KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY.
COLLEGE OF HEALTH SCIENCES
DEPARTMENT OF MOLECULAR MEDICINE

DESCRIPTIVE EPIDEMIOLOGY OF NEOPLASTIC BREAST LESIONS AND MOLECULAR CHARACTERISTICS OF BREAST CANCER IN GHANA

A Thesis Presented to the

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BY
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DECLARATION

This research work described in this thesis was carried out at the Department of Molecular Medicine, School of Medical Sciences, College of Health Sciences, Kwame Nkrumah University of Science and Technology. This work has not been submitted for any other degree.

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DEDICATION:

This thesis is dedicated to my wife Cecilia, late mother Mrs. Ruth A Ghartey and breast cancer victims all over the world. My Dad and siblings have always supported me. I dedicate this work to them as well.

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LIST OF ABBREVIATIONS

ANDI: ABERRATIONS OF NORMAL DEVELOPMENT AND INVOLUTION

APES: AMINO-PROPYL-TRI-ETHOXY-SALINE

BRCA1: BREAST CANCER ASSOCIATED GENE 1

BRCA2: BREAST CANCER ASSOCIATED GENE 2

BSE: BREAST SELF EXAMINATION

CKDs: CYCLIN DEPENDENT KINASES

DAB: DIAMINOBENZIDINE

DCC: DEXTRAN COATED CHARCOAL ASSAY

DCIS: DUCTAL CARCINOMA IN SITU

ER: OESTROGEN RECEPTOR

FCCs: FIBROCYSTIC CHANGES

FISH: FLOURESCENT IN SITU HYBRIDISATION

FNAC: FINE NEEDLE ASPIRATION CYTOLOGY

GP: GENERAL PRACTITIONER

HER 2: HUMAN EPIDERMAL GROWTH FACTOR RECEPTOR 2

HPV : HUMAN PAPILOMA VIRUS

HRP: HORSERADISH PEROXIDASE

IHC: IMMUNOHISTOCHEMICAL

KATH: KOMFO ANOKYE TEACHIN HOSPITAL

Ki67: TUMOUR PROLIFERATIVE INDEX PROTEIN

KTH: KORLE-BU TEACHING HOSPITAL

NCC: NUMBER OF CYCLES OF CHEMOTHERAPY

NHIS: NATIONAL HEALTH INSURANCE SCHEME

PBS: PHOSPHATE BUFFERED SALINE

PCNA: PROLIFERATIND CELL NUCLEAR ANTIGEN

PFA: PARAFORMALDEHYDE

PR: PROGESTERONE RECEPTOR

PWB: PUBLIC WELL BEING

SHBG: SEX HORMONE BINDING GLOBULIN

TS: TUMOUR STAGE

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ABSTRACT

Background

Breast cancer is emerging as a major health problem in Ghana. It is the leading cause of cancer-related deaths among women in Ghana. Its nationwide prevalence in Ghana was unknown at the time of this study (2004 -2008). Surgical oncologists reported a low response rate to anti-oestrogen therapy and survival for breast cancer in Ghana.

Objective

Investigating the descriptive epidemiology of neoplastic breast lesions and profiling clinically important biomolecules of breast cancer in Ghana, forms the core objective of this research work.

Method

Over 44,482 females were screened manually for neoplastic breast lesions and educated on breast cancer with a view to enhance early detection. Hence, no rigid selection criteria for subjects were employed during the study period; 2004 to 2008. A new device called the Breastlight; was evaluated as a novel adjunct to early detection of tumour related angiogenesis from 2007 – 2008. Archival Breast cancer specimen (N=33) were analysed for clinically important bio-molecules. These were obtained at random from breast cancer patients seen at leading hospitals. Oestrogen receptor (ER), Progesterone Receptor (PR), HER-2 and Ki67 levels in Paraffin embedded breast cancer specimen were assayed using the Immunohistochemical (IHC) method. Existing data on ER/PR (N=228) from leading hospitals was reviewed.

