BREAST CANCER MOLECULAR SUBTYPES AND ITS ASSOCIATED RISK FACTORS AMONG PATIENT WITH BREAST CANCER ATTENDING AT MUHIMBILI NATIONAL HOSPITAL.

Georvin Marco Haule, MD

Masters of Medicine (General Surgery) Dissertation Muhimbili University

of Health and Allied Sciences

October, 2024

Muhimbili University of Health and Allied Sciences

School of Medicine

BREAST CANCER MOLECULAR SUBTYPES AND ITS ASSOCIATED RISK FACTORS AMONG PATIENT WITH BREAST CANCER ATTENDING AT MUHIMBILI NATIONAL HOSPITAL

By

Georvin Marco Haule

A Dissertation Submitted in (Partial) fulfillment of the Requirements for the Degree of Masters of Medicine in Surgery of

Muhimbili University of Health and Allied Sciences

October, 2024

CERTIFICATION

I, the undersigned, hereby certify that I have thoroughly reviewed and recommend for acceptance by Muhimbili University of Health and Allied Sciences the dissertation entitled**"** Breast cancer molecular subtypes and its associated risk factors among female patient with breast cancer attending at MNH" submitted in partial fulfillment of the requirements for the degree of Masters of Medicine (GeneralSurgery) at Muhimbili University of Health and Allied Sciences (MUHAS) ,DaresSalaam,Tanzania.

_____

Prof.M Mchembe

(Supervisor)

_____

Date:

_____

Dr.Yonah Ringo

(Co-Supervisor)

_____

Date:

DECLARATION AND COPYRIGHT

I, Georvin Marco Haule, here by declare that the dissertation submitted by me is entirely my own work, and I have not presented it before to any other university or institution for any degree or qualification. I acknowledge that any ideas or materials obtained from other sources have been duly acknowledged in the dissertation. I further affirm that I will not submit this dissertation to any other university or institution for a similar or any other degree award.

Signature…… Date……

The present dissertation is subject to copyright protection under the Berne Convention, the Copy right Act of 1999, and other national and international laws concerning intellectual property. Without the express written consent of the Directorate of Postgraduate Studies acting on behalf of the author and Muhimbili University of Health and Allied Sciences, this work may not be reproduced in whole or in part using any means, except for short extracts for the purposes of fair dealing, research, or private study, as well as for critical scholarly review
or discussion, provided that appropriate acknowledgement is given.

ACKNOWLEDGEMENT

I want to start by expressing my gratitude to the all-powerful God, who has given me life and everything I own. My sincere gratitude goes out to my supervisors, Prof. Mchembe and Dr. Y. Ringo, for their invaluable counsel and mentorship during the planning, development, and execution of this research project. I would like to express my gratitude to the Muhimbili University of Health and Allied Sciences (MUHAS) for their sponsorship and funding of this research, which allowed for its completion.

I also want to express my profound gratitude to the MNH and MUHAS surgery department faculty members for their continuous support and direction during the entire study process.

I would want to express my gratitude to my fellow MUHAS surgical residents, who have been excellent colleagues during our training. Through all of the difficulties we have encountered, we have helped and encouraged one another.

Lastly, I would want to sincerely thank my parents for their unwavering support in my academic and personal development, as well as their guidance, commitment, and prayers. Their constant encouragement has given me more strength than inspiration during my academic career.

.

DEDICATION

I dedicate my dissertation to my family, who have supported and inspired me throughout my academic career. My foundation has been built upon the unshakable love and support of my parents, Mr. Marco Simon Haule and Mrs. Agnes Mwangamba.This accomplishment is equally theirs and mine, and I will always be appreciative of their love and support.

TABLE OF CONTENT

CERTIFICATION i

DECLARATION AND COPYRIGHT ii

ACKNOWLEDGEMENT iii

DEDICATION iv

LIST OF ABBREVIATIONS ix

OPERATIONAL DEFINITION: x

ABSTRACT xi

CHAPTER ONE. 1

1.0 INTRODUCTION: 1

1.1 Background. 1

1.2 Statement of the problem: 4

1.3 Conceptual framework ofassocition between risk for breast cancer and molecular subtypes 5

1.4 Rationale 6

1.5 Questions 6

1.5.1 Main research question 6

1.5.2 Specific research questions 6

1.6 Broad objective 6

1.6.1 Specific objectives 7

1.7LITERATURE REVIEW 8

1.7.1 Clinicopathological characteristics of breast cancer molecular subtypes 8

1.7.2Luminal A subtype and (modifiable and non modifiable)risk factors for breast cancer. 10

1.7.3Luminal B and(modiafiable and non modifiable)risk factors for breast cancer 11

1.7.4 HER2/neu andmodifiable and non modifiable risk factors for breast cancer 12

1.7.5Triple negative subtype and modifiable and non modifiable risk factor for breast cancer. 14

CHAPTER TWO....17

2.0 METHODOLOGY 17

2.1 Study design....17

2.2 Study area 17

2.3 Study population....17

2.4 Eligibilitycriteria.....17

2.4.1Inclusion criteria.....17

2.4.2 Exclusion criteria....17

2.6 sample size calculation. 18

2.7 Recruitment of study participants....18

2.8 Data collection procedure. 18

2.9 Data analysis....18

2.10 Variables....19

2.10.1Dependent variables:....19

2.10.2 Independentvariables:....19

2.11 Study limitations and mitigation....19

2.12 Ethical considerations.....19

2.13.Dissemination of the research findings....20

CHAPTER THREE 21

3.0RESULTS 21

3.1.Social demographic characteristics of the study participants 21

3.2. clinical pathological characharistics of the study participants. 23

3.3. Clinico pathological characheristic among breast cancer molecular subtypes. 25

3.3Association between breast cancer molecular subtypes and modifiable risk factor for breast cancer 28

3.4 Association between molecular subtypes and non modifiable risk factors 32

CHAPTER FOUR 35

4.0 DISCUSION 35

CHAPTER FIVE 40

5.0 ;LIMITATION S,CONCLUSION AND RECOMMENDATIONS. 40

5.1 Study limitations 40

5.2 Conclusion 40

5.3 R eccomendations 40

REFERRENCES; 41

APPENDIXI: 49

APPENDIX II 55

APPENDIX III. 60

APPENDIX IV; 62

LIST OF TABLES

Table 1: Social demographic characteristics of the participants n=168 22
Table 2: Clinico pathological characheretics of the study participants. 25
Table 3: pathological characteristics of breast cancer molecular subtypes. 26
Table 4: Multinomial Logistic regression analysis of breast cancer molecular subtypes and modifiable risk factors. 30
Table 5: Multinomial Logistic regression analysis of breast cancer molecular subtypes and non modifiable risk factors 34

LIST OF FIGURES

Figure 1: Conceptial framework 5

LIST OF ABBREVIATIONS

BC	Breast cancer
BRCA 1	Breast cancer gene 1
BRCA 2	Breast cancer gene 2
BMI	Body mass index
CK	Cytokeratin
CI	Confidence interval
ER	Estrogen receptor
GLOBOCAN	Global Cancer Observatory
HER2/neu	Human epidermal growth factor receptor
MNH	Muhimbili National Hospital
MUHAS	Muhimbili University of Health and Allied Sciences.
PR	Progesterone receptor
PTEN	Phosphatase and tensin homolog
TNBC	Triple negative breast cancer
TP53	Tumor protein 53

OPERATIONAL DEFINITION:

Luminal A is a type of breast cancer that express gene that closely overlapping with gene for normal breast luminal epithelia, high gene expression of hormonal receptor and its related gene.

Luminal B is a type of breast cancer that express luminal cytokeratin gene, moderate to low expression of hormone receptors and related genes.

Her 2 enriched is a molecular subtype of breast cancer that has low expression of hormonal receptor and associated genes, high expression of proliferation gene and diffuse TP53 mutation

Triple negative-breast tumor is type of breast tumor that express gene that closely related to gene expressed with basal epithelial cell and cytokeratin (CK5/6) of the normal breast tissue with a low expression of hormonal receptor ER/PR and related genes, low expression of HER2/neu.

ABSTRACT

Background; Breast cancer is the malignant neoplastic changes that arise from epithelia cells of ducts and lobules of the breast. Breast cancer comprises number of breast tumor subtypes that differ in molecular and cellular origin with different treatment and prognosis.

Molecular subtypes such as triple negative subtype, they have poor prognosis, and limited treatment options. This highlights the importance of identifying risk factors associated with specific molecular subtype.

In developing countries, the incidence of breast cancer is expected to increase further due to westernization of life style that led to increased risk for breast cancer, however, there is limited number of studies that have been done in Africa addressing the association between molecular subtypes and risk factors for breast cancer with inconsistent findings.

Aim; The study was aimed to determine the association between risk factors for breast cancer and breast cancer molecular subtypes among female patients with breast cancer at Muhimbili national hospital from march 2023 to April 2024.

Methodology; This was a hospital based. Prospective analytical cross-sectional study. Risk factors and other patient information were collected through interviewing the patient and histological information from medical records through a structured questionnaire. Odds ratio was calculated by Multinomial logistic regression to assess association as well to control for confounding variable, p value <0.05 was considered significant were computed by SPSS version 27.

Results: The study enrolled 168 female patients with histological confirmed breast cancer, with the mean age at diagnosis of 49±11.2 years,78% (132) were diagnose at the age above 40 years, 54% (91) had ER receptor positive,43% (73) were positive for Progesterone receptor, Her 2 enriched receptor positive by 43% (73), majority 87% (155) of the patients, Ki 67 was not done.

Invasive ductal carcinoma was prevalent 64% (107). Luminal A was the most common subtypes and grade II were the leading histological finding 38.1% (61), while 64% (59) of the total patients enrolled in the study, the pathological grade was not documented, Breast Cancer stage III was highly observed by 43% (73) while stage II and I were the least encountered.

Molecular subtype had revealed association with modifiable risk factors for breast cancer among patients with alcohol use (P 0.04, AOR 0.22,95%CI 0.52-0.94), history of contraceptive use (P 0.028, AOR 0.365, 95%CI 0.149-0.895) and normal body mass index (P 0.031, AOR 3.66,95%CI 1.13-11.3) were negatively associated Luminal B compared with luminal A, Age at menarche above 12 years have revealed significant negative association with Triple negative subtypes (P 0.013, AOR 0.167, 95%CI ,0.41-0.69) and Her 2 subtypes (P 0.014, OAR 0.147,95% CI 0.32-68), other modifiable and non-modifiable risk factors were not associated with breast cancer molecular subtypes.

Conclusion: Alcohol intake and history of contraceptive use had shown to have protective effect on luminal B compared to luminal A, while Body mass index had positive association with luminal B, however other modifiable risk factors were not associated with breast cancer molecular subtypes.

Age at menarche above 12 years has protective effect on Tripple negative and Her 2 molecular subtypes while other non- modifiable risk factors were not associated with breast cancer molecular subtypes.

Recommendation: Large sample size and multicenter study may help in addressing inconsistencies and risk factors with less prevalence.

Addressing one molecular subtype will increases the accuracy of ascertaining the association with risk factors.

Improvement in investigating patient with breast cancer especially KI 67 will help in subtyping luminal A and Luminal B breast cancers.

CHAPTER ONE.

1.0 CHAPTER ONE:

1.1 Background.

Breast cancer is the malignant neoplastic changes that arise from epithelia cells of ducts and lobules of breast. Breast cancer comprises number of breast tumor subtypes that differ in molecular and cellular origin with different prognosis and treatment (1).

Globally breast cancer is the world's most prevalent cancer and the leading cause of cancer death in women and incidence have increased by 20 % since 2018 Breast cancer accounting for 23% of the total cases and 14% of the cancer deaths(1).

In Africa breast cancer is the most prevalent cancer in African female with high mortality and low survival rates (2).About 186,598 patients with breast cancer were diagnosed in Africa ,with 85,787 related deaths from 2020 GLOBOCAN data. In Sub Saharan African (SSA) the incidence is rising compared to developed countries, and the prevalent is approximated to increase by factor of 2 up to 2050 (3).

In addition to that most of SSA countries lack cancer registries where by only 43% of WHO member state in SSA countries have cancer registries, that led the true disease burden to remain unclear (3).

In East Africa the incidence of breast cancer is about one-third of regions composed of high-income countries with standardized mortality rate of 15.4 per 100,000 and 37.7% of 5 year survival rates of patient with breast cancer (3)(4).

