CLINICOPATHOLOGICAL PRESENTATION OF BREAST CANCER PATIENTS WITH DIFFERENT MOLECULAR SUBTYPES AT MUHIMBILI NATIONAL HOSPITAL

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By

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A Dissertation Submitted in (Partial) Fulfillment of the Requirements for the Degree of Master of Medicine (General Surgery) of

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October, 2021

CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance by Muhimbili University of Health and Allied Sciences a dissertation entitled; **‘‘Clinicopathological presentation of breast cancer patients with different molecular subtypes at Muhimbili National Hospital’’,** in (partial) fulfillment of the requirements for the degree of Master of Medicine (General Surgery) of Muhimbili University of Health and Allied Sciences.

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Dr. A. M. Kategile

(Supervisor)

Date: _____

_____

Dr. Caspar Haule

(Supervisor)

Date: _____

DECLARATION AND COPYRIGHT

I, **Dr**. **Ferdinand Sylvester Matemu,** declare that this **dissertation** is my original work and that it has not been presented and will not be presented to any other university for a similar or any other degree award.

Signature…… **Date**……

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First and foremost, praises and thanks must go to the almighty God, for his showers of abundant blessings through-out the time that I have been working by sweat and blood to accomplish this work. With the guidance of his Holy Spirit, I have seen this work through successfully.

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Last but not least, may I express my heartfelt gratitude to my family and friends at large for their close support, prayers and encouragement throughout my research work.

DEDICATION

This study is wholeheartedly dedicated to my beloved parents, Mr & Mrs. S. A. Matemu; this is because they have all been a special source to me for inspiration and strength and have always provided me with moral, spiritual, emotional and financial support.

I also dedicate this study to Bochi Hospital Ltd for their support to my career development.

Last but not the least, I dedicate this to my friends and colleagues for their support throughout the project.

ABSTRACT

Background: Breast cancer is among the most common (2nd to cervical cancer) incident cancer among women worldwide. In Tanzania, the current proportion of breast cancer is up to 27.76/100,000 population (Globocan 2020). However, mortality is very high in Tanzania due to late-stage disease or aggressive tumour types. Tanzania also is facing a challenge of inadequate resources which then has created a gap in the description of clinicopathological presentations of breast cancer. Prior local studies had some limitations including; exclusion of males, small sample size (n ≈70) and incomplete IHC**.**

Objectives: To analyze the clinicopathological presentation of breast cancer patients with different molecular subtypes at MNH.

Materials and Methods: This descriptive retrospective cross-sectional Hospital based study included a total of 446 patient data files (male & female) between 2014 and 2019 at MNH. Patients diagnosed with epithelialized breast cancer and complete IHC were included. All patients with incomplete IHC data were excluded. Data was collected using a structured questionnaire and analyzed using SPSS.

Results: In this Study 446 patients met the inclusion criteria including females (98.4%) and 1.6% were males. Most patients came with duration of chief complaint of 24 months (35.9%) and most presented with stage 3 (36.8%) and stage 4 (59.9%). The most common histology type was IDC (83.2%). However, among patients with IDC 36.3% presented with Luminal A and 34.3% presented with Triple negative molecular subtypes. Lastly, the most prevalent molecular subtype was Luminal A (36.3%); followed by Triple negative (34.3%), Luminal B (15%) and lastly HER-2 enriched (14.3%). Nevertheless, Luminal A and Triple negative molecular subtypes are common in patients with stage 3 & 4 disease.

Conclusion: Late presentation was a challenge and was associated with stage 3 & 4 disease and Luminal A and Triple negative subtypes. Lumina A and Triple negative were the commonest molecular subtypes. IDC histology type was the commonest.

Recommendations: With all these findings a larger multicenter study is needed.

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ACRONYMS AND ABREVIATIONS

- **ER-** Oestrogen Receptor
- **HER-2** – Human Epidermal growth factor Receptor 2
- **IBC-** Inflammatory Breast Cancer
- **MNH-** Muhimbili National Hospital
- **MRM-** Modified Radical Mastectomy
- **ORCI-** Ocean Road Cancer Institute
- **PR-** Progesterone Receptor

DEFINITION OF TERMS

- **BREAST CANCER**
  - Cancer is a group of diseases in which cells in the body grow, change, and multiply out of control. Usually, cancer is named after the body part in which it originated. Thus, breast cancer refers to the erratic growth and proliferation of cells that originate in the breast tissue. A group of rapidly dividing cells may form a lump or mass of extra tissue [47]
- **IMMUNOHISTOCHEMISTRY**
  - Immunohistochemistry (IHC) is used to characterize intracellular proteins or various cell surfaces in all tissues. Individual markers or more often panels of various marker proteins can be used to characterize various tumour subtypes, confirm tissue of origin, distinguish metastatic from primary tumour and provide additional information which may be important for prognosis, predicting response to therapy or evaluating residual tumour post-treatment. [48]
  - The most common immunohistochemical breast cancer prognostic and therapeutic markers used include: estrogen receptor, human epidermal growth factor receptor-2, Ki-67, progesterone receptor, and p53.[48]
- **LUMINAL A**
  - Is a molecular subtype of breast cancer where hormone-receptor positive (oestrogen-receptor and/or progesterone-receptor positive) and HER2 negative. [49]
- **LUMINAL B**
  - Is a molecular subtype of breast cancer where hormone-receptor positive (oestrogen-receptor and/or progesterone-receptor positive), and HER2 positive. [49]
- **HER-2 RICH**
  - Is a molecular subtype of breast cancer where hormone-receptor negative (oestrogen-receptor and progesterone-receptor negative) and HER2 positive. [49]

- **BASAL-LIKE (TRIPLE NEGATIVE)**
  - Is a molecular subtype of breast cancer where hormone-receptor negative (oestrogen-receptor and progesterone-receptor negative) and HER2 negative. [49]

CHAPTER ONE

1.1 Introduction

Breast cancer is the most common incident cancer among women worldwide with more than 1 million new cases diagnosed every year [1].

Breast cancer varies across the world between races and regions [2,3]. In the U.S., African Americans have lower incidence rates but higher mortality than Whites [2], a pattern attributed to a higher aggressiveness of disease [4] and socio-economic disparities [4,5] among African Americans. African American women also have higher incidence rate of more aggressive forms of breast cancer, such as inflammatory breast cancer (IBC) than Whites [6].

Within Africa, in spite of the low incidence of breast cancer, the mortality from this disease continues to be extremely high with survival much below that seen in other parts of the world [7]. This problem has been attributed to the fact that most developing countries have low or inadequate resources (funds and technology) to deal with breast cancer (recommended standard approach in management).

In Tanzania, the most recent report by WHO- International Agency for research on cancer (Globocan 2020), has shown that the current proportion of breast cancer is up to 27.76/100,000 population (female and male) [43].

Currently, approximately 80% of women diagnosed with breast cancer are diagnosed at advanced stages of disease and have limited access to early detection, diagnosis and treatment services. Consensus findings from a variety of retrospective studies point to late stage at diagnosis, with the majority of patients presenting with stage III or IV disease. [9]

A recent prospective study based at MNH and Tumaini Hospital, collected data on tumor stage, type and nodal status from 348 women, aged 28 to 79 years old with stage I-III breast cancer undergoing modified radical mastectomy. The majority of patients (83.7%) presented with stage III disease and 16.3% with stage II. [10]

Other studies have reported similar stage distributions: 5.2% with stage II disease, 57% with stage III and 37.5% with stage IV; [11] and 32.1% with stage III and 57.8% with stage IV [12].

However according to current advancement in management of breast cancer there has been an increase demand in obtaining clinicopathological molecular subtypes of all cases diagnosed with breast cancer so as to determine treatment modality and prognosis factors.[49] In addition, testing of HER-2 is performed to select patients who will benefit from Trastuzumab therapy. [20,21].

Several studies have shown that the benefit from hormonal therapy is proportional to the hormonal receptor levels [22]. HER-2 is an oncogene which belong to a family of epidermal growth factors and is amplified in 14-25% of cases of breast cancers [23-24]. Amplification for this gene leads to the expression of a trans-membrane protein which can be detected by immunohistochemistry. The current management of the breast cancer involves the use of Trastuzumab for patients with amplification of this gene [25].

