

**CORRELATION BETWEEN RECEPTOR STATUS AND THE  
PRESENCE OF AXILLARY LYMPH NODE METASTASIS IN  
BREAST CANCER IN KENYATTA NATIONAL HOSPITAL**

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**H58/68560/2011**

A dissertation presented in part fulfilment of the requirements for the award of the degree of Master of Medicine in General Surgery of the University of Nairobi.

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## **CANDIDATE'S DECLARATION**

I hereby declare that this research is my own original work and has not been presented for a degree or any award in any other University.

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## **ABBREVIATIONS**

<b>AIs</b>	Aromatase Inhibitors
<b>AJCC</b>	American Joint Committee on Cancer
<b>ASCO</b>	American Society of Clinical Oncology
<b>CAP</b>	College of American Pathologists
<b>DFS</b>	Disease Free Survival
<b>ER/PR</b>	Estrogen and Progesterone hormonal receptors
<b>ERC</b>	Ethics Research Committee
<b>H&amp;E</b>	Haematoxylin and Eosin
<b>HER-2</b>	Human Epidermal Growth Factor receptor two
<b>IARC</b>	International Agency for Research of Cancer
<b>KNH</b>	Kenyatta National Hospital
<b>MRM</b>	Modified Radical Mastectomy
<b>MS excel</b>	Microsoft excel
<b>NPY</b>	Neuropeptide Y receptor
<b>OS</b>	Overall Survival
<b>Pcr</b>	Pathologically complete response
<b>RFS</b>	Recurrence Free Survival
<b>SERMs</b>	Selective Estrogen Receptor Modulators
<b>SOPs</b>	Standard Operating Procedures
<b>SPSS</b>	Statistical Package for Social Sciences
<b>TMA</b> s	Tissue Microarrays
<b>TNBC</b>	Triple Negative Receptor Breast Cancers
<b>TNM</b>	Tumour Node metastasis
<b>UON</b>	University of Nairobi

## **ABSTRACT**

**Background:** Breast cancer is the second most common malignancy in Kenyan women. Breast cancer survival is linked to early detection, timely and appropriate treatment. Furthermore, survival is dependent on stage and biological behaviour of the tumour. Expression of hormonal receptors by breast cancer cells is one of the key factors in the management of this disease because of its impact on metastatic spread, recurrence, disease free survival and overall survival. It however remains unclear how different molecular subtypes of breast cancer impact on axillary node involvement.

**Objective:** This study seeks to determine the correlation between expression of hormonal receptors, Human epidermal growth factor receptor-2 and Ki-67 protein in breast cancer with that of axillary lymph node status in breast carcinoma.

**Methodology:** This was a prospective cross sectional study carried out over 9 months at Kenyatta National Hospital (KNH) surgical wards and histopathology laboratory. Using a consecutive sampling method, patients with a histological diagnosis of breast cancer and scheduled to undergo a modified radical mastectomy (MRM) were recruited. Data collected included age at diagnosis, parity, menopausal status, clinical examination findings, stage of the disease clinically and pathologically and hormonal receptor status. Data was managed using MS Excel and analyzed using SPSS version 21.0. Continuous data is presented as means and categorical variables as proportions. All statistical tests were conducted at 5% level of significance. The data is presented in form of tables, bar charts and pie charts.

**Results:** The results of this study show that the most common molecular subtype of breast cancer in Kenyan women who present at the Kenyatta National Hospital for treatment is Luminal A followed by TNBC. Luminal A disease was spread almost evenly across all age groups while TNBC disease was most commonly diagnosed in younger premenopausal women. In this study most patients presented with T3 disease with a higher number of them being Luminal A followed by TNBC disease which were prevalent across all T size stages. Luminal A disease has the highest number of lymph node positive disease tumours but TNBC and HER-2 enriched disease have a higher propensity for nodal involvement in breast cancer. However, our study shows no correlation between hormonal receptor positivity or negativity and expression of HER-2 or lack of it, and nodal status in invasive breast cancer.

## **1.0 INTRODUCTION**

Globally, breast cancer in women is the most common malignancy accounting for 11.9% of the world's total cancer cases with an incidence rate of 34 per 100000 constituting 12% of the total cancer incidence among women(1). According to the GLOBOCAN 2013 database generated by the International Agency for Research of Cancer (IARC), breast cancer incidence almost doubled in 2012 compared to 2008. An estimated 133,000 new breast cancer cases were recorded in Africa in 2012 accounting for 27.6% of all cancer cases (2). East Africa reports a cumulative risk of above 4% (3).

Breast cancer growth, spread and management is hormone dependent. Guidelines written jointly by the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) recommend the routine analysis of estrogen and progesterone receptors(ER/PR) in all breast cancers (4). This is because their presence determines prognosis and selection of appropriate treatment. The success of hormonal therapies such as selective estrogen receptor modulators (SERMs) and aromatase inhibitors (AIs) is limited to patients whose cancers express ER/PR. Low grade tumours are more likely to be ER/PR positive and tend to have a better prognosis unlike those with a negative ER/PR status that tend to be larger, higher stage tumours with poorly differentiated morphology and an increased number of axillary lymph node metastases (5, 6).

Multifocal disease, high grade disease, large tumours and lymphovascular invasion have been described as predictors of axillary node metastasis but the impact of tumour molecular subtype on axillary node involvement has not been correlated yet (7, 8). Lymph nodes metastasis is the most important prognostic factor in patients with operable breast cancer. The number of positive lymph nodes correlates directly to local and distant recurrence. The five year Overall Survival(OS) is 82.8% in lymph node negative disease but drops to 73% in patients with one(1) to three(3) positive lymph nodes, reducing further to 45.7% in patients with four(4) to twelve(12) positive nodes and 28.4% in those with more than 13 positive lymph nodes (9, 10). Rates of axillary node involvement in the different molecular breast cancer subtypes based on their combined immunohistochemistry(IHC) expression of hormonal receptors and HER-2 status have not been systematically described and

controversies about the role of lymph node involvement as an intrinsic characteristic of breast cancer genetics remains (11).

## **1.1 LITERATURE REVIEW**

### **1.1.1 Role of Estrogen and Progesterone Receptors in Breast cancer biology**

Expression of ER/PR is the most reliable predictive factor for response to hormonal therapy due to their correlation with the neoplastic grade of the tumour (5, 6). Determination of ER/PR status prior to therapeutic intervention is standard practice and can be achieved by IHC which is relatively inexpensive and available in Kenya. PR is expressed in 60-70% of breast cancers with a higher positive rate noted in elderly or postmenopausal women. Loss of PR expression by tumour cells is associated with a poorer prognosis as it is a marker of a functional ER (12). Prolonged exposure to estrogen is critical to the development of breast carcinoma. ER is a nuclear transcription factor that binds Estrogen Responsive Elements and recruits co-factors like neuropeptide Y receptor (NPY) that facilitate gene transcription resulting in estrogen induced neoplastic ductal cell proliferation (13,14). Progesterone binds PR in a similar manner as ER but there is pre-receptor regulation characterized by cytoplasmic metabolism limiting its interactions with PR. This shows that the ER/PR receptors work in tandem in the tumour biology of breast carcinoma but a positive PR status does not appear to have an independent predictive value when the ER status is known (15).