In Tanzania, breast cancer is the second prevalent cancer after cervical cancer among women, over the past two decade , there is increased in incidence rate of 7.2 and predicted to increase by more than 80% in 2030 ·About half of women diagnosed with breast cancer in Tanzania die of the disease as majority of them present with advanced stage, approximately 80% of BC diagnosed in late stage (5)(6).

Breast cancer classification is essential because it dictate the management of patients , In traditional classification of breast cancer was based on morphological classification, however , patients with breast cancer of the same stage, histological type and histological grade show differences in prognosis, survival of patients , recurrence and response to treatment that warrant more investigation (7).

Advancement of gene expression profiling technique and immunohistochemistry analysis are used for breast cancer molecular classification; however, immunohistochemistry is the most used in clinical practice. Molecular classification is essential for targeted therapy and it is more importantly because individualized treatment programs now have become possible with the implementation of this classification (7,8,9).

According to St. Gallen consensus, there are four major type of breast cancer molecular subtypes such as, luminal A , Luminal B , HER2 enriched and Triple negative or basal like subtype, while the fifth group is closely resembling with Luminal A subtype (8,9).

Luminal A, express gene that closely overlapping with gene for normal breast luminal epithelium with high gene expression of hormonal receptor and its related genes furthermore it has low expression of ki 67 and cytokeratin.ER/PR positive, HER2/neu negative on its biological properties, also is the most prevalent subtype and comprises almost half of invasive breast cancer, and histologically it includes tubular carcinoma, cribriform carcinoma, low grade invasive ductal carcinoma and classic lobular carcinoma. This group of breast cancer respond to hormonal therapy and chemotherapy, overall this kind of molecular subtype has good prognosis in comparison with other subtypes(9,10,11).

Luminal B subtype is the molecular subtype that express luminal cytokeratin gene with moderate to low expression of hormone receptors and related genes. Biologically it is defined as ER/PR positive with variation in HER2/neu expression and express higher ki 67 than Luminal A and approximately comprises above 15% of all invasive breast cancer and it has high histologic grade compared to Luminal A. It includes invasive ductal carcinoma such as Micropapillary carcinoma and NOS. This type of breast cancer has less response to hormonal

therapy and also display variations to chemotherapy response, futhermore,its prognosis is not good as compared to luminal A (10–12).

HER2/neu subtype has high expression of HER2/neu gene and its associated receptors and low expression of hormonal receptor and associated genes with high expression of proliferation gene index ki 67 and are more related to diffuse TP53 mutation. Moreover , it has negative ER/PR and positive HER2/neu on its biological properties ,with high histologic grade and nodal positivity on management .It responds to targeted therapy especially trastuzmab also it respond to chemotherapy ,however, it shows unfavorable prognosis (1)(10).

Triple negative subtype comprise with breast tumors that express genes that are closely related to genes expressed by basal epithelial cell and cytokeratin (CK5/6) of the normal breast tissue with a low expression of hormonal receptor ER/PR and related genes, also it has low expression of HER2/neu receptor and associated genes, moreover most of tumor in this molecular subtype has ER,PR and HER2/neu receptor negative on its biological features with high proliferation index, ki 67,diffuse TP53 mutation, and abnormality in BRCA1 gene, however 20 percent of invasive breast cancer it consist of high grade invasive ductal carcinoma, NOS, Metaplastic carcinoma and Medullary carcinoma(13)(14).

Triple negative subtype is not sensitive to hormonal or targeted therapy ,therefor with that insensitivity has made chemotherapy to remain the main treatment option and they had shown to be more sensitive to platinum chemotherapy and poly ADP ribrose polymerase (PARP) inhibitors, however ,TNBC has worse prognosis (2)(12)(14)(15).

Risk factors for breast cancer are well established, these risk factors are hypothesized to be associated with specific molecular subtypes. The risk factors for breast cancer are grouped into modifiable and non-modifiable risk factors, to start with non-modifiable risk factor are, old age , female sex , genetic mutation such as BRCA1/2 ,TP53 and PTEN ,history of breast cancer in the family, previous history of breast cancer ,breast density, previous radiation exposure , non-cancerous or benign breast disease, early menarche, late menopause and ethnicity /race, modifiable risk as breast feeding , number of parity, early first full pregnancy, use of

contraceptive , hormonal therapy, alcohol consumption, physical exercise , obesity , overweight and cigarette smoking (2)(15)(16).

1.2 Statement of the problem:

Breast cancer is the world's most prevalent cancer and the leading cause of cancer death similarly in Africa, breast cancer is the most prevalent cancer among African women (17).

Breast cancer molecular subtypes have unique etiology due to differences in genetic, environmental and life risk factor (17)(27).Risk prediction model were developed from European women ,studies have shown they have poor prediction among African women this highlighted the need for investigating African women so as to develop tool that is tailored to African women (19)(18).

Treatments offered to patient with breast cancer and the response to treatment are dictated by molecular subtypes ,TNCB and HER2/neu enriched molecular subtypes present in late stage and are more aggressive compared to other subtypes with limited treatment options (17).

In Tanzania, the incidence of breast cancer is predicted to increase by more than 80% by 2030 this may be due to westernization in term of lifestyles such as delayed pregnancies, reduced breastfeeding, low age at menarche, lack of physical activity, and poor diet (14).

In addition to that, the study done in Tanzania revealed that modifiable risk factors such as obesity in young age, cigarette smoking and alcohol use, increases risk for developing breast cancer, understanding the association between modifiable risk factor and molecular subtypes is necessary for preventive measures (20).

Only a single study have been done addressed the association of reproductive risk factors and breast cancer molecular subtype ,however other risk factors were not encountered (6).

Limited number of studies have been done in Africa, that investigated the relationship between molecular subtypes and specific risk factors. Inconsistence observation from different countries in East Africa (6)(19)(19) and in West Africa on association of risk factors and molecular subtype such as breast feeding (18)(22),obesity and over weight (13)(30)(31)(35)(25)(26).

1.3 Conceptual framework for association between risk for breast cancer and molecular subtypes.

From the epidemiological studies revealed that there is relationship between breast cancer molecular subtypes and risk factors for developing breast cancer,

Modifiable and non-modifiable risk factor are well established that they have causal effect on breast cancer, regardless of the differences in histological types and grade of cancer, the risk factors for breast cancer determine the type of molecular subtypes. Therefore, the risk factors for breast cancer are independent factors and breast cancer molecular subtypes are the dependent factors for studying association between risk factors for breast cancer and molecular subtypes.

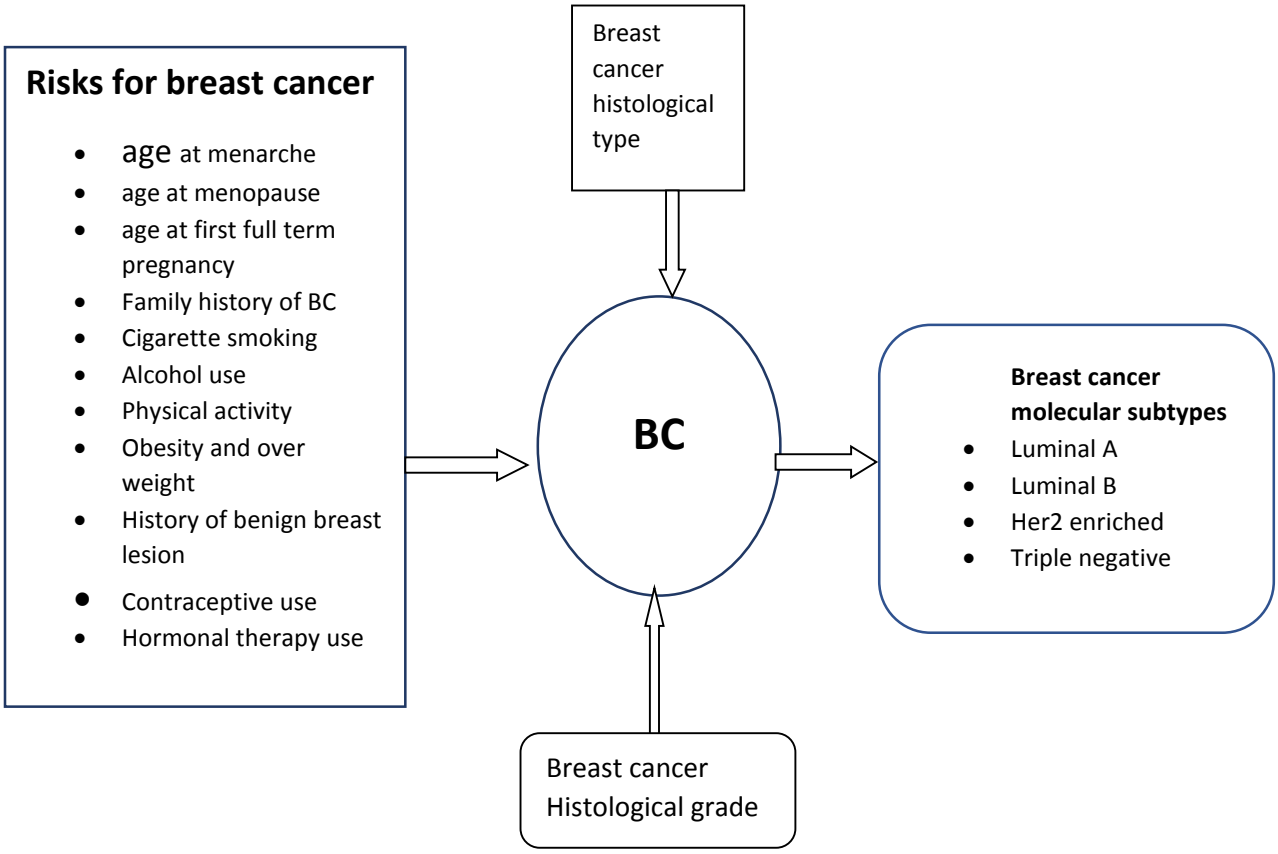


Figure 1: Conceptual framework

1.4 Rationale

This study will add knowledge on relationship between molecular subtypes and breast cancer risk factors among Tanzanian women.

Will be useful for developing of risk predictive model tool that is tailored for African women and in specific Tanzanian women.

Establishment of preventive strategies especially for risk factors associated with breast cancer molecular subtypes with poor prognosis and limited treatment options.

Reveal the etiological heterogeneity in breast cancer molecular subtypes among Tanzanian women.

1.5 Questions

1.5.1 Main research question

What are the risk factors for breast cancer associated to each breast cancer molecular subtypes among female patient with breast cancer at MNH?

1.5.2 Specific research questions

This research aims at answering the following questions;

i. What are the clinicopathological profile of breast cancer molecular subtypes among patient with breast cancer at MNH?
ii. What are the modifiable risk factors for breast cancer associated with each breast cancer molecular subtype among patient with breast cancer at MNH?
iii. What are the non-modifiable risk factors for breast cancer associated with each molecular subtypes among patient with breast cancer MNH?

1.6 Broad objective

To determine the association between risk factors for breast cancer and molecular subtypes among female patient with breast cancer at MNH.

1.6.1 Specific objectives

i. To describes clinicopathological profile of breast cancer molecular subtypes among patient with breast cancer at MNH.

ii. To determine association between modifiable risk factors for breast cancer and molecular subtypes among patient with breast cancer at MNH.

iii. To establish association between non modifiable risk factors for breast and each molecular subtype among patient with breast cancer at MNH.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Clinicopathological characteristics of breast cancer molecular subtypes

Invasive ductal carcinoma is the most frequently histological type ,accounting approximately 84.7% of cases ,from study conducted Brazil, similar findings were revealed from studies done in South America, Malaysia, as well as comparative study from Iraq and British,(27),(28).In Africa ,invasive ductal carcinoma is a leading diagnosed breast cancer revealed from different studies (29)(20).

In united State of America, luminal A is commonly diagnosed molecular subtypes, account about more than half of cases and Triple negative molecular subtype (TNBC) was second prevalent subtypes, while non luminal HER2/neu were the least prevalent molecular subtypes. This was consistent with the observation from Asia and South America studies, involving 6 countries ,shows Luminal A is prevalent subtype(30),(31),(32).

In contrary, a study conducted in Brazil have shown lumina B HER2 negative is leading diagnosed molecular subtype(33)(34), In consistency of prevalence was noted in different countries, reveals different results as HER2/neu molecular subtype is more frequently diagnosed in Malaysia (28). Luminal A ,was predominantly associated with lobular carcinoma , while Luminal B and HER2/neu were predominantly diagnosed with ductal carcinoma shown from study conducted among Turkish women(34). In East Africa, a study conducted in Rwanda has shown the same observation(35).Further more study from Kenya reveals luminal A and luminal B were most frequently diagnosed subtypes, while TNBC was the second prevalent subtype and HER2/neu was the least prevalent molecular subtypes(19)(36).However ,in study from West Africa, had different observation , TNBC were the most frequently diagnosed molecular subtypes(29).