In poor resource settings, these markers are not routinely tested and it is therefore impossible to select the patients who will benefit from adjuvant therapy. Currently there is a need to maintain routine testing of these markers in some poor resource settings as a mode of therapeutic selection in patients with breast cancer, and the status of these markers should be investigated in different regions of Africa [26,27].

Few studies around the world and in Tanzania have shown the relationship between breast cancer; immunohistochemistry (IHC) hormonal receptor subtyping and treatment and prognostic factors.

A retrospective study was performed to explore the relationship between molecular subtypes and clinicopathological features of breast cancer in Chinese women. [20]

Six hundred and twenty-eight Chinese women with breast cancer were classified into four molecular subtypes according to their oestrogen receptor (ER), progesterone receptor (PR) and Her-2 status. The prevalence rate of each molecular subtype was analysed. Relationship between the subtypes and clinicopathologic features was determined. The

distribution of molecular subtypes was as follows: luminal A 46.5%, luminal B 17.0%, basal 21.5%, HER2/neu 15.0%. [20]

It was suggested that there existed close relationship between molecular subtypes and clinicopathological features of breast cancer.

These findings are very important for understanding the occurrence, development, prognosis and treatment of breast cancer in Chinese population [20].

In Tanzania, one study evaluated 60 cases of breast cancer for PR and ER status, where the trend was poor expression of these markers with only 26.7% the patients were expected to benefit from hormonal therapy. The patients were of young age with an advanced stage at the time of presentation [28].

The same trend was observed in Kenya where the patients had an advanced stage of the disease with a low percentage likely to be hormonal sensitive in all stages of the disease [29].

However, one study showed a high level of HER-2 over-expression (20.26%) and was common in Grade III invasive ductal cancers [23]. Other studies have shown a higher rate of HER-2 gene amplification in ductal cancers compared to lobular cancers; those tumours had a high histological grade with negative ER and PR status [22,30].

With regard to age, studies have shown the trend of negative receptor status at a young age. Women over 40 years are more likely to benefit from adjuvant endocrine therapy with low recurrence, whereas younger women have a high prevalence of HER-2 over expression and a low 5-years survival rate [30].

Few studies in Tanzania have shown that most patients report with late-stage breast cancer disease; however, little is known about Clinicopathological presentation in terms of molecular subtypes among men and women diagnosed with Breast Cancer.

1.2 Literature Review

Gene-expression profiling has had a considerable impact on our understanding of breast cancer biology. During the last 15 years, 5 intrinsic molecular subtypes of breast cancer (Luminal A, Luminal B, HER2-enriched, Basal-like and Claudin-low) have been identified and intensively studied. [18]

Within hormone receptor-positive and HER2-negative early breast cancer, the Luminal A and B subtypes predict 10-year outcome regardless of systemic treatment administered as well as residual risk of distant recurrence after 5 years of endocrine therapy. Within clinically HER2-positive disease, the 4 main intrinsic subtypes can be identified and dominate the biological and clinical phenotype. [18]

From a clinical perspective, patients with HER2+/HER2-enriched disease seem to benefit the most from neoadjuvant trastuzumab, or dual HER2 blockade with trastuzumab/lapatinib, in combination with chemotherapy, and patients with HER2+/Luminal A disease seem to have a relative better outcome compared to the other subtypes. Finally, within triple-negative breast cancer (TNBC), the Basal-like disease predominates (70–80%) and, from a biological perspective, should be considered a cancer-type by itself. [18]

Importantly, the distinction between Basal-like versus non-Basal-like within TNBC might predict survival following neo-adjuvant multi-agent chemotherapy, bevacizumab benefit in the neoadjuvant setting (CALGB40603), and docetaxel vs. carboplatin benefit in first-line metastatic disease (TNT study). [18]

Overall, this data suggests that intrinsic molecular profiling provides clinically relevant information beyond current pathology-based classifications and therefore it is important and worth studying. [18]

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Six hundred and twenty-eight Chinese women with breast cancer were classified into four molecular subtypes according to their estrogen receptor (ER), progesterone receptor (PR) and Her-2 status. The prevalence rate of each molecular subtype was analysed. Relationship between the subtypes and clinicopathologic features was determined. The distribution of molecular subtypes was as follows: luminal A 46.5%, luminal B 17.0%, basal 21.5%, HER2/neu 15.0%. [20]

The subtypes had no significant difference under different menopausal status. However, in the age-specific groups, the age group of ≤35 years was more likely to get basal cell-like cancer (36.9%). Statistically significant differences were found among molecular subtypes by age, nuclear grade, tumour size, lymph node (LN) metastasis, tumour stage by American Joint Committee on Cancer (AJCC), radiotherapy but not by chemotherapy, types of surgery. [20]

After adjusting for several relative confounding factors, the basal subtype more likely had lower nodal involvement in both the incidence of LN metastasis (≥1 positive LN) and incidence of high-volume LN metastasis (≥4 positive LN). The HER2/neu subtype had higher nodal involvement in the incidence of high-volume LN metastases. After adjusting for relative confounding factors, the HER2/neu subtype more likely had higher AJCC tumour stages. [20]

It was suggested that there existed close relationship between molecular subtypes and clinicopathological features of breast cancer. In addition, the breast cancer subtypes have been proven to be an independent predictor of LN involvement and AJCC tumour stage. These findings are very important for understanding the occurrence, development, prognosis and treatment of breast cancer in Chinese population. [20]

Another study done in Thai-women documented on breast cancer subtypes based on ER, PR and HER-2 status in Thai women, where expression of these subtypes may not be similar to those evident in Western women. [19]

During 2009 to 2010, histological findings from 324 invasive ductal carcinomas (IDC) at Siriraj Hospital were studied. Various subtypes of IDC were identified according to expression of ER, PR and HER-2: luminal-A (ER+; PR+/-; HER-2-), Luminal-B (ER+; PR+/-; HER-2 +), HER-2 (ER-; PR-; HER-2+) and basal-like (ER-; PR-; HER-2-). As

well, associations of tumour size, tumour grade, nodal status, angiolymphatic invasion (ALI), multicentricity and multifocality with different breast cancer subtypes were studied. Of 324 IDCs, 143 (44.1%), 147 (45.4%), 15 (4.6%) and 12 (3.7%) were T1, T2, T3 and T4, respectively. Most tumours were grade 2 (54.9%) and had no nodal involvement (53.4%). According to ER, PR and HER-2 status, 192 (59.3%), 40 (12.3%), 43 (13.3%) and 49 (15.1%) tumours were luminal-A, Luminal-B, HER-2 and basal-like subtypes. HER-2 subtype presented with large tumour ($p=0.04$, ANOVA). Luminal-A IDC was associated with single foci ($p<0.01$, $\chi 2 \chi 2$). HER-2 and basal-like subtypes were likely to have high tumour grade ($p<0.01$, $\chi 2 \chi 2$). In addition, HER-2 subtype had higher number of nodal involvement ($p=0.048$, $\chi 2 \chi 2$). [19]

In conclusion, the luminal-A subtype accounted for the majority of IDCs in Thai women. Percentages of HER-2 and basal-like IDCs were high, compared with a recent study from the USA. The HER-2 subtype was related with high nodal invasion. The findings may highlight biological differences between IDCs occurring in Asian and Western women. [19].

A prospective study of 129 breast cancer patients in Kijabe, Kenya revealed that 66% of patients were ER and PR-negative; HER-2 status was tested in 34 women with a finding that 44% were TN [36]. Notably, this study may have underestimated the incidence of TN disease as the authors considered a score of IHC 2+ as being HER-2 positive despite that only one quarter of IHC 2+ results are indeed HER-2 positive as confirmed by FISH [37].

Other trials in Kenya and Uganda showed elevated rates of TN disease and basal-like marker expression ranging from 28% to 36% [38–40]. Studies in Tanzania, which only had the capacity to test ER and PR expression, showed rates of hormone receptor negativity exceeding approximately 50% and above consistent with an increased proportion of basal-like and/or TN breast cancer in this region. [36]

Another study done in Uganda where by Pathology reports for 2000–2004 from Nsambya Hospital, reporting invasive breast carcinoma, provided 45 microscopically confirmed cases. [34]

Results were; 73% of patients were 50 years or younger.

