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**SCHOOL OF MEDICINE**

**DEPARTMENT OF SURGERY**

**Effect of Oestrogen Receptor Status of Women with Breast Cancer Treated  
With Tamoxifen at University Teaching Hospital and Cancer Diseases  
Hospital**

**By**

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Dissertation submitted in the partial fulfillment of the University of Zambia for the Award of  
the degree Master of Medicine in General Surgery

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### **CERTIFICATION**

This is to certify that this dissertation entitled *Effect of oestrogen receptor status of women with breast cancer treated with tamoxifen at UTH and CDH* by Dr. Nkuliyingoma Anastase is now ready for examination.

Supervisor: Dr Chadwick L. T. Ngwisha

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Date.....

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### **CERTIFICATE OF APPROVAL**

This dissertation by Dr. Anastase Nkuliyingoma is approved in partial fulfillment of the requirements for the award of Master of Medicine in General Surgery by the University of Zambia.

**Examiner's Signature**

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I, hereby declare that this dissertation has never to my knowledge been previously published in part or in full for a diploma or a degree at any University. Acknowledgement for referenced materials has been appropriately made.

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Signature (Student)..... Date.....

Signature (Supervisor)..... Date.....

Signature (Co-supervisor)..... Date.....



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## **DEDICATION**

To my wife Esperance and children (Corrine and Aline) for their patience and support during my time away from home for training.

## ABBREVIATIONS

UTH	:	University Teaching Hospital
ER	:	Oestrogen (Estrogen) Receptor
PR	:	Progesterone Receptor
ERP(ER+)	:	Oestrogen Receptor Positive
ERN (ER-)	:	Oestrogen Receptor negative
PRP (PR+)	:	Progesterone Receptor positive
PRN (PR-)	:	Progesterone Receptor negative
HER2	:	Human Epidermal Growth Factor Receptor.
CDH	:	Cancer Diseases Hospital
ASCO /CAP	:	American Society of Clinical Oncology-College of American Pathologists.
UNZAREC	:	University of Zambia Research Ethics Committee
SERMS	:	Selective Estrogen Receptor Modulators
IARC	:	The International Agency for Research on Cancer the specialized cancer agency of the World Health Organization, today released the latest data on cancer incidence, mortality, and prevalence worldwide
UNZA	:	University of Zambia
QOL	:	Quality of Life

## DEFINITIONS OF TERMS

**Breast cancer:** a growth of abnormal cells usually within the ducts or lobules of the breast (Abu and Alkaissi, 2007).

**Hormones** are substances that function as chemical messengers in the body. They affect the actions of cells and tissues at various locations in the body, often reaching their targets through the bloodstream. The hormones oestrogen and progesterone are produced by the ovaries in pre-menopausal women and by some other tissues, including fat and skin, in both pre-menopausal and post-menopausal women. Oestrogen and progesterone can also promote the growth of some breast cancers, which are called hormone-sensitive (or hormone-dependent) breast cancers (National Cancer Institute, 2012).

**Receptor:** refers to the proteins found on the surface of many breast cancer cells.

**Hormone receptors:** these are molecules that can bind to a specific hormone. Receptors for peptide hormones tend to be found on the plasma membrane of cells, whereas receptors for lipid-soluble hormones are usually found within the cytoplasm. Hormone receptors receive growth signals from the hormones oestrogen and progesterone.

**Adjuvant therapy:** administration of cytotoxic chemotherapy or the use of ablative or additive endocrine therapy after primary surgery of breast cancer to kill or inhibit clinically occult micro metastasis (National Cancer Institute, 2012).

**Adjuvant therapy for early-stage breast cancer:** Research has shown that women treated for early-stage ER-positive breast cancer benefit from receiving at least 5 years of adjuvant hormone therapy. Adjuvant therapy is treatment given after the main treatment (surgery, in the case of early-stage breast cancer) to increase the likelihood of a cure.

Adjuvant therapy may include radiation therapy and some combination of chemotherapy, hormone therapy, and targeted therapy. Tamoxifen has been approved by the FDA for adjuvant hormone treatment of pre-menopausal and post-menopausal women (and men) with ER-positive early-stage breast cancer (National Cancer Institute, 2012).

**Tamoxifen:** a synthetic drug used to treat breast cancer and infertility in women. It acts as an oestrogen antagonist. It is given to pre- and post-menopausal women to lower the risk of hormone-receptor positive breast cancer and to prevent its recurrence (National Cancer Institute, 2012).

Tamoxifen itself is a prodrug, having relatively little affinity for its target protein, the oestrogen receptor. It is metabolized in the liver by the cytochrome P450 isoform CYP2D6 and CYP3A4 into active metabolites such as 4-hydroxytamoxifen (afimoxifene) and N-desmethyl-4-hydroxytamoxifen (endoxifen) which have 30-100 times more affinity with the oestrogen receptor than tamoxifen itself. These active metabolites compete with oestrogen in the body for binding to the oestrogen receptor. In breast tissue, 4-hydroxytamoxifen acts as an oestrogen receptor antagonist so that transcription of oestrogen-responsive genes is inhibited (National Cancer Institute, 2012).

QOL: generally consists of a number of domains including physical functioning, psychological well-being (such as levels of anxiety and depression), and social support. In this study, two clinical characteristics namely axillary lymph nodes and chest-ray appearance have been added to make a modified QOL scoring (the lower score being 5 and 17 being the highest). The lower is the scoring, the worst is the outcome and the high score represents the good outcome (Sheila Perry, 2007).

## Abstract

The main objective of this study was to determine the prevalence of oestrogen receptor-negative breast cancers at UTH and CDH and its impact on tamoxifen management. The specific objectives of the study were to determine the prevalence of oestrogen receptor negative of breast cancer patients; to determine other clinical and epidemiological characteristics of these patients with ER negative breast cancer; and to determine the short term (six months) clinical outcome of oestrogen receptor negative breast cancer patients on tamoxifen therapy following mastectomy. The study combined cross sectional and prospective observational designs. The immunostaining include breast cancer histologically confirmed in participants not pre-treated with tamoxifen at UTH and CDH. Forty-six female breast cancer patients were recruited for this study. The impact of ER negative and management of breast cancer with tamoxifen was measured through changes observed in QOL(EuroQol Group's EQ5D) questionnaire combined with two clinical characteristics that were investigated namely chest radiography and axillary lymph nodes in relation to ER negative. The study has revealed a higher prevalence of oestrogen receptor negative among the participants. The data demonstrates that ER positive tumours accounted for about 45.7% of all tumours, whereas 54.3% were ER negative tumours. Therefore, in this study the ER positive rate was lower than that ER negative. The results about the quality of life tended to slight increase between the first and second visit and significantly decline between the second and third visits. Total quality of life in the patients declined significantly at the third visit. This trend was noted in both the ER negative and ER positive groups. But the worst results were observed in the ER negative group. Lastly, the results revealed a significant association between diabetes mellitus and ER status; patients who were diabetic were likely to be ER negative. The study has established that although the quality of life declined significantly in all the participants, the ER negative proved to be worse. These results suggest that tumour with ER negative; adjuvant tamoxifen therapy may have no benefit.

## CHAPTER 1: INTRODUCTION

### 1.0 Overview

This chapter presents the background of the study. Areas covered include the statement of the problem, objectives of the study, hypothesis, justification of the study, and limitations and delimitations of the study.

### 1.1 Background of the Study

In this study, receptors refer to the proteins found on the surface of many breast cancer cells. These receptors enable the cells to receive signals to grow (National Cancer Institute, 2014; Breastcancer.org, 2014). In mammals, two ER subtypes, ER $\alpha$  and ER $\beta$ , have been characterized and, it appears that ER $\alpha$  is the most important estrogen receptor (Treves et al, 2003).

Adjuvant endocrine therapy is usually offered after surgery and radiotherapy for breast cancers. It is effective only among patients with hormone receptor-expressed tumors, such as oestrogen receptor (ER) positive and/or progesterone receptor (PR)-positive (Abu and Alkaissi, 2007; National Cancer Institute, 2014). It can reduce the risk of deaths due to breast cancer and recurrence in those patients, who have been widely confirmed for breast cancer through many clinical trials (National Cancer Institute, 2014; Treves, 2003). It has been reported that after a long-term follow-up, more than 34% of significant reduction in the relative risk for recurrence and death was observed in patients with adjuvant hormonal therapy (National Cancer Institute, 2014). Thus, the ER and PR status of breast tumours are now routinely determined, so that physician can suggest an appropriate endocrine therapy for the patient. In general, it has been reported that more than 67% of breast cancers are sensitive to tamoxifen therapy (Harris et al., 2000; Bowa, 1999).