Luminal A was frequently found among women who are above 45 years of age , while Luminal B subtypes were more commonly found in young age ,this was consistent with results from

Asia (30)(32)(9).Study done in Iraq show TNBC and HER2/neu were more prevalent in young age (37)(38).A study conducted in West Africa shows that majority of patient with TNBC had less than 40 years of age (29).

Luminal A shown to have features with less aggressive presentation, such as smaller in size, low grade, and early age at time of diagnosis, while other molecular subtypes, such as Luminal B, TNBC, and HER 2/neu, found to have more aggressive manifestations, such as late stage and high grade tumors (30), similar findings were found in different studies done in Brazil, Iraq, and Asia (32)(9)(37), as well as in a Turkish study that showed most Turkish women with locally advanced diseases had luminal A, while locally advanced diseases were luminal B/HER2/neu (29).In Africa ,A study conducted in Kenya has shown HER2/neu and TNBC associated with higher grade and majority presented with pathological grade three ,with similar observation was made,HER2/neu and TNBC present with poor prognosis from other African countries(30)(36).

A study from Brazil shows that majority of patient were diagnosed with higher stage, most were diagnosed with stage II B and higher stage, as well as T2 status was frequently encountered among Luminal A ,this observations were consistent with the studies conducted in South America and India (27)(28)(38)(33).In contrary from previous observation , T1 status was frequently encountered in Turkish women among luminal A patient (34).However, In East Africa , majority of patients about 90% had tumor size of more than 2cm and T2 status was the leading tumor status, compared with higher T status these were revealed from a study conducted in Kenya (19), On other hand study from Rwanda have shown majority of patients were diagnosed with stage III and IV(35)(35).

TNBC and HER2/neu , were more commonly associated with lymphovascular invasion, while Luminal A was less likely to involve lymphovascular invasion as per Turkish study, luminal A was predominantly metastasis to the bone, while TNBC and HER2/neu were involved with lymphnode metastasis (34) also study from India reveals that TNBC was more likely to present with lymphnode involvement (39).A study done in Kenya shows lymphnode was not different with regard to molecular subtypes (36).

2.2Luminal A subtype and (modifiable and non-modifiable) risk factors for breast cancer.

Majority of Studies reveal Luminal A subtypes is the most prevalent subtype compared to other breast cancer molecular subtypes and most of the reproductive risk factors for breast cancer are associated with luminal A subtypes(40).

Age at menarche is inversely associated with luminal A subtypes, women who attained menarche at the age less than 12 year were associated with high risk for developing luminal A breast cancer(18) ,this observation is consistent with findings from study done in Asia , shows early age at menarche is linked with increased risk for luminal A subtypes(22).In Africa ,a study from Kenya revealed similar observation, on other hand ,an thetical result was shown from study done in Uganda where by early age at menarche is not associated with luminal subtype (19).

Women with a first full-term pregnancy at age 30 years or beyond are more likely to develop luminal A subtype breast cancer from studies done in America and Asia (19,20).Furthermore, similar findings were revealed from the review of study done in United State of America (USA)(42)and from multicentric study conducted in Northern and Eastern China(41). Contrary result was observed from study done in Europe, which has revealed that there is no association of age at first complete term pregnancy with BC molecular subtypes(18).In East Africa, Tanzania women who had their first full-term pregnancy at 30 years old or more has increased risk of luminal A molecular subtype(6) ,this observation was inconsistent with a study conducted in Uganda, had revealed that there is no association between molecular subtypes and risk for breast cancer(21).

Parity shows strong inversely association with luminal A , where by women with more than two children have lower risk for developing luminal A subtype from studies conducted in America and Scotland(13) ,this finding was consistent with the review from 22 cohort studies and case control studies(43). In other hand, different observation were found from different countries, that there is no relationship between parity and molecular subtype in Italian women(33)and East African women from Uganda revealed no association of parity and breast cancer molecular subtypes(44).However, from study done in Tanzania reveals parity has strong inversely association with luminal A molecular subtype(6).

Increased body mass index (BMI) have shown to increase risk of luminal A as per review of nine (9) cohorts studies in European countries(33),also in East Asian women with obesity and overweight had increased risk for luminal subtypes especially luminal A(45).On other hand, inconsistency was shown from the study done in China, that shows high body mass index(BMI) in premenopausal has protective effect on luminal A subtypes(46).

Late menopause is associated with increased risk for luminal A subtypes from study done in China (45),similar observation was revealed from 10 studies from review of 19 studies.

In West African , perimenopausal women with high Body mass index they have reduced risk for luminal A and luminal B subtype as well as HER2/neu enriched from study done in Nigeria (25).Contrary results were shown from East Africa ,where by obesity and overweight were not associated with risk of any molecular subtype from studies done in Kenya and Uganda (44)(19), while study done in Tanzania ,body mass index (BMI) status was not analyzed for association(6).

Female who didn't breastfeed have increased risk of both luminal A and ER- PR tumors(45).In East Africa, shows female who had never breastfed are more likely to have luminal A subtype than non-luminal A subtypes, However in study done in Kenya and Uganda have revealed no association between breast feeding and molecular subtypes(22,25).

Women who ever drunk alcohol , have increased risk of having luminal A subtype and luminal B subtype than other subtypes from European study, on other hand, cigarette smoking was not associated with all subtypes(33),the same observation was revealed from America (47),

Breast cancer molecular subtypes are associated with smoking , several studies have shown there is an association between smoking and breast cancer molecular subtypes ,study from America ,shows that ,smoking increases risk of developing luminal A subtypes among female whom are smoker compared to non-smoker (48),similar finding was observed in a study undertaken in Norway (47).

2.3Luminal B and modifiable and non-modifiable risk factors for breast cancer

Breast feeding For longer duration at least 12 months have a significant protective effect for developing luminal B breast cancers from cohort study done in Europe (49).

Female with a first complete term pregnancy at old age were more likely to develop luminal B ,as well as luminal A subtypes BC ,this was shown from large cohort consortium in European women (33), Contrary with a East African study from Tanzania revealed women who had first term pregnancy under 30 years of age were more like to have luminal B molecular subtypes (6) There is increased risk for developing luminal B subtype for the women with increased age at menopause ,this have been shown from European women as per cohort consortium (33).

Different findings was observed , thus age at menarche, parity, and age at first full-term pregnancy did not affect luminal B breast cancers in cohort of premenopausal women from Italy(49),similar result was revealed from study done in Europe (33).

In East African ,women from Kenya with low parity were more likely to be luminal B subtype (19) ,Ina thetical result from Uganda ,found that parity has no association with molecular subtypes (21).

Perimenopause and pre-menopausal women with high body mass index were less like to have luminal B subtype from West Africa,(25).

Hormone therapy (HT) user, have stronger associations with longer durations on hormonal therapy with increased risk for luminal B from study done in America (22), on other hand East Africa study from Kenya reveals that there is no association between luminal B and Her2 enriched with hormonal therapy (19).

Women who smoke cigarette were associated with increased risk for luminal B

from studies conducted from America and Norway (47) ,with consistent findings from systemic review study from China , also revealed smoking is associated with ER positive breast cancer (50).On the contrary with several studies have shown there is no association between smoking and breast cancer molecular subtypes(51)(52).

From study conducted in Norway and America , they have shown there is association between alcohol intake and luminal A breast molecular subtypes, women who take alcohol had increased chance of developing luminal like breast cancer(47)(48).

2.4 HER2/neu and modifiable and non-modifiable risk factors for breast cancer.

Early age at menarche is positively related with increased risk of HER2/neu subtypes from review study from Europe (42),similar observation was revealed from White and African

American women(42). Women who attained menarche at older age was not associated with decreased risk of HER2/neu compared to luminal A molecular subtypes from study done in China (41).On top of that , younger age at menarche was not associated with breast cancer molecular subtype (18).

Using hormone replacement therapy were related to the risk of HER2/neu enriched breast cancer. However ,the risk increased with current use of hormone therapy(HT) ,with stronger associations for longer durations on hormonal therapy (19,22).

HER2/neu subtypes is more prevalent in post-menopausal women on hormonal therapy revealed from study done in Asia and Scotland(41)(7),with similar findings from review study done in Europe (43).On other hand, In East Africa ,a study from Kenya reveals that there is no association between HER2/neu enriched with hormonal therapy (19).

Contraceptive drugs are also associated with risk for HER2/neu molecular subtype, using contraceptive drugs for more than 5 years were strongly associated with increased risk of Her 2 subtype also it is strongly associated with the increased risk for triple negative subtype (53).

Parity is associated with high risk of HER2/neu breast cancer, particularly women who are more than para three with complete term pregnancies compared to women who are null parous a study in Mexico and America ,similar observation from study done in United kingdom (18,27,31),from a review study ,few studies revealed consistent findings and contrary result was shown from 11 studies that there is no association between parity and HER2/neu molecular subtype (42).

In East Africa ,study done in Kenya shows postmenopausal women with high body mass index (BMI) were related with increased risk for developing HER2/neu subtypes, this was inconsistent with the study from West Africa in Nigeria, where by obese patient were less like to have Her 2 molecular subtype (25)(24). Furthermore ,study from Uganda revealed no association between body mass index (BMI) and molecular subtype heterogeneity (44).

Cigarette smoking increases risk for HER2/neu molecular subtypes ,this was revealed from study conducted in United State of America , women who ever smoked have higher chance of having HER2 positive (48),similar findings from systemic review shows smoking is associated with ER positive breast molecular types but was not associated with ER negative breast

cancer(50).On other hand, Different studies shows there is no significant association between smoking and breast cancer molecular subtypes(52)(51).

Female who drinks alcohol were not associated with increased risk of developing HER2 subtypes, this has been shown from the study done in Norway, while other luminal subtypes were associated with alcohol intake(47).

4.5Triple negative subtype and modifiable and non-modifiable risk factor for breast cancer.

Triple negative molecular subtype is prevalent diagnosed at the early age or among youth population, with poor response to treatment and has limited treatment option that can be offered to the patient, majority of patients with triple negative breast cancer they present in late stage of breast cancer. In identifying risk factors associated with triple negative molecular subtype will help on minimizing the burden of disease through early detection and prevention (30-32).

Obesity and overweight is positively associated with increased risks for developing triple negative subtype from America and Asia women,(13)(30-32), However similar finding was observed in Scotland that obesity in premenopausal women associates with high chance to develop TNCB(13). In Africa there is inconsistent findings from the study done in East Africa and West Africa from Nigeria have revealed that obese patients were less likely to have triple negative or Her2 molecular subtype from Keyan women ,contrary with results from West African women where by high body mass index were significantly associated with increased risk for triple negative cancer from the study done in Nigeria(24)(25).

Early age at menarche less than 13 years has positive association with TNCB .In studies done in north America, Latin America countries and East Asia(55)(45)(54).On other hand, different observation was revealed inversely association of age at menarche and risk for TNBC from review study (42).

TNBC cases were more likely to have a younger age at first birth and high parity when compared to luminal A-like cases from the study done in United kingdom from Scotland(56) , Moreover the finding was consistent with the study from Africa America and Hispanic origin women (54).

History of breast cancer in family is associated with increased risk for all BC subtypes with highly association with TNBC(54).Furthermore, large cohort study from European women revealed family history had risk for all type of BC molecular subtype with strong association with luminal B(33).

Earlier age at first full-term pregnancy were associated with increased risk of TNBC(54), also a review of studies shown increased like hood for developing TNCB for women with short duration between age at menarche and first full term pregnancy . On other hand inconsistent results were found from Asia and America reveals no association between age of menarche and first term pregnancy with TNCB(18).

Women whom breast feed at least six months have lower risk of developing TNBC. Increase in duration of breast feeding is inversely related with decreased risk for developing TNBC from studies done in America and Asia (54).However, A review of 11 studies reveal similar observation that breast feeding have decreased risk for developing TNBC. Moreover there is 50% decreases risk for TNBC for women who breast feed at least for 12 months (42).

From 9 cohort studies consort in European countries ,women with old age and high parity were more associated with TNBC(23).

Smoking was not associated with TNCB molecular subtypes from study conducted in American(48)(51), Similar findings were shown from a study conducted in Europe(47)(7)(52),in contrast ,smoking was associated with ER positive breast cancer ,from systemic study from China(50),and from study conducted in America (57).

