec, followed by denaturation at 95°C for 20 sec and probe annealing at 35°C for 30 sec. Melting curve analysis was performed by heating the samples from 35°C to 80°C using a 0.1°C/sec ramp rate and fluorescence acquisition. The ParaDNA software (version 1.6.0.27) automatically analyzed the melting curves and computed the associated *BRCA1/2* genotypes (**Figures 2A–K**) within approximately one hour. Automated software calls were assessed using the ParaDNA Data Review software to examine sample melting curves. Once the BRCA 1.0 Research Assay's performance was confirmed using the specific controls, germline DNA of 50 hormone receptor-positive BC patients previously analyzed with the 70-gene MammaPrint assay, was genotyped. Reference sequences used for *BRCA1* and *BRCA2* analyses were GenBank NM_007294.4 (*BRCA1*) and NM_000059.3 (*BRCA2*).

The genotyping calls generated by the BRCA 1.0 Research Assay were confirmed using alternative methods including real-time PCR conducted by means of the Roche LightCycler hybridization and simple assay systems (18) and/or Sanger sequencing using the primer sets listed in **Table 2** and the BigDye® Terminator v3.1 Cycle Sequencing Kit (Thermo Scientific Corp., Waltham, MA) on an ABI Genetic Analyzer. The electropherograms were analyzed by visual inspection and aligned to the reference sequences.

The data were analyzed and described using cross-tabulation and frequency tables analyzed using the STATISTICA package. One-way ANOVA was used to compare the average age between subgroups. The significance level was set at 0.05 for the determination of statistical significance.

RESULTS

The BRCA 1.0 POC Research Assay was first standardized in the laboratory by using ten control samples before commencing testing of the study cohort. These samples represented each of the eight selected *BRCA1/2* founder/recurrent SA pathogenic variants, evaluated together with a no template and two variant-negative controls. All samples were genotyped using 3-color, 4-tube multiplex assays after adding the extracted DNA to each of the four plate wells. The test duration from sample-to-result was approximately one hour, excluding previously performed DNA extraction and quantification. The ParaDNA software automatically analyzed the multiplex melt curve data and generated genotype calls (**Figures 2A–K**). There was a 100% concordance between the genotyping calls obtained by the ParaDNA instrument and software and those identified using alternative methods. All the samples and negative controls were correctly assigned using 2 ng of input DNA only.

Once the BRCA 1.0 POC Research Assay's analytical performance was confirmed, germline DNA of 50 BC patients was genotyped. Melt curve analyses indicated *BRCA2* c.7934delG in five of the patients (**Figure 3A**). Detection of *BRCA2* c.7934delG (rs80359688) using the BRCA 1.0 POC Research Assay was confirmed by DNA sequencing for these patients (**Figure 3B**). The electropherogram indicated a single base deletion, which resulted in a shift of the reading frame, prematurely truncating the associated peptide (**Figure 3B**). The BRCA 1.0 POC Research Assay initially failed partly for a single sample (1/50, 2%) as no results were obtained for four of the eight variants tested (two wells). Repeat of the assay resulted in successful genotyping of all eight *BRCA1/2* variants, indicating a user set-up error. Homozygous variant-negative (reference) samples generated a single melting peak (**Figures 2J, K**), whereas heterozygous variant-positive samples yielded two peaks (**Figures 2A–H**). All five *BRCA2* c.7934delG cases exhibited an additional melting peak at 50.0°C (**Figure 3A**).

The performance of the BRCA 1.0 POC Research Assay was further evaluated by analyzing the same samples using real-time PCR on the Roche LC480 real-time PCR instrument. Melting peak data were assessed manually to calculate melting peak temperatures for each of the eight *BRCA1/2* founder variants. All 50 samples were assigned the correct automated software calls using 2 ng to 62.5 pg of extracted input DNA. No false-negative or false-positive real-time PCR results were obtained with either the laboratory-based LightCycler or portable ParaDNA device using the same HyBeacon probes.