Hormone receptors receive growth signals from the hormones, oestrogen and progesterone. These hormones may be both absent and if HER2 is also-negative then breast cancer is called "triple-negative". This is considered to be more aggressive and difficult to treat than hormone-receptor-positive breast cancer (National Cancer Institute, 2014).

Triple -negative breast cancer is estimated to be about 20% of histologically confirmed breast cancer. These types of cancers will not respond to hormonal therapies (including

tamoxifen neither to medications that target HER2, such as Herceptin). However they can be treated with chemotherapy and radiation therapy (National Cancer Institute, 2014).

Out of all breast cancers, 20-30% of them are HER2 receptors (human epidermal growth factor receptor) positive. Normally, these receptors receive signals that stimulate the growth of breast cancer cells, thus the more HER2 receptors, the more stimulation resulting in increased breast cancer growth (National Cancer Institute, 2014). This means that hormonal therapies and HER2-targeted therapies have to interfere with these signals and stop cancer cells from getting the messages to grow (National Cancer Institute, 2014).

Tamoxifen is one of the hormonal therapies given to pre- and post-menopausal women to lower the risk of hormone-receptor positive breast cancer and to prevent its recurrence (National Cancer Institute, 2014). Different markers determine or predict the clinical outcomes and the prognosis expected from treatment as these markers stimulate the growth of the cancer cells; the main hormones being oestrogen, progesterone and HER2. In this study, the emphasis is on the oestrogen status.

## **1.2 Statement of the Problem**

From the literature review, oestrogen negative breast cancer constitutes about 20-30% of all breast cancer cases. Furthermore, it is known that oestrogen negative breast cancer patients do not benefit from the use of tamoxifen in the management this type of breast cancer. CDH offers treatment of breast cancer with hormonal therapy (tamoxifen) with no information about the oestrogen receptor status on majority of patients. This may therefore pose a problem to patients who may be ER negative.

## **1.4 Study Objectives**

The main objective of this study was to determine the prevalence of oestrogen receptor negative breast cancers at UTH/CDH and its impact on use of tamoxifen in management. The specific objectives of this study were:

1. To determine the prevalence of each receptor status (ER, PR, HER2) in breast cancer patients.
2. To determine other clinical and epidemiological characteristics of these patients with ER negative breast cancer.



3. To determine the short term (six months) clinical outcome of oestrogen receptor negative and oestrogen receptor positive breast cancer patients on tamoxifen therapy following mastectomy in relation with the quality of life score (QOL).

## 1.5 Hypothesis

The proportion of oestrogen receptor negative breast cancers at UTH/CDH is greater than 30% and these patients have a poor response to tamoxifen post mastectomy leading to a poor clinical outcome.

### **Primary Outcome**

The proportion of ER negative in breast cancer is greater than 30% of women presenting to UTH and CDH with histological confirmed breast cancer and treatment with tamoxifen may not be necessary.

### **Secondary Outcome**

A comparison of short term clinical outcome is worse in oestrogen receptor negative patients as opposed to oestrogen receptor positive patients on tamoxifen therapy.

## 1.6 Study Justification

There is a paucity of data on the profile of breast cancer molecular characteristics in relation with hormone receptors. This implies that CDH is empirically subjecting this population of patients to tamoxifen treatment, a practice that is not evidence based.

For this reason post mastectomy adjuvant management with tamoxifen may not be appropriate or effective in the category of patient with negative oestrogen receptor. This is a big gap that needs to be addressed in order to improve management of post mastectomy patients, and justifies the need for this study. This research will help highlight the role of immunohistochemistry in management of breast cancer at UTH and CDH.

## 1.7 Limitations of the Study

The limitations of this study were: participants in this study were drawn from UTH and CDH and the sample was purposeful selected; therefore limiting the extent to which the findings may be generalized to other parts of the Zambia. Furthermore, the study was conducted over a short period of six months.

## **CHAPTER 2: LITERATURE REVIEW**

### **2.0 Overview**

This chapter covers a review of literature on relevant research related to the study. The Chapter is divided into the following sections: oestrogen, progesterone, and breast cancer; and Immunohistochemistry studies in Africa.

### **2.1 Oestrogen, Progesterone, and Breast Cancer**

Most of studies have associated the outcome of treatment using tamoxifen with the immunohistochemical characteristics of each breast cancer. It was in the early 1960s that the radio-labeled oestrogen was first observed to be preferentially concentrated in the oestrogen target organs of animals and also in human breast cancers. These observations gave rise to the concept of an “oestrogen receptor” (Roy and Othieno, 2011). Since then it has become clear that human breast cancers are dependent on oestrogen and progesterone or both, for growth. From this concept hormonal therapy manipulation targeting the receptor started and the drugs used are called selective oestrogen receptor modulators (SERMs) (Harris et al., 2000). Tamoxifen belongs to this group of drugs. ER-positive cancers respond to anti-oestrogen therapies like tamoxifen, a drug that works by blocking the oestrogen receptors on the breast tissue cells and slowing their oestrogen-fuelled growth. However tamoxifen is ineffective on oestrogen hormone-receptor-negative breast cancer (Li et al., 2009). Although the older endocrine therapies included surgical ablative procedures and high dose hormones in the early 1970s, current guidelines call for the determination of ER and PR status in all primary invasive breast cancers. Adjuvant endocrine therapy should be considered only in ER positive patients (Roy and Othieno, 2011). In the 2000s, the gold standard for adjuvant endocrine therapy was tamoxifen, and the duration of treatment was 5 years. After 5 years of adjuvant tamoxifen treatment, the reduction in annual rate of recurrence and mortality was 41% and 34%, respectively (National Cancer Institute, 2014). Furthermore, the reduction in contralateral breast cancer was 39%, and the 15-year absolute reduction in recurrence and

mortality was 12% and 9.2%, respectively, which were significantly different from patients not receiving tamoxifen (National Cancer Institute, 2014).. The basis for choice of tamoxifen use is found on the recognition of ER receptors presence or absence. Detection of ER receptors is done by immunoassays.

Worldwide, breast cancer is the commonest cancer of women in developed countries, representing 16% of all female cancers and accounting for 25% of mortality worldwide (Miller, 2010). The average annual incidence ranges from 10 to 80 per 100,000 females (Bowa, 2008).

Whilst 75% of women with breast cancer have cancer cells that are oestrogen-receptor-positive about 65% of these have cells that are both oestrogen and progesterone-receptor positive (Miller, 2010), 20-30 % of the breast cancers have many HER2 receptors (human epidermal growth factor receptor) that receive signals and stimulate the growth of breast cancer. Hence the hormonal therapies are to also target HER2 and interfere with these signals and stop cancer cells growth (National Cancer Institute, 2014). The incidence of cancer worldwide is expected to rise to 26.4 million with 17 million deaths by 2030 (Miller, 2010). According to GLOBOCAN 2012 (IARC's online database), providing the latest estimates for 28 types of cancer in 184 countries worldwide with a rise to 14.1 million new cases, breast cancer is 1.7 million (11.9%). The risk of breast cancer in lifetime, in the United States is said to be 1 in 8 (12.5%) with a 1 in 35 (3%) chance of death (Miller, 2010).

United States has the highest annual incidence rates 128.6 per 100,000 in whites and 112.6 per 100,000 among African Americans and it is reported that the mortality rates are the second most common cause of deaths among women (15%) after lung cancer (Breastcancer.org, 2011)

In United Kingdom it is estimated that there are 45,000 newly diagnosed cases and 12,500 deaths per year. In 60% of these cases, tamoxifen was used as treatment, the drug become ineffective in 35 % (Breastcancer.org) (Walker, 2008).

Regionally, the epidemiological data is scarce but in South Africa, the 2009 statistics indicate that 1 in 29 women per annum are diagnosed with breast cancer (CANSA CARE, 2009).

In Zambia, the true incidence of breast cancer is not known. Breast cancer is said to be the second commonest cancer in Zambia after cervical cancer as indicated by CDH records of 2008. The total number of cancers treated amounted to 1204; out of this number, breast cancer cases were 205 (17.02%) while 304(25.23%) cases were of cervical cancer (CDH, 2009).

In the department of Surgery (UTH) morbidity and mortality statistics, an average of 4 to 5 mastectomies is weekly done due to breast cancer. And hence 20 mastectomies are done per month, taking up 23% of all cancers in department of surgery (CDH, 2009). In a study conducted at UTH, and observed that the proportional incidence of breast cancer was 8.6% (Bowa et al, 2009).