Alcohol intake is not associated with TNCB,as well other molecular subtypes, this was revealed from the studies conducted in Norway and USA, that there is no statistical significant between alcohol and ER negative breast cancer(47,48).

Among women who used hormonal replacement therapy, study has found there is no association with TNBC. However hormonal replacement therapy was significantly associated with increased risk for luminal A subtypes from the research done in America (7).

Physical exercise reduce the risk for developing breast cancer as well associated with reduced like hood of triple negative breast Cancer from study conducted in Europe(47).In Africa from study conducted in Nigeria, women who exercise were associated with less likely to develop Triple negative breast cancer compared to women who are physically inactive were associated with high prevalence of TNBC(58).

CHAPTER THREE

3.0 METHODOLOGY

3.1 Study design

A Prospective hospital based analytical cross-sectional study, was carried for 9 months from July 2023 to March 2024 where by female patients with histological confirmed to have BC admitted in the ward or seen as an outpatient at Muhimbili National hospital were included in the study.

3.2 Study area

The study was carried out at Muhimbili national hospital in surgical wards and clinics under the department of General Surgery. Muhimbili national hospital is the national referral hospital and University teaching hospital with capacity of admitting 1500 patients as per bed capacity, and serving 2000 outpatients per week. A department of General Surgery with the capacity to attend about 35 breast cancer patients as in patient and as outpatient in a month.

3.3 Study population

The study was carried out among female patients with BC cancer who were admitted in surgical ward and seen as outpatients at surgical clinic.

3.4 Eligibility criteria.

3.4.1Inclusion criteria.

I. Female patient with a histological confirmed breast cancer with lobular or ductal breast cancer attending at surgical clinic or admitted in surgical department at MNH.

3.4.2 Exclusion criteria.

I. Female patient with confirmed mesenchymal breast cancer.

3.6 sample size calculation.

The sample size will be estimated by using the following formula for randomly sampling cross sectional study.

$$n=\frac{Z^2P(1-P)}{D2}$$

Prevalence of breast cancer (P) = 9.9% (Tanzania)

Marginal error (D)= 5%

Z score = 1.98

$n= (1.98)^2(0.099) \times (1-0.099)/ (0.05)^2$

n= 156

3.7 Recruitment of study participants

Data collection was commenced with identification of female patients with histological confirmed breast cancer attending at MNH, who were admitted in surgical wards and those who were seen directly on their respective days of clinic, simple randomly sampling technique was used to select available patients in the ward or at the clinic, and those who met inclusion criteria were enrolled in the study.

8.8 Data collection procedure.

Demographic data and risk for breast cancer data were collected by filling the structured questionnaires through interview in ward or at clinic. Histological data was obtained from the medical records. Data collection tool wase validated prior to actual research data collection.

3.9 Data analysis

Data extracted from the structured questionnaires were entered into excel sheet, cleaned and checked for consistency, then analysis was computed using the Statistical Package for Social Sciences (SPSS) version 27. Data were described and summarized by using the measures of central tendency categorical variable and continuous variable were described as mean, and

frequency distribution table accordingly and multinomial logistic regression was used to determine odd ratio and control for confounding factors from independent variable. Luminal A subtype was the reference group.

3.10 Variables

3.10.1Dependent variables:

Outcomes were categorical and polychotomous variable such as luminal A, Luminal B, Her2 enriched and triple negative.

These were obtained from immunohistochemistry results and those patients with ER+, PR + He2 - or ER+/PR+plus Her 2 – were group as luminal A, those with both or one of ER/+PR plus Her 2 – were group as Luminal B and Triple negative with ER-, PR- and Her 2 -.

3.10.2 Independent variables:

Risk factor for breast cancer such as, age at first full term pregnancy, parity status, age at menarche, age at menopause, history of breast cancer in first degree relative, personal history of benign breast disease, oral contraceptive use, use of post-menopausal hormone therapy, Body mass index, alcohol consumption, and cigarette smoking status were independent factors

3.11 Study limitations and mitigation

Difficult in differentiating between luminal A and luminal B which are non HER2 due unavailability of KI 67 Cellular maker for proliferation.

Unavailability of regents for detection of cytokeratin 5/6, thus difficult in detecting basal like tumor from triple negative tumors.

Failure of patient to recall some information needed for the study will be one of the limitations of this study.

3.12 Ethical considerations.

The ethical clearance for conducting this study was sought from the MUHAS Institutional Review Board (Ref. No. DA.282/298/01.C/1816) after approval from the Department of

General Surgery. Permission to collect data was requested from the Muhimbili national hospital research and consultancy unit. The study was conducted with respect to all ethical principles of doing medical research. Informed consent was obtained given and client names was not written on the information gathering tool for the reason of confidentiality.

3.13. Dissemination of the research findings

After data collection and analysis, findings were presented at the department of General surgery then disseminated to the Dean of the school of medicine and Director of Postgraduate studies. The findings were also given to the teaching, research, and consultancy coordination unit of MNH. The research report will be deposited into the MUHAS Institution repository. Manual script prepared for publication in the local and international journals.

CHAPTER FOUR

4.0RESULTS.

4.1. Social demographic characteristics of the study participants

This study enrolled 168 female patients with breast cancer attended at MNH, with the mean age at diagnosis of 49±11.2 years,78% (132) were diagnose at the age above 40 years, large number of participants had primary level of education 60.7% (102), 10.2 % (17) had no formal education, while 23.4% 9(37) and 5.4% (9) had secondary level and college level education respectively. Married patients were leading in number, 65.5% (110), followed by unmarried 17.9% (30), the rest were widowed, divorced, and cohabiting by 10.7% ,3.6%and 1.8% respectively, most of the study participants were living in urban areas 64.4% (108), while the rest living in rural area accounted by 35% (60).

The mean age at menarche was 13 years, majority 79.2% (122) attained menarche at 12 years and above, 20.8% (32) they didn't recall their age at menarche, there was no participant who have early menarche. Among post menopause women majority 61% (96) attained menopause at the age less than 55 years and only one participant has delayed menopause, with 45.6±4.5 years as mean age at menopause and 38.8.3% (69) were pre-menopausal.

Contraceptive drugs were used by almost half of the participant 48.2% (82).Only one patient among post-menopausal women had history of hormonal replacement therapy ,11.9% (20) had physical exercise .Also the mean age at first full term pregnancy was 22.8±4 years, many of the patients 91.6%(141) had first term pregnancy bellow 30 years. Multiparous with 3 or more than 3 deliveries were the leading observation 53.6% (90), follow by those who delivered twice 36.9% (60) and null parous encountered by 9.5% (16),

Among participants majority had breastfed 3 children and more than 3 children for more than 12 months.

The study participants 88.1% (145) had no history of alcohol use , History of breast cancer in the family was encountered by 22% (38). Only one patient had history of smoking cigarettes,

while the rest have never smoked, familiar history of breast lesion, the table below summarized social demographic characteristics among participants.

Table 1: Social demographic characteristics of the participants n=168

Variable		Frequency	Percent %
Age at diagnosis (49 +11.2)	Less than 40	36	22
	40 and above	132	78
Education status	No formal	18	10.2
	Secondary level	39	23.4
	College level	9	5.4
	Primary	102	60.7
Employment status	Farmer	45	26.8
	Employed	83	49.5
	House wives	39	23.2
Marital status	Not married	30	17.9
	Married	110	65.5
	Cohabiting	3	1.8
	Widowed	18	10.7
	Divorced	6	3.6
Residency	Urban	108	64.4
	Rural	60	35.6
Age at menarche	12 years and above	122	79.2%
	I don't remember	46	20.8%
Age at menopause	less than 55 years	96	61.0%
	55 years and above	1	0.6%
	not attained menopause	59	38.3%

History of physical exercises	Yes	20	11.9
	No	148	88.1
History of using oral contraceptives	yes	82	48.2%
	No	86	51.8%
Parity	Null porous	16	9.5
	Less 3	62	36.9
	Equal and above 3	90	53.6
Age at first term pregnancy	Below 30	141	91.6%
	30 and above	13	8.4%
Number of breastfed children	<3children	62	36.9%
	≥3 children	90	53.6.3%
History of using HRT	yes	1	0.6%
	No	167	99.4%
Family history of breast cancer	yes	38	22.4%
	No	130	77.6%
History of alcohol drinking	yes	23	13.7
	No	145	86.3

4.2. clinical pathological characteristics of the study participants.

Molecular receptors status of patients was distributed as 54% (91) had ER receptor positive,43% (73) were positive for Progesterone receptors with similar observation was noted on Her 2 enriched receptor positive by 43% (73), While only 7.7% (13) had positive ki 67 with the ki 67

receptor positive more than 14 percent and only 1 had less than 14%, while majority 87% (155) of the patients Ki 67 was not done.

Invasive ductal carcinoma had high incidence 64% (107) among the participants while invasive lobular carcinoma was observed by 37% (61). Luminal A molecular subtypes was the most frequently diagnose in this study 32.7% (55), luminal B was a second prevalent from this study by 26.8%,triple negative 23.8% (40) and Her2 enriched was the least diagnosed16.1%(28) .Breast cancer grade II were the leading histological findings 38.1%(61) , then was followed by breast cancer grade III by 16.1% (27), grade I had 10% (18).However majority of patients tumor grade were not documented to their histology results accounted by 64% (59) of the total patients enrolled in the study.

Breast cancer clinical stage III was highly observed by 43% (73) followed by cancer stage IV 35.1% (59) while stage II and I were the least encountered by 16% (28) and 4.8% (8) respectively.

Locally advanced breast cancer was leading by 72% (121), followed by metastatic breast cancer 17.3% (29) and early breast cancer was less encountered by 4.8% (8). T4 disease was highly observed in the study 64.9% (109), this was followed by T3 by 25% (42) while Tx 1.8% (3) and T1 had 0.6% (1).

Table 2: Clinical pathological characteristics of the study participants.

variable		N	P(%)
Estrogen receptor	positive	90	54
	negative	78	46
Progesterone receptor	positive	73	43
	negative	95	57
Her 2 enriched receptor	positive	73	43
	negative	96	57
Ki67	Done	13	7.7
	Not done	155	87
Molecular subtype	luminal A	55	32.7
	luminal B	45	26.8
	Her	28	16.1
	triple negatives	40	23.8
Histological type	invasive ductal carcinoma	107	64
	invasive lobular carcinoma	61	37
Grade	I	18	10.7
	II	64	38.1
	III	27	16.1
	Not documented	59	64.9
Clinical stage	I	8	4.8
	II	28	16.7
	III	73	43.5
	IV	59	35.1
Clinical stage classification	early BC	18	10.7
	local advanced breast	121	72.0
	metastatic breast cancer	29	17.3
T status	T1	1	0.6
	T2	13	7.7
	T3	42	25.0
	T4	109	64.9
	TX	3	1.8

4.3. Clinical pathological characteristic among breast cancer molecular subtypes.

Invasive ductal carcinoma was prevalent among all molecular subtypes.T4 disease was prevalent among studied population as well as among all molecular subtype, where patient with Luminal A 72% (40) had T4 disease, those patient with luminal B as well T4 disease was mostly T status diagnosed 55.6% (25), similar observation was noted among patient with Her 2 disease

63% (17), also about more than half of patients whom had triple negative disease had T4 disease 67.5% (27).

Stage three disease was prevalent among all molecular subtypes, among luminal A patient's clinical stage 3 accounted for 53.8% (28), luminal B had 40.9% (18) of stage 3, Her 2 enriched had 34.6% (9) and also stage 3 disease was leading among with triple negative subtypes by 32% (13).

Majority of the patient presented with late disease and locally advanced being the commonest presentation that cut across all molecular subtypes,78.8% (41),68.2% (30),57.7% (15) and 75% (30) were prevalent among patient with Luminal A, Luminal B, Her 2 and Triple negative respectively.

Grade II histological result was the commonest among the studied patient, as well was the leading diagnosis among each molecular subtypes by 57.1% (20), 51.9% (14),64.7(11) and 62.1(18) among Luminal A, luminal B, Her 2 enriched and triple negative respectively.

Table 3: pathological characteristics of breast cancer molecular subtypes.