BRCA2 c.7934delG was the only pathogenic variant detected in 10% of the 50 cases studied (n=5). Patients carrying this variant were diagnosed at a significantly younger age than variant-negative individuals (p=0.03), with mean ages of 41.60 ± 6.58 and 51.77 ± 9.83, respectively (**Figure 4**). Previous review of their histopathology reports indicated ductal/carcinoma of no special type in all five *BRCA2* c.7934delG carriers. Two cases had a low-risk MammaPrint profile for BC metastasis (luminal A) supporting omission of chemotherapy, whereas three had a high-risk profile (luminal B) as supported by molecular subtyping using the 80-gene BluePrint assay.

DISCUSSION

In this retrospective study, the results obtained with the rapid BRCA 1.0 POC Research Assay in patients with early-stage

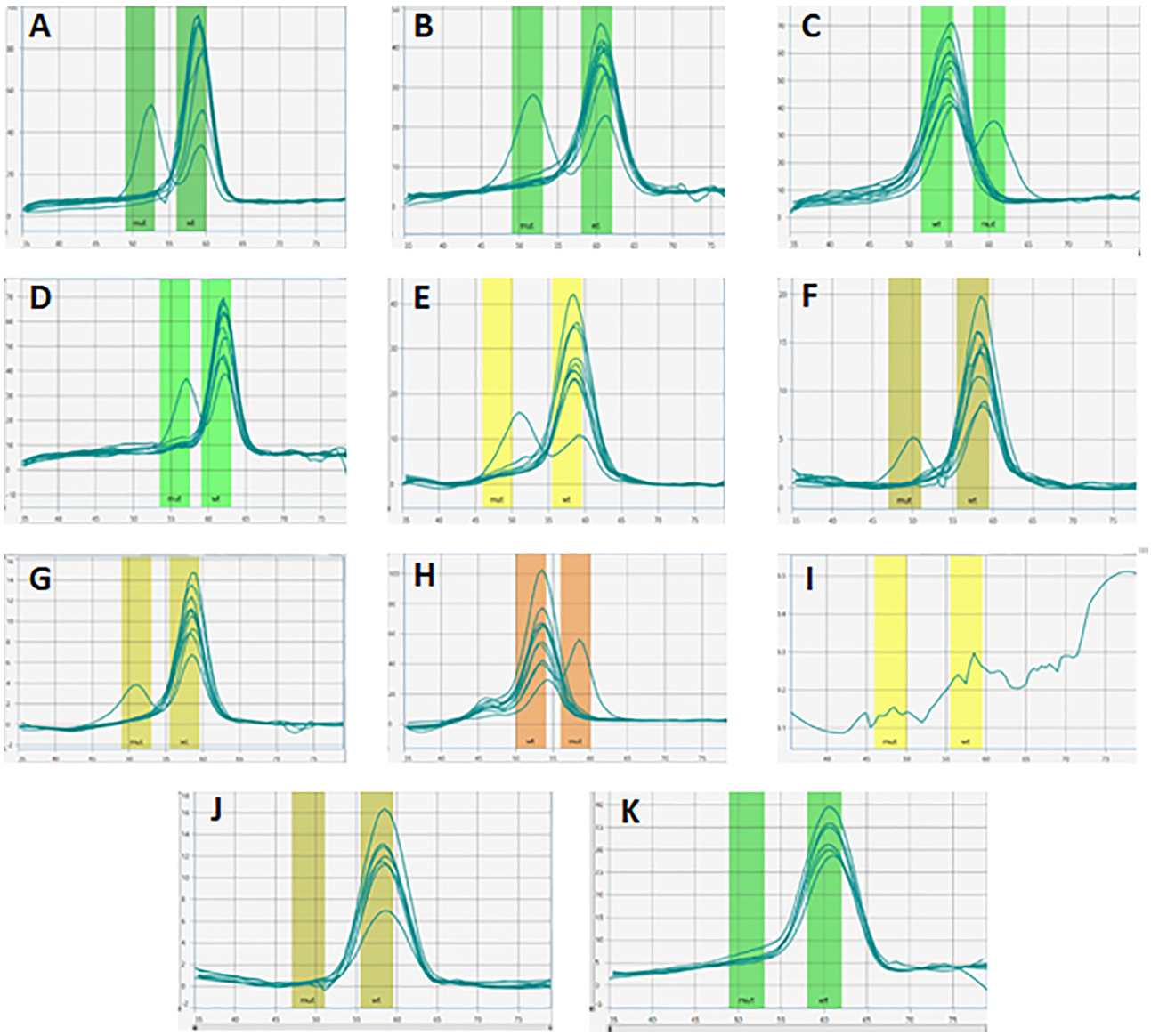

FIGURE 2 | *BRCA1/2* genotyping of control samples using the BRCA 1.0 POC assay. Melting curve analysis of founder mutations *BRCA1* c.68_69delAG, c.1374delC, c.2641G>T, c.5266dupC and *BRCA2* c.5771_5774delTTCA, c.5946delT, c.6447_6448dupTA, c.7934delG **(A–H)**. By using the HyBeacon FAM probe (green), melting peaks were correctly detected for pathogenic variants *BRCA1* c.1374delC (rs397508862) **(A)**, *BRCA2* c.6447_6448dupTA (rs397507858) **(B)**, *BRCA1* c.2641G>T (rs397508088) **(C)**, *BRCA1* c.5266dupC (rs80357906) **(D)**, while the CAL 560 probe (orange) detected *BRCA2* c.5771_5774delTTCA (rs80359535) **(E)**, *BRCA2* c.5946delT (rs80359550) **(F)**, *BRCA2* c.7934delG (rs80359688) **(G)** and the CAL 610 probe identified the pathogenic variant *BRCA1* c.68_69delAG (rs80357914) **(H)** with fluor red dye on the controls with known *BRCA1/2* variants. No peaks were detected in **(I)**, confirming the absence of amplification in the blank sample containing no template DNA. The absence of a second melting curve in **(J)** and **(K)** was expected and confirmed the negative controls as samples without any specific founder/recurrent pathogenic variants.