Histologic types were invasive ductal carcinoma (78%) and ‘‘good’’ prognosis types (11%). Overall, 40% were grade 3, but 48% of invasive ductal carcinomas were grade 3. Oestrogen receptor was positive in 60% overall and in 51% of invasive ductal carcinomas. HER2/neu was overexpressed in 11%; 36% were ‘‘triple’’ negative (oestrogen receptor, progesterone receptor, HER2/neu negative). [34].

Another study was done in Tanzania [36] whereby, In the study a total of 52 cases of breast cancer in north-western Tanzania were investigated. Patients' mean age at diagnosis was 49 years. The majority of the tumours was invasive ductal carcinoma 47 (90.4%) and 40 (76.9%) were of histological grade III. Thirty-eight (73.1%) of the patient had lymph node metastasis at the time of diagnosis and 36 (69.2%) were at clinical stage III. Only 3 (5.8%) patients were in clinical stage I. [36]

There was a tendency of a low level of expression of the receptors, whereby Oestrogen Receptor (ER) positive tumours were 17 (32.7%), progesterone receptor (PR) positive tumours were 22 (42.3%), and HER-2 positive tumours were 12 (23.1%). Triple negative tumours constituted 20 (38.4%) of the patients. Most of the tumours (75%) showed high proliferation by Ki-67. Lymph node metastasis was more common in Triple Negative and HER enriched tumours. [36]

Another study [42] whereby, In this study data was abstracted from the medical records of all breast cancer patients attending Ocean Road Cancer Institute (ORCI) over a 2-year period from July 2007 to June 2009.

Tumour tissue paraffin blocks were collected for all patients with available tissues for the determination of oestrogen receptor (ER) and progesterone receptor (PR). Among the 488 patients, stage was determined for 356 patients, 90.7% of whom presented in LS. Of the 57 tumour tissues, 49.1% were ER−/PR−. Patients with ulceration (OR = 4.97; 95% CI = 1.07, 23.04; p = 0.04) and peau d'orange (OR = 6.78; 95% CI = 1.48, 31.17; p = 0.01) were more likely to present in LS rather than ES. However, this study never addressed HER 2-receptor type and the relationship between clinicopathological presentation and molecular subtypes was a limitation for this study. [42]

Another study was done in Tanzania [44]. In this study a total of 384 patients were studied. The median age was 45 years (range 21 to 78 years). The male to female ratio was 1: 46.8.

Most of the patients were premenopausal (63.8%) and presented late with advanced breast cancer disease. Majority of patients (63.0%) presented with stage III disease. Lymph node and distant metastasis at the time of diagnosis was reported in 70.8% and 21.4% of patients, respectively. [44]

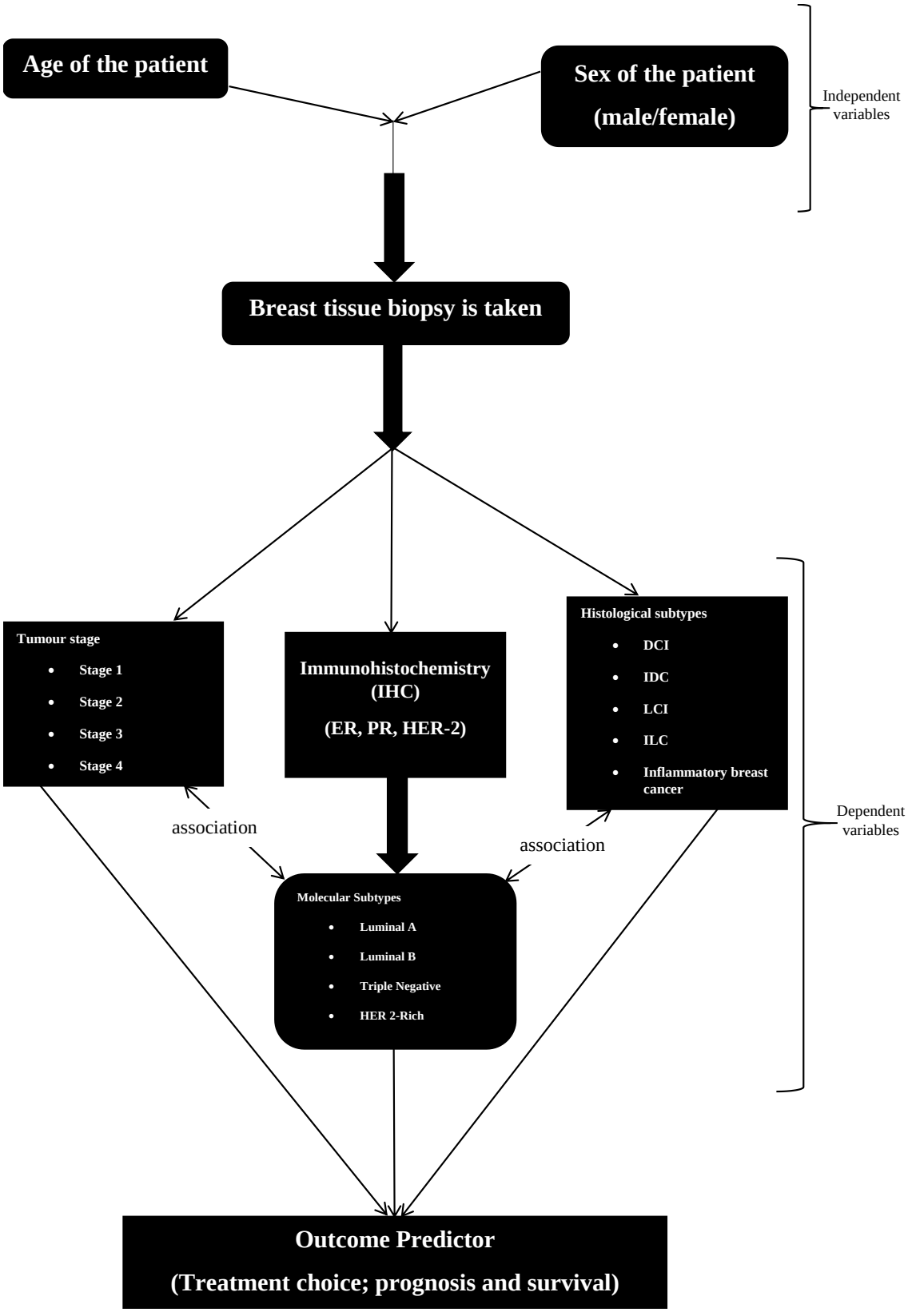
Invasive ductal carcinoma (91.7%) was the most frequent histopathological type and most patients (63.8%) had poorly differentiated tumour. Patients with tumour size greater than 6cm had significantly high rate of lymph node metastasis (P=0.001) and presence of necrosis within the tumour (P=0.012) compared to patients with tumour size less than 6cm in diameter. Patients younger than 45 years had significantly high rate of lymph node metastasis compared to the patients above this age (P=0.011). Mastectomy was the main modality of treatment that was used in 99.5% of the patients. Adjuvant chemotherapy and radiotherapy was reported in 44.8% and 11.7% of patients, respectively. Hormonal therapy (tamoxifen) was given postoperatively to all patients.This study however never addressed the issue of ER; PR; and HER 2 and their clinical subtypes. [44]

Another study done in Tanzania [45]. In this study A total of 348 patients were admitted with breast cancer including 86 patients (with 16 from TH having similar demography and presentation) meeting inclusion criteria. Age-range at diagnosis was 28–79 years, mean 52.1 years. Most (89 %) attained menarche after 11 years. About 56 % were postmenopausal. The majority (78 %) were multiparous with positive family history in 14.1 and 37.6 % used hormonal contraceptives. [45]

About 27.1 % were social alcohol drinkers. The majority (61 %) had T4b disease, 75.6 % had positive axillary nodes including 42.7 % with 4–9 involved nodes (N2). The commonest (91.9 %) histological type was invasive ductal carcinoma. Lobular, medullary and mucinous carcinomas were rare. Most (83.7 %) of our patients presented with stage III and the rest stage II. Intermediate- and high-grade tumours accounted for 73.5 %. Following MRM, 25 % of our patients had positive surgical margins and similarly for the base.This study however did not address the ER, PR and HER 2 subtypes and the study had only 86 patients meeting the inclusion criteria. However, the study did not address breast cancer in male patients. [45]