## **2.2 Immunohistochemistry Studies in Africa**

In Africa, a few studies related to immunohistochemistry have been conducted. In the year 2008, in Kenya, Bird PA et al studied ER/PR immunohistochemistry on 120 women with breast cancer and they observed that only 24% of women had ER positive tumours while 66% were negative for both ER and PR receptors. They found that the outcome was not associated with stage, age of patient, parity, neither menopausal status nor the node metastasis. In their conclusive remarks, the breast cancer was likely to be less sensitive to tamoxifen as hormone treatment. For these authors, ER/PR determination for each breast cancer should be a priority in the control of breast cancer (Bird and Houssami, 2008).

In a Ugandan study of 40 breast cancer female patients, Indrjit et al found that 40% were ER negative (Roy and Othieno, 2011). This high rate of ER negative is also reported in Central Sudanese women in whom 64% were ER positive and 67% PR positive out of 113 patients (Awadelkarim et al, 2011). In Nigeria, Clement A Adebamowo et al studied 192 female breast cancer and found 65, 1% ER positive, 54.7% were PR positive and 79.7% were HER2 negative, thus more 34% were ER negative (Adebamowo, 2008). In another study done in Nigerian and Senegalese black women by Huo D et al revealed that these breast cancers were predominantly triple negative as only 24% of 378 patients were ER positive while PR positive accounted for 20% as positive and HER2 positive proportion was 17% (Huo et al., 2009).

A Similar study was done in South Africa and has shown the concordance of hormone receptor status with 74% of 976 post-menopausal patient being PR positive and 88% for ER positive (Regan et al., 2006).

### **2.3 Outcomes studies on treatment with tamoxifen in relation with ER status.**

In study conducted by Yang et al looking at the benefit of tamoxifen in ER negative identification of breast cancer biomarkers was of great significance and the determination of the response to hormonal therapy was a key point (Yang et al., 2012). In that study, high-grade tumours with ER negative /PR positive, adjuvant tamoxifen therapy may have no survival benefit, whereas for the patients with low-grade ER negative /PR positive tumors, adjuvant tamoxifen therapy is highly suggestive (Yang et al, 2012).

In the present study, identification of breast cancer biomarkers was of great significance, and determination of the response to hormonal therapy was a key point to survival.

In a large series of primary invasive breast cancer with long-term follow-up, the clinical outcome showed that double-positive breast cancer had the best outcome followed by single-positive tumors; the double-negative phenotype had the worst outcome, which was consistent with the results from this study. In that study the double-negative phenotype had the worst outcome (Rakha et al., 2007).

Osborne et al. demonstrated that double-positive tumors had higher response rate when compared with ER positive/PR negative- tumours. Loprinzi et al also showed that PR level was responsive to the treatment benefit

In a Taiwan, the ER positive rate was lower than that reported in Western countries However, single-positive and double-negative tumours accounted for 21% and 25% of the tumours, respectively, which is consistent with data from other countries .ER-positive status included both ER+/PR+ and ER+/PR- phenotypes meaning 25% of breast cancers were ER-/PR-, and therefore, adjuvant hormone therapy was not indicated, the worst disease-free survival and overall survival was observed and consistent with previous studies (Osborne et al., 2005).

In Chinese study, breast cancer patients with ER-/PR+ tumours were mainly pre-menopausal and younger in age. They received less benefit from adjuvant tamoxifen therapy (Yu et al., 2008).

The study of Yang et al demonstrated an association with better outcome for the ER+/PR- phenotype when compared with the double-negative group. There was no significant difference between the ER-/PR+ and double-negative groups in overall and disease-free survival.

But in a study by Rakha et al. , patients with single-positive tumors (ER+/PR- and ER-/PR+) had prognostic and predictive differences in overall survival and disease-free interval when compared with patients who had double-positive tumours. Unfortunately the study had only six patients treated with adjuvant tamoxifen after surgery in the ER-/PR+ group. Possibly the small number of cases in the group may have led to the false significant correlation and may not reflect the real response to hormonal therapy (Rakha, 2007)

In Taiwanese study, the ER-/PR+ group, 97 cases were treated with tamoxifen and 31 cases were left untreated with tamoxifen. Tamoxifen demonstrated little benefit in this group when compared with the double-negative tumours, and the result was consistent with a previously reported study (Rakha, 2007).

Type 2 diabetes is a serious health problem that affects more than 7% of adults in developed countries. Up to 16% of patients with breast cancer have diabetes, and two major risk factors for type 2 diabetes-old age and obesity-are also associated with breast cancer. Three mechanisms have been postulated to associate diabetes with breast cancer: activation of the insulin pathway, activation of the insulin-like-growth-factor pathway, and regulation of endogenous sex hormones. Comparative cohort studies and case-control studies suggest that type 2 diabetes may be associated with 10-20% excess relative risk of breast cancer (Wolf, 2005).

In relation of diabetes mellitus and its influence on the breast cancer, it has been observed that the pre-existing diabetes was significantly associated with all-cause mortality in six of seven studies. The patients with breast cancer and diabetes had a significantly higher all-cause mortality risk compared with their no diabetic counterparts and three of four studies found pre-existing diabetes to be associated with more advanced stage at presentation (Wolf, 2005).

For Sub Saharan Africa the true data is not available and in Zambia the prevalence is, as by International Diabetes Federation (IDF), estimated to be 3, 1 % (M Ng, andu 2014).

## **CHAPTER 3: METHODOLOGY**

### **3.0 Overview**

This chapter presents the research methodology adopted in this study. The chapter discusses the study design, sample size and sampling techniques, inclusion and exclusion criteria, biopsies collection and sectioning of specimens, and data analysis. Other issues discussed include variables of the study, assessment of ER/PR and HER2 status, ethical issues and risk mitigation.

### **3.1 Study design**

In view of specific objectives the study type was a mixture of a cross-sectional and a prospective observational study (cohort). This study was conducted for a period of 18 months (August 2012 to April 2014) and 46 breast cancers have been studied in women with invasive breast cancer.

### **3.2 Study site**

This study was conducted in University Teaching Hospital and Cancer Diseases Hospital, Lusaka, Zambia.

### **3.3 Study population**

The participants were female adults aged 18 years (age of consent for surgery) and above who were referred to UTH and/or Cancer Diseases Hospital from anywhere within Zambia were the core target of this research. None of them had a surgical oophorectomy.

### **3.4 Case definition**

The study was conducted on outpatients who presented with histologically confirmed invasive breast cancer and tamoxifen susceptible.

### **3.5 Sample Size and Sampling Procedure**

Purposive (convenient) sampling was used to select participants. Participants with histologically proven breast cancer were recruited from at the University Teaching Hospital and Lusaka.



The target population who attend health care at the Cancer Diseases Hospital was estimated at 480 over a period of two years at a rate of 20 per month, with the expected frequency (prevalence) of 50% and using a precision of 5% which brings worst acceptable results at 55%. The sample size was determined using the (10 per week x 52, 520  $\approx$  10% so  $\pm$ 480) parameters and formula below:

1. Population Size =480
  2. Margin of Error (Confidence Interval) = +/- 5%
  3. Confidence Level = 95% confident.
  4. Standard of Deviation = .5 – this is the most forgiving number and ensures that your sample will be large enough.
- 95% – Z Score = 1.96

Formula

$$n = \frac{N}{1 + Ne^2}$$

Where:

n= sample size

N=population

e=confidence level

$$n = \frac{480}{1 + 480 * 0.05^2}$$

$$= 218$$

It was estimated that in three months the sample would be approximately 218/4 = 54. The target sample size was 54.

### 3.7 Inclusion and Exclusion criteria

The following criteria were used to select participants for inclusion in the study:

- a) Female 18 year old and above.
- b) Informed consent to participate in the study.
- c) Histologically confirmed breast cancer.
- d) Tamoxifen susceptible (not allergic to tamoxifen).
- e) Primarily of breast origin
- f) Not a recurrence of breast cancer
- g) Sound mental health

Each participant selected was assigned an identification number to avoid duplication of participants (where one participant could be entered more than once).

The following criteria were used to exclude cases from the study:

- a) Male
- b) Female below 18years old of age
- c) Not consented to participate
- d) Not confirmed histologically as breast cancer.
- e) Breast cancer reported as secondary
- f) Recurrence of breast cancer
- g) Mental illness
- h) Surgical oophorectomy

### 3.8 Variables of the Study

The following variables were identified as dependent and independent variables, respectively:

#### **Dependent**

- Improved Quality of Life (measured by Quality of Life Score). See appendix 6

**Independent** affecting quality of life score as defined in appendix 6.

- ER receptor status (ER /PR negative or positive)
- Tamoxifen.
- Age.
- Histological type:
  - Invasive ductile carcinoma
  - Invasive lobular carcinoma

### 3.9 Biopsies collection, Tissue processing, Sectioning and Staining

Each participant was informed of the modality and after she was assessed for study inclusion, tissue biopsy was taken from patient for histopathology and immunohistochemical staining to determine the estrogen status (ER status). Techniques of biopsy, namely excision and incision, were used in this research for diagnosis of cancer. None of the patients had undergone neo-adjuvant therapy, none were recurrences.