hormone receptor-positive BC showed a relatively high *BRCA1/2* founder variant detection rate (10%). This finding justifies screening of both familial and sporadic cases for germline *BRCA1/2* variants, and not only triple-negative BC when tumor type is considered in *BRCA1/2* risk prediction algorithms. Since the BRCA POC 1.0 Research Assay is inexpensive and can be manufactured locally, it may in future be utilized for all SA BC patients, irrespective of age, family history, ethnicity, tumor type or recurrence risk profile. Comparison with standard laboratory-based assays using stored DNA samples showed 100% concordance between the portable screening device and the various laboratory-based methods previously standardized. As the ParaDNA workflow is an integrated system from sample collection to result

TABLE 2 | Oligonucleotide primers used for conventional polymerase chain reaction application and Sanger sequencing of *BRCA1* (NM007294.4) and *BRCA2* (NM000059.3) gene regions spanning the nucleotide positions of eight pathogenic founder/recurrent mutations previously identified in the multi-ethnic South African population.

Gene	Region	Variant	Primer[a]	Oligonucleotide primers (5' to 3')	Size (bp)
BRCA1	Exon 2	c.68_69delAG p.(Glu23ValfsX17)	F	TGTGTTAAAGTTCATTGGAACA	149
		[Jewish, European]	R	CATAGGAATCCCAAATTAATACA	
	Exon 10	c.1374delC (p.Asp458GlufsX17)	F	TCGCATGCTCAGAGAATCC	400
		[Afrikaner]	R	TGTGGCTCAGTAACAAATGCTC	
	Exon 10	c.2641G>T (p.Glu881Ter)	F	GCTCAGTATTTGCAGAATAC	253
		[Afrikaner]	R	GCTTATCTTTCTGACCAACC	
	Exon 19	c.5266dupC (p.Gln1756ProfsX74)	F	AGTCAGAGGAGATGTGGTCAATGG	236
		[Ashkenazi Jewish]	R	GTGGTTGGGATGGAAGAGTGAA	
BRCA2	Exon 11	c.5946delT (p.Ser1982ArgfsX22)	F	CGAGGCATTGGATGATTCAGAG	394
		[Ashkenazi Jewish]	R	GAGCTGGTCTGAATGTTCGTTAC	
		c.6447_6448dupTA (p.Lys2150IlefsX19)	F	GAGAAACCCAGAGCACTGTG	404
		[Mixed Ancestry]	R	CTAAGATAAGGGGCTCTCCTC	
		c.5771_5774del TTCA (p.Ile1924ArgfsX38)	F	CGAGGCATTGGATGATTCAGAG	394
		[Xhosa, Mixed Ancestry]	R	GAGCTGGTCTGAATGTTCGTTAC	
	Exon 17	c.7934delG (p.Arg2645AsnfsX3)	F	GTAGTTGTTGAATTCAGTATC	354
		[Afrikaner, Mixed Ancestry]	R	TGGCAACTGTCACTGACAAC	