		Immune Histochemistry Class			
		luminal A ***n, (%)***	**luminal B** ***n, (%)***	**Her-2** ***n, (%)***	**triple negatives** ***n, (%)***
Histological type	invasive ductal carcinoma	31 (57.4)	27(60.0)	18(66.7)	29(72.5)
	invasive lobular carcinoma	23(42.6)	18(40.0)	9(33.3)	11(27.5)
T status	1	0(0.0)	1(2.2)	0(0.0)	0(0.0)
	2	3(5.5)	6(13.3)	1(3.7)	3(7.5)
	3	8(14.5)	12(26.7)	7(25.9)	10(25.0)
	4	40(72.7)	25(55.6)	17(63.0)	27(67.5)
	X	1(1.8)	0(0.0)	1(3.7)	0(0.0)
	Not documented	3 (5.5)	1 (2.2)	1 (3.7)	0 (0.0)
Stage of the disease	I	3(5.8)	3(6.8)	1(3.8)	1(2.5)
	II	4(7.7)	12(25.0)	4(15.4)	8(20.0)
	III	33(53.8)	18(40.9)	9(34.6)	13(32.5)
	IV	17(32.7)	12(27.3)	12(46.2)	18(45.0)
Stage classification	early breast cancer	4(7.7)	9(18.2)	3(11.5)	2(5.0)
	local advanced breast cancer	46(78.8)	30(68.2)	15(57.7)	30(75.0)
	metastatic breast cancer	7(13.5)	6(13.6)	8(30.8)	8(20.0)
Pathological Classification	I	6(17.1)	7(25.9)	2(11.8)	3(10.3)
	II	20(57.1)	14(51.9)	11(64.7)	18(62.1)
	III	9(25.7)	6(22.2)	4(23.5)	8(27.6)

4.3Association between breast cancer molecular subtypes and modifiable risk factor for breast cancer.

Strength of association between modifiable risk factor was computed by adjusted odds ratio from multinomial logistic regression with luminal A as reference group.

Luminal B molecular subtype had a protective effect compared with luminal A among patient who had history of alcohol use (P 0.04, AOR 0.22,95% CI 0.52-0.94).

Patient with history of contraceptive use were less likely to have luminal B subtypes compared with luminal A (P 0.028, AOR 0.36, 95%CI 0.149-0.895), study have shown that Patient with normal weight were likely to be luminal B than Luminal A. (P 0.031, AOR 3.66,95% CI 1.13-11.3)

Luminal B was more likely to develop compared with Luminal A among patients who had breastfed less than 3 children (OAR 1.22, P 0.66, 95% CI 0.49-3.0). Participants with history of being overweight were more likely to have Luminal B in comparison with luminal A (AOR 1.19, P 0.74, 95%CI 0.45-3.15),

Age at first full term pregnancy at 30 years or above were less likely to have luminal A compared with B (P 0.25, AOR 2.89, 95%CI 0.47-17). Women whom had age at menopause below 55 years were likely to develop her 2 enriched compared to luminal A, (P 0.223, AOR 1.61, 95% CI 0.7-2.12).

Her 2 enriched molecular subtype is not associated with modifiable risk factors for breast cancer whereby all modifiable risk factors were not statistically significant associated .Those who had history of alcohol use were unlikely to have Her 2 enriched subtypes compared to luminal A (p 0.24 ,AOR 0.37 , 95% CI 0.73-1.915), Participants with first term pregnancy at the age less than 30 years were less likely to be Luminal B than Luminal A but this observation was not statistically significant (P 0.95 ,AOR 0.941 , 95% CI 0.14-6.4),The study showed that women who had breastfed less than 3 children for more than 1 year have protection against Her 2 enriched subtype (p 0.24 ,AOR 0.54 , 95% CI 0.18-1.5). Being overweight was shown to be associated with higher risk for Her 2 enriched cancer than Luminal A breast cancer (p 0.62,

AOR 1.32, 95% CI 0.43-4.03). Also having history of overweight during puberty had protective effect on Her 2 molecular subtypes compared with patients with luminal A (p 0.95, AOR 0.968, 95% CI 0.32-12.89). Women who had been on contraceptive medication were less likely to have Her2 enriched breast cancer compared to Luminal A patients (p 0.398, AOR 0.065, 95% CI 0.24-1.77).

Triple negative molecular subtype had higher likely hood to develop compared to Luminal A for women who had their first full term pregnancy at the age less 30 years however the association was not statically significant (P 0.26, AOR 2.78 95% CI 0.46-6.1). Alcohol had shown protective effect on Triple negative subtype compared to Luminal A (P 0.192, AOR 0.43, P., CI 0.12-1.53).

Patient with history of contraceptive use were less like to have luminal B subtype compared with luminal A (P 0.73, AOR O.85,95% CI 0.35-2.1). In this study it has been observed that normal BMI were less likely to have triple negative than Luminal A (P 0.64, AOR 0. 84, 95%CI 0.31-2.3). Participants with history of being overweight had 1.02 odd to have triple negative than luminal A. (P 0.97, OAR 1.02, 95%CI 0.35-2.7).

Table 4 below is the summary of association between molecular subtypes and modifiable risk factors for breast cancer.

Table 4: Multinomial Logistic regression analysis of breast cancer molecular subtypes and modifiable risk factors.

Molecular subtypes	Variable	P_{adj}	AOR	95% Confidence Interval for Exp(B)	
				Lower Bound	Upper Bound
luminal B	**Age at first full term pregnancy**				
	<30 years	.254	2.892	.467	17.896
	≥30 years		Ref		
	Breast feeding				
	Breastfed <3 children	.662	1.222	.497	3.001
	Breastfed ≥3 children	.	**Ref**	.	.
	History of being overweight				
	Yes	.724	1.191	.451	3.149
	No	.	**Ref**	.	.
	History of alcohol drinking				
	Yes	**.041**	.222	.052	.943
	No	**.**	**Ref**	.	.
	History of contraceptive use				
	Yes	**.028**	.365	.149	.895
	No	.	**Ref**	.	.
	BMI				
	Normal	**.031**	3.662	1.130	11.874
	Overweight	.	**Ref**	.	.
Her 2 enriched	Age at first full term pregnancy				
	<30 years	.951	.941	.140	6.347
	≥30 years				
	Breast feeding				
	< 3 children	.261	.540	.184	1.581
	≥3 children	.	**Ref**	.	.
	History of being overweight				

	Yes	.953	.968	.324	2.890
	No	.	**Ref**	.	.
	History of alcohol consumption				
	Yes	.238	.374	.073	1.915
	No	.	**Ref**	.	.
	History of contraceptive use				
	Yes	.398	.648	.237	1.773
	No	.	**Ref**	.	.
	BMI				
	Normal	.627	1.319	.432	4.028
	Overweight	.	**Ref**	.	.
Triple negative	Age at first full term pregnancy				
	<30 years	.264	2.783	.461	16.788
	≥30 years				
	Breast feeding				
	< 3 children	.776	1.141	.460	2.826
	≥3 children	.	**Ref**	.	.
	History of being overweight				
	Yes	.974	1.016	.388	2.664
	No	.	**Ref**	.	.
	History of alcohol drinking				
	Yes	.192	.428	.120	1.533
	No	.	**Ref**	.	.
	History of contraceptive use				
	Yes	.737	.857	.349	2.105
	No	.	**Ref**	.	.
	BMI				
	Normal	.640	2.729	.381	4.812
	Overweight	.	**Ref**	.	.

4.4 Association between molecular subtypes and non-modifiable risk factors.

Luminal B molecular subtypes had increased risk compared with luminal A among patients who have attained menopause below the age at 55 years (P0.124, OAR 1.67,95%CI 0.16-1.7). Women who were diagnosed with breast cancer at the age less than 40 years were less likely to have luminal B in comparison with Luminal A (P 0.82, OAR 0.7195% 0.36-1.4).

Luminal B molecular subtype had high like hood to develop than luminal A breast cancer among patients with the age at menarche more than 12 years. (P 0.884, OAR 1.147,95%CI 0.18-7). Among patients who had familial history of breast cancer in first degree relative had increased likely hood of developing Lumina B compared to patients with luminal A (P 0.37, AOR 1.579, 95% CI 0.40-4.79).

Her2 enriched had shown that age at menarche above 12 years was associated with decreased risk of Her 2 enriched compared to Luminal A (P 0.014, OAR 0.147,95% CI 0.32-68).

Women with familial history of breast cancer in the first degree relative were highly like to be of Her 2 molecular subtypes than luminal A, however this observation was non statistically significant (P 0.60, OAR 1.844,95%CI 0.40-4.8). Her 2 enriched had higher likely hood compared with luminal A among postmenopausal women who attained menopause at the age below 55 years (P 0.462, OAR 11,23,95%CI 0.71-2.12).

The age at diagnosis below 40 years was associated with increased risk of developing Her 2 enriched than Luminal A patients, but this finding was not significant (P 0.134, OAR 3.02,95%CI 0.7-12.8).

This study has revealed that the age at menarche above 12 years have protective effect on developing Triple negative molecular subtype compared to the Luminal A (P 0.13, AOR 0.167, 95%CI ,0.41-0.69).

Triple negative subtype had higher likely hood to develop compared to Luminal A among Patients with the age at diagnosis below 40 years, however, the observed association was not statistically significant. (P 0.285, OAR 1.84, 95%C 0.60-5.66),

Post menopausal women who attained menopause at the age below 55 had increased risk of having triple negative subtype compared women with luminal A subtype (P 0.103, AOR 1.480,95% CI 0.92-2.4).

Table 5: Multinomial Logistic regression analysis of breast cancer molecular subtypes and non-modifiable risk factors

Breast cancer molecular subtypes.			**P-value**	**Odds ratio**	**95% Confidence Interval for odds ratio.**	
					Lower Bound	**Upper Bound**
	Age at menopause	< 55		1.617	1.617	1.617
Luminal B			0.123			
		≥55		Ref		
	Age at diagnosis	>40	.822	.710	.036	14.077
		≥40				
				Ref		
	Age at menarche	≥12 years	.884	1.147	.181	7.255
		< 12 years		Ref		
	Familial hx of BC					
		Yes	.374	1.574	.579	4.276
		No		Ref		
Her-2	Age at menopause	< 55	.462	1.227	.711	2.117
		≥55			.	.
				Ref.		
	Age at diagnosis	< 40	.134	3.022	.712	12.831
		> 40	.	Ref.	.	.
	Familial hx of BC					
		Yes	.602	1.391	.403	4.798
		No				
	Age at menarche	>12	**.014**	.147	.032	.679
		< 12	.	ref	.	
Triple negative.	Age at diagnosis	< 40	.285	1.844	.601	5.656
		> 40	.	.	.	.
	Age at menarche	≥12 years	**.013**	.167	.041	.689
		< 12 years	.	.	.	.
	Age at menopause	< 55	.103	1.480	.923	2.374
		≥55		Ref		

CHAPTER FIVE

5.0 DISCUSION

This study had shown that invasive ductal carcinoma had higher incidence 64% (107) among the participants while invasive lobular carcinoma was observed by 37% (61). Similar observation was encountered from study conducted in Brazil showed that invasive ductal carcinoma is the most frequently histological type ,accounted by 84.7% of cases, also similar findings were revealed from studies done in South America and Malaysia, as well as comparative study from Iraq and British,(27),(28) .In Africa ,invasive ductal carcinoma is a leading diagnosed breast cancer was revealed from different studies (29)(20).

T4 disease was prevalent among study participants as well as among all molecular subtypes this findings were inconsistence with a study conducted from Brazil and Rwanda shows that majority of patient were diagnosed with T status higher than T2 , this observations were consistent with the studies conducted in South America and India (27)(28)(38)(33)(35)(35), inconsistence observation was noted from East Africa ,that majority of patients about 90% had tumor size of more than 2cm and T2 status was the leading tumor status, compared with higher T status these were revealed from a study conducted in Kenya (19). Also study from Turkey revealed T1 status was frequently encountered (34).These differences arisen due to different health care seeking behavior , knowledge and availability of screening programs.

Grade II histological result was the commonest among the studied patients, as well it was the leading diagnosis among each molecular subtypes ,similar observation had shown that Luminal B , Her 2 enriched and Tripple negative had histological grade II prevalent ,also similar findings were found in different studies done in Brazil, Iraq, and Asia(30), (32)(9)(37).In Africa a study conducted in Kenya has shown HER2/neu and TNBC associated with higher tumor grade where by majority presented with grade three ,with similar observation was made that HER2/neu and TNBC present with poor prognosis from other African countries (30)(36).

Majority of the patients presented with late disease and locally advanced being the commonest presentation that cut across all molecular subtypes while from Turkish study had shown that

most Turkish women with localized/early diseases had luminal A, while locally advanced diseases were luminal B and HER2/neu (29) (30).