### **1.1.2 Role of Human Epidermal Growth Factor Receptor 2 in Breast Carcinoma**

Interaction between Human Epidermal Growth Factor receptor two (HER-2) and ER is critical to the pathogenesis of breast carcinoma and disease resistance to therapeutic intervention (16). HER-2 activates multiple intracellular signaling cascades leading to a post translational modification of ER with a resultant increase of intracellular signaling pathways that potentiate HER-2 activity and down regulate the synthesis and expression of HER-2 (17, 18). This negative feedback is the hallmark of tumours that over express the HER-2 protein and is associated with high-grade disease and nodal metastases. Over expression of HER-2 occurs in approximately 20 % of breast cancers and was initially associated with aggressive disease before the development of HER\_2 targeted therapies.

## **1.2 Triple Negative Receptor Breast Cancer**

Triple-negative receptor breast cancers(TNBC) account for approximately 20 percent of breast cancers diagnosed worldwide and is more commonly diagnosed in younger women

less than 40 years of age compared with hormone receptor positive breast cancer (19). TNBC has a genetic signature of basal-like tumors associated with aggressive histological features. Several studies have also noted that TNBC is more common in black women than white women (20, 21). It has been postulated that breast cancer in Sub-Saharan Africa is receptor poor but studies in Kenya and Nigeria have demonstrated similar trends of receptor positive disease and TNBC to those of Caucasian and African-American women (22, 23).

### **1.3 Overall Survival, Disease Free survival and Recurrence Free survival in relation to breast carcinoma hormonal receptor Status**

Studies have established a relationship between hormone receptor levels and patient outcomes in terms of OS, disease-free survival(DFS), recurrence free survival(RFS) and five-year survival which are positively associated with ER/PR status in breast cancer (24,25). Women with stage I ER-positive breast cancer who receive no systemic therapy after surgery have a 5 to 10 percent lower likelihood of recurrence at five years than those with ER-negative tumours (26, 27). As the length of follow-up time increases, the advantage of ER-positivity in regards to RFS diminishes (28, 29). This may be a reflection of improvements in adjuvant chemotherapy that benefits those with HER\_2 gene amplification and TNBC over time or new mutations within tumour of ER negative subsets of tumour cells. ER status is also associated with site specific metastatic spread. ER-positive tumours are more likely to develop metastases in bone, soft tissue and the reproductive/genital tracts while ER-negative tumours are more likely to metastasize to the brain and liver which are sites that are associated with shorter overall survival (30). The five-year DFS for women with hormone receptor positive breast cancer is 70 percent for those who get hormonal therapy after surgery against 69 percent for those who receive hormonal therapy alone with a five-year OS of 94 percent versus 80 percent respectively for each group (31). Achieving a pathologically complete response (pCR) after neoadjuvant therapy in patients who present with locally advanced disease especially in TNBC is associated with improvement in disease-free survival (DFS) (32,33).

Despite TNBC having a higher likelihood to achieve an excellent pCR after chemotherapy, it still has a poorer overall outcome in comparison to patients with hormone receptor-positive breast cancer (34, 35). This “paradoxical improvement” of TNBC is explained by the overall better prognosis associated with hormone receptor-positive disease following surgical treatment, which is improved with the addition of adjuvant hormonal therapy over the next 5

to 10 years after diagnosis. Most breast cancer recurrences commonly occur within the first five years of diagnosis, particularly with TNBC or HER2-positive disease. The five-year DFS in TNBC is 67 percent for women who get surgery after neoadjuvant therapy against a 35 percent DFS for those who get neoadjuvant therapy only. The five year OS is 79% against 69 percent for each group respectively. For HER-2 receptor positive breast cancers the ten year OS rate for patients who received adjuvant chemotherapy only after surgery is 75 percent against the group that received chemotherapy and HER-2 targeted therapy whose OS is 84 percent with an improvement in DFS for this group being placed at 74 percent (36).

#### **1.4 The role of axillary lymph node status in Breast cancer**

Breast carcinoma will typically spread first to the axillary lymph nodes. This makes axillary lymph node assessment an integral component in the staging, prognosis, and treatment of invasive breast cancer. Axillary lymph node assessment is done via sentinel node biopsy or axillary lymph node dissection. Nodal status in the different molecular subtypes of breast cancer is the most robust factor correlated to OS and RFS (10, 37). The 5-year OS rate for patients with LN metastasis is 40% less than in patients without metastasis (38-40). Luminal A tumours have the lowest risk of lymph node metastasis while the luminal B and HER-2 have the highest risk of lymph node metastasis with the greatest number of T1 and T4 tumours respectively. (37,41). In patients with an ER/PR+, Her2- status, the incidence of lymph node metastasis increases as the tumour increases in size but in TNBC disease, lymph node metastasis will occur irrespective of tumour size (42). For women with node positive disease the molecular subtypes of luminal B and HER2 have the worst RFS rates amongst all node positive cases. We can therefore infer that by knowing the tumour size and intrinsic molecular subtype, lymph node metastasis can be predicted prior to surgery.

## **2.0 STUDY JUSTIFICATION**

Breast cancer is the second most frequently diagnosed malignancy in Kenyan women and the leading cause of cancer death among women globally. (1) Genomic profiling has demonstrated the presence of discrete breast tumour subtypes with distinct clinical behaviour that generally align with the presence or absence of ER/PR and HER-2 (43). There are few studies done in the Kenyan population that describe the incidence of expression of hormonal receptors in breast cancer but they do not look at the incidence of breast cancer subtypes defined by the joint expression of hormonal receptors and HER-2 status. Although the incidence of breast cancer is lower in black women, they have worse outcomes than Caucasian and Hispanic women when evaluation of outcome is done independent of stage at diagnosis (44, 45). The factors that contribute towards the survival disparities observed between black women and Caucasian women are thought to be biologic and social. It is therefore important to determine if genetic differences in these groups of women play a role. This may change the approach to treatment and therefore decrease the discrepancies noted in survival outcome. This study seeks to determine if there is a correlation of breast cancer receptor status and the presence of axillary nodal metastasis and if expression of receptors has an impact on rates of nodal involvement with a view to improving patient management.

## **2.1 RESEARCH QUESTION**

Is there an association between hormone receptor status and axillary lymph node status in invasive breast cancer in Kenyan women?

## **2.2 Broad objective**

To determine the association between invasive breast cancer receptor status with the presence of axillary lymph node metastasis among Kenyan women.

## **2.3 Specific objective**

1. To classify invasive breast carcinoma using morphology and immunohistochemistry (ER, PR, HER-2) in tissues obtained by modified radical mastectomy in Kenyan women
2. To establish pathologic axillary lymph node metastasis of invasive breast carcinoma in breast cancer patients

3. To correlate ER/PR/HER-2 and ki-67 receptor status of breast carcinoma with the presence of axillary lymph node involvement

## **3.0 MATERIALS AND METHODS**

### **3.1 Study design**

This is a cross sectional study that was conducted over 9 months

### **3.2 Study site**

Study was conducted at KNH surgical wards and Histopathology Laboratory

### **3.3 Study population**

Female patients over the age of 18 years with a histological diagnosis of breast cancer

### **3.4 Study Participants**

Patients were enrolled consecutively by the Principal investigator assisted by a trained study assistant who is defined as a medical officer with a minimal qualification of MBChB. The assistant was well informed of what the study entails and information that needed to be collected to make the study a success.