[a]*F, forward; R, reverse.*

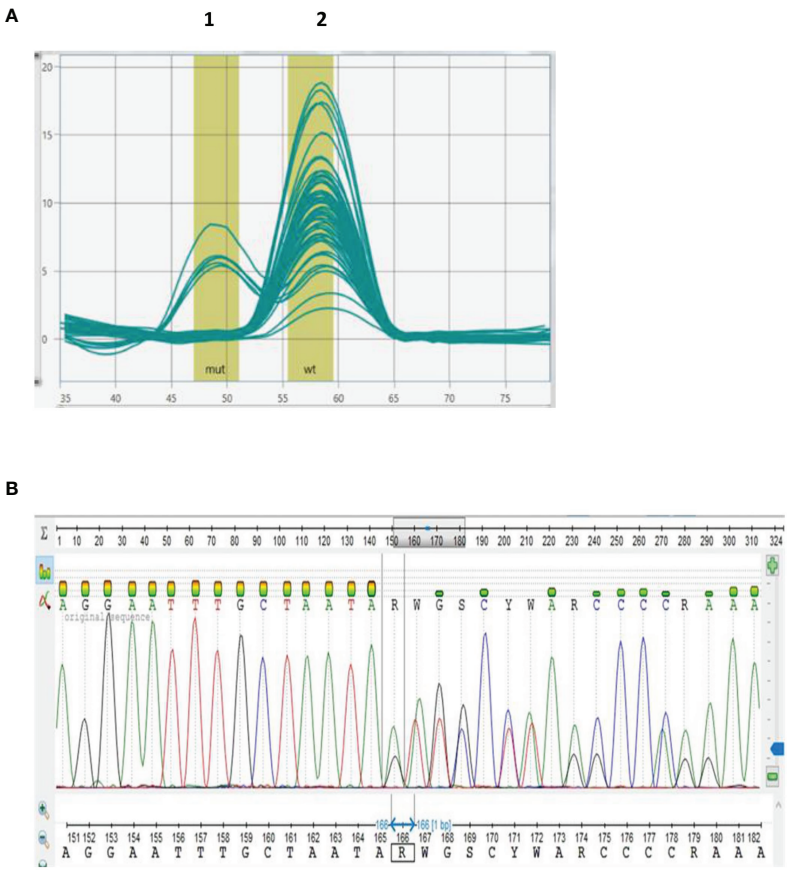

FIGURE 3 | Genotyping of breast cancer patient samples using the BRCA 1.0 POC Research Assay designed to detect eight *BRCA1/2* founder/recurrent South African pathogenic variants. Melting curve analysis of *BRCA2* c.7934delG **(A)** with the CAL 560 probe indicating the presence of a second melting peak (peak 1) for six samples (five patients and the positive control). No pathogenic variants were detected in 45 DNA samples (peak 2). Detection of *BRCA2* c.7934delG was confirmed by Sanger sequencing **(B)**.