Another study [46] found that, A total of 218 cases were confirmed to be carcinoma including 70 meeting inclusion criteria. Age at diagnosis ranged 18–75 years and mean age was 48.36 years. Majority (64.3%) were in the 36–55 years' age-group. Histologically, most (88.6%) women had invasive ductal carcinoma including 43.1% of intermediate grade. A great majority (78%) were stage three. Due to logistical constrains, 75.7% (n = 53/70) cases where immunostained for hormones including 43.4% (ER+), 26.4% (PgR+), and 28% (ER+/PgR+). Furthermore, 65.7% (n = 46/70) cases were immunostained for HER-2 and 15.2% (n = 7/46) were positive, 45.6% were triple negative(ER-,PgR-,HER2-), 23.9% (ER+,PgR+,HER2-) or luminal B, 2.2% (ER+,PgR-,HER2+),13% (ER-,PgR-,HER2+) and 15% (ER+,PgR-,HER2-) with none being triple positive. This study however had only few cases (70 cases only) meeting the inclusion criteria and males with breast cancer were excluded from the study. [45]

1.3 Conceptual Framework



1.4 Problem Statement

The Incidence of breast cancer in Tanzania is reported to be low while mortality is reported to be very high due to the fact that; most patients present with late-stage disease or they may present with breast tumour types that are very aggressive.

In this era of advanced technology in medical care, Tanzania has been facing inadequate resources to fully analyze and provide a description on the clinicopathological presentation and molecular subtypes of breast cancer patients. Such challenges among them is failure of most patients to finance histopathological tumor studies and immunohistochemistry (IHC).

Several studies conducted in Tanzania have failed to provide a good description due to a number of limitations including; having few cases (average of 75) meeting inclusion criteria, excluding cases of male breast cancer and some cases in previous studies despite meeting inclusion criteria they couldn't afford the cost of ER, PR & HER2 Immunohistochemistry and hence there is missing data.

These issues must be addressed to close the gap and draw a more realistic picture on the description of clinicopathological presentation and molecular subtypes of breast cancer patients.

1.5 Rationale

Molecular breast cancer subtypes provide us with clinically relevant information beyond the current pathology-based classification and therefore it is important and worth studying.

The determination of clinicopathological molecular subtyping allows us to determine patients who will benefit from Hormonal Therapy; targeted therapy (transtuzmab) and also helps in classification of breast cancer into different tumour types, treatment (chemotherapy; radiotherapy; surgery) and prognosis.

In Tanzania there is little information about molecular subtyping of breast cancer; whereby most studies focused on hormonal receptors ER and PR; some studies excluded completely male patients with breast cancer; some studies did not address HER 2-receptor subtype and all studies conducted in Tanzania had very few (an average of 75) cases participating in the study.

Therefore, there is a gap of information concerning studies on ER, PR and HER-2 subtyping and hence this study is worth doing to analyze and provide a better description on how breast cancer patients (male & female) present in terms of their clinicopathological characteristics. These are useful in helping health care personnel to decide the mode of treatment to be provided to these patients such as when to do surgical intervention; when to give chemotherapy, radiotherapy, immunotherapy and hormonal therapy. Molecular subtyping and TNM staging of breast cancer also can be used to forecast prognosis and may influence decision to do palliative care

This study is going to draw a picture of the description of the clinicopathological presentation of breast cancer patients with different molecular subtypes in Tanzania and help providing useful reference in the management, prognosis and palliation of patients with breast cancer in Tanzania.

1.6 Research Question

What is the relationship between clinicopathological presentation and molecular subtypes of breast cancer patients in Tanzania?

1.7 Research Objectives

1.7.1 Broad Objectives

- To analyze the clinicopathological presentation of breast cancer patients with different molecular subtypes at MNH.

1.7.2 Specific Objectives

- To categorize different breast cancer stages in patients with different breast cancer molecular subtypes.
- To distinguish histological types in patients with different breast cancer molecular subtypes.
- To calculate the prevalence of Luminal A; Luminal B; Triple negative and HER-2 rich subtypes in breast cancer patients.

CHAPTER TWO

2.0 METHODS

2.1 Study Design

- The study design was a descriptive hospital based cross-sectional study.

2.2 Study Area

The study was set at Muhimbili National Hospital (MNH) which is the largest and oldest hospital in the country and also serves as a teaching hospital to MUHAS. It serves as the apex of referral services in the country making it appropriate to recruit enough sample size. With over 1500 beds and over 1000 OPD visits daily, with over 60 female surgical beds even makes it more conducive. It is also the main gate pass for patients diagnosed with breast cancer from other referral hospitals in the country to Ocean Road Cancer Institute hence cases will be easy to pick.

2.3 Study Population

- All patients diagnosed with breast cancer at MNH between 2014 and 2019.

2.4 Study Sample

- All patients diagnosed with breast cancer and have clinical immunohistochemistry (IHC) results.

2.5 Study Duration

- The study was conducted from June 2020 to August 2021.
- The study was held among all patients diagnosed with epithelialized breast cancer by clinical TNM staging system; histology and immunohistochemistry at MNH between 2014 to 2019.

2.6 Inclusion Criteria

- All patients clinically diagnosed with breast cancer and with immunohistochemistry (IHC) results.

- All patients with histopathological epithelialized breast cancer (DCIS; IDC; LCIS; ILC)

2.7 Exclusion Criteria

- All patients with incomplete data for histopathology and IHC.
- All patients with recurrent breast cancer whereby IHC results have changed.
- All patients diagnosed with non-epithelialized breast cancer (breast sarcoma; breast lymphoma).

2.8 Sample Size

- All patients diagnosed with breast cancer at MNH between 2014 and 2019.
- A pilot study was conducted and data registry had an average of 270 patients with complete data. The sample size required was calculated by using a single standard proportion formula (Kirk wood, 2003)
- According to 2020 WHO- Globocan report; the proportion of breast cancer in Tanzania was 27.76/100,000 population [43].
- Using the following formula to calculate the sample size:

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

Where:

N= my sample size

Z= 95% confidence interval = 1.96

D= 5%

P= 27.76% (2020 WHO-GLOBOCAN report [43])

N= 308

$$n = \frac{(1.96)^2 \times 27.76(100 - 27.76) = 308}{(5)^2}$$

Therefore, the calculated minimum sample size =308 patients

Assuming non-response rate of 10%;

Adjusted sample size, n' will be: n x adjusted factor

308x 100/100-10

308 x 100/90

342 patients

Hence sample size for the study was approximated to be 342 patients, this study however managed to recruit 446 patients.

2.9 Sampling Technique

- Data and information of all patients who meet the inclusion criteria was recruited in the study by using convenience non-probability sampling technique.

2.10 Data Collection Method

- Electronic medical records search for all women and men diagnosed with breast cancer based on their IHC results.
- Data from case files and record books was extracted for the same purpose.
- Patients' records were extracted from Hospital medical records from 2014 to 2019 data records for all male and female patients diagnosed with breast cancer and with their respective results for Histological type and Immunohistochemistry.
- IHC results were obtained from records for ER, PR and HER-2 status of the respective patient. This information was processed and molecular classification was assigned for each patient whereby:
    - Luminal A = ER Positive; PR Positive/Negative; HER-2 Negative
    - Luminal B = ER Positive; PR Positive; HER- 2 Positive
    - Triple Negative/Basal like = ER Negative; PR Negative; HER-2 Negative
    - HER-2-enriched = ER Negative; PR Negative; HER-2 Positive
- Data collected included IHC reports, histopathology reports as well as clinical notes on tumour staging and this Information was then entered into structured questionnaires that were given serial numbers in addition to hospital numbers for systematic record keeping.

2.11 Data Analysis

- Data was checked for completeness, coded, entered and analyzed by SPSS version 24. The sample demographic characteristics of the participants was described using frequency distribution and percentages. Continuous variables were summarized to means, range and standard deviations, while categorical variables into proportions.
- Data was analysed to reflect and show the association between breast cancer Molecular subtypes against tumour stage; tumour histological types and lymph node status using the Chi square test with significance level.