In this study it has been found that luminal B had protective effect compared with Luminal A among breast cancer patients with history of alcohol consumption , this observation was statically significant with P value of 0.04 , inconsistence findings were revealed from study conducted in Norway America and Europe, they have shown women who take alcohol had increased chance of developing luminal B breast cancer (33), (47)(48) (47).This inconsistences had arisen due to they differ in ethnicity.

Body mass index was associated with luminal B , study have shown that Patient with normal weight were likely to be luminal B than Luminal A .This observation was consistence with the conducted from Europe ,revealed increased body mass index (BMI) have shown to increase risk of luminal A than luminal B as per review of nine cohorts studies in European countries(33),also in East Asian women with obesity and overweight had increased risk for luminal subtypes especially luminal A (45).On other hand, inconsistence was shown from the study done in China, that shows high body mass index(BMI) in premenopausal has protective effect on luminal A subtypes than B(46).

In this study we found contraceptive use, familial history of breast cancer in the first degree relative and age at menarche above 12 years they had positive association with luminal B however these observations were not statistically significant. However observation from Italy and Europe had shown that the age at menarche did not affect luminal B breast cancers in cohort study (33)(49), also similar observation was pinpointed out from study done in Uganda where by early age at menarche was not associated with luminal subtype (19).

The age at first full term pregnancy is not associated with luminal B in this study, this findings was consistent with cohort study that the age at first full-term pregnancy did not affect luminal B breast cancers in (49), However different results was noted from the study done in Europe as female with a first complete term pregnancy at old age were more likely to develop luminal B subtypes BC (33) and the results from East African study from Tanzania revealed that women who had first term pregnancy under 30 years of age were more likely to have luminal B

molecular subtype (6) However findings from this study was not statistically significant , this can be explain due to small sample size of the study.

Her2 enriched from this study had shown that age at menarche above 12 years was associated with decreased risk of Her 2 enriched compared to Luminal A (P 0.014 OAR 0.14795% CI 0.32-68).This observation was consistence with European study**,** early age at menarche is positively related with increased risk of HER2/neu subtype (42), However study done in China have revealed different findings from this study that Women who attained menarche at older age was not associated with decreased risk of HER2/neu compared to luminal A molecular subtypes (41)(18).a

Body mass index, history of breast cancer in a family , history of contraceptive use, age at diagnosis ,breast feeding , age at first full term pregnancy and alcohol use were not stastically significant associated with Her2 enriched breast cancer molecular subtype , this was congruent with the study done from Norway and Uganda (47)(44). Contrary findings from European and American studies had revealed that women who ever drunk alcohol , had increased risk of having luminal A subtype and luminal B subtype other than other subtypes (33),(47) also in study from Kenya shows postmenopausal women with high body mass index (BMI) were related with increased risk for developing HER2/neu subtype, this was inconsistent with the study conducted from West Africa in Nigeria, where by obese patients were less likely to have Her 2 molecular subtype (25)(24).Inconsistence observations from this study regarding Her 2 enriched molecular subtypes with the risk factors for breast cancer was due to geographical and ethnicity differences.

This study has revealed that age at menarche above 12 years has protective effect on developing Triple negative molecular subtype compared to the Luminal A (P 0.013,AOR 0.167, 95%CI ,0.41-0.69), this observation was keeping in line with the study from North America and Asia that early age at menarche less than 13 years ,has positive association with TNCB ,(55)(45)(54).On other hand , different observation was revealed inversely association of age at menarche and risk for TNBC from review study (42). On other hand inconsistent results were

found from Asia and America reveals no association between the age at menarche and age first term pregnancy with TNCB(18).

Triple negative has not shown significant association with modifiable risk factor for breast cancer such as, history of alcohol use, history of contraceptive use, age at first term

Pregnancy, body mass index, parity and physical exercise. These findings from this study was inconsistence with the observations from America and Asia, study revealed obesity and overweight is positively associated with increased risks for developing triple negative subtype ,(13)(30-32), similar findings was also observed in Scotland that obesity in premenopausal women associates with high chance to develop TNCB(13) and observation from West African women where by high body mass index were significantly associated with increased risk for triple negative cancer (24)(25).

From this study alcohol had no association with Triple negative breast cancer , similar observation was seen in Norway and USA studies that Alcohol intake is not associated with TNCB ,as well as other molecular subtypes, that there is no statistically significant between alcohol and ER negative breast cancer (47,48),Different observations from this study was spotted by the study done in European women (33),(54).

The age at diagnosis had no statistically significance with triple negative subtype from this study, contrary result was revealed that Triple negative molecular subtype was prevalent diagnosed at the early age or among youth population (30-32).

Furthermore this study showed no association between triple negative and history of breast cancer in the family while study from Europe ,shown that history of breast cancer in family is associated with increased risk for all BC subtypes with highly association with TNBC(54),(33).

Age at first full term pregnancy had no association with TNBC from this study was different from the study done in United kingdom had revealed that TNBC cases were more likely to have a younger age at first birth and high parity when compared to luminal A-like cases (56) ,Moreover the findings was consistent with the study from Africa America and Hispanic origin

women (54). In addition to that Asia and America had reveals no association between age of menarche and age at the first term pregnancy with TNCB(18).

Result from this study had failed to reveal association between TNBC and breast feeding , this was out of phase with the observation from American and Asian women, whom breastfed at least six month have lower risk of developing TNBC (54),also a review of 11 studies reveal similar observation that breast feeding have decreased risk for developing TNBC (42).

CHAPTER SIX

6.0; LIMITATIONS, CONCLUSION AND RECOMMENDATIONS.

6.1 Study limitations

Recall bias was noted from study participants where by remote information were not able to recall.

Being a single centered study, result from this study cannot be generalized to the general population

Rarely risk factors were not captured among enrolled participants due to small sample size.

Ki 67 was not used on differentiating between Luminal A and Luminal B molecular subtypes,

6.2 Conclusion.

Alcohol intake and history of contraceptive use had shown to have protective effect on luminal B compared to luminal A, while Body mass index had positive association with luminal B, however other modifiable risk factors were not associated with breast cancer molecular subtypes.

Age at menarche more 12 years has protective effect on Tripple negative and Her 2 molecular subtypes while other non- modifiable risk factors were not associated with breast cancer molecular subtypes.

6.3 Recommendations

Large sample size and multicenter study may help in addressing inconsistencies and risk factors with less prevalence.

Addressing one molecular subtype will increases the accuracy of ascertaining the association with risk factors.

Improvement in investigating patient with breast cancer especially KI 67 will help in subtyping luminal A and Luminal B breast cancers.

REFERRENCES;

1. Sharma R. Breast cancer burden in Africa: evidence from GLOBOCAN 2018. Public Heal | [Internet]. 2020;43(4):763–71. Available from: https://doi.org/10.1093/pubmed/fdaa099

2. Sharma R. Breast cancer burden in Africa: evidence from GLOBOCAN 2018. J Public Health (Oxf) [Internet]. 2021 Dec 1 [cited 2022 Oct 13];43(4):763–71. Available from: https://pubmed.ncbi.nlm.nih.gov/32657321/

3. Anyigba CA, Awandare GA, Paemka L. Minireview Breast cancer in sub-Saharan Africa: The current state and uncertain future. Exp Biol Med. 2021;246:1377–87.

4. Ezeome ER, Yawe KDT, Ayandipo O, Badejo O, Adebamowo SN, Achusi B, et al. The African Female Breast Cancer Epidemiology Study Protocol. Front Oncol. 2022 Apr 13;12:1291.

5. Mansouri H, R. Mwakigonja A, A. Rodgers , Faith, A. Mpolya E, Elingarami S. A Review on the Status of Breast Cancer Care in Tanzania. Multidiscip Cancer Investig [Internet]. 2020 Jul 1 [cited 2022 Oct 14];4(3):5–14. Available from: http://mcijournal.com/article-1-267-en.html

6. Rweyemamu LP, Akan G, Adolf IC, Magorosa EP, Mosha IJ, Dharsee N, et al. The distribution of reproductive risk factors disclosed the heterogeneity of receptor-defined breast cancer subtypes among Tanzanian women. BMC Womens Health [Internet]. 2021;21(1):1–13. Available from: https://doi.org/10.1186/s12905-021-01536-6

7. Turkoz FP, Solak M, Petekkaya I, Keskin O, Kertmen N, Sarici F, et al. Association between common risk factors and molecular subtypes in breast cancer patients. Breast [Internet]. 2013 [cited 2022 Oct 18];22(3):344–50. Available from: https://pubmed.ncbi.nlm.nih.gov/22981738/

8. Al-thoubaity FK. Molecular classification of breast cancer: A retrospective cohort study. Ann Med Surg [Internet]. 2020 Jan 1 [cited 2022 Oct 18];49:44. Available from:

/pmc/articles/PMC6926136/

9. Sun N, Gao P, Li Y, Yan Z, Peng Z, Zhang Y, et al. Screening and Identification of Key Common and Specific Genes and Their Prognostic Roles in Different Molecular Subtypes of Breast Cancer. Front Mol Biosci. 2021 Feb 11;8:15.

10. Rouzier R, Perou CM, Symmans WF, Ibrahim N, Cristofanilli M, Anderson K, et al. Breast Cancer Molecular Subtypes Respond Differently to Preoperative Chemotherapy. Clin Cancer Res [Internet]. 2005 Aug 15 [cited 2022 Oct 18];11(16):5678–85. Available from: https://aacrjournals.org/clincancerres/article/11/16/5678/185584/Breast-Cancer-Molecular-Subtypes-Respond

11. Eliyatkin N, Yalcin E, Zengel B, Aktaş S, Vardar E. Molecular Classification of Breast Carcinoma: From Traditional, Old-Fashioned Way to A New Age, and A New Way. J Breast Heal. 2015;11(2):59–66.

12. Mohammed AA. The clinical behavior of different molecular subtypes of breast cancer. Cancer Treat Res Commun [Internet]. 2021 Jan 1 [cited 2022 Oct 18];29. Available from: https://pubmed.ncbi.nlm.nih.gov/34624832/

13. Turkoz FP, Solak M, Petekkaya I, Keskin O, Kertmen N, Sarici F, et al. Association between common risk factors and molecular subtypes in breast cancer patients. The Breast. 2013 Jun 1;22(3):344–50.

14. Łukasiewicz S, Czeczelewski M, Forma A, Baj J, Sitarz R, Stanisławek A. Breast Cancer—Epidemiology, Risk Factors, Classification, Prognostic Markers, and Current Treatment Strategies—An Updated Review. Cancers (Basel) [Internet]. 2021 Sep 1 [cited 2022 Oct 18];13(17). Available from: /pmc/articles/PMC8428369/

15. Pandit P, Patil R, Palwe V, Gandhe S, Patil R, Nagarkar R. Prevalence of Molecular Subtypes of Breast Cancer: A Single Institutional Experience of 2062 Patients. Eur J Breast Heal. 2020;16(1):39–43.

16. Yin L, Duan JJ, Bian XW, Yu SC. Triple-negative breast cancer molecular subtyping and treatment progress. Breast Cancer Res [Internet]. 2020 Jun 9 [cited 2022 Oct 18];22(1):1–13. Available from: https://breast-cancer-research.biomedcentral.com/articles/10.1186/s13058-020-01296-5

17. Mccarthy AM, Friebel-Klingner T, Ehsan S, Welch M, Chen J, Kontos D, et al. Relationship of established risk factors with breast cancer subtypes. 2021;

18. Brouckaert O, Rudolph A, Laenen A, Keeman R, Bolla MK, Wang Q, et al. Reproductive profiles and risk of breast cancer subtypes: A multi-center case-only study. Breast Cancer Res [Internet]. 2017 Nov 7 [cited 2022 Oct 20];19(1):1–12. Available from: https://breast-cancer-research.biomedcentral.com/articles/10.1186/s13058-017-0909-3

19. Sayed S, Fan S, Moloo Z, Wasike R, Bird P, Saleh M, et al. Breast cancer risk factors in relation to molecular subtypes in breast cancer patients from Kenya. Available from: https://doi.org/10.1186/s13058-021-01446-3

20. Onyango L, Id A, Rutashobya AK, Lutainulwa EW, Mwanga AH, Kivuyo SL. The effect of reproductive, hormonal, nutritional and lifestyle on breast cancer risk among black Tanzanian women: A case control study. 2022; Available from: https://doi.org/10.1371/journal.pone.0263374

21. Galukande M, Wabinga H, Mirembe F, Karamagi C, Asea A. Difference in Risk Factors for Breast Cancer by ER Status in an Indigenous African Population. Artic ID [Internet]. 2013;2013. Available from: http://dx.