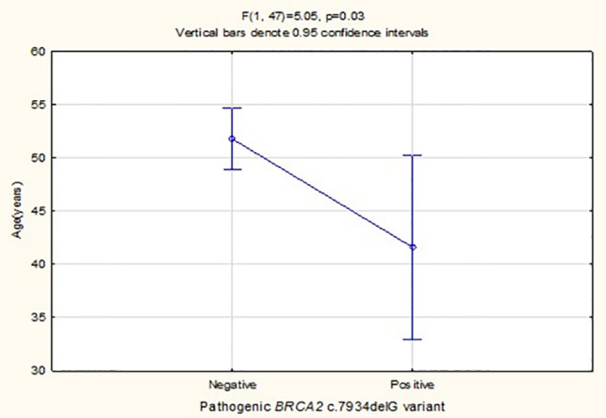

FIGURE 4 | Comparison of the mean age between the five BC patients identified with *BRCA2* c.7934delG founder/recurrent pathogenic variant *versus* the 45 non-mutation carriers.

generation, separate DNA extraction can be eliminated in future with use of the sample collector also applied in forensics (23). Direct application using fresh cheek swabs/saliva as the preferred sample type performed excellent during the test development and optimization process (17). This makes POC testing using the ParaDNA device ideal for application of robust, first-tier targeted genetic tests in any clinic in Africa with access to personal or online genetic/genomic counseling support.

Africa is the second-largest continent, globally representing 14% of the world's population (24). Although economic growth was stable between 2018 – 2019, the estimated 3.4% growth of countries such as SA, Egypt and Nigeria were below the decadal average of 5% for the continent. The number was predicted to increase to 3.9% during 2020, before the outbreak of the coronavirus disease 2019 (COVID-19) pandemic (25). The realization that Africa could benefit from the application of genomic medicine was captured in a policy paper composed by 38 researchers across the continent (26). This framework for the implementation of genomic medicine in Africa aims to reduce the disease burden by translating genomic research information into clinical application using PSGT as one of the proposed implementation strategies. With sufficient evidence for actionability, genomic medicine involving test panels such as our BRCA POC Research and COVID-19 screening assays using the same ParaDNA device, was fast-tracked in SA for test development and validation. Due to Africa's extreme diversity, a "one size fits all" healthcare approach is not appropriate. By providing guidance, the WHO Regional Office for Africa aims to ensure that no one is left behind as the continent progresses towards sustainable and equitable health (27).

Implementing *BRCA1/2* targeted testing at POC is ideal for African countries for which an increased frequency of founder/recurrent actionable pathogenic variants have been identified through the years (28–30). For SA, the eight variants covered in the BRCA 1.0 POC Research Assay include three highly prevalent Ashkenazi Jewish/European founder variants of global relevance. *BRCA1* c.68_69delAG (rs80357914) has also been identified at an increased frequency in Egypt and Morocco. This highlights the value of the assay that can be adapted and redesigned according to each countries' needs, depending on their familial BC mutation spectrum. *BRCA2* c.7934delG represents the most common SA founder variant. Therefore, it was not surprising that this single-base deletion was detected in our study cohort from a non-rural, private healthcare setting, at a 10% rate comparable to the 7.9% carrier status reported in the most extensive SA study published to date (151/1906) (18). Since *BRCA1/2* genetic testing could decrease mortality from breast, prostate, gynecological and some other cancers, and help inform therapy, there is a need to develop or adjust tools to enable targeted treatment and optimal care for all cancer patients (31). The updated pathology-adjusted Manchester score frequently used in SA for estimating the threshold for *BRCA1/2* probability (32), would be more effective in the SA population if patients with hormone receptor-positive BC (linked to the *BRCA2* c.7934delG founder variant in our study cohort) is considered for testing similarly to the inclusion of triple-negative BC. This will also apply to the use of other risk stratification tools such as CanRisk (https://www.phpc.cam.ac.uk/pcu/research/research-groups/cancer-group/canrisk/), which incorporates scientific discoveries in both cancer genomics and epidemiology. A genetic counseling toolkit enabling *BRCA1/2* founder/recurrent variant testing at the POC may add significant value, especially when incorporating the assessment of critical co-morbidities impacting on cancer risk and the option for NGS in eligible *BRCA1/2* founder variant-negative cases.