### **3.5 Inclusion criteria**

1. Female patients with breast cancer 18 years and above
2. Histologically confirmed diagnosis of breast carcinoma that is Stage I, II or III
3. Patients scheduled to undergo an MRM and give informed consent

### **3.6 Exclusion Criteria**

1. Patients with Stage Four(IV) breast cancer
2. Patients who do not wish to participate in the study or do not sign consent
3. Patients with a previous history of axillary lymph node dissection
4. Patients who have had neoadjuvant therapy
5. Patients with recurrent disease following previous treatment



### 3.7 Sample size calculation

According to KNH data from hospital records, an estimated number of 100 fully worked up breast cancer patients are seen annually. Therefore, out of this population a representative sample was drawn and the sample size calculation obtained using the formula for finite population (46).

The calculation was as follows:

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

Where

$n'$  = sample size with finite population correction,

$N$  = size of the target population = 100,

$Z$  =  $Z$  statistic for 95% level of confidence = 1.96,

$P$  = Estimated proportion of breast cancer patients with hormone receptors= 40% (47)

$d$  = margin of error = 5%

= **79** breast cancer patients were sampled

### 3.8 DATA COLLECTION

Patients with histologically confirmed breast cancer who were admitted to the general surgical wards for MRM were recruited. Recruitment was verbal and the participants were duly informed of the nature and purpose of the study. For the patients who agreed to participate in the study, informed consent was obtained and they were subsequently enrolled into the study. Data collected include age at diagnosis, parity, menopausal status, clinical examination findings and stage of the disease. This data was entered in a pretested data collection form partially adapted from the Tumour Node metastasis (TNM) staging from the American Joint Committee on Cancer (AJCC) Staging Manual, Seventh Edition (2010) on diagnosis of breast cancer. (Appendix 1) The histology specimens were handled no different than others in KNH. The cost of hormonal receptors and HER-2 were funded by the principal researcher because they are not routinely covered by the normal KNH processing system. In the histopathology laboratory all MRM specimens were evaluated by pathologists.

Sensitization of Lead surgeons and Pathologists and residents was done through presentation at the Breast Clinic, The Tumour Board and Grand Round conferences and through notices in the General Surgical wards.

### **3.8.1 Laboratory Procedures**

Following gross examination and selection of tissues for evaluation, the specimen was preserved for at least six hours in 10% neutral buffered formal saline then processed for up to eight hours to dehydrate the tissues and impregnate the cells with molten paraffin wax to prevent tissue degradation. The tissue was then filled with warm wax and transferred to a stainless steel mould where the molten paraffin wax was allowed to set to give the tissue support and shape and allow for sectioning. The wax impregnated tissue was then allowed to cool and solidify and then sectioned using a microtome to produce thin slices of tissue about one cell thick (about 3-4 micrometers). The thin sections were then floated on warm water so that they can be easily maneuvered and transferred to glass slides. The tissue section was then stained with Haematoxylin and Eosin stain (H&E) and the slide covered with a thin layer of glass. The slides were then evaluated microscopically by pathologists. Pathological analysis for tumour size (maximum diameter of the invasive tumour or the largest component if multiple foci present), morphology, lymphovascular invasion and presence of axillary lymph node involvement was done.

Immunohistochemistry was performed using tissue microarrays (TMAs). The use of TMAs drastically reduces costs and reduces tissue wastage. To make a TMA, the pathologist evaluated each slide per case and selected the most representative slide for the purposes of the study. The region of interest was marked using a pen directly under the microscope then the paraffin blocks corresponding to these marked slides were retrieved from the archive. A quick comparison between block and slide was made. Thereafter, using a designed tissue array, the donor block was punched out in the estimated region of interest and transferred onto a recipient TMA block. The tissue microarray slides generated from the paraffin-embedded tissue blocks were deparaffinised and rehydrated for 5 min, washed and incubated overnight at 4°C with primary antibodies against ER (1: 50 dilution; Dinona), PR (1: 100 dilution; Dinona) and HER-2(1: 250 dilution; DAKO). After a second incubation with a biotinylated anti-goat antibody, slides were incubated with peroxidase-labelled streptavidin and the reaction products visualized by immersing the slides in diaminobenzidine tetrachloride and counterstaining with Harris haematoxylin. Cases were considered positive for ER or PR when strong nuclear staining was observed in at least 10% of tumour cells examined and positive for HER-2 in cases of strong (3+) membranous staining in at least 10% of tumour cells but scores of 0 to 2+ were regarded as negative. The 2013 St Gallen

Conference refined the criteria to identify different molecular subtypes of breast cancer by use of the Ki-67 level and expression of PR. Luminal A disease is defined as ER positive, HER2 negative, Ki-67 low, and PR high; Luminal B disease(HER-2 negative) is defined as ER positive, HER2 negative, and either Ki-67 high or PR low; Luminal B-like (HER2 positive) is defined as ER positive, HER2 over expression, any Ki-67, and any PR; HER2 positive disease is defined as HER2 over-expression with absent ER and PR ; and triple negative disease as ER, PR absent and HER2 negative. These definitions have been used in this study to further refine the different molecular breast cancer subtypes.

### **3.9 QUALITY ASSURANCE**

All reagents were prepared in accordance with standard operation procedures (SOPs) and with the manufacturer's instructions. The fixation was made immediately using 10% neutral buffered formal saline and tissue processed using standard histology preparation protocols. The TMAs were prepared using standard array templates. Immunohistochemistry was performed using standard protocols and positive controls were included. All the stained sections were reported by an experienced consultant pathologist. All positive and 10% of negatives smears, randomly picked, were re-examined by an independent pathologist. A status conference between the pathologists was called to establish a consensus if discordance was noted in the tissue examination.

### **3.10 DATA ANALYSIS**

Data collected was entered and managed in an MS Excel spread sheet. Data was analyzed using SPSS version 21.0. Patient characteristics were summarized using age, parity and menopausal status; continuous data was presented as means and categorical variables as proportions. Prevalence of hormone receptor expression was presented as a proportion of all breast cancer patients. Axillary lymph node involvement was presented as a percentage and associated with hormone receptor status using chi-square test of associations. Odds ratios were calculated and presented as estimates for relative risk of axillary involvement in patients with hormones receptor expressions. All statistical tests will be conducted at 5% level of significance.

### **3.11 Results dissemination**

Results of this study have been published as a dissertation in part fulfillment of the degree Masters in Medicine in General Surgery, and have been disseminated to the thematic head of department of General Surgery at KNH and to the overall head of surgery KNH. Copies have also been availed to the UoN department of surgery, College of Health Sciences library and to The Ethics and Research Committee of KNH/UON. The findings of this study will be disseminated in seminars, conferences and workshops. Manuscripts will be submitted for publication in peer reviewed journals.

### **3.12 ETHICAL CONSIDERATION**

This study commenced after approval from the Department of Surgery UoN and the UoN-KNH ERC. All tissue biopsy samples were carefully used to make imprints smears to avoid risks that can be caused by repeat of procedure. Patient privacy and confidentiality was strictly observed. All results of imprint cytology were communicated to the attending surgeon and discordances were managed.

The patient received a pre-consent counselling on the study after which an informed consent was obtained from them.

With a signed informed consent the patient was enrolled into the study.

Patients were not coerced to enroll as patients in the study. Non-participation did not affect such a patient's care in the hospital.