22. Palmer JR, Viscidi E, Troester MA, Hong CC, Schedin P, Bethea TN, et al. Parity, lactation, and breast cancer subtypes in African American Women: Results from the AMBER Consortium. J Natl Cancer Inst [Internet]. 2014;106(10). Available from: https://academic.oup.com/jnci/article/106/10/dju237/927797

23. Tamimi RM, Colditz GA, Hazra A, Baer HJ, Hankinson SE, Rosner B, et al. Traditional Breast Cancer Risk Factors in Relation to Molecular Subtypes of Breast Cancer.

24. Islam T, Matsuo K, Ito H, Hosono S, Watanabe M, Iwata H, et al. Reproductive and hormonal risk factors for luminal, HER2-overexpressing, and triple-negative breast cancer in Japanese women. Ann Oncol [Internet]. 2012 Sep 1 [cited 2022 Oct 5];23(9):2435–41. Available from: http://www.annalsofoncology.org/article/S0923753419351841/fulltext

25. Akinyemiju T, Jones K, Gupta A, Oyekunle T, Saraiya V, Deveaux A, et al. Association of body composition with odds of breast cancer by molecular subtype: analysis of the Mechanisms for Established and Novel Risk Factors for Breast Cancer in Nigerian Women (MEND) study. BMC Cancer. 2021 Dec 1;21(1).

26. Islam T, Matsuo K, Ito H, Hosono S, Watanabe M, Iwata H, et al. Reproductive and hormonal risk factors for luminal, HER2-overexpressing, and triple-negative breast cancer in Japanese women. Ann Oncol [Internet]. 2012 Sep 1 [cited 2022 Oct 18];23(9):2435–41. Available from: http://www.annalsofoncology.org/article/S0923753419351841/fulltext

27. de Almeida LM, Cortés S, Vilensky M, Valenzuela O, Cortes-Sanabria L, de Souza M, et al. Socioeconomic, Clinical, and Molecular Features of Breast Cancer Influence Overall Survival of Latin American Women. Front Oncol. 2022;12(March):1–15.

28. Pan JW, Zabidi MMA, Ng PS, Meng MY, Hasan SN, Sandey B, et al. The molecular landscape of Asian breast cancers reveals clinically relevant population-specific differences. Nat Commun [Internet]. 2020;11(1):1–12. Available from: http://dx.doi.org/10.1038/s41467-020-20173-5

29. National Association of Resident Doctors of Nigeria. Nigerian Journal of Medicine. Med J. 2015;24(1):17–27.

30. Llanos AAM, Chandwani S, Bandera E V, Hirshfield KM, Lin Y, Ambrosone CB, et al. Cancer Causes Control. 2015;26(12):1737–50.

31. Almeida AP, Reis M, Miranda De Sousa Teixeira C, Lima De Medeiros AR, Zolinda K,

Chaves C, et al. Sociodemographic and Clinical-pathological Study of Molecular Subtitles of Breast Carcinoma in a Reference Unit of Maranhão Estudo sociodemográfico e clínico-patológico de legendas moleculares de carcinoma de mama em uma unidade de referência do Maranhão. Rev Bras Ginecol Obs [Internet]. 2020 [cited 2023 Mar 14];42(12):820–8. Available from: https://doi.org/

32. Su Y, Zheng Y, Zheng W, Gu K, Chen Z, Li G, et al. Distinct distribution and prognostic significance of molecular subtypes of breast cancer in Chinese women: A population-based cohort study. BMC Cancer. 2011;11.

33. Morra A, Jung AY, Behrens S, Keeman R, Ahearn TU, Anton-Culver H, et al. Breast cancer risk factors and survival by tumor subtype: Pooled analyses from the breast cancer association consortium. Cancer Epidemiol Biomarkers Prev [Internet]. 2021 Apr 1 [cited 2022 Oct 20];30(4):623–42. Available from: https://aacrjournals.org/cebp/article/30/4/623/670709/Breast-Cancer-Risk-Factors-and-Survival-by-Tumor

34. Dokcu S, Caparlar MA, Başçeken Sİ, Eroglu A. Distribution and Clinicopathological Characteristics of Breast Cancer Molecular Subtypes in Turkish Women. Eur J Clin Med. 2022;3(6):14–20.

35. Ntirenganya AF, Twagirumukiza JD, Bucyibaruta G, Byiringiro F, Rugwizangoga B. Predictors of molecular subtypes in women with breast cancer in. 2022;79(4):65–77.

36. Mbchb GSM, Sayed S, Chauhan R, Fcps SP, Aga A. Gakinya et al.,2010_BC molecular subtypes & clinocopathological xristics at AKUH,N. 2010;5(January):19–24.

37. Alwan NAS, Tawfeeq FN, Muallah FH. Breast Cancer Subtypes among Iraqi Patients: Identified By Their ER, PR and HER2 Status. J Fac Med Baghdad. 2018;59(4):303–7.

38. Mugada V. Association between sociodemographic factors and estrogen receptor-positive, progesterone receptor-positive breast cancer subtypes: A cross-sectional study. Makara J Heal Res. 2020;24(2).

39. Nabi MG, Ahangar A, Wahid MA, Kuchay S. Clinicopathological comparison of triple negative breast cancers with non-triple negative breast cancers in a hospital in North India. Niger J Clin Pract. 2015;18(3):381–6.

40. Popli P, Gutterman EM, Omene C, Ganesan S, Mills D, Marlink R. Receptor-Defined Breast Cancer in Five East African Countries and Its Implications for Treatment: Systematic Review and Meta-Analysis. JCO Glob Oncol. 2021 Dec 16;(7):289–301.

41. Yang XR, Sherman ME, Rimm DL, Lissowska J, Brinton LA, Peplonska B, et al. Differences in Risk Factors for Breast Cancer Molecular Subtypes in a Population-Based Study. Cancer Epidemiol Biomarkers Prev [Internet]. 2007 Mar 1 [cited 2022 Oct 18];16(3):439–43. Available from: https://aacrjournals.org/cebp/article/16/3/439/260456/Differences-in-Risk-Factors-for-Breast-Cancer

42. Anderson KN, Schwab RB, Elena Martinez M, Elena Martínez M. Reproductive Risk Factors and Breast Cancer Subtypes: A Review of the Literature. Breast Cancer Res Treat. 2014;144(1):1–10.

43. Anderson WF, Rosenberg PS, Prat A, Perou CM, Sherman ME. How Many Etiological Subtypes of Breast Cancer: Two, Three, Four, Or More? JNCI J Natl Cancer Inst [Internet]. 2014 Aug 1 [cited 2022 Oct 18];106(8). Available from: https://academic.oup.com/jnci/article/106/8/dju165/910468

44. Galukande M, Wabinga H, Mirembe F, Karamagi C, Asea A. Difference in Risk Factors for Breast Cancer by ER Status in an Indigenous African Population. ISRN Oncol. 2013 Jun 25;2013:1–5.

45. Zhang L, Huang Y, Feng Z, Wang X, Li H, Song F, et al. Comparison of breast cancer risk factors among molecular subtypes: A case-only study. Cancer Med [Internet]. 2019 Apr 1 [cited 2022 Oct 1];8(4):1882–92. Available from: https://onlinelibrary.wiley.com/doi/full/10.1002/cam4.2012

46. Yang XR, Sherman ME, Rimm DL, Lissowska J, Brinton LA, Peplonska B, et al. Differences in Risk Factors for Breast Cancer Molecular Subtypes in a Population-Based Study. Cancer Epidemiol Biomarkers Prev [Internet]. 2007 Mar 1 [cited 2022 Oct 1];16(3):439–43. Available from: https://aacrjournals.org/cebp/article/16/3/439/260456/Differences-in-Risk-Factors-for-Breast-Cancer

47. Ellingjord-Dale M, Vos L, Hjerkind KV, Hjartåker A, Russnes HG, Tretli S, et al. Alcohol, physical activity, smoking, and breast cancer subtypes in a large, nested case–control study from the Norwegian Breast Cancer Screening Program. Cancer Epidemiol Biomarkers Prev. 2017;26(12):1736–44.

48. Carey LA, Perou CM, Livasy CA, Dressler LG. in the Carolina Breast Cancer Study. Jama. 2010;29(1):25–32.

49. Giudici F, Scaggiante B, Scomersi S, Bortul M, Tonutti M, Zanconati F. Breastfeeding: A reproductive factor able to reduce the risk of luminal B breast cancer in premenopausal White women. Eur J Cancer Prev [Internet]. 2017 [cited 2022 Dec 26];26(3):217–24. Available from: https://journals.lww.com/eurjcancerprev/Fulltext/2017/05000/Breastfeeding__a_reproductive_factor_able_to.6.aspx

50. He Y, Si Y, Li X, Hong J, Yu C, He N. The relationship between tobacco and breast cancer incidence: A systematic review and meta-analysis of observational studies. Front Oncol. 2022;12(September):1–18.

51. Jahshan A, Aoun M, Dekhne N, Taranikanti V, Ph D. Relationship Between Smoking and Triple-Negative Breast Cancer : A Retrospective Analysis. :3.

52. Goldvaser H, Gal O, Rizel S, Hendler D, Neiman V, Shochat T, et al. The association between smoking and breast cancer characteristics and outcome. BMC Cancer.

2017;17(1):1–8.

53. Barańska A, Dolar-Szczasny J, Kanadys W, Kinik W, Ceglarska D, Religioni U, et al. Oral Contraceptive Use and Breast Cancer Risk According to Molecular Subtypes Status: A Systematic Review and Meta-Analysis of Case-Control Studies. Cancers (Basel) [Internet]. 2022;14(3). Available from: https://doi.org/10.3390/cancers14030574

54. Chen L, Li CI, Tang MTC, Porter P, Hill DA, Wiggins CL, et al. Reproductive factors and risk of luminal, HER2-overexpressing, and triple-negative breast cancer among multiethnic women. Cancer Epidemiol Biomarkers Prev. 2016 Sep 1;25(9):1297–304.

55. Romieu I, Biessy C, Carayol M, His M, Torres-Mejía G, Ángeles-Llerenas A, et al. Reproductive factors and molecular subtypes of breast cancer among premenopausal women in Latin America: the PRECAMA study On behalf of the PRECAMA team*. Available from: www.nature.com/scientificreports/

56. Chitkara A, Mesa-Eguiagaray I, Wild SH, Hall PS, Cameron DA, Sims AH, et al. Reproductive history differs by molecular subtypes of breast cancer among women aged ≤ 50 years in Scotland diagnosed 2009–2016: a cross-sectional study. Breast Cancer Res Treat [Internet]. 2022 Sep 18 [cited 2022 Oct 20];196(2):379–87. Available from: https://link.springer.com/article/10.1007/s10549-022-06721-1

57. Butler EN, Tse C-K, Elizabeth Bell M, Conway K, Olshan AF, Troester MA. Cancer Causes Control. 2016;27(6):775–86.

58. Bigman G, Adebamowo SN, Yawe KDT, Yilkudi M, Olaomi O, Badejo O, et al. Leisure-time physical activity is associated with reduced risks of breast cancer and triple negative breast cancer in Nigerian women. Cancer Epidemiol. 2022 Aug 1;79:102195.

APPENDIXI:

ENGLISH VISION QUESTIONNARE

Interview parameters

1. Questionnaire number……...

2. Date of interview …….

3. Name of interviewer……...

4. Name of ward/ clinic ……

5. Patient file number……

6. Patient histology result ……..

7.PatientImmunohistochemistiry result……

8. Patient clinical stage and grade……

Respondent characteristics

1. In which date, month and year were you born? ……..Age (years)

2. What is the highest level of education you attained?

   A No formal education

   B. Primary education

   C. Secondary education (O-level)

   D. Advanced secondary education(A-level)/college(certificate)

   E. Diploma/Advanced diploma

   F. University (degree/masters/PHD etc)

3. Place of Residence

   A .region …….

   B. Town /village ……..

4. What is your occupation?
   A. Agriculture / Farming
   B. Business / Trade
   C. Housewife
   D. Civil servant
   E. Other (Specify)……

5. What is your marital status?
   A. Single
   B. Married
   C. Cohabiting
   D. Widow
   E. Divorced/Separated

6 .How old were you whenmenstral flow start/ you began to menstruate? .....