The new BRCA 1.0 POC assay can serve multiple purposes. Not only may patients and their close relatives become aware of being at increased risk of developing various cancer types, pathogenic *BRCA1/2* variants are also treatment targets for poly ADP-ribose polymerase (PARP) inhibitors (33). Clinical complications related to anti-cancer treatment regimens (34–36) furthermore led to the development of genomic assays such as the 70-gene MammaPrint microarray with level 1A evidence of clinical utility for prediction of chemotherapy benefit (37). Microarray analysis using tumor samples reformed our past understanding of BC as a single disease to a complex disorder consisting of at least four major subtypes, namely luminal A, luminal B, HER2-enriched and basal-type. Currently, tumor pathology including ER, PR and HER2 status is used routinely in SA as a proxy for identifying these subtypes, which may result in misclassification and inappropriate treatment in a subgroup of patients (20). While IHC assessment of ER, PR and HER2 status proved valuable in the private sector for selecting early-stage BC patients in SA for cost-effective use of the MammaPrint test generally performed on RNA extracted from surgical biopsies (19), post-surgery identification of a pathogenic *BRCA1/2* variant in germline DNA is a major concern. A bilateral mastectomy would be most effective in these cases as defective *BRCA1/2* genes increase the risk of a

second breast primary, as well as other secondary cancers, which in turn are likely to metastasize and may be treatment-resistant (38–40). Although expensive, tumor gene profiling is currently reimbursed by private medical schemes in SA after careful patient selection using tools such as the MammaPrint pre-screen algorithm (19). The cost-benefit potential of selective MammaPrint testing was recently confirmed in a study of approximately 600 tumor samples of SA patients with early-stage BC, by employing a chemotherapy de-escalation strategy through clinical risk stratification (41). While toxicity profiles make hormone therapies an attractive option, the standard of healthcare on the African continent is reflected by the lack of ER, PR and HER2 assessment in many state institutions managing the disease (3, 42). This limitation was highlighted by Torrorey-Sawe et al. (3), as IHC was not determined to assess hormone receptor status in a relatively large proportion of study participants enrolled in a Kenyan whole exome sequencing study, initially focused on *BRCA1/2* for return of research results. This finding raised awareness for potential chemotherapy over-treatment in African patients with early-stage BC, which needs to be addressed in the future as part of the WHO's development goals for the continent (27).

Although the small number of 50 study participants is a limiting factor, the samples available for this evaluation in patients with hormone receptor-positive breast cancer, are considered sufficient to support analytical validation and clinical utility of the BRCA 1.0 POC Research Assay as the primary aim achieved. Implementation of POC testing will decrease turn-around time and testing costs, as *BRCA1/2* founder variant testing at a reference laboratory in SA currently costs approximately ZAR 1500 to ZAR 2500, depending on the number of variants tested for according to ethnic/population group (43). In contrast, the BRCA 1.0 POC Research Assay's projected cost once commercialized has been estimated at approximately ZAR 1000, with the option to adjust the design periodically to incorporate new actionable research data obtained for genes involved in hereditary breast and ovarian cancer syndrome. Regarding genetic counseling, a consultation session currently costs between ZAR 500 and ZAR 1300, depending on the duration. When performed in parallel with POC, the total cost should not exceed that of the current first-tier test alone (ZAR 2500). The BRCA POC Research Assay results will also help identify patients in need of more comprehensive hereditary breast and ovarian cancer screening using affordable NGS panels for *BRCA1/2* founder variant-negative patients (approximately ZAR 8000 in the state sector). As NGS analysis using an extended gene panel has been proposed to replace *BRCA1/2* founder variant testing in SA (43), the risk-benefit analysis recently performed helped pave the way forward (18). It provided insight into genetic professionals' view for the future and confirmed the importance of a first-tier test now possible at POC.