Participation in this study did not attract extra cost to the medical care of the participants.

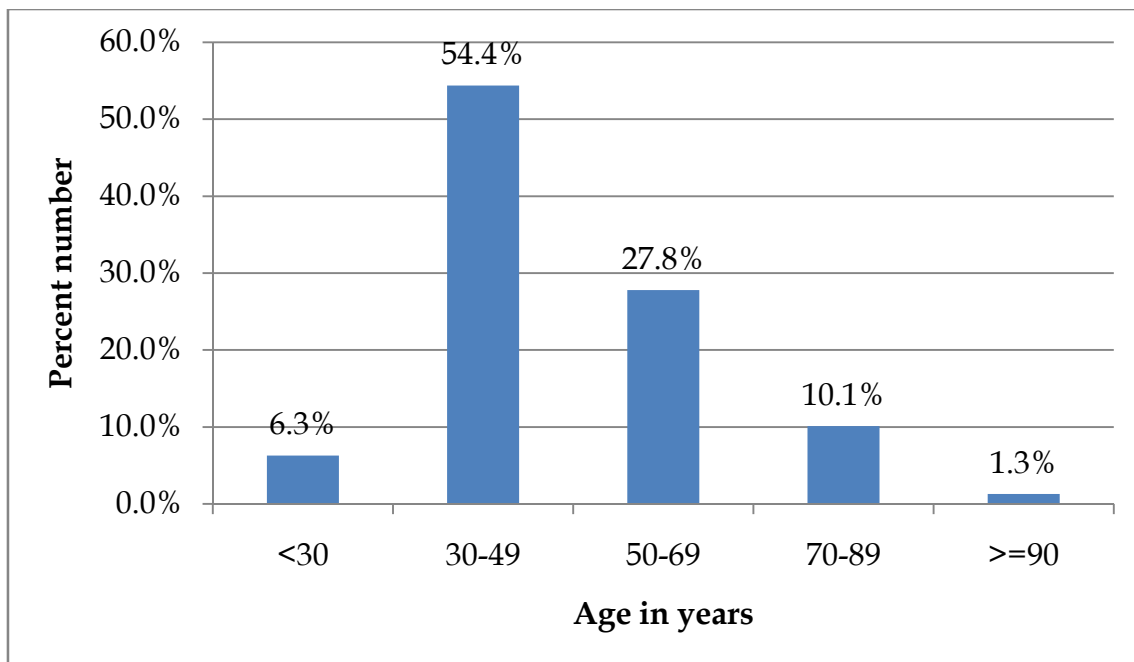
Patients' hospital file number was included into the data sheet to facilitate easy tracing and capture missed information during data collection.

The data sheet was kept safely with the researcher and confidentiality maintained throughout. Electronic data file generated was encrypted with a password only availed to the research team. Any hard copy research data was kept in a safe locked cabinet only accessed by the research team. The collected data will be destroyed after completion of this study.

## 4.0 RESULTS

The study cohort comprised of 79 women with invasive ductal breast cancer who underwent a modified radical mastectomy with a mean age of 48 years (SD=14.5). Majority (54.4%) of the patients were of the age group 30-49 years.

**Figure 1: Age distribution**



The mean age at which women got their first child was 23 years and the mean parity was seven children (SD=2.8). Most (64.6%) of women included in the study were pre-menopausal and 35.4% of them were post-menopausal. All women had a palpable breast lump and most were T3 (55.7%).

**Table 1: Patient characteristics**

Variable	Frequency (%)
Mean age (SD)	48.3 (14.5)
Mean parity (SD)	6.5 (2.8)
Median (IQR)	7 (6-8)
Mean age at first child (SD)	23.2 (4.1)
Menopausal status	
Pre-menopausal	51 (64.6)
Postmenopausal	28 (35.4)

In the clinical nodal status, most were classified as palpable and mobile at 41.8% and 19% as palpable and fixed. In the pathological staging of nodal disease, 38% of patients were pN2. Majority (48.1%) of patients presented at stage III disease. The histology of the tumour was ductal carcinoma in 91.1% and lymph vascular invasion was found in 83.5% of all specimens submitted.

**Table 2: Clinicopathological characteristics of invasive ductal breast carcinoma**

Variable	Frequency (%)
Size of breast lump	
T1	15 (19.0)
T2	20 (25.3)
T3	44 (55.7)
Palpable lymph nodes	
Mobile	33 (41.8)
Fixed	15 (19.0)
Not palpable	31 (39.2)
Stage	
1	8 (10.1)
2	33 (41.8)
3	38 (48.1)
Pathological classification of nodal involvement	
0	23 (29.1)
1	15 (19.0)
2	30 (38.0)
3	11 (13.9)
Lymphovascular invasion	
Yes	66 (83.5)
No	13 (16.5)
Ductal histology	72 (91.1)
Lobular histology	5 (6.3)
Variants-mucinous ductal	2 (2.5)

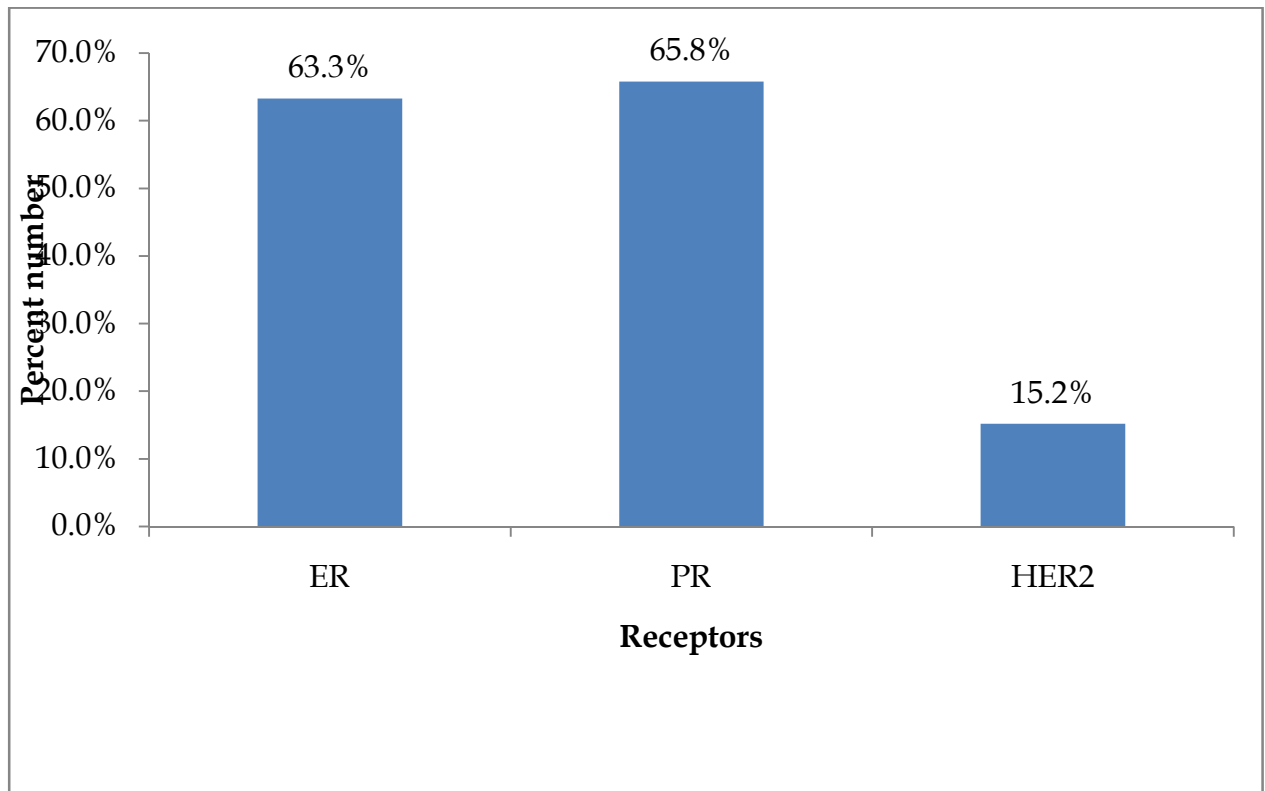
TMA technology was used to do hormonal receptor status and HER-2 expression for all specimens submitted. Patients with ER and HER-2 positive disease were 63.3% and 15.2% of respectively (table 3). When molecular classification was used to further classify patients on hormonal receptor status and HER-2 expression in breast cancer disease, 58.2 and 27.8% were found to be Luminal A and TNBC disease respectively (figure 3).