Biopsy tissues were placed in 10% neutral-buffered formalin for fixation for 3 hours. The tissues were thereafter processed as follows in an automated tissue processor; further fixation

was allowed for another 3 hours in 10% neutral buffered formalin, then tissues were dehydrated in graded ethanol in ascending order i.e., 70% ethanol for 1 hour, 80% ethanol for 1 hour, 90% ethanol for 1 hour, then 3 changes of absolute ethanol for a total of 6 hours. After dehydration, biopsy tissues were cleared in xylene (3 changes of xylene) for 6 hours then they were infiltrated / impregnated with molten paraffin wax for 4 hours. After impregnation, tissues were taken out from the processor and put in an embedding center for blocking or embedding. Embedding is the placing of processed biopsy tissue in a mould with their labels and then fresh melted wax is poured in it and allowed to settle and solidify. After the blocks were completely cooled, they were trimmed and sectioned on a microtome and 3 micron thick sections were obtained and floated on microscope slides and then they were placed on a hot plate for 30 minutes in order to fix. After fixation, the slides were stained using the heamatoxylin and eosin (H and E) stain for histopathological diagnosis of cancer. The heamatoxylin and eosin procedure was done as follows; taking sections to water i.e.; passing tissues in 2 changes of xylene for deparaffinization, 2 changes of ethanol for dehydration and then water for 2 minutes in each. After that the tissues were stained in Mayer's heamatoxylin for 15 minutes, then excess heamatoxylin was washed in water, then the tissues were differentiated using 1 % acid-alcohol for 30 seconds, then tissues were blued in tap water for 5 minutes after that they were rinsed in distilled water. After rinsing the tissues were stained with eosin for 5 minutes and then rinsed again in distilled water. After that the tissues were dehydrated in ethanol, cleared in xylene and mounted with dibutylphthalate xylene (DPX). The tissues were then allowed to dry and reading of slides on a microscope was done by the pathologist.

For Immunohistochemistry the following was the procedure; after taking sections to water, the sections were placed in a solution of methanol and hydrogen peroxide for 20 minutes for endogenous peroxidase blocking, thereafter the tissues were rinsed in distilled water and then antigen retrieval was done using pre-heated citrate buffer in a pressure cooker for 20 minutes. The tissues were then allowed to cool at room temperature for 30 minutes, then blocking was done using normal goat blocking solution, thereafter the tissues were incubated using three primary antibodies, namely; estrogen-B1 (PPG510), progesterone (PgR636) and HER2-PY-1248) from Dako for 1 hour. After primary antibody incubation, tissues were rinsed in phosphate-buffered saline (PBS) FOR 5 Minutes, and then they were incubated with biotinylated secondary antibody for 30 minutes, and then rinsed in PBS for 5 minutes. The

tissues were then incubated with avidin-biotin complex (ABC) solution for 30 minutes and then rinsed with PBS for 5 minutes. After that the tissues were visualized using a( diaminobenzidine (DAB) substrate and chromogen. The tissues were then rinsed in distilled water then stained with Mayer's heamatoxylin for 20 seconds, then blued in tap water for 2 minutes, thereafter treated with ammonia for 10 seconds. The tissues were then dehydrated in ethanol, cleared in xylene and mounted with dibutylphthalate xylene (DPX). After allowing the slides to dry, they were examined on microscope by the pathologist to determine the ER, PR and HER2 status.

### **3.10 Assessment of ER/PR and HER2 status**

The biochemical assays for ER and PR are quantifiable, and are expressed as fmol/mg cytosol protein. There are other modalities for scoring ER and PR. The H-score uses the percentage of cells stained as weak, moderate or strong, the sum of these gives an overall maximum score of 300. Originally a cut-off point of 120 is to distinguish positive and negative. In this study, the aspect of score did not apply as the only interest was to test the positivity or negativity of ER/PR/HER2 in the specimen.

### **3.11 Data and Analysis**

Data from questionnaire was analysed using the Statistical Package for the Social Sciences (SPSS), version 20. Where comparative tests were needed, quantitative variables were compared between groups using Student's t-test (for continuous variables). The significance level was set at  $p = 0.05$ . Results were expressed as means with standard deviations (SDs) for continuous variables; counts and percentages for categorical variables.

### **3.12 Ethical Issues**

Written ethical approval was sought from the Biomedical Ethics Committee of the University of Zambia. Permission to conduct this research within UTH and CDH was obtained from the administrations of UTH and CDH.

Informed consent (written) was obtained from every eligible participant and confidentiality was assured. Those unable to read were given clear verbal explanation in their native language about consent and they ascertain that they understood by the thumb print. No form of pressure was exercised on the participant to give consent. The participant was at liberty to abstain from participation in the study and to withdraw consent at any time. For strict

confidentiality each specimen was given a number for identification. The samples were processed at UTH pathology laboratory.

### **3.13 Risk and mitigation**

There are no risks encountered in taking part in this study, except it involved a minor surgery on the affected breast and/or lymph node.

## CHAPTER 4: RESULTS

### 4.0 Overview

This chapter presents the findings of the study. The chapter is organized according to the objectives of the study, and includes a summary and a conclusion. The main objective of the study was to determine the prevalence of oestrogen receptor-negative breast cancers at UTH/CDH and its impact on tamoxifen management. The specific objectives of the study were:

1. To determine the prevalence of oestrogen receptor status(ER) of breast cancer patients.
2. To determine clinical and epidemiological characteristics of these patients with ER negative breast cancer.
3. To determine the short term (six months) clinical outcome of oestrogen receptor negative and oestrogen receptor positive breast cancer patients on tamoxifen therapy following mastectomy.

### 4.1 Prevalence of oestrogen receptor negative breast cancer patients

The first objective of this study was to determine the prevalence of oestrogen receptor negative and that of oestrogen receptor positive. Table 1 shows the prevalence of oestrogen receptor status breast cancer in these patients. Twenty-one (45.7%) were oestrogen positive whilst 25 participants (54.3%) were oestrogen negative. The results indicate that there is a higher percentage of oestrogen receptor negative than oestrogen positive. Regarding progesterone receptor, 24 (52.2%) participants were positive whilst 22 (47.8%) were negative. And HER2 receptor, two (4.3%) participants were positive whilst 44 (95.7%) were negative.

Table 1: Prevalence of oestrogen receptor negative breast cancer patients

Receptor	Receptor status	Frequency	Percentage (%)
Oestrogen receptor	Positive	21	45.7
	Negative	25	54.3
Progesterone receptor	Positive	24	52.2
	Negative	22	47.8
HER2 receptor	Positive	2	4.3
	Negative	44	95.7
Chemotherapy treatment	Yes	46	100.0
Tamoxifen therapy	Yes	46	100.0

## 4.2 Characteristics of the patients

Table 2 below presents the biographical data of the participants. There were 46 participants involved in this study. Seven (15.2%) had attained primary education, 25 (54.3%) had attained secondary education, and 14 (30.4%) had not attained formal education. Only one participant was using contraceptives and only one participant was smoking tobacco. Furthermore, only nine (19.6%) participants were drinking alcohol.

Table2: Biographical characteristics of the subjects

Variable	Values	Frequency (n=46)	Percentage
Educational level	Primary	7	15.2
	Secondary	25	54.3
	None	14	30.4
Contraceptives use	No	45	97.8
	Yes	1	2.2
Smoke	No	45	97.8
	Yes	1	2.2
Drink alcohol	No	37	80.4
	Yes	9	19.6

The analysis was conducted to assess whether there was an association between age (age< 50 *Versus* >50) and ER/PR status (positive *versus* negative); using a Chi Square test at a significance level of 0.05. Tables 3 and 4 present the findings related to it. Twenty-six participants were aged below 50 years while 20 participants were aged 50 years and above. Sixteen participants who were aged below 50 years were ER negative while nine participants who were aged 50 years or above were ER negative (Table 3). The Chi Square test results ( $\chi^2=1.246$ ;  $df=1$ ;  $p=.264$ ,  $p<0.05$ ) show that there was no association between age and ER status of the participants.