2.12 Study Limitations

- The study was a retrospective one and therefore incomplete data from files and record books was a challenge during data collection.

2.13 Study Mitigations

- All patients whose information on a particular variable were incomplete, had to be excluded in the analysis of that particular variable.

2.14 Ethical Consideration

- The study did seek ethical approval from MUHAS IRB and separate permission from MNH consultancy bureau to use its patients. The study protocol followed the ethical guidelines of the 1975 Helsinki Declaration.
- Approval of the study was sought from MUHAS research ethics committee.
- Confidentiality was maintained during and after the study by safely keeping the study materials.
- A waiver of informed consent was obtained from respective institution IRB.
- Only personnel directly involved with the research were granted access to the data. Information gathered shall be used only for purposes of research and resultant publication.
- All the research data, software and hardcopies which will be used in the study, will be handled to MUHAS authority after the study for publication.

2.15 Study Variables

- **Independent variables**
  - ER
  - PR
  - HER-2
  - Sex
  - Age
- **Dependent variables**
  - Tumour histology type
  - Tumour stage
  - Luminal A
  - Luminal B
  - HER-2 enriched
  - Triple Negative
- **Level of Measurement**
  - Quantitative (numerical) discrete
    - Age
  - Categorical Nominal
    - Sex with 2 levels 1=male and 2= female
    - Tumour histology type with 5 levels 1= DCIS; 2= IDC; 3= LCIS; 4= ILC; 5= Inflammatory carcinoma
    - Tumour stage with 4 levels 1= stage 1; 2= stage 2; 3= stage 3; 4= stage 4
    - Tumour molecular subtype with 4 levels 1= Luminal A (ER Positive; PR Positive and HER-2 Negative); 2= Luminal B (ER Positive; PR Positive and HER-2 Positive); 3= Triple negative (ER Negative; PR Negative and HER-2 Negative); 4= HER-2 Rich (ER Negative; PR Negative and HER-2 Positive)

2.16 Results Dissemination

Findings of the study shall be presented to the department of surgery MNH, and at local and international conferences. Similarly, publication in regional or international journal will be done.

Hard and soft copies will be made available to both the department and university for deposit into the repository.

Attempts to publish the report in local and international journals will be made.

CHAPTER THREE

3.0 RESULTS

In this Study a total of 560 patients data files were recruited and reviewed; among the recruited files only 446 patients' data files met the inclusion criteria. Among those 439 were females, making about 98.4% and on the other end there were only 7 males (1.6%) in the study. (see **Table 1**). This shows that female gender is associated with increased risk of developing breast cancer as compared to male gender.

Table 1: Description of participants according to Gender distribution

GENDER DISTRIBUTION

	Frequency	***Percent***
Male	7	*1.6*
Female	439	*98.4*
Total	446	*100.0*

Upon assessment of age distribution among the 446 patients recruited in the study, the median age was found to be 50 years with age range of 27 to 89 years. (see **Table 2 & Figure 1**).

Table 2: This table shows description of age distribution among the participants in this study.

Statistics

Age in Years	
Mean	51.40
Median	50.00
Mode	42

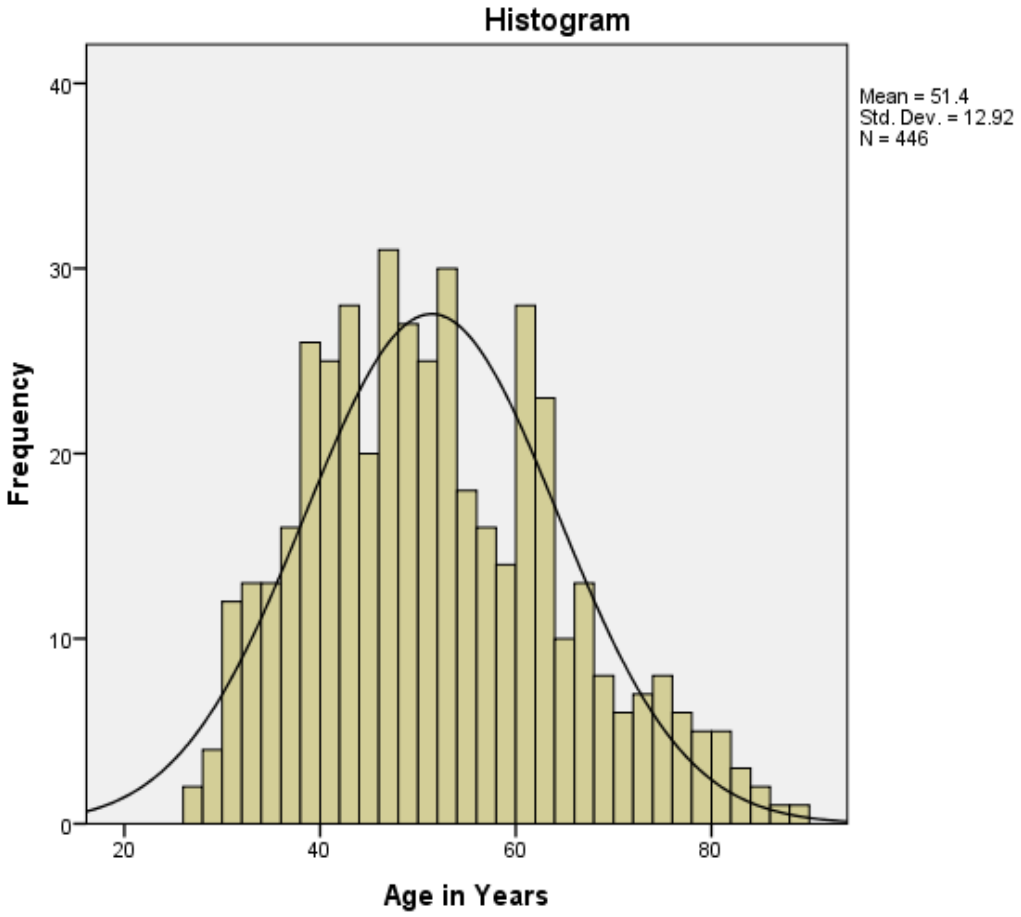


Figure 1: This Histogram gives a good picture of age distribution among the 446 patients who were recruited in this study having mean age of 51.4 years

Among other risk factors in female patients, being post-menopausal pose rather an increased lifetime risk for breast cancer; this study has showed that out of 439 female patients in the study 287 were post-menopausal (65.4%) and 152(34.6%) were pre-menopausal. This shows that breast cancer is common among post-menopausal females. (See **Figure 2**).

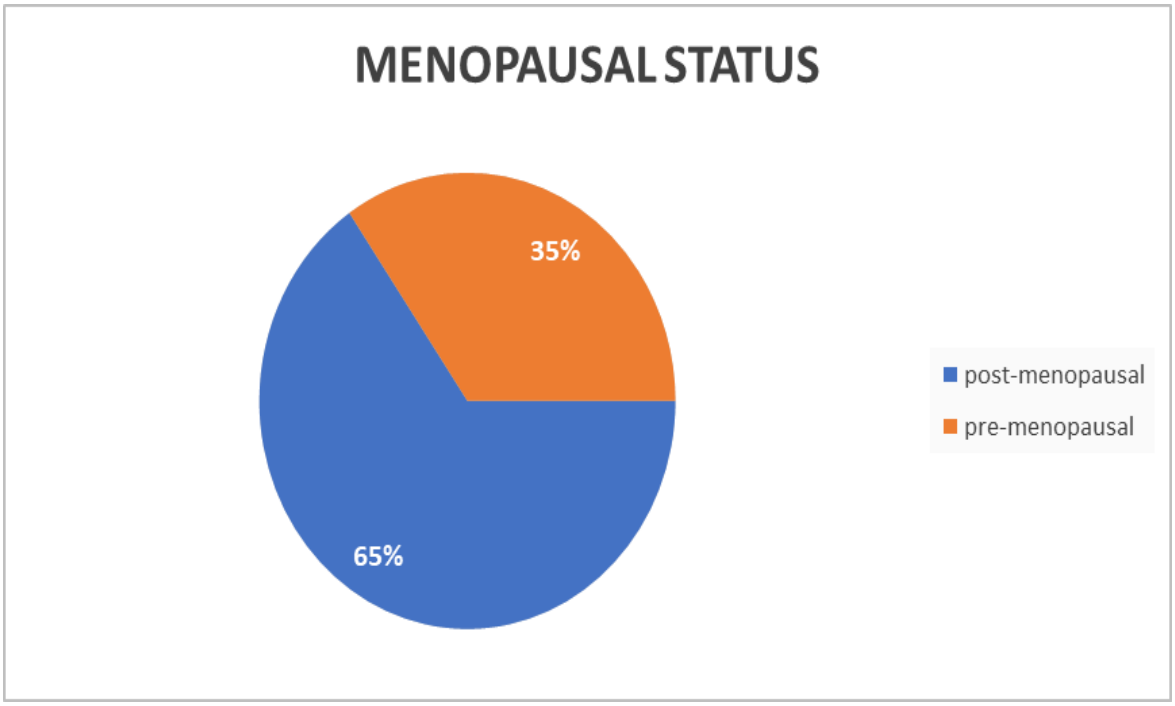


Figure 2: This pie chart clearly shows the fact that in this study breast cancer was seen more among post-menopausal women (65.4%).