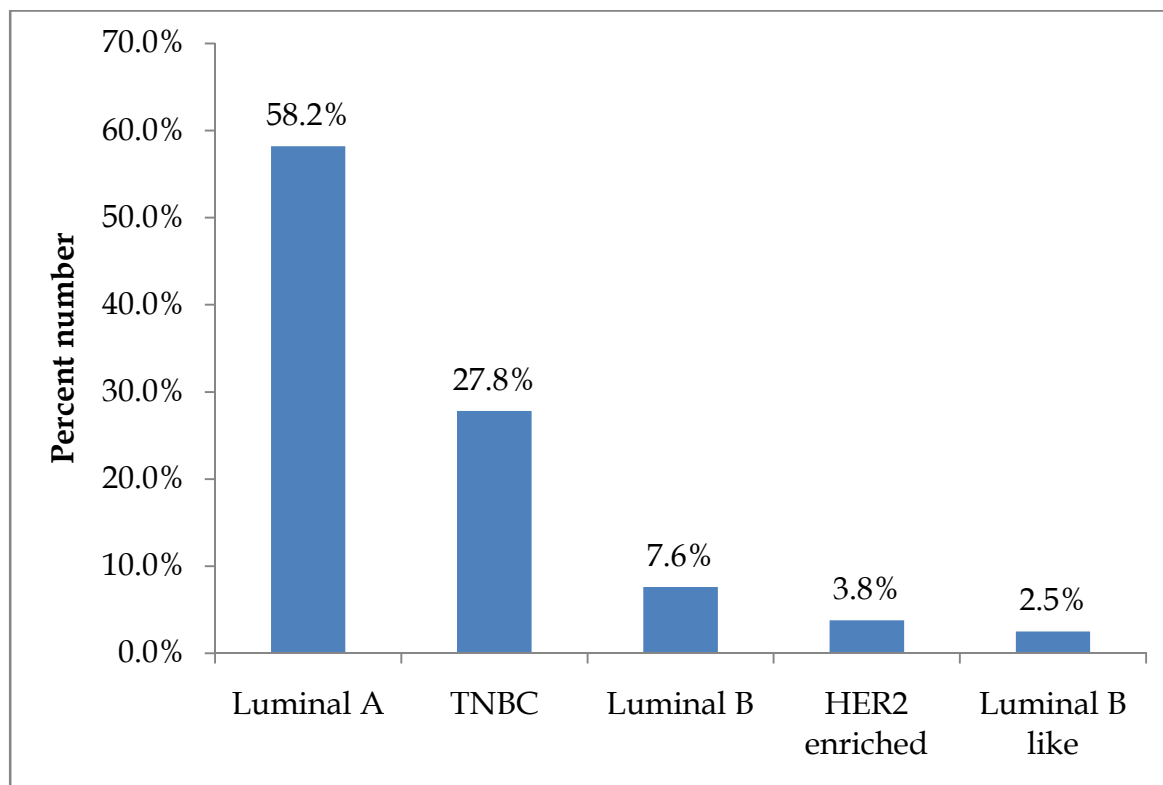
**Table 3: Hormonal Receptor Status**

Variable	Frequency (%)
ER	
Positive	50 (63.3)
Negative	29 (36.7)
PR	
Positive	52 (65.8)
Negative	27 (34.2)
HER2	
Positive	12 (15.2)
Negative	67 (84.8)

**Figure 2: Hormonal Receptor status**



**Figure 3: Molecular classification of breast cancer**



Luminal A disease had the highest percentage of nodal involvement of all patients with nodal disease followed by TNBC at 57.1% and 28.6% respectively. However when nodal involvement was analysed group by group, it was found to be highest in the HER-2 disease group at 100% followed by TNBC disease at 72.7%.(table 5) Luminal A disease was 58.8% and 57.1% in the pre-menopausal and post-menopausal groups respectively while TNBC disease was 29.4% and 25% in the pre-menopausal and post-menopausal groups respectively. Luminal B disease was 3.9% and 14.3% in the pre-menopausal and post-menopausal groups respectively. Basal like disease (Luminal B Like) was 2%in both the pre-menopausal and post-menopausal groups. HER-2 enriched disease was 5.9% in the premenopausal group with no patient in the post-menopausal group (table 6).These findings were however not statistically significant ( $p=0.05$ ).

**Table 4: Association between hormonal receptors and nodal involvement**

Variable	N	Nodal involvement		P value
		Yes (%)	No (%)	
Luminal A	46	32 (57.1)	14 (60.9)	0.760
Luminal B	6	4 (7.1)	2 (8.7)	0.813
LuminalB like	2	1 (1.8)	1 (4.3)	0.510
HER2 enriched	3	3 (5.4)	0 (0.0)	0.552
TNBC	22	16 (28.6)	6 (26.1)	0.823

**Table 5: Association between hormonal receptors and nodal involvement in the different molecular subtypes**

Variable	N	Nodal involvement		P value
		Yes (%)	No (%)	
Luminal A	46	32 (69.6)	14 (30.4)	0.760
Luminal B	6	4 (66.7)	2 (33.3)	0.813
Luminal B like	2	1 (50)	1 (50)	0.510
HER2 enriched	3	3 (100)	0 (0.00)	0.552
TNBC	22	16(72.7)	6 ( 27.3)	0.823

**Table 6: Association between hormonal receptors and menopausal status**

Variable	N	Menopausal status		P value
		Pre	Post	
Luminal A	46	30 (58.8)	16 (57.1)	0.885
Luminal B	6	2 (3.9)	4 (14.3)	0.096
Luminal B like	2	1 (2.0)	1 (2.0)	0.663
HER2 enriched	3	3 (5.9)	0 (0.0)	0.549
TNBC	22	15 (29.4)	7 (25.0)	0.676

**Table 7: Association between age and different molecular subtypes of Breast carcinoma**

	Age in years				P value
	<30	30-49	50-69	>=70	
Luminal A	1 (20.0%)	27 (62.8%)	12 (54.5%)	6 (66.7%)	0.339
Luminal B	1 (20.0%)	0 (0.0)	4 (18.2)	1 (11.1)	<b>0.011</b>
Luminal B Like	0 (0.0)	1 (2.3)	1 (4.5)	0 (0.0)	1.000
HER2 enriched	0 (0.0)	3 (7.0)	0 (0.0)	0 (0.0)	0.749
TNBC	3 (60.0)	12 (27.9)	5 (22.7)	2 (22.2)	0.424