Table 3: Age of participants and their ER status

	Oestrogen receptor		Total
	Positive	Negative	
<50	10(38.5%)	16(61.5%)	26
≥50	11(55%)	9(45%)	20
Total	21	25	46

Some suggestion was noted that breast cancers diagnosed at younger than age 40 or older than age 80 had a higher proportion of missing ER status than did patients aged 40 to 80, but the difference was not statistically significant.

Table 4: Chi-Square Tests on Age and ER Status

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	1.246 <sup>a</sup>	1	.264		
Continuity Correction <sup>b</sup>	.669	1	.413		
Likelihood Ratio	1.249	1	.264		
Linear-by-Linear Association	1.219	1	.270		
N of Valid Cases	46				

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 9.13.

b. Computed only for a 2x2 table

### 4.3 Short term clinical outcome of ER breast cancer patients on tamoxifen therapy following mastectomy

Analysis was conducted to establish short term clinical outcomes of ER among breast cancer patients on tamoxifen therapy following mastectomy. The results showed that the average quality of life of the patients at the first, second and third visits was 13.50, 13.54, and 12.67, respectively (Figure 1). Paired samples t test was conducted to establish whether quality of life had improved after the first visit, at significance level of 0.05. The results showed that there was no significant difference in the quality of life of patients between the first and second visit ( $t=-0.437$ ;  $df=45$ ;  $p=0.642$ ). However, there was a significant difference in the average quality of life in the patients between the second and third visits ( $t=8.279$ ;  $df=45$ ;  $p=0.001$ ). In other words, the total quality of life in the patients declined significantly at the third visit.



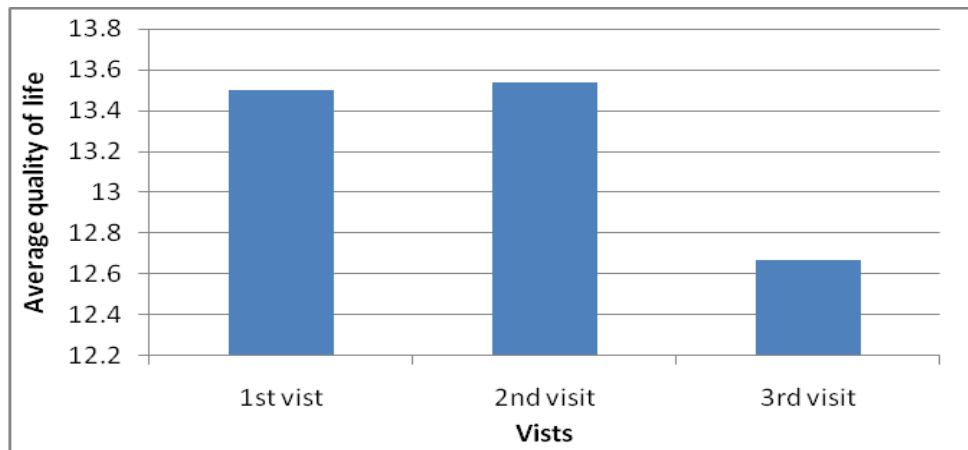


Figure 1: Average quality of life among the patients at the three visitations

#### 4.3.1 Quality of Life and ER status

Regarding the QOL, the analysis was conducted to establish the quality of life for separate ER negative (ERN) and ER positive (ERP). Tables 3-5 present the findings. The results show that the quality of life for ER positive was 13.48 at first visit, 13.52 at second visit, and 12.76 at third visit. Furthermore, the results show that the average quality of life for ER negative was 13.52 at first visit, 13.56 at second visit, and 12.60 at third visit. Similarly, these results show that the quality of life tended to decline between the second and third visits. Paired samples t tests were conducted at a significance level of 0.05 to establish whether there was any significant difference in the quality of life between the visits in each separate group of participants (ERN and ERP). The results showed that there was no significant difference in average quality of life between the first visit and the second visit in the ERP group ( $t=-.295$   $df=20$ ;  $p=0.771$ ). However, there was a significant decline in the quality of life between the second and third visit in the ERP group ( $t=6.478$ ;  $df=24$ ;  $p=0.001$ ).

Furthermore, the results showed that there was no significant difference in the average quality of life between the first visit and the second visit in the ERN group ( $t=-.371$ ;  $df=24$ ;  $p=0.714$ ). However, there was a significant decline in the average quality of life between the second and third visit in the ERP group ( $t=13.668$ ;  $df=24$ ;  $p=0.001$ ).

These results showed that the quality of life tended to slight increase between the first and second visit but significantly declined between the second and third visits. This trend was noted in both the ERP and ERN groups.

Table 1: Descriptive statistics of average quality of life among ERN and ERP participants at 1st, 2nd and 3rd visitations

Estrogen receptor			Mean	N	Std. Deviation
Positive	Pair 1	Quality of life at first visit	13.48	21	.750
		Quality of life at second visit	13.52	21	.814
	Pair 2	Quality of life at second visit	13.52	21	.814
		Quality of life at third visit	12.76	21	.831
	Pair 3	Quality of life at first visit	13.48	21	.750
		Quality of life at third visit	12.76	21	.831
Negative	Pair 1	Quality of life at first visit	13.52	25	.823
		Quality of life at second visit	13.56	25	.870
	Pair 2	Quality of life at second visit	13.56	25	.870
		Quality of life at third visit	12.60	25	.866
	Pair 3	Quality of life at first visit	13.52	25	.823
		Quality of life at third visit	12.60	25	.866

Table 2: Paired Samples Correlations of quality of life at 1st, 2nd and 3rd visitations

Oestrogen receptor			N	Correlation	Sig.
Positive	Pair 1	Quality of life at first visit & Quality of life at second visit	21	.554	.009
	Pair 2	Quality of life at second visit & Quality of life at third visit	21	.785	.000
	Pair 3	Quality of life at first visit & Quality of life at third visit	21	.512	.018
Negative	Pair 1	Quality of life at first visit & Quality of life at second visit	25	.799	.000
	Pair 2	Quality of life at second visit & Quality of life at third visit	25	.918	.000
	Pair 3	Quality of life at first visit & Quality of life at third visit	25	.772	.000

Table 3: Paired Samples Test regarding quality of life

Oestrogen receptor		Paired Differences				t	df	Sig. (2-tailed)
		Mean	Std. Deviation	95% Confidence Interval of the Difference				
				Lower	Upper			
Positive	Quality of life at first visit - Quality of life at second visit	-.048	.740	-.384	.289	-.295	20	.771
	Quality of life at second visit - Quality of life at third visit	.762	.539	.517	1.007	6.478	20	.001
	Quality of life at first visit - Quality of life at third visit	.714	.784	.358	1.071	4.176	20	.001
Negative	Quality of life at first visit - Quality of life at second visit	-.040	.539	-.262	.182	-.371	24	.714
	Quality of life at second visit - Quality of life at third visit	.960	.351	.815	1.105	13.668	24	.001
	Quality of life at first visit - Quality of life at third visit	.920	.572	.684	1.156	8.048	24	.001

#### 4.3.2 Quality of Life and Chest X-ray features

More analysis was conducted to establish whether there was any significant difference in the quality of life regarding chest x ray changes between the second and third visit in each category of ER status. These changes were based on the chest x ray's opacity appearance. The more extended opacity, the worse outcome was considered. A Chi Square Goodness of Fit test was conducted at a significant level of 0.05. Tables 6, 7 and 8 present the findings. The findings indicate that in the ERP category five participants had worse X-ray features at the second visit (Table 6) and 19 worse X-ray features at the third visit (Table 7). The Chi square test results in Table 9 show that there was a significant worsening of X-ray features between the second and third visits ( $\chi^2=13.762$ ;  $df=1$ ;  $p=0.001$ ). Similarly, the findings

indicate that in the ERN category three participants had worse X-ray features at the second visit (Table 6) and 22 worse X-ray features at the third visit (Table 7). The Chi square test results in Table 9 show that there was a significant increase worsening of X-ray features between the second and third visits ( $\chi^2=14.440$ ;  $df=1$ ;  $p=0.001$ ). In summary, in both ER statuses the findings have shown that the quality of life with regard to X-ray changes significantly declined at the third visit. However, the worst results were observed in the ERN group.