7. Was your menses regular at 18 years of age?
   A. Yes
   B. No

8. Do you still have menses?
   A. Yes
   B. No

9. If no, how did menopause occur? .....

10. How old were you when menopause occurred? …….

11. Have you used hormonal therapy drugs during menopause ?

    A. Yes

    B. No

12. How long did you use the following medicines during menopause?

    A. ……..Months 49

    B. …….years 50

13. Have you ever used following medicines?

    A. Contraceptives

        i. Yes

        ii. No

    B. Hormones for treatment of infertility

        i. Yes

        ii. No

Pregnancy, parity and breastfeeding

14. Have you ever been pregnant?

    A. Yes

    B. No

15. If YES, how many times? ..... (Include live birth, still birth, miscarriages and abortions)

16. How old were you, when you were pregnant for the first time? ……..

17. How many times have you given birth? ..... (include still births after 8th month)

18. How old were you when you gave birth for the first baby? ..... (Include still birth after 8th month)

19. Did you breast-feed?

A. Yes (Go to question 21)

B. No (Go to question 23)

20. If YES, how many children? ……..

21. How long did you breast-feed, including mixed feeding?

A. 1st baby ……..

B. 2nd baby ……....

C. 3rd baby …….

D. others specify …….

22. History of breast lumps/ surgeries

A. Yes

B. No

23. History of obesity during adolescence

A. Yes

B. No

24. there is any one who have or had been diagnosed with breast cancer in family

A. No (If unsure or could not remember)

B. Yes (Mention the relationship with the patient)

i. Father

ii. Mother

iii. Siblings

iv. Grandmother / Grandfather

v. Other relative ……

Smoking status

25. Do you smoke/ have you ever smoked cigarette in the past?

A. Yes

B. No (Go to question 29)

26. If yes, How old were you when you started to smoke? .....

27. How many cigarettes do you smoke a day? …….

28. For howlong have /had you smoked cigarrate……

28. Did / does anyone smoke inside your living apartment?

A. Yes

B. No (Go to question 32)

29. How long does/did someone inside your apartment smoke...

A. …….Months

B. …….Years

30. How many hours per day do/did you spend in smoke filled apartment...

31. Does/ did someone smoke at your work place? ...

A. Yes

B. No

32. How long do you work at smoke filled work place? .....

Alcohol use

33. Do/did you drink alcoholic beverages?

A. Yes ……

B. No

34. How old were you when you started drinking habitually? .....

Physical activity status.

35. Do you exercise ?

A.Yes

B. No

36. For how long do you exercise per week?

A. less than 3hour per week

B. more than 3hours per week

Body mass index

37. Weight (kg) of respondent ……

38. Height of respondent ……..

39. Have you ever received radiation therapy involving the chest ?

A. yes specify ,how old were are you …..

B. No

APPENDIX II

SWAHILI VISION QUESTIONNAIRE

I. Taarifa za mtafiti

1. Namba ya dodoso…….....

2. Tarehe ya mahojiano ……

3. Jina la mtafiti……

4. Jina la wodi…….

5. Namba ya mgonjwa…….

6. Majibu ya kipimo a)Kansa b) Hana kansa…(Aina ya kansa……)

7.Majibu ya immune histochemisrty ……..

II. Taarifa binafsi za mshiriki

1.Ulizaliwa tarehe ngapi? Siku…. / mwezi…/Mwaka (Jaza umri kwa 12 miaka) …….

2. Unakiwango gani cha elimu?

A. Sikuwahi kwenda shule

B. Elimu ya msingi

C. Elimu ya sekondari (kidato cha nne)

D. Elimu ya sekondari (kidato cha sita)

E. Chuo ngazi ya Stashahada / chuongaziyacheti

F. Chuo kikuu (Shahada/uzamili/uzamivu)

3. Unaishi wapi?

A.mkoa…….

B.Jijini/ Mjini / Kijijini

4. Unajishughuli shananini?

A. Kilimo

B. Biashara

C. Mama wanyumbani

D. Mwajiriwa

E. Nyinginezo (Taja)……

5. Hali yandoa

A. Sijaolewa

B. Nimeolewa

C. Ninaishi na mwanaume ila hatujafunga ndoa

D. Mjane

E. Tumeachana/Tumetengana

6. Umeanza kupata hedhi ukiwa na umri wa miaka mingapi?.....….

7.Wakati unaumri wa miaka18,mzunguko wahedhi ulikuwa wakawaida ?

8. Bado unapata hedhi? ……

A. Ndio

B.Hapana

9. Kama hapana, ukomo wahedhi ulitokeaje?**.....**

10. Ulikuwa na umri gani ukomo wa hedhi ulipotokea……

11. Umewahi kutumia dawa zakuongeza vichocheo vyamwili vya kike kukinzana naukomo wahedhi?

A. Ndiyo

B. Hapana

12. Kama ndiyo umetumia kwa miaka mingapi?.....

13. Umewahi kutumia dawa zifuatazo?

A. Dawa za uzazi wa mpango

i. Ndiyo

ii. Hapana

B. Dawa za matibabu ya ugumba

i. Ndiyo

ii. Hapana

14. Umewahi kuwa mjamzito?

A. Ndiyo

B. Hapana ….nenda swali la 24

15. Kama ndiyo, marangapi?.....(inahusisha mimba zilizoharibika)

16. Ulikuwa naumri gani ulipokuwa mjamzito kwamaraya kwanza?.....

17. Ulijifungua? ……(inahusisha mimba Zaidi ya miezi 8)

A. Ndiyo

B. Hapana

18. Umejifungua marangapi?.....(inahusisha mimba zilizoharibika baada yamiezi 8)

19. Ulikuwa na umri gani ulipojifungua kwa mara ya kwanza?.....

20. Je, ulinyonyesha?

A. Ndiyo

B. Hapana

21. Uli /umenyonyesha watoto wangapi?.....

22. Umenyonyesha kwa muda gani? (inahusisha unyonyeshaji na chakula)

A. Mtoto wa kwanza ……..

B. Mtoto wa pili……

C. Mtoto watatu……

23. Unahistoria ya uvimbe kwenye matiti?

A. Ndiyo

B. Hapana

24. Unahistoria ya kuwa mnene wakati wa balehe yako?

A. Ndiyo

B. Hapana

25.Kuna mtu yeyote kwenye familia anaumwa/alishakuwa na saratani ya matiti Kuna ndugu yako amewahi kupatikana na kansa ya ziwa?

A. Hapana

B. Ndio (Taja uhusiano wako na huyo ndugu)

i. Baba

ii. Mama

iii. Bibi / Babu

iv. Ndugu mwingine (taja)……

26. Je umewahi/ unavuta sigara?

A. Ndiyo

B. Hapana …. Nenda swali la 31

27. Kama ndiyo, ulikuwa na umri wa miaka mingapi ulipoanza kuvuta sigara?.....(miaka)

29..Ulikuwa/unavuta sigara ngapi kwasiku?.....

30. Umewahi kuishi na mtu anayevuta sigara ndani ya nyumba moja?

A. Ndiyo ……..

B. Hapana…..nenda swali la 34

31. Kama ndiyo, umeishi naye Kwa muda gani?

A. Miezi……

B. Miaka ……

32. Ulikuwa / unakaa muda gani ndani ya nyumba yenye moshi?.....(masaa/siku)

33. Kuna mtu anavuta sigara kazini kwako?

A. Ndiyo

B. Hapana……nenda swali la 35

34..Unafanya kazi katika mazingira ya moshi wa sigara kwa muda gani kwasiku?...miaka?....

A. Ndiyo

B. Hapana nenda swali la 38

35. Ulianza kunywa pombe ukiwa unaumri wa miaka mingapi?.....

36. Je,huwa unafanya mazoezi …?

A. Ndio

B. Hapana

37. Unatumia muda gani kufanya mazoezi ?.

A .zaidi ya masaa matatu kwa wiki

B. pungufu ya masaa matatu kwa wiki

APPENDIX III.

Informed Consent form

Consent to participate in the study

I ,Dr.Georvin Marco,am undertaking a study on assessment of association between risk factors for breast cancer and breast cancer molecular subtypes among female patient with breast cancer at MNH

Aim of the study

The purpose of the study is to determine the association between risk factors for breast cancer and breast cancer molecular subtypes among female patient with breast cancer at MNH
As well as partial fulfillment for the completion of the Masters of medicine in General surgery.

How to participate

The data will be gathered through an interview utilizing a structured questionnaire if you accept to take part in this study.

Confidentiality

The data collected will be kept private. On any study document, identification numbers shall be used in place of names..

Participation and Withdrawal Rights

participation in this study is optional. You have the option of taking part in this study or not. If you choose not to take part in the study, there are no consequences, and your kid will continue to get the necessary normal hospital treatment.

Benefits

The information collected will to establish the association of risk factors for breast cancer and molecular subtypes, which will be of paramount important on prevention strategies targeting the type with poor prognosis and limited treatment option.

Tel. +255763023674. The supervisors of this research Prof. M. Mchembe phone no: +2556241317Please get in touch with me directly or one of the following if you have any questions.

You should get in touch with the study's principal investigator, Dr. Georvin Marco, at The Muhimbili University of Health and Allied Sciences, P. O. Box 65001, Dar es Salaam, if you have any questions at +255763023674, Prof.M.Mchembe and Dr. Ringo phone no: +255756680746.

OR

In case you have questions about your rights of participation in this study, you may contact Dr. Bruno Sunguya, The Director of Research and publications, P. O. Box 65001 DSM. Telephone: +255 022 2152489.

Participant agreement.

I ……. have read the contents in this form. My questions have been answered. I am willing to participate in this study.

Signature of participant ……Date…….……

Signature of Researcher ……. Date…….

APPENDIX IV;

SWAHILI VISION INFORMED CONSENT.

FOMU YA RIDHAA YA KUSHIRIKI KATIKA UTAFITI.

CHUO KIKUU CHA AFYA NA SAYANSI SHIRIKISHI MUHIMBILI.

KURUGENZI YA TAFITI NA UCHAPISHAJIRIDHAA YA KUSHIRIKI KWENYE UTAFITI.

Mimi Dk.Georvin Marco, mwanafunzi wa shahada ya uzamili katika upasuaji.

Nafanya utafiti kuhusu uhusiano wavihatarishi vya ugonjwa wa saratani ya Matiti na aina kimolekuliya saratani ya maziwa.

DHUMUNI LA UTAFITI

Kutambua vihatarishi mbalimbali vinavyo sababisha aina ya molikuliya saratani ya matiti.

Saidia, upatikanaji wa taarifa utakao tumika katika uandaajiwa mkakati wa kuzuia saratani ya matiti.

Kama hitaji kuhitimu shahada ya uzamili katika upasuaji.

JINSI YA KUSHIRIKI

Ukikubali kushiriki taarifa zako zitachukuliwa kwanjia ya mahojiano.

USIRI

Taarifa zote zitakazokusanywa kupitia dodoso hili zitakuwa ni siri. Majina yako

Hayatatumika wala namba yako ya faili badala yake tutatumia namba yako yautambulisho yautafiti.

UHURU WA KUSHIRIKI NA HAKI YA KUJITOA

Kushiriki kwenye utafiti huu ihiari. Unaweza kushiriki au kukataa kushiriki. Hakuna adhabu kwa uhamuzi wa kutoshiriki katika utafiti huu.

FAIDA

Matokeo ya utafiti huu yatasaidia kuongeza uelewa wavi hatarishi vya saratani ya matiti. Ambao utasaidia katika mpango mkakati wa kuzuia saratani ya matiti.

Pia itasaidia kuanzisha mipango mkakati ya kuzuia viashiria hatarishi vya ugonjwa saratani ya matiti.

MAWASILIANO

Kwa mawasiliano na maswali kuhusu utafiti huu, unaweza wasilianana mtafiti mkuu Dkt.Georvin Marco kwasimu +255763023674 nabaruapepe ,georvinmarco@gmail.com, Chuo Kikuu cha Afya na Sayansi Shirikishi Muhimbili, S. L. P. 65001, Dar es Salaam.
Pia unaweza wasiliana na wasimamizi wangu; Prof M.Mchembe ,+25754292586, na Dr.Y.E.Ringo: +255756680746. Kwa maswali kuhusu haki ya ushiriki katika utafiti huu unaweza kuwasiliana na Dr. Bruno Sunguya ,Mkurugenzi wa UtafitinaUchapishaji, S.L.P 65001, Dar es Salaam. Simu+255 022 2152489.

Tafadhali weka sahihi Kama umekubali kushiriki kwenye huu uutafiti.

Mimi..... nimesoma na kuelewa maelezo ya fomu hii na nimekubali kushiriki katika utafiti huu.

Sahihi ya mshiriki…… Tarehe……..

Sahihi ya mtafiti……. Tarehe……...……