**Table 8: Association between size of Lump and Molecular subtypes of Breast Cancer**

	Size of the lump				P value
	T1b	T1c	T2	T3	
Luminal A	3 (100.0)	5 (41.7)	10 (50.0)	28 (63.6)	0.241
Luminal B	0 (0.0)	2 (16.7)	0 (0.0)	4 (9.1)	0.316
Luminal B Like	0 (0.0)	1 (8.3)	1 (5.0)	0 (0.0)	0.236
HER2 enriched	0 (0.0)	0 (0.0)	1 (5.0)	2 (4.5)	1.000
TNBC	0 (0.0)	4 (33.3)	8 (40.0)	10 (22.7)	0.381

Most patients were found to have Luminal A disease across all age brackets followed by TNBC disease (table7). HER-2 enriched disease was only found in the 30-49years age bracket. Luminal B disease was prevalent in the women more than fifty years old and this was statistically significant ( $p=0.01$ ).

Most women presented with T3 disease and most of these were Luminal A disease(table8).It is postulated that because this study was carried out in a general hospital, most patents presented with late stage disease rather than early stage disease. These results were however not statistically significant ( $p=0.05$ ).

## 5.0 DISCUSSION

Breast carcinoma will typically spread first to the axillary lymph nodes making axillary lymph node assessment an integral component in the staging, prognosis, and treatment of invasive breast cancer. Axillary lymph node status is the most robust factor correlated to OS and RFS in the different molecular subtypes of breast cancer (10, 37). The 5-year OS rate for patients with LN metastasis is 40% less than in patients without metastasis (38-40). The number of axillary lymph node metastases is used as an important prognostication factor when deciding on indications for adjuvant therapy. It is on this premise that the 2013 St Gallen Conference consensus made the conclusion that node positive Luminal A breast cancer can in most cases be successfully treated with endocrine therapy alone. Chemotherapy is used to supplement endocrine therapy in cases of high bulk disease in this group of patients.

The results of this study show that the most common molecular subtype of breast cancer in Kenyan women who present at the Kenyatta National Hospital for treatment is Luminal A followed by TNBC. This data is supported by other studies that reported that the breast cancer molecular subtypes pattern in African women mimics that of the western world where receptor positive disease is the most common molecular subtype (22, 48). This puts into dispute data that reports that TNBC disease is the most common molecular subtype amongst black women (49).

Luminal A disease was spread almost evenly across all age groups while TNBC disease was most commonly diagnosed in younger premenopausal women. This is similar to data derived from the SEER California database that showed that African-American women were twice as likely to be diagnosed with Luminal A breast cancer but TNBC has a higher incidence in African-American women (50, 51). Data derived from the population-based North Carolina Breast Cancer cohort showed that premenopausal African-American woman had higher rates of TNBC than the postmenopausal African-American woman (52). In this study most patients presented with T3 disease with a higher number of them being Luminal A followed by TNBC disease which were prevalent across all T size stages. Of the patients who had nodal disease (57.1%), most had Luminal A disease followed by TNBC disease. A similar trend is reported in literature in which patients with Luminal A disease have an increased incidence of lymph node metastasis as the tumour increases in size (41).

In TNBC disease, the risk of nodal involvement is higher and metastasis to the lymph node occurs irrespective of tumour size(37).Nodal involvement rates in this study is similar to other studies that showed high rates of lymph node positivity in hormonal receptor positive disease than in hormonal receptor negative disease (53, 54). When nodal status was compared within the different molecular subtypes, HER-2 enriched disease followed by TNBC had the highest rates of nodal involvement at 100% and 72.7% respectively. These results are comparable to those in previous studies showing that Luminal A tumours have the lowest risk of lymph node metastasis despite it having the highest number of tumours that have node positive disease while HER-2 disease is reported in literature as having the highest risk of lymph node metastasis and higher T size stage tumours (37, 41-42).

This study has demonstrated that the most common molecular subtype of breast cancer across all age groups is Luminal A disease. TNBC, Basal-like and HER-2 enriched disease commonly occur in premenopausal women with TNBC disease commonly picked up in women less than 50 years of age. Luminal A disease has the highest number of lymph node positive disease tumours but TNBC and HER-2 enriched disease have a higher propensity for nodal involvement in breast cancer. However, our study shows no correlation between hormonal receptor positivity or negativity and expression of HER-2 or lack of it, and nodal status in invasive breast cancer. This trend has also been demonstrated in previous studies. (55, 56)

## **5.2 STUDY LIMITATION**

The main drawback of this study was the small sample size and limited follow-up of patients. A larger long-term multicentre study would help to elucidate whether the pattern of disease progression is different in our cohort of patients from other population groups. It will also help to see if the lymph node status in the different molecular subtypes has a correlation to OS/DFS in our cohort of patients. Ki67 was also not assessed in all of our patients and so the categorization of molecular types was made by use of the PR in patients who did not get it tested. This may have skewed our data in the molecular subtypes

### **5.3 CONCLUSION**

This study has demonstrated that the most common molecular subtype of breast cancer across all age groups is Luminal A disease followed by TNBC. TNBC disease however, has a higher incidence in women less than 50 years of age. Majority of the patients presented with T3 disease with a higher number of them being Luminal A followed by TNBC disease. Luminal A disease has the highest number of lymph node positive disease tumours but when a comparison of the lymph node status was carried out within the different molecular subtypes, HER-2 enriched disease followed by TNBC disease had a higher risk for nodal involvement in breast cancer. However, our study shows no correlation between hormonal receptor positivity or negativity and expression of HER-2 or lack of it, and nodal status in invasive breast cancer.



## STUDY TIMELINE

ACTIVITY	Feb 2015	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan 2016	Feb	Mar	Apr	May
Proposal development																
Ethical Approval																
Data Collection																
Data Analysis																
Dissertation Writing and presentation																

## STUDY BUDGET

Components	Unit of Measure	Duration/ Number	Cost (kshs)	Total (Kshs)
<b>Personnel</b>				
Research Assistant	1Pax	14 Days	1,500.00	21,000.00
Statistician				30,000.00
<b>Printing</b>				
Consent Form	1 Copy	3 Pages	10.00	30.00
Questionnaires	1 Copy	3 Pages	10.00	30.00
Final Report	1 Copy	80 Pages	10.00	800.00
<b>Photocopying</b>				
Consent Form	90 Copies	3 Pages	3.00	810.00
Questionnaires	90 Copies	3 pages	3.00	810.00
Final Report	5 Copies	80 pages	3.00	1,200.00
Final Report Binding	6 Copies	1	500.00	3,000.00
<b>Laboratory Cost</b>				
	86 Samples	1	2,700.00	232,200.00
<b>Other costs</b>				
ERC Fees				2,000.00
<b>Total</b>				<b>291,880.00</b>

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## APPENDICES

### Appendix I : Data Sheet Tool Collection

Patient Hospital Number .....

Study Number .....

1. Age .....
2. Parity..... Age at when first child was obtained.....
3. Menopausal Status: Premenopausal.....  
Postmenopausal.....