Table 4: X-ray at second visit

Oestrogen receptor		Observed N	Expected N	Residual
Positive	worse	5	10.5	-5.5
	same as at diagnosis	16	10.5	5.5
	Total	21		
Negative	worse	3	12.5	-9.5
	same as at diagnosis	22	12.5	9.5
	Total	25		

Table 5: X-ray at third visit

Oestrogen receptor		Observed N	Expected N	Residual
Positive	worse	19	10.5	8.5
	same as at diagnosis	2	10.5	-8.5
	Total	21		
Negative	worse	22	12.5	9.5
	same as at diagnosis	3	12.5	-9.5
	Total	25		

Table 6: Test Statistics: X-Ray visits

Oestrogen receptor		x-ray at second visit	x-ray at third visit
Positive	Chi-Square	5.762 <sup>a</sup>	13.762 <sup>a</sup>
	df	1	1
	Asymp. Sig.	.016	.001
	Point Probability	.019	.000
Negative	Chi-Square	14.440 <sup>b</sup>	14.440 <sup>b</sup>
	df	1	1
	Asymp. Sig.	.000	.001
	Point Probability	.000	.000

a. 0 cells (0.0%) have expected frequencies less than 5. The minimum expected cell frequency is 10.5.

b. 0 cells (0.0%) have expected frequencies less than 5. The minimum expected cell frequency is 12.5.

#### 4.3.3 Quality of Life and number of axillary lymph nodes

Here the analysis was conducted to establish whether there was any significant difference in the quality of life regarding the increase in axillary nodal numbers between the second and third visit in each category of ER status. The number of lymph nodes counted using manual palpation was used in judgment of improvement or worsening. Hence the higher was the actual number counted the worse was the outcome. A Chi Square Goodness of Fit test was conducted at a significant level of 0.05. Tables 9, 10 and 11 present the findings. The findings indicate that in the ERP category four participants had an increase in the nodal numbers at the second visit (Table 9) and 18 participants had an increase in the number of nodes at the third visit (Table 10). The Chi square test results in Table 11 show that there was a significant increase in the number of nodes between the second and third visits ( $\chi^2=10.714$ ;  $df=1$ ;  $p=0.001$ ). Similarly, the findings indicate that in the ERN category two participants had an increase in the number of nodes at the second visit (Table 9) and 23 participants had an increase in the number of nodes at the at the third visit (Table 10). The Chi square test results in Table 11 show that there was a significant increase in the number of participants with an increase in nodal number between the second and third visits ( $\chi^2=14.440$ ;  $df=1$ ;  $p=0.001$ ). In summary, in both ER statuses the findings have shown that the quality of life with regard an increase in the number of axillary lymph nodes significantly increased at the third visit. However, the worst results were observed in the ERN group.

Table 7: Nodal number at second visit

Oestrogen receptor		Observed N	Expected N	Residual
Positive	Increased	4	10.5	-6.5
	same as at diagnosis	17	10.5	6.5
	Total	21		
Negative	Increased	2	12.5	-10.5
	same as at diagnosis	23	12.5	10.5
	Total	25		

Table 8: Nodal number at third visit

Oestrogen receptor		Observed N	Expected N	Residual
Positive	Increased	18	10.5	7.5
	same as at diagnosis	3	10.5	-7.5
	Total	21		
Negative	Increased	23	12.5	10.5
	same as at diagnosis	2	12.5	-10.5
	Total	25		

Table 9: Test Statistics

Oestrogen receptor		Nodal number at second visit	Nodal number at third visit
Positive	Chi-Square	8.048 <sup>a</sup>	10.714 <sup>a</sup>
	df	1	1
	Asymp. Sig.	.005	.001
Negative	Chi-Square	17.640 <sup>b</sup>	17.640 <sup>b</sup>
	df	1	1
	Asymp. Sig.	.000	.001

a. 0 cells (0.0%) have expected frequencies less than 5. The minimum expected cell frequency is 10.5.

b. 0 cells (0.0%) have expected frequencies less than 5. The minimum expected cell frequency is 12.5.

#### 4.4 Diabetes Mellitus and ER status

Statistical tests were conducted to establish whether there was an association between ER status of the participants and their diabetic status. Table 12 below presents a summary of the results. Analysis was conducted to establish there was an association between diabetes and ER status among the participants. Chi Square tests were conducted at a significant level of 0.05. The results revealed a significant association between diabetes mellitus and ER status (Fishers' Exact test=5.381; df=1; p=0.027). In other words, there was an association between diabetes mellitus and ER status; patients who were diabetic were likely to be ER negative.

Table 10: Oestrogen receptor and Diabetes mellitus

			Diabetes mellitus		Total
			No	Yes	
Oestrogen receptor	Positive	frequency	20	1	21
		Expected frequency	16.9	4.1	21.0
	Negative	frequency	17	8	25
		Expected frequency	20.1	4.9	25.0
Total		frequency	37	9	46
		Expected frequency	37.0	9.0	46.0

Table 11: Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)
Pearson Chi-Square	5.381 <sup>a</sup>	1	.020	.027
Continuity Correction <sup>b</sup>	3.789	1	.052	
Likelihood Ratio	6.093	1	.014	.027
Fisher's Exact Test				.027
Linear-by-Linear Association	5.264 <sup>c</sup>	1	.022	.027
N of Valid Cases	46			

a. 2 cells (50.0%) have expected count less than 5. The minimum expected count is 4.11.

b. Computed only for a 2x2 table

c. The standardized statistic is 2.294.

Furthermore, a statistical test was conducted to establish whether there was an association between patients' total quality of life and their diabetic status. An independent samples t test was conducted at  $\alpha = 0.05$ . The results were not significant ( $t=1.361$ ;  $df=44$ ;  $p=0.180$ ). In other words, there was an association between diabetes mellitus and the patients' total quality of life.

## 4.6 Summary of the Findings

This chapter has presented the findings of the study. The main objective of the study was to determine the prevalence of oestrogen receptor-negative breast cancers at UTH/CDH and its impact on tamoxifen management. The specific objectives of the study were to determine the prevalence of oestrogen receptor status(ER/PR/HER2) of breast cancer patients; to determine other clinical and epidemiological characteristics of the patients with ER negative breast cancer; and to determine the short term (six months) clinical outcome of oestrogen receptor negative and oestrogen receptor positive breast cancer patients on tamoxifen therapy following mastectomy.

The study has revealed a high prevalence of oestrogen receptor negative among the participants (54.3%). The results the quality of life tended to slight increase between the first and second visit but significantly declined between the second and third visits. This trend was noted in both the ER positive and ER negative groups. In other words, the total quality of life in the patients declined significantly at the third visit. This trend was noted in both the ER positive and ER negative groups. Further results revealed that in both ER statuses that the quality of life with regard to chest x-ray changes significantly declined at the third visit; while the worst results were observed in the ER negative group. Similarly, in both ER statuses the findings revealed that the quality of life with regard to an increase in the number of nodes significantly increased at the third visit; and the worst results were observed in the ER negative group. Lastly, the results revealed a significant association between diabetes mellitus and ER status; patients who were diabetic were likely to be ER negative.



## CHAPTER 5: DISCUSSIONS

### 5.0 Overview

This chapter discusses the findings of the study. The target sample size was 54, but data was collected from 46 participants who were readily available, giving a response rate of 85.2% making it a large number and statistically acceptable response rate. Thus there was 14.8% attrition due to non-availability of anticipated participants. The chapter is divided into the following sections: prevalence, clinical outcome with the use of tamoxifen, considering the QOL parameters, and the impact of diabetes mellitus.

### 5.1 Prevalence of oestrogen receptor negative breast cancer patients

During the organization of this study it was evident that there is a paucity of literature on breast cancer in the Zambian setting, as well as in the rest of Sub Sahara Africa. This makes it difficult to make a conclusive comparison on the protocol to use in line of biomarkers determination. The first objective of the study was to determine the prevalence of oestrogen receptor negative, eventually that of oestrogen receptor positive, PR and HER2 statuses of patients. The study has identified breast cancer biomarkers of ER, PR and HER2. Of all the participants in this study, the results have established that ER positive tumours accounted for about 45.7% of all tumours, whereas 54, 3% were ER negative tumours. Therefore, ER positive rate was lower than ER negative rate. ER negative breast cancers were predominant in this study. However adjuvant endocrine therapy was provided in all cases and at the end of the observation there was no benefit from tamoxifen therapy in this group of participants(ER negative). It is important to note that although these findings may not be generalized to all health centers; it might be prudent to consider it as an eye opener since these participants were referral from different parts of the country in Zambia. It is good to note the higher rate of ER negative reported in this study is comparable to the findings reported in other countries in literature review and this could impact treatment decisions. With the input from all stakeholders, it may be possible to make it easy to have ER status testing available and affordable to all to avoid patients being treated empirically with tamoxifen. If the situation remains as it is now (empirical use of tamoxifen) then only the minority is benefiting because ER positive rate is lower, 21 (45.7%) in this study.

Regarding progesterone receptor, 24 (52.2%) participants were positive whilst 22 (47.8%) were negative. Regarding HER2 receptor, two (4.3%) participants were positive whilst 44

(95.7%) were negative. Looking at these results, there is a need to have further studies to have a picture of what happens in other phenotypes of patients, some of which are triple negative breast cancer in our setting.