However, regarding the time of presentation of chief complaints, the study has revealed the fact that most patients had their duration of chief complaints up to 24 months (about 35.9%); where by the median duration of chief complaints was 12 months (about 15.2%). This clearly shows that most patient came in for medical attention with late presentation. (See **Table 3**)

Table 3: This table provides a clear picture of duration of chief complaints among the 446 patients that were recruited in this study.

Duration of Chief Complaint (Breast Mass) in Months

Duration of C/C	Frequency	Percent
2	1	.2
3	47	10.5
4	52	11.7
5	11	2.5
6	43	9.6
7	19	4.3
8	10	2.2
9	14	3.1
10	6	1.3
12	68	15.2
18	1	.2
24	160	35.9
36	12	2.7
48	2	.4
Total	446	100.0

Late presentation of chief complaints has been seen as a huge challenge in this study; however other studies in literature review have shown that late presentation has a strong association with advanced stage of breast cancer disease and contributes markedly in rendering patients with poor prognosis and hence high morbidity and mortality rate.

In this study we have tried to address this challenge by comparing the duration of chief complaints (breast mass) against TNM staging of the patients in the study; as a result, the study has shown that among patients with TNM Stage 4 disease 130 of them (about 49.7%) presented with chief complaints of breast mass for 24 months and among those with Stage 3 disease 17.7% presented with chief complaint of breast mass for 24 months. This show that there is a relationship between late presentation of chief complaints and advanced breast cancer TNM stage. (See **Table 4**)

Table 4: This table shows the relationship between duration of chief complaints against TNM disease stage, showing that late presentation is associated with advanced disease stage.

Duration of Chief Complaint (Breast Mass) in Months * TNM Stage of the Disease Crosstabulation						
		TNM Stage of the Disease				Total
		Stage 1 Disease	Stage 2 Disease	Stage 3 Disease	Stage 4 Disease	
Duration of Chief Complaint (Breast Mass) in Months	2	0	0	1	0	1
	3	0	7	25	15	47
	4	1	4	24	23	52
	5	0	0	7	4	11
	6	0	1	23	19	43
	7	0	1	7	11	19
	8	0	0	4	6	10
	9	0	0	8	6	14
	10	0	0	1	5	6
	12	0	0	28	40	68
	18	0	0	0	1	1
	24	0	1	29	130	160
	36	0	0	7	5	12
	48	0	0	0	2	2
Total		1	14	164	267	446

The study has also addressed the fact that breast cancer is strongly associated with some of the risk factors. The study has shown the age of 20 years (35%) as the Median age at first pregnancy; among women in the study who had children 36.3% had at least 3 children and about 77.6% of the women with children breast fed them for 2 years duration. However, the Median age at Menopause was 50 years and also it has been shown that 84.5% of women in the study have been using some form of contraception and 83% denied family history of breast cancer. Therefore, there is a strong association between breast cancer and use of contraceptives. (See **Table 5**)

Table 5: This table shows different risk factors that were expressed by participants of this study.

Statistics

	Age at first Pregnancy in Years	Number of Pregnancies	Duration of Breast feeding in Years	History of Using Contraceptives	Age at Menopause in Years	Family History of Breast Cancer
	20.24	3.90	1.19	1.12	49.82	1.83
Median	20.00	4.00	1.00	1.00	50.00	2.00
Mode	20	3	1	1	50	2

This study also addressed the TNM stage at which the participants presented with on their first visit; the study has shown that, majority of patients were diagnosed with disease TNM Stage 3 and 4 at their first visit, that is 36.8% of patients presented with stage 3 and 59.9% presented with stage 4 respectively. This shows the fact that most patients presented with locally advanced and metastatic breast cancer disease. Most literature have described this as late disease presentation. (see **Table 6 and Figure 3**)

Table 6: This table shows the fact that most patients in this study came with late breast cancer disease presentation.

TNM Stage of the Disease	**Frequency**	**Percent**
Stage 1 Disease	1	.2
Stage 2 Disease	14	3.1
Stage 3 Disease	164	36.8
Stage 4 Disease	267	59.9
Total	446	100.0

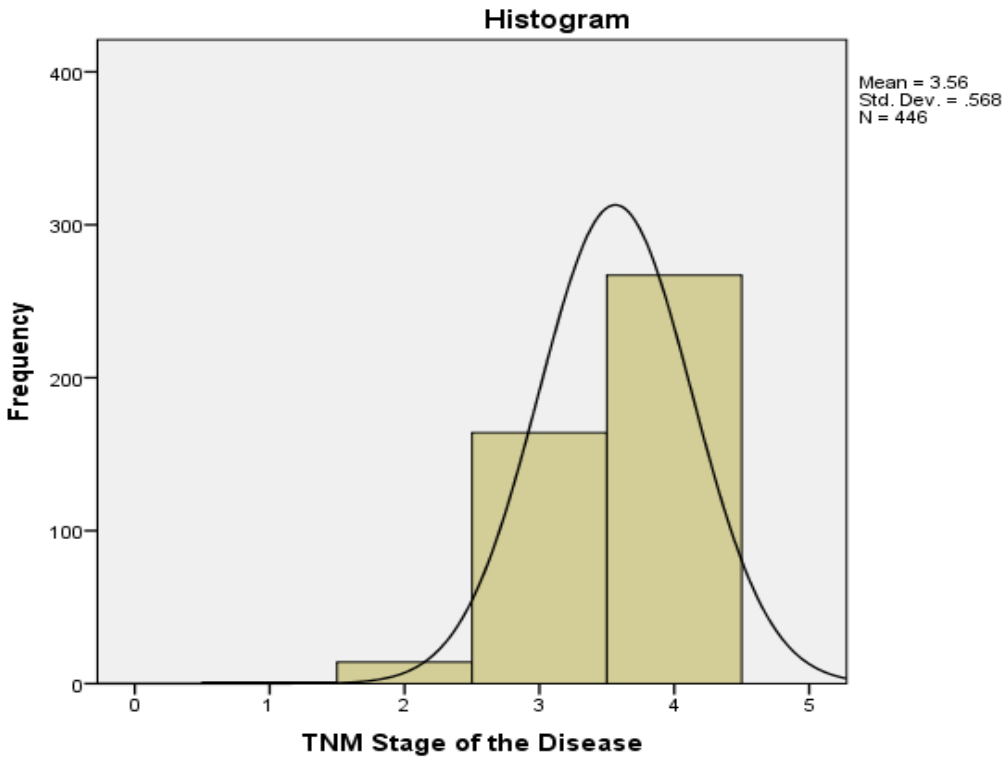


Figure 3: This Histogram shows a clear picture that most patients in this study presented with TNM stage 4 breast cancer disease (about 59.9%).