NB: Menopause is defined as having amenorrhoea for period of at least twelve consecutive months in a woman who is not expectant and is not on contraception.

4. Size of breast lump:- (Tick as appropriate)
  - T1a .....
  - T1b .....
  - T1c .....
  - T2 .....
  - T3 .....
5. Palpable lymph nodes (Tick as appropriate)
  - Not palpable .....
  - Mobile .....
  - Fixed .....
6. Stage of disease (Tick as appropriate)
  - Stage I.....
  - Stage II.....
  - Stage III.....

7. Pathological classification of tumour morphology: (Tick as appropriate)
1. Invasive ductal carcinoma .....
  2. Invasive Lobular carcinoma .....
  3. Others.....(specify)

8. Pathological classification of nodal involvement: (Tick as appropriate)

Pn1- 1 to 3 axillary lymph nodes .....

Pn2-4 to 9 axillary lymph nodes .....

Pn3- 10 or more axillary lymph nodes .....

9. Lymphovascular Invasion : (Tick as appropriate)

1. Yes

2. No

10. Hormonal Receptor Status: (Tick as appropriate)

ER Positive .....

ER Negative .....

PR Positive .....

PR Negative .....

HER-2 Positive .....

HER-2 Negative .....

## **Appendix II:STATEMENT OF CONSENT**

### **Part 1; INFORMATION SHEET**

#### **Introduction**

My name is Dr. Marilyn Akinyi Omondi, a postgraduate student at the University of Nairobi, school of medicine department of surgery, pursuing masters of medicine in general surgery. I am carrying out a study to determine hormonal receptor status patterns of breast cancer and its correlation with axillary lymph node metastasis.

Cancer is now a common problem in our society. This could be because of increases screening possibly due to better understanding of the subject by clinicians or due to a more educated society that is interested in good health and well-being. This study seeks to look at women above eighteen years of age who present with features of breast cancer at the breast clinic at Kenyatta National Hospital and determine pathological patterns that can be later used in management of the breast cancer patient. This will contribute in filling in knowledge gaps in our patient population and improve patient management. Please feel free to seek any clarification on the above.

#### **Confidentiality**

The information obtained will be treated with confidentiality and will be available to the principal investigator and authorized study team. Your name will not be used; instead you will be assigned a number. You are entitled to be treated with dignity and respect.

#### **Sharing Results**

Information from this study will be shared with Breast Surgeons and Oncologists, clinicians and other relevant health care workers, policy makers within KNH/UON and ministry of health.

### **Cost and compensation**

There will be no extra cost incurred for participating in this study nor is there compensation offered.

This proposal has been reviewed and approved by UON/KNH ethics committee. This is a committee tasked with making sure that research participants are protected from harm. In case you have further questions on this research please feel free to contact any of the below clinicians and institutions for further clarification.

### **Who to contact:**

#### **Secretary**

KNH-UON; ERC

PO BOX 20723-00202, KNH, Nairobi, Kenya

Phone Number; +254-020-2726300-9 ext. 44355

Email; [KNHplan@ken.healthnet.org](mailto:KNHplan@ken.healthnet.org)

### **Principal researcher**

#### **Dr. Marilyn Akinyi Omondi**

Department of Surgery, School of Medicine, University of Nairobi

P.O. Box 19676-00202 KNH, Nairobi, Kenya

Mobile no. 0722986777

University of Nairobi

**Supervisors;**

**Dr Dan Kiptoon**

MBChB (UON), MMed (Gen surG.) (UON),

Lecturer and Consultant General Surgeon

Department of surgery, UON.

Signature .....Date.....

**Dr Daniel Ojuka**

MBChB (UON), MMed (Gen surG.) (UON), FCS (ECSA)

Lecturer and Consultant General Surgeon

Department of surgery, UON.

Signature .....Date.....

**Dr Edwin Walong**

MBChB (U.O.N), MMed Pathology (U.O.N), FC Path (ECSA)

Lecturer and Consultant Pathologist

Department of Pathology, University of Nairobi

Signature .....Date.....

**PART 2; CONSENT CERTIFICATE**

**Title: CORRELATION OF INVASIVE BREAST CANCER RECEPTOR STATUS WITH AXILLARY LYMPH NODE METASTASIS: CERTIFICATE OF CONSENT**

If able to read and write

I have read the above information, or it has been read to me. I have had the opportunity to ask questions about it which been answered to my satisfaction. I consent voluntarily to participate as a study participant in this research.

Print Name of Participant \_\_\_\_\_

Signature of Participant \_\_\_\_\_

Date \_\_\_\_\_

If unable to read and write

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions and answered to my satisfaction. I confirm that the individual has given consent freely.

Print Name of witness \_\_\_\_\_

Thumb print of participant

Signature of witness \_\_\_\_\_

Date \_\_\_\_\_

**PART 3; STATEMENT BY RESEARCHER**

I have accurately read out the information sheet to the participant, and to the best of my ability made sure that the participant understands that the following will be done:

- Refusal to participate or withdrawal from the study will not in any way compromise the care of treatment.
- All information given will be treated with confidentiality.
- The results of this study might be shared with relevant healthcare as well as policy makers and also published in relevant medical journals.

I confirm that the participant was given an opportunity to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

A copy of this Informed Consent Form has been provided to the participant.

Name of researcher/person taking consent \_\_\_\_\_

Signature of researcher/person taking consent \_\_\_\_\_

Date \_\_\_\_\_

### **AppendixIII: FOMU YA MAKUBALIANO YA KUJIUNGA NA UTAFITI**

Fomu hii ya makubaliano inahusisha wagonjwa ambao wanahudumiwa kwenye kitengo cha upasuaji katika hospitali ya KNH. Unaalikwa kujiunga na utafiti wa **“CORRELATION OF BREAST CANCER RECEPTOR STATUS WITH AXILLARY LYMPH NODE MESTASTASIS”**

**Mtafitii mkuu**

**Dr. Marilyn Akinyi Omondi**

Chuo kikuu cha Nairobi, shule ya utabibu idara ya upasuaji

Fomu hii ya makubaliano ina sehemu tatu:

1. Habari itakayo kusaidia kukata kauli
2. Fomu ya makubaliano (utakapo weka sahihi)
3. Ujumbe kutoka kwa mtafiti

**Sehemu ya kwanza;**

**Ukurasa wa habari**

Kwa majina mimi ni Dr. Marilyn Akinyi Omondi, niko katika chuo kikuu cha Nairobi, shule ya utabibu, idara ya upasuaji ambapo nasomea upasuaji. Utafiti wangu inahusia” **Nia ya utafiti “CORRELATION OF BREAST CANCER RECEPTOR STATUS WITH AXILLARY LYMPH NODE MESTASTASIS”**

Saratani imekuwa tatizo kubwa katika jamii yetu ya kiasia. Yawezekana ni kwa sababu madaktari wameweza kuielewa ugonjwa huu vizuri na wameweza kuwapata wagonjwa hawa wa saratani mapema ama kutokana na jamii yetu kuelimika zaidi na kutaka kutunza afya zao zaidi. Utafiti huu inataka kuangalia saratani ya matiti ya wanawake waliotimiza umri ya miaka kumi na nane wanaokuja kuonekana katika kliniki ya matiti katika Hospitali ya Taifa ya Kenyatta. Tunataka kuangalia mwelekeo wa ugonjwa huu katika wanawake ambao wamethibitshwa kuwa na saratani ya matiti katika nyama iliyopimwa kwenya maabara .Hii itachangia katika mapengo ya maarifa katika idadi ya wanawake wanaouguwa na saratani ya



matiti na kuboresha huduma ya wagonjwa hawa. Tafadhali jisikie huru kuuliza kwa ufafanuzi yoyote juu ya utafiti huu .