## **5.2 Characteristics of the patients**

Regarding other characteristics, there is an impressive aspect that may be good to observe. This is despite not statistically correlated (possibly due to the small sample size) in this study, there is a remarkable evidence of high proportion of ER negative breast cancer, and with the demographic analysis related to age it is important to note on basis of the number of ER that the older age ( $\geq 50$  years) constituted 45% of the ER negative group whilst the younger group ( $< 50$  years) constituted 61.5%. So this leads to an assumption that when making the decision based on the age of the patient, in our setting, and in absence of the biomarkers (ER status) it may be possible to be biased and consider that most of younger women fall into ER negative group and hence other options of treatment than tamoxifen to be considered.

## **5.3 Short term clinical outcome of ER breast cancer patients on tamoxifen therapy following mastectomy**

As already stated, the other objective of the study was to establish short term clinical outcomes of ER among breast cancer patients on tamoxifen therapy following mastectomy. All participants were treated with tamoxifen. Tamoxifen demonstrated little benefit in the ER negative group when compared with the ER positive participants based on the pre-determined QOL scores. This result was consistent with reported studies in literature review. Patients with ER positive tumours had better clinical outcomes; patients with ER negative tumours experienced the worst outcome. As in literature review, the breast cancer is a common health issue associated with the poor prognosis not only due to late presentation but; as shown in this study; the ER status has an important role when it comes to hormonal management with tamoxifen.

The study has established that although the quality of life declined significantly in all the participants, the ER negative proved to be worse. These results suggest that tumours with ER negative; adjuvant tamoxifen therapy may have no benefit.

Although tamoxifen may have been effective in some ER negative participants with possibly relative dependence on histological grade, randomized clinical trials are needed to understand

the real treatment effect of hormonal therapy on ER negative tumours. To end the practice based on assumption, we certainly need to do ER profile on all our patients to avoid disadvantaging those patients with oestrogen negative breast cancer who could then be treated with other appropriate regimens.

The intrinsic biological factors (receptor status) should not exclusively be considered as the sole cause of poor prognosis as other factors such as advanced stage at diagnosis also play an important role in the end outcome. This implies that benefit from adjuvant treatment options in advanced invasive breast cancer (even if ER positive) may be limited or if treatment carried on them, the expected end result may out rightly not be seen.

### **5.3.1 Quality of Life and ER status**

In this study the quality of life for separate ER negative and ER positive was investigated. The results showed that the quality of life tended to slightly increase between the first and second visit but significantly decline between the second and third visits. This trend was noted in both the ER positive and ER negative categories but the worst results were observed in the ER negative group. Based on these findings as valid evidence, it is likely that high biologically aggressive breast cancer (ER negative) occurring in our setting and it explains the poor outcome. This calls for a change in our protocol of treatment.

### **5.3.2 Quality of Life and Chest X-ray features**

Furthermore the analysis was conducted to establish whether there was any significant difference in the quality of life regarding chest x-ray changes between the second and third visit in each category of ER status. In both ER statuses the findings showed that the quality of life with regard to chest x-ray changes significantly declined at the third visit. However, the worst results were observed in the ER negative group. This shows how ER negative breast cancer is more aggressive.

### **5.3.3 Quality of Life and axillary lymph nodes numbers**

Regarding the axillary nodes, the analysis was conducted to establish whether there was any significant difference in the quality of life regarding the increase in nodal numbers between the second and third visit in each category of ER status. In both ER statuses the findings have shown that the quality of life with regard in increase of the number of nodes significantly increased at the third visit and the worst results were observed in the ER negative category.

In regard to the above three observations related to the QOL, the analysis of each clinical characteristic namely chest x- ray and axillary lymph nodes, indicates that the trend is worse for the ER negative treated with tamoxifen. The principle of using a drug, tamoxifen, is expected to be associated with the good outcome but this is not the case of ER negative in this study, hence it requires a review of our protocol to provide an appropriate clinical care to these patients in our setting.

Since there are no benefits to use tamoxifen on the ER negative patients, the best option would to use the group of other drugs to provide care to these patients.

#### **5.4 Diabetes Mellitus and ER status**

In relation to the diabetes mellitus, the statistical tests were conducted to establish whether there was an association between ER status of the participants and their diabetic status. Table 12 below presents a summary of the results. The results revealed a significant association between diabetes mellitus and ER status; there was an association between diabetes mellitus and ER status; patients who were diabetic were likely to be ER negative. Therefore, it should be a mandatory test for everyone who is newly diagnosed with breast cancer to know her diabetes mellitus status as this could greatly improve the clinical outcome of the would be in need of both diseases care.

## CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

### 6.0 Overview

This chapter presents the conclusion and recommendations.

### 6.1 Conclusion

The proportion of ER negative at UTH / CDH is greater than 30%. This study has shown that the number of participants is ER negative and hence this may be a reality that requires having further input to determine the protocol of approach in our setting.

Furthermore, like other studies in literature review, in this study it has come out that the clinical outcome is worse in ER negative patients than ER positive patients on tamoxifen therapy. Simply saying there is no benefit to use tamoxifen in ER negative.

Since these findings are comparable to other findings in literature, it is imperative that ER status determination be done before administering tamoxifen to all patients in order to effectively deal with the breast cancer using hormonal manipulation as mode of treatment. The findings are highly showing the possibility that the patients in our setting have aggressive breast cancer (ER negative) and less susceptible to conventional hormonal treatment namely tamoxifen. In other way, it will more be beneficial to consider the protocols based on breast cancer immunohistochemical phenotype.

The results were not significant in the four of the clinical characteristics except between diabetes mellitus and ER status (Fishers' Exact test=5.381; df=1; p=0.027). More studies in literature have proved it and this one is also showing the same picture. So it is beneficial to take a complete medical history from our patients to have reliable and sound information concerning other conditions that a patient may be a victim of. The diabetes mellitus may not be the only one affecting the outcome of breast cancer but this is a call to our clinical care provider. By this study, it should not exclusively be that other diseases are not important but simply be a matter of further studies to clarify the influence of each one as many factors may have contributed to current observation in this study.

## 6.2 Recommendations

The following are the recommendations:

1. Post mastectomy tamoxifen use should not be based on assumption that every patient is predominantly ER positive. The ER status has to be pre-determined before the use of hormonal treatment based on tamoxifen and the availability of the drugs with better clinical outcome for ER negative patients must be ensured.
2. ER status and diabetes mellitus require an in depth analysis for better understanding of what we offer to our patients who may have both ailments. Furthermore, it is important to make it mandatory to test for diabetes mellitus in a newly diagnosed breast cancer as this could greatly improve the clinical outcome of the would be in need of both diseases care.

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## **Appendix 1: Information Sheet**

### **Consent form**

Why are we giving you this form?

The main purpose of this form is to give beforehand information about the named research study to give you a clear any doubts and ask any questions you may have about this study. Thus giving you insight as to whether you would like to take part or not in the study to determine the prevalence of oestrogen receptors-negative breast cancers at UTH/CDH with a view of improving post mastectomy management with tamoxifen.

### **Voluntary Participation:**

Your participation in this study is voluntary. You are free to withdraw from the study at any time if you wish to do so.

### **Who is carrying out this study?**

I am a student /Doctor training to specialize in General Surgery at the University of Zambia, School of Medicine, and Department of Surgery. In partial fulfillment of the program study I am expected to undertake a research in any area of health care that will contribute to the provision of quality health care and improve on the body of knowledge.

### **Background Information**

As aforementioned, you are being requested to participate in this research study, where we want to determine the prevalence of oestrogen receptors-negative breast cancers at UTH/CDH with a view of improving post mastectomy management with tamoxifen. With this information, we will be able to make relevant recommendations, policies and interventions at UTH/CDH to improve our service delivery to breast cancer patients.

Your participation and provision of this information will be very vital to us and we sincerely request you to take part.

### **What takes place in this Research study?**

You will be interviewed when necessary in confidence and all the information will be dealt in the same manner.

**Possible problems**

As a participant you will be asked to voluntarily undergo one of the surgical procedures (excision or incision biopsy).

We believe that all the processes involved will be harmless to you. However some of few expected problems may be a bit of bleeding and possible allergies due to anesthetic drug but these would be handled professionally. If we notice anything suspicious to you as a result of any procedure, during and after collection of information, we will let you know and further facilitate your seeking of appropriate medical interventions and treatment.

**Benefits:**

There are no monetary benefits that will be given in exchange for information obtained. However taking part in this study will generate information that will contribute to the provision of quality health services.