The results obtained in this study supports incorporation of germline *BRCA1/2* testing early in the treatment planning of all BC patients in SA (18), including those opting for gene profiling using MammaPrint. Although MammaPrint is not available to BC patients in the public sector at present the prioritization of clinically high-risk patients for testing, such as those with node-positive (1–3) early-stage BC, could result in safe avoidance of chemotherapy and its associated side effects in approximately 50% of eligible BC patients (37, 41). By performing the BRCA 1.0 POC Research Assay in conjunction with transcriptional gene profiling, unnecessary medical expenditure may be further reduced. Therefore, health economic studies are warranted to determine potential cost-benefits from performing genetic counseling combined with rapid *BRCA1/2* POC testing, compared to usual care. By pro-actively positioning POC testing as a genetic counseling tool, we envisage a future where BC patients will have access to personalized genomic medicine across the continuum of cancer care, as illustrated in **Figure 5**. All BC patients will benefit from genetic counseling at the POC, as *BRCA1/2* variant carriers with a high-risk MammaPrint 70-gene profile are at increased risk for both local and distant/metastatic recurrence of their cancer. Those patients with a low-risk transcriptional gene profile may be unaware of the presence of a possible *BRCA*/other germline variants unrelated to risk for metastasis assessed by MammaPrint. This was evidenced by delayed detection of the *BRCA2* c.7934delG variant (initially in tumor DNA using NGS) in one of our study participants diagnosed with metachronous bladder cancer four years after receiving a low-risk MammaPrint result (44). Integration of germline DNA testing and tumour genetics is therefore essential for optimal treatment of patients at increased risk of secondary cancers and BC recurrence (45, 46). Validation of genomic medicine test panels and transfer of actionable gene variants to POC devices offers a flexible platform for adding modifiable environmental exposure data to inform intervention and prevention efforts towards global health (47–49). For genomic medicine to become a reality in Africa, a screening algorithm standardized by the Department of Health needs to be implemented to ensure adherence to set standards for optimal care provided to all BC patients (50).

In conclusion, this study is the first to comprehensively investigate the cost-saving potential and clinical value of *BRCA1/2* POC testing. By using HyBeacon probe technology, the *BRCA1/2* test was transferred from a laboratory-based assay requiring sample transport (extra cost and risk of sample mix-up) and batching (expensive as multiple control reactions are required) to a rapid, robust assay performed at POC on the portable ParaDNA device. By performing targeted genotyping by trained healthcare professionals as a first-tier test at the POC in parallel to genetic counseling as a feasible option for all histologically confirmed breast/ovarian cancer patients, sample collection and testing can be moved out of a tertiary healthcare setting to currently unreached communities. This will reduce loss to follow-up and create the ability to improve care by delivering on-demand psychosocial support directly to the patient and indirectly to the community, where needed. Our findings provided proof of the BRCA 1.0 POC Research Assay's analytical performance, while the clinical utility was evidenced by reaching the 10% threshold for cost-effective variant detection in patients with hormone receptor-positive BC, not currently