As a tool for decision making on mode of treatment; prediction of prognosis and survival of patients with breast cancer; this study has also addressed the relationship between, breast cancer TNM Staging and breast cancer molecular classification among the clinicopathological presentation of breast cancer; Upon the comparison between TNM staging and tumour molecular subtypes, the study shows that majority of patients presented with Luminal A and Triple negative molecular sub-types at stage 3 and 4 disease; that is 37.7% of patient with Luminal A subtype presented with stage 3 disease and 58% of patients with Luminal A presented with stage 4 disease. On the other hand, 35.3% of patient with Triple negative subtype presented with stage 3 disease and 62.7% of patients with Triple negative presented with stage 4 disease. Therefore, in this study it is shown that there is a strong association between late (advanced)-stage breast cancer disease and molecular subtypes (Luminal A and Triple negative). (See **Table 7 and Figure 4**)

Table 7: This table shows that most patients presenting with TNM stage 3 & 4 also present with Luminal A and Triple Negative molecular subtypes.

TNM Stage of the Disease * Tumour Molecular Subtype Crosstabulation						
		Tumour Molecular Subtype				Total
		Luminal A	Luminal B	Triple Negative	HER-2 Rich	
TNM Stage of the Disease	Stage 1 Disease	1	0	0	0	1
	Stage 2 Disease	6	3	3	2	14
	Stage 3 Disease	61	24	54	25	164
	Stage 4 Disease	94	40	96	37	267
Total		162	67	153	64	446

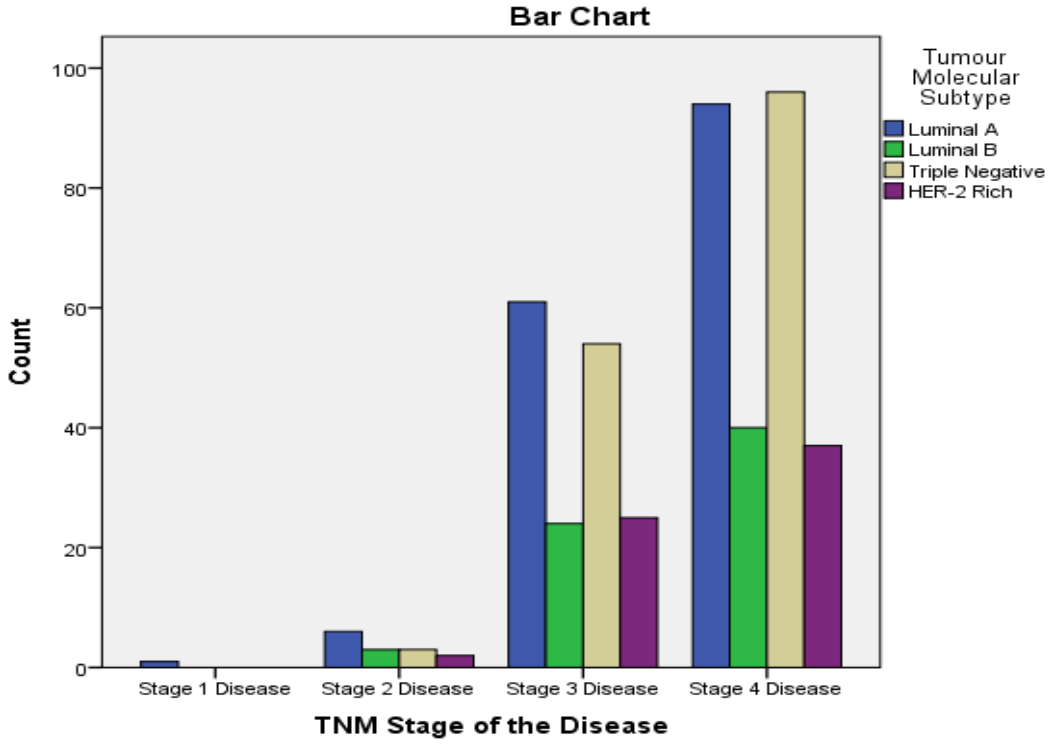


Figure 4: This bar chart shows a strong relationship between TNM stage 3&4 and Luminal A and Triple Negative molecular subtypes

However, upon comparison of TNM staging with gender, the study shows that most male patients presented with late-stage disease; that is 42.9% of all males presented with stage 3 disease and 42.9% presented with stage 4 disease; on the other end, 36.7% of females presented with stage 3 disease and 60.1% presented with stage 4 disease. (See **Table 8 and Figure 5**)

Table 8: This table shows again the fact that breast cancer is more common in Female gender compared to male gender and the fact that in the study late breast cancer disease presentation is in both genders.

TNM Stage of the Disease * Gender Crosstabulation

		Gender		Total
		Male	Female	
TNM Stage of the Disease	Stage 1 Disease	0	1	1
	Stage 2 Disease	1	13	14
	Stage 3 Disease	3	161	164
	Stage 4 Disease	3	264	267
Total		7	439	446

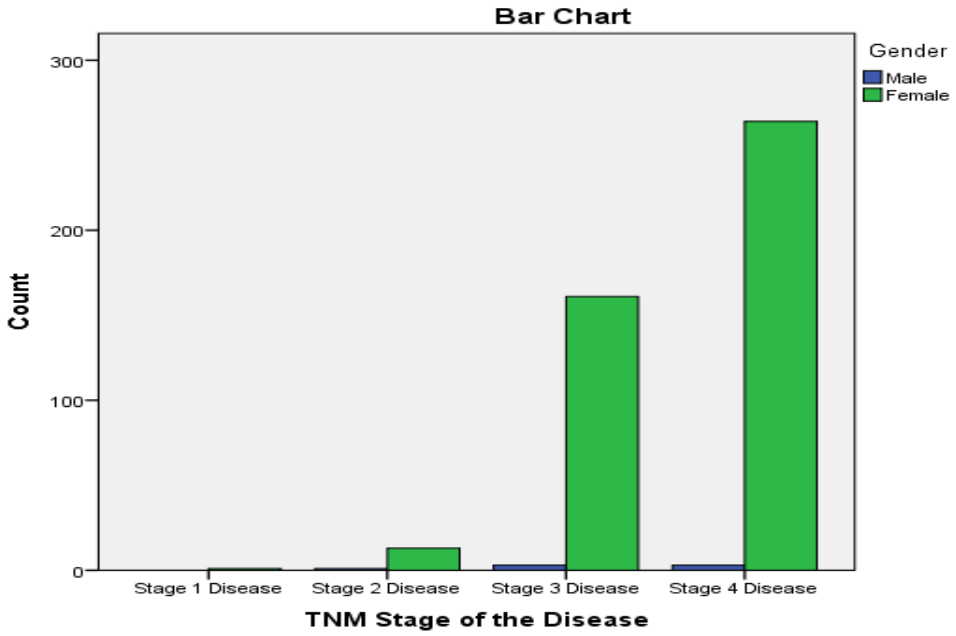


Figure 5: This Figure shows again the fact that breast cancer is more common in Female gender compared to male gender and the fact that in the study late breast cancer disease presentation is in both genders.

Among the clinicopathological presentations of breast cancer, this study addressed the relationship between Tumour histology type and molecular subtypes of breast tumour. The study showed that 83.2% of the tumours were Invasive ductal carcinoma followed by Invasive Lobular carcinomas (14.6%). On the other end most tumours had their molecular subtypes as Luminal A (36.3%) and Triple negative (34.3%). Upon comparison the study shows that IDC histology type is the most common followed by ILC with Luminal A followed by Triple negative molecular subtypes respectively. (See **Table 9, Table 10, and Table 11**).

Table 9: This table shows that the common histology for breast cancer among the 446 patients in the study was Invasive Ductal Carcinoma (83.2%).

Tumour Histopathology Type	*Frequency*	*Percent*
Ductal Carcinoma in Situ	1	.2
Invasive Ductal Carcinoma	371	83.2
Invasive Lobular Carcinoma	65	14.6
Inflammatory Carcinoma	9	2.0
Total	446	100.0

Table 10: This table shows that in the study most patient presented with molecular subtypes Luminal A (36.3%) and Triple negative (34.3%).

Tumour Molecular Subtype	*Frequency*	*Percent*
Luminal A	162	36.3
Luminal B	67	15.0
Triple Negative	153	34.3
HER-2 Rich	64	14.3
Total	446	100.0

Table 11: This table shows a strong association between Invasive Ductal Carcinoma histology type and molecular subtypes Luminal A and Triple negative.