### **Aina ya utafiti**

Utafiti huu utaangalia mgonjwa ambaye amethibitshwa kuwa na saratani ya matiti kwenye maabara. Ambapo nyama itakayopimwa itatatua jinsi ambavyo saratani hii inavyoenea katika mwili.

### **Usiri na hadhi**

Habari ambayo tutapata kutoka kwa utafiti huu ni ya siri na itakuwa wazi kwa mtafiti mkuu na watafiti wasaidizi. jina lako halitatumika ila utapewa nambari. Kwa wakati wa utafiti utashughulikiwa kwa hadhi na heshima.

### **Ugavi wa matokeo**

Matokeo ya utafiti itasambazwa kwa madaktari na wahusika wengine kwenye kitengo ya afya. Habari hii pia itasambazwa kwa wapanga sera kwenye hospitali na wizara ya afya.

### **Gharama na fidia**

Hakuna gharama zaidi katika kushiriki katika utafiti huu. Pendekezo limechunguzwa na kupewa kibali na kamati ya chuo kikuu cha Nairobi ikishirikiana na hospitali ya Kenyatta. kamati ina jukumu ya kuhakikisha ya kwamba washiriki kwenye utafiti, haki yao imelindwa.

### **Wasiliana na:**

#### **Mtafiti mkuu**

#### **Dr. Marilyn Akinyi Omondi**

Kitengo cha upasuaji, shule ya utabibu, chuo kikuu cha Nairobi

Anwani 19676-00202 KNH, Nairobi, Kenya

Nambari ya simu 0722986777

**Chuo kikuu cha Nairobi; wasimamizi**

**Dkt. Dan Kiptoon**

MBCChB (UON), MMed (Gen surG.) (UON),

Mhadhiri na daktari wa upasuaji

Kitengo Cha upasuaji, UON.

**Dkt. Daniel Ojuka**

MBCChB (UON), MMed (Gen surG.) (UON), FCS (ECSA)

Mhadhiri na daktari wa upasuaji

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**Dr Edwin Walong**

MBCChB (U.O.N), MMed Pathology (U.O.N),FC Path(ECSA)

Mhadhiri

Kitengo cha Pathology, University of Nairobi

Shauku kuhusu maadili ya utafiti wasiliana na;

**Katibu**

KNH-UON; ERC

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Nambari ya simu; +254-020-2726300-9 ext. 44355

Barua pepe; KNHplan@ken.healthnet.org

## **SEHEMU YA 2: FOMU YA MAKUBALIANO**

### **Wanaojua kusoma na kuandika**

Nimeelezwa utafiti huu kwa kina. Nimekubali kushiriki utafiti huu kwa hiari yangu. Nimepata wakati wa kuuliza maswali na nimeelewa kuwa iwapo nina maswali zaidi, ninaweza kumuuliza mtafiti mkuu au watafiti waliotajwa hapo awali.

Jina la Mshiriki \_\_\_\_\_

Sahihi ya mshiriki \_\_\_\_\_

Tarehe. \_\_\_\_\_

### **Kwa wasioweza kusoma na kuandika:**

Nimeshuhudia kusoma na maelezo ya utafiti huu kwa mshiriki. Mshiriki amepewa nafasi ya kuuliza maswali. Nathibitisha kuwa mshiriki alipeana ruhusa ya kushiriki bila ya kulazimishwa.

Jina la shahidi \_\_\_\_\_

Alama ya kidole ya mshiriki

Sahihi la shahidi \_\_\_\_\_

Tarehe. \_\_\_\_\_

### **SEHEMU YA 3: UJUMBE KUTOKA KWA MTAFITI**

Nimemsomea mshiriki Ujumbe kiwango ninavyoweza na kuhakikisha kuwa mshiriki amefahamu yafuatayo:

- Kutoshiriki au kujitoa kwenye utafiti huu hautamzuia kupata matibabu.
- Ujumbe kuhusu majibu yake yatahifadhiwa kwa siri.
- Matokeo ya utafiti huu inaweza chapishwa kutoa habari kuhusu wanawake wanaougua na saratani ya matiti iliyoenea kwenye makwapa ambapo mkia wa titi hupatikana.

Ninathibitisha kuwa mshiriki alipewa nafasi ya kuuliza maswali na yote yakajibiwa kikamilifu. Ninahakikisha kuwa mshiriki alitoa ruhusa bila ya kushurutishwa.

Mshiriki amepewa nakala ya hii fomu ya makubaliano.

Jina la mtafiti \_\_\_\_\_

Sahihi ya Mtafiti \_\_\_\_\_

Tarehe \_\_\_\_\_

## Appendix IV: KNH/UON-ERC LETTER OF APPROVAL



**UNIVERSITY OF NAIROBI**  
COLLEGE OF HEALTH SCIENCES  
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Twitter: @UONKNH\_ERC [https://twitter.com/UONKNH\\_ERC](https://twitter.com/UONKNH_ERC)



**KENYATTA NATIONAL HOSPITAL**  
P O BOX 20723 Code 00202  
Tel: 726300-3  
Fax: 725272  
Telegrams: MEDSUP, Nairobi

Ref: KNH-ERC/A/399

25<sup>th</sup> September 2015

Dr. Omondi Akinyi Marilyn  
Reg. No.58/68560/2011  
Dept. of Surgery  
School of Medicine  
University of Nairobi

Dear Dr. Omondi

**Research Proposal: "Correlation between receptor status and the presence of axillary lymph node metastasis in breast cancer in Kenyan women in Kenyatta National Hospital (P302/05/2015)"**

This is to inform you that the KNH/UoN-Ethics & Research Committee (KNH/UoN-ERC) has reviewed and **approved** your above proposal. The approval periods are 25<sup>th</sup> September 2015 – 24<sup>th</sup> September 2016.

This approval is subject to compliance with the following requirements:

- a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
- b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH/UoN ERC before implementation.
- c) Death and life threatening problems and serious adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH/UoN ERC within 72 hours of notification.
- d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH/UoN ERC within 72 hours.
- e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
- f) Clearance for export of biological specimens must be obtained from KNH/UoN-Ethics & Research Committee for each batch of shipment.
- g) Submission of an *executive summary* report within 90 days upon completion of the study. This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/or plagiarism.

For more details consult the KNH/UoN ERC website <http://www.erc.uonbi.ac.ke>

Protect to discover

Yours sincerely,



**PROF. M.L. CHINDIA**  
**SECRETARY, KNH/UON-ERC**

- c.c. The Principal, College of Health Sciences, UoN
- The Deputy Director CS, KNH
- The Chairperson, KNH/UoN-ERC
- The Assistant Director, Health Information Dept. KNH
- The Dean, School of Medicine, UoN
- The Chairman, Dept. of Surgery, UoN
- Supervisors: Dr. Dan Kiptoon, Dr. Daniel Ojuka, Dr. Edwin Walong, Dr. Mary Mungania

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