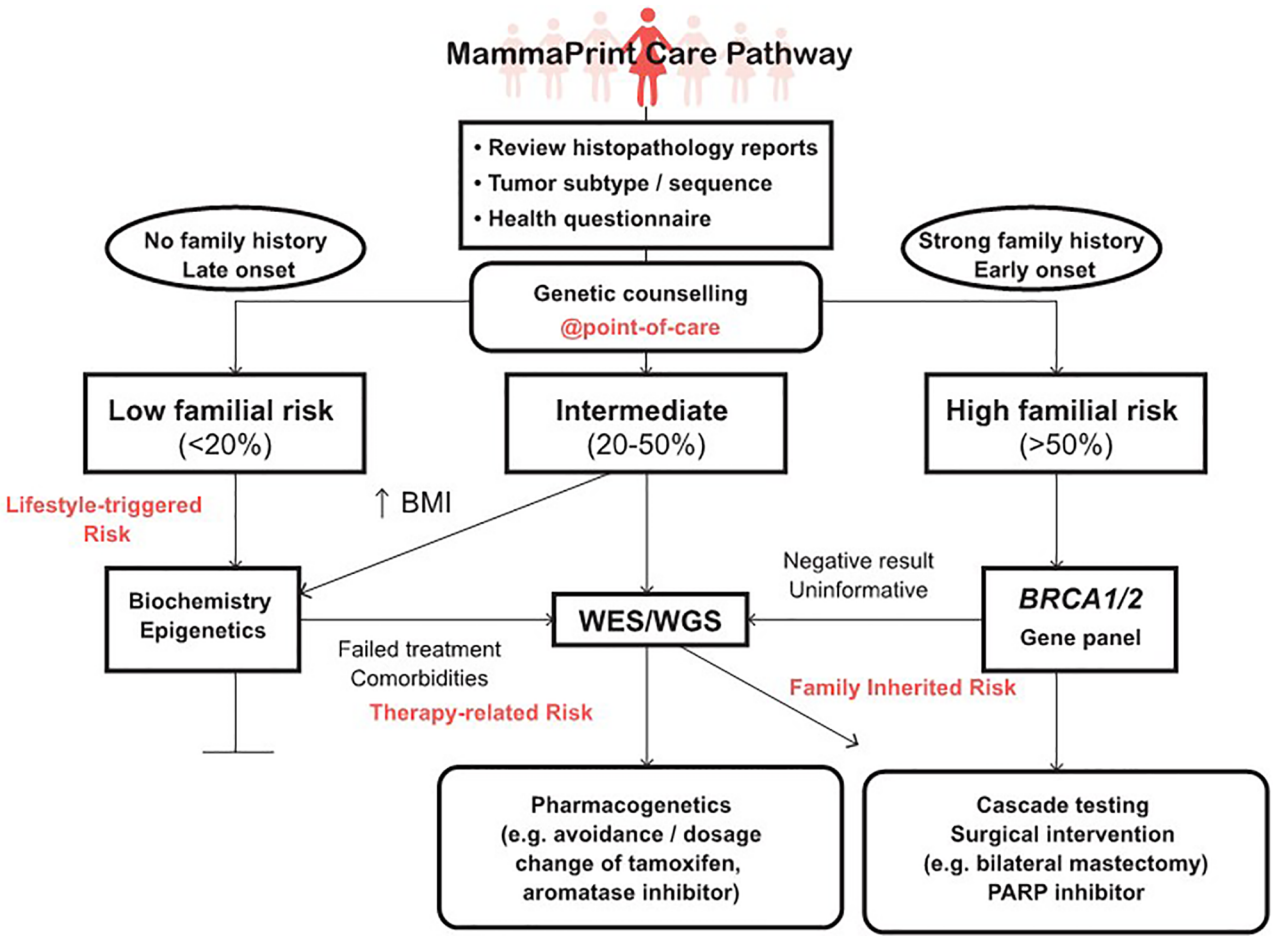

FIGURE 5 | Incorporation of genetic counseling into the MammaPrint care pathway extending from point-of-care germline DNA testing to advanced genomic technologies, including clinical assessments such as body mass index (BMI) and whole exome/genome sequencing (WES/WGS) using a pathology-supported genetic testing approach.

considered for routine *BRCA1/2* testing in SA. Once regulatory authorities have approved on-site BRCA POC testing, this model may be presented to policymakers for wider implementation of oncogenomic medicine in Africa.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation. The clinical characteristics of the 50 breast cancer patients screened were previously reported by the first author at http://etd.cput.ac.za/handle/20.500.11838/3080.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Health and Wellness Sciences Research Ethics Committee of the Cape Peninsula University of Technology in Cape Town (CPUT/HW-REC 2018/H10), the Ethics Committee of the Faculty of Health Sciences, University of the Free State (UFS-HSD2019/1835/291001), and approved as a sub-study under reference number N09/06/166 by the Health and Research Ethics Review Committee of Stellenbosch University, SA. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

LM, KG, and MJK made substantial contributions to the conception, design and completion of this project involving both germline and tumor genetics. LM obtained ethics approval, selected the data for analysis and performed the genetic studies together with KM, AP, and DF. DF developed and manufactured the assay used in this study and provided

training and expert guidance throughout the project's conception and execution. RT-S reviewed the histopathology reports, and MK performed the statistical analysis. FP and PE provided clinical oversight and verified genetic results obtained in this study against their patients' positive and negative test results reported elsewhere. NM obtained inter-institutional ethics approval, verified the analytical assay validation, and framed the study in relation to past achievements and the present standard of care. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: Author DF was employed by company LGC Limited. MJK is a non-executive director and shareholder of Gknowmix (Pty) Ltd. that is involved with the development of the POC 1.0 BRCA Research Assay.

The remaining 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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