Tumour Histopathology Type * Tumour Molecular Subtype Crosstabulation

		Tumour Molecular Subtype				Total
		Luminal A	Luminal B	Triple Negative	HER-2 Rich	
Tumour Histopathology Type	Ductal Carcinoma in Situ	1	0	0	0	1
	Invasive Ductal Carcinoma	129	56	129	57	371
	Invasive Lobular Carcinoma	30	8	21	6	65
	Inflammatory Carcinoma	2	3	3	1	9
Total		162	67	153	64	446

CHAPTER FOUR

4.0 DISCUSION

This study was focusing on analyzing the clinicopathological presentation of patients presenting with breast cancer (male & female) at MNH. In the study we found that, most patients presented with breast cancer stage 3 (36.8%) and stage 4 (59.9%) (advanced breast cancer disease), and among these patients there was a close relationship with duration of chief complaint of 24 months (49.7% with stage 4 and 17.7% with stage 3) and therefore late presentation was common among patients. The findings of this study clearly portray a mirror image of what most breast cancer patients present in Tanzania with majority presenting with advanced stage disease. These findings are consistent with other local study [44], which found that, the male to female ratio was 1: 46.8. Most of the patients were premenopausal (63.8%) and presented late with advanced breast cancer disease. Majority of patients (63.0%) presented with stage III disease. Lymph node and distant metastasis at the time of diagnosis was reported in 70.8% and 21.4% of patients, respectively. Nevertheless, this study found that breast cancer is common in female patients (98.4%) and was least common in male patients (1.6%); making a ratio of 1:62.7.

However, this study also found that breast cancer is more common among patients with advanced age (mean age of 51 years) for both male and female patients. Nevertheless, among women in the study, breast cancer was common in post-menopausal women (65.4%). The findings clearly paint a picture that, in Tanzania having age >50 years and being post-menopausal has a close relationship with breast cancer. These findings were similar to one local study [45], which found that, mean age was 52.1 years and most (56 %) were postmenopausal.

Regarding histopathological presentation, our study found that, the most common breast cancer epithelialized histopathological type was Invasive Ductal Carcinoma (IDC) (83.2%) followed by Invasive Lobular Carcinoma (ILC) (14.6%). However, there was a relationship between IDC and Molecular subtypes, whereby, among patients with IDC 36.3% presented with Luminal A and 34.3% presented with Triple negative molecular subtypes.

Luminal A (36.3%) and Triple negative (34.3%) were the most common molecular subtypes. Nevertheless, Luminal A and Triple negative molecular subtypes were seen to be common in patients with stage 3 & 4 disease whereby 37.7% of patients with stage 3 disease presented with Luminal A; 35.3% of patients with stage 3 presented with Triple negative; 58% of patients with stage 4 disease presented with Luminal A and 62.7% of patients with stage 4 disease presented with Triple negative molecular subtype.

The immediate above findings in this study, has rather depicted a new picture on how most breast cancer patients present in Tanzania. The findings show a pattern of clinicopathological presentation whereby, the common histological type is Invasive Ductal Carcinoma and the most common molecular subtypes are Luminal A and Triple Negative.

However, these findings were also seen in prior local studies, but all had limitations which renders them to have inadequate information. The 1st study [45] only showed that the most common histological type was IDC (91.9%). The 2nd study [46], despite its limitations, the findings were; histologically, most (88.6%) women had invasive ductal carcinoma including 43.1% of intermediate grade. A great majority (78%) were stage three. Due to logistical constrains, 75.7% (n = 53/70) cases where immunostained for hormones including 43.4% (ER+), 26.4% (PgR+), and 28% (ER+/PgR+). Furthermore, 65.7% (n = 46/70) cases were immunostained for HER-2 and 15.2% (n = 7/46) were positive, 45.6% were triple negative (ER-,PgR-,HER2-), 23.9% (ER+,PgR+,HER2-) or luminal B, 2.2% (ER+,PgR-,HER2+),13% (ER-,PgR-,HER2+) and 15% (ER+,PgR-,HER2-) with none being triple positive.

In spite of all the above findings, this study also showed that there is a close relationship between breast cancer and the use of contraceptives. It is shown that the use of contraceptives is common among female breast cancer patients (84.5%). In Tanzania most women at their reproductive age use contraceptives for family planning and birth control. The findings in this study rather portrays a controversial picture regarding the relationship between the use of contraceptives and breast cancer in women. These findings were consistence with a prior study [45], whereby, among 86 female patients who met inclusion criteria, the majority (78 %) were multiparous and (37.6 %) used hormonal contraceptives.

Overall, despite its limitations, this study was able to describe clinicopathological presentation of breast cancer patients at MNH and has drawn a picture on how breast cancer patients present in Tanzania. Nevertheless, these findings are statistically insignificant and therefore we recommend a large prospective multicenter study.

CHAPTER FIVE

5.1 Conclusion

- Most patients presented with breast cancer stage 3 (36.8%) and stage 4 (59.9%) (advanced breast cancer disease), and among these patients there was a close relationship with duration of chief complaint of 24 months (49.7% with stage 4 and 17.7% with stage 3) and therefore late presentation was common among patients.

- The most common breast cancer epithelialized histopathological type was Invasive Ductal Carcinoma (IDC) (83.2%) followed by Invasive Lobular Carcinoma (ILC) (14.6%). However, there was a relationship between IDC and Molecular subtypes, whereby, among patients with IDC 36.3% presented with Luminal A and 34.3% presented with Triple negative molecular subtypes.

- Lastly, the most prevalent (commonest) molecular subtype was Luminal A (36.3%); followed by Triple negative (34.3%), Luminal B (15%) and lastly HER-2 enriched (14.3%). Nevertheless, Luminal A and Triple negative molecular subtypes are common in patients with stage 3 & 4 disease whereby 37.7% of patients with stage 3 disease presented with Luminal A; 35.3% of patients with stage 3 presented with Triple negative; 58% of patients with stage 4 disease presented with Luminal A and 62.7% of patients with stage 4 disease presented with Triple negative molecular subtype.

5.2 Limitations

This study being a hospital-based retrospective one it was hindered by a number of limitations during data collection as highlighted below:

- Most of the files that were reviewed had insufficient documentation on patient details of important breast cancer risk factors.

- Some of the breast cancer patients couldn’t afford paying for the immunohistochemistry staining for ER, PR and HER2.

- For patient seen at OPD with only electronic files had poor documentation and missing of important information.

5.3 Recommendations

A large multicenter prospective study is needed to analyze and understand the clinicopathological presentation of breast cancer in males and females in Tanzania.

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APPENDICES

Appendix I: Data Collection Tool

- **Patient information**
  - Serial Number ……
  - MRN …….
  - Sex of patient……. (M/F)
    - Male (M)
    - Female (F)
  - Age of Patient ……. Years.
- **Duration of Chief Complaint**
  - Time of onset of symptoms ……
- **Obstetric and Gynaecological history**
  - Age at first pregnancy…….
  - Number of pregnancies …….
  - Duration of breast feeding ……
  - History of using contraception …….
  - Age at menopause ……
- **Family History**
  - Family history of breast cancer (yes/no)
- **Tumour characteristics**
  - TNM status …….
  - Tumour stage ……...
    - Stage 1
    - Stage 2
    - Stage 3
    - Stage 4
  - Surgical status (done/Not done)
  - Type of Surgery …….
    - MRM
    - Lumpectomy (Partial Mastectomy)

- Simple Mastectomy
- Total Mastectomy with Axillary clearance
- Conservative breast surgery (WLE/Quadrantectomy)
- Toilet Mastectomy
- Skin Sparing Mastectomy

- Post-Operative TNM Classification …….
- Chemotherapy/Radiotherapy/Hormonal Therapy status …….
  - Adjuvant
  - Neo-Adjuvant
  - Palliative Chemotherapy/Radiotherapy/Immunotherapy
- Tumour histological type ……
  - Ductal carcinoma in situ.
  - Invasive ductal carcinoma.
  - Lobular carcinoma in situ.
  - Invasive lobular carcinoma.
  - Inflammatory carcinoma.
- Tumour molecular subtypes ……
  - Luminal A (ER Positive; PR Positive and HER-2 Negative)
  - Luminal B (ER Positive; PR Positive and HER-2 Positive)
  - Triple negative (ER Negative; PR Negative and HER-2 Negative)
  - HER-2 Rich (ER Negative; PR Negative and HER-2 Positive)