It is hoped that the study will help reduce over and under assuming that all the breast cancers are oestrogen positive as far as tamoxifen treatment is concerned in post mastectomy at UTH/CDH. The health problems of none availability of oestrogen receptor profile will be identified and quantified and a provision will be put in place for advising and counseling about treatment of patients who will be found ER negative and needing medical attention during the time of the study.

**Confidentiality**

Your personal details will not be made public by the investigator. A code number will be used to identify the research information gathered during this study from you. All information will be stored in a secure place. Information from this study may be used for research purposed and may be published, however your name will not be made public by the investigators. It is possible that, after the study is over, we may want to look again at the laboratory and interview record data collected during this study to help us answer other questions. If this is the case, your name will still not be made public again by the investigators. You are not required to write your name or initials on the questionnaire to give identity.

**Payment for the research related injury**

Should a problem arise in relation to any procedure (excision or incision and allergies to anesthetic drug) resulting from this study, the researcher should be notified on +260977940055. And you will be facilitated to seek and receive medical care from the health facility. Breast biopsy is a very minor surgery, no debilitating injury expected to occur for

compensation as it will be done professionally, on the basis of informed consent and voluntary.

### **Dissemination of research findings**

The primary target audience of our study is the health care providers and the various local and international organizations fostering advocacy on the plight of the breast cancer patients. Last but not the least, research findings will be submitted to various publishing agents and dissemination institutions for access to the public.

### **Consent Formalities**

I..... (Participant's name) have been informed about the study. I volunteer to participate in the study. A copy of this form signed by me and one of the study investigators is being given to me.

Signature/ Thumb .....

Date (D/M/Y) .....

### **Interviewer**

I have explained this research study to the participant. I am available to answer any questions now or in the future regarding the study and her rights.

Investigator (printed names) .....

Signature .....

Date (D/M/Y ) .....

### **Witness**

I ..... (Witness name) witnessed the above taking place with good faith and certify being true.

Date .....

Signature .....

## Appendix 2: Confounding Factors

HIV status

*Chronic medical disease*

: Diabetes

: Hypertension

: TB

### Appendix 3: Allred Score

The Allred score is a composite of the percentage of cells that stained and the intensity of their staining. The percentage of cells staining is classified from 0 through 5, and the intensity of cells staining is rated as 1, 2 or 3. Then if you add, for example, 5 and 3 together, you have an Allred score of 8. In order to initiate therapy, the cutoff we use for positivity would be over one-third of the cells staining strongly or over two-thirds staining moderately

Allred Score for ER Status (0-8)*			
% Staining Score	Proportion of Positive staining cells	Intensity Score	Average intensity of positively stained cells
0	None	0	None
1	<1/100	1	Weak
2	1/100 to 1/10	2	Intermediate
3	1/10 to 1/3	3	Strong
4	1/3 to 2/3		
5	>2/3		
*Allred Score = % Staining Score + Intensity Score			
Derived from: Harvey JM et al. Estrogen receptor status by immunohistochemistry is superior to the ligand-binding assay for predicting response to adjuvant endocrine therapy in breast cancer. J ClinOncol 1999;17(5):1474-81. Abstract			

## Appendix 4: H-score

The H-score is a method of assessing the extent of nuclear immunoreactivity, applicable to steroid receptors. The score is obtained by the formula:

3 x percentage of strongly staining nuclei + 2 x percentage of moderately staining nuclei + percentage of weakly staining nuclei, giving a range of 0 to 300.

## Appendix 5: Quality-adjusted Life-year

By definition the quality-adjusted life-year (QALY) is a generic measure of health-related quality of life that takes into account both the quantity and the quality of life generated by interventions administered by the caring staff. Thus, an intervention that results in a patient living for an additional five years rather than dying within one year, but where quality of life fell from 1 to 0.6 generates five years extra life with a quality of 0.6 ( $= 3.0$ ), less one year of reduced quality ( $1 - 0.6$ ) ( $= 0.4$ ), so the (net) QALYs generated by the intervention are  $3.0 - 0.4$  ( $= 2.6$ ). Another way of determining the weight or impact associated with a particular health state is to use standard descriptive systems such as the EuroQol Group's EQ5D questionnaire, which categorizes health states according to dimensions namely mobility, self-care, usual activities (e.g. work, study, homework or leisure activities), pain/discomfort and anxiety/depression as it the case in this study.



## Appendix 6: Quality Of Life Score

This Instrument was adapted from the Quality Adjusted Life Year (QALY)

### **Mobility:**

Have no problems in walking about.....	3
Have some problems in walking about.....	2
Confined to bed.....	1

### **Self-care:**

Have no problems with self-care.....	3
Have some problems washing or dressing myself.....	2
Unable to wash or dress myself.....	1

### **Usual Activities (e.g. work, study, housework)**

Have no problems with performing my usual activities.....	3
Have some problems with performing my usual activities.....	2
Unable to perform my usual activities.....	1

### **Pain/Discomfort:**

Have no pain or discomfort.....	3
Have moderate pain or discomfort.....	2
Have extreme pain or discomfort.....	1

### **Anxiety/Depression.**

Not anxious or depressed.....	3
Moderately anxious or depressed.....	2
Extremely anxious or depressed.....	1

### **Chest x-ray**

Worse.....	0
Same as at diagnosis date.....	1

**Nodal number**

Increased.....	0
Remains same as at diagnosis date.....	1

## Appendix 7: Questionnaire Form

### Demographics

#### [A] Personal Details

1. Age:  (i) (ID no)   
(ii) Birth order   
(iii) NO of siblings

3. Level of education: primary ☐ secondary ☐ none ☐

4. Residential place? .....

#### (B) Socio-Economic Factors

1. Parity  age at first delivery (if has child)

2 Age at menarche (first periods)

3 Age at Menopause

2. Contraceptive use? Yes ☐ No ☐ Type oestrogen alon ☐ oestrogen-progest ☐

4. Do you smoke? Yes ☐ No ☐

a. Tobacco  How many (per day)

B Cannabis  How many (per day)

5 Do you drink alcohol? Yes ☐ No ☐

If, yes, type: Chibuku ☐ Kachasu ☐ others ☐

How many bottles per day

6 Do you suffer of any of the following diseases?

##### *a) Diabetes mellitus*

Yes ☐

NO ☐

##### *b) HIV*

i. Yes ☐

ii. No ☐

**c) TB**

i. Yes ☐

ii. No ☐

**d) Hypertension**

i. Yes ☐

ii. No ☐

**C Breast Cancer data**

What is the size of the lump at the diagnosis date?

i. 15mm ☐

ii. 15-20mm ☐

iii.  $\geq 20$ -35mm ☐

iv.  $\geq 35$ -50mm ☐

v.  $\geq 50$ mm ☐

What is the nodal status at date of diagnosis?

i. Local ☐

ii. Regional ☐

iii. Generalized ☐

How many nodes by palpation?

1 ☐

2 ☐

3 ☐

4 ☐

5 ☐

What is the grade?

a) Well differentiated (low grade), ☐

☐

b) Moderately differentiated (intermediate grade),

c) Poorly differentiated (high grade),

☐

What is the type of surgery offered?

i. Simple mastectomy?

☐

ii. Radical mastectomy?

☐

What is your receptor status?

**a) Oestrogen:**

i. Positive

☐

ii. Negative

☐

**b) Progesterone**

i. Positive

☐

ii. Negative

☐

**c) HER2**

i. Positive

☐

ii. Negative

☐

Type of treatment

Chemotherapy:

i. Yes

☐

ii. No

☐

Radiotherapy.

i. Yes

☐

ii. No

☐

Hormonal (Tamoxifen)?

Yes ☐ No ☐ Combination of above? Yes ☐ No ☐

Has the treatment improved the **QUALITY OF LIFE**?

**Mobility:**

Have no problems in walking about.....	3
Have some problems in walking about.....	2
Confined to bed.....	1

**Self-care:**

Have no problems with self-care.....	3
Have some problems washing or dressing myself.....	2
Unable to wash or dress myself.....	1

**Usual Activities (e.g. work, study, housework).**

Have no problems with performing my usual activities.....	3
Have some problems with performing my usual activities.....	2
Unable to perform my usual activities.....	1

**Pain/Discomfort:**

Have no pain or discomfort.....	3
Have moderate pain or discomfort.....	2
Have extreme pain or discomfort.....	1

**Anxiety/Depression.**

Not anxious or depressed.....	3
Moderately anxious or depressed.....	2
Extremely anxious or depressed.....	1

**Chest x-ray**

Worse.....	0
Same as at diagnosis date.....	1

**Nodal number**

Increased.....	0
Remains same as at diagnosis date.....	1