Results

54.84% of those diagnosed with breast cancer among screened subjects were pre-menopausal. Among pre-menopausal breast cancer victims; the most common age at detection was 39 years. Among post-menopausal breast cancer victims; the most common age at detection was 54 years. Average age at detection for breast cancer among screened subjects was 42.59 years. Prevalence of neoplastic breast lesions was 3.4%. Prevalence rate for breast cancer in Ghana ranged from 0.41% – 1.11% (95% confidence interval) among females aged 18 to 80 years in Ghana (black Africans). ER, HER-2 and Ki67 levels in breast cancer tumours (n=33) were profiled as follows; ER positives (18.18%): biochemical subtypes; ER+/HER-2-/Ki67+ (1) = 3.03%, ER+/HER-2+/Ki67+ (2) = 6.06% and ER+/HER-2-/Ki67- (3) = 9.09%. ER negatives (81.82%): biochemical subtypes; ER-/HER-2+/Ki67+ (22) = 66.67%, ER-/HER-2-/Ki67+ (4) = 12.12% and ER-/HER-2-/Ki67- (1) = 3.03%. Triple negative Breast cancers occurred at a rate of 15.15%. There were two biochemical sub-types; [ER-(PR-)/HER-2-/Ki67+] occurred at a rate of 12.12% and [ER-(PR-)/HER-2-/Ki67-] (which could be referred to as 'quadruple negative') was 3.03% of the cases.

Conclusion

Ghanaian women (black Africans) may develop breast cancer some 10 to 15 years earlier than Caucasians. Late stage presentation, unfavourable tumour characteristics and biomolecule profiles make them more aggressive and are unlikely to respond to hormonal therapy. However, they have a high tendency to respond to immunotherapy as indicated by high rate of HER-2 protein expression (66.67 % + 6.06 % = 72.73 %).

CHAPTER ONE

INTRODUCTION

1.0. INTRODUCTION

Cancer of the breast and medical history span ancient times. Through the centuries the female breasts have been acclaimed universally as a symbol of beauty, sexuality and infant nutrition. The vast majority of the lesions that occur in the breast are benign. The term "benign breast diseases" encompasses a heterogeneous group of lesions that may present a wide range of symptoms or may be detected as incidental microscopic findings. The incidence of benign breast lesions begins to rise during the second decade of life and peaks in the fourth and fifth decades, as opposed to malignant diseases, for which the incidence continues to increase after menopause, although at a less rapid pace (Donegan, 2002; London et al., 1992; McDivitt et al., 1992; Morrow, 1992; Shaaban et al., 2002).

Much concern is given to malignant lesions of the breast because breast cancer is highly destructive of women in their prime of life. However, benign lesions of the breast are far more frequent than malignant ones (Bartow et al, 1987; Caleffi et al., 2004; Cole et al, 1978; Cook and Rohan, 1985; Fitzgibbons et al, 1998; Hutchison et al, 1980; Kelsey and Gammon, 1990; La Vecchia et al, 1985; Sarnelli and Squartini, 1991). In some instances women feared breast cancer more than they wanted to preserve their breasts. Hence, they underwent mastectomy at their own insistence. Many women now believe that their breasts should be preserved in the presence of breast cancer.

Among women around the globe, breast cancer is both the most common cancer and the leading cause of cancer-related deaths. With 1 million new cases in the world each year,

breast cancer is the commonest malignancy in women and comprises 18% of all female cancers (Sarnelli and Squartini, 1991., Swerdlow et al, 2001). Women in economically disadvantaged countries have a lower incidence of breast cancer, but poorer survival rates for the disease relative to women in affluent countries (Anderson et al., 2003).

Breasts are oestrogen dependent organs, which undergo changes throughout a woman's life. These changes are generally classified as physiological, aberrations of normal development and involution (ANDI) or malignant. For reasons that are not fully understood the mammary epithelial cells behave in an aberrant manner. The cells of the breasts become disturbed and local control mechanisms fail to operate. This results in an increase in some breast cell types, followed by derangement of both structure and function (Moolgavkar et al., 1980).

Age adjusted incidence and mortality for breast cancer varies by up to a factor of five between countries. The difference between Far Eastern and Western countries is diminishing but is still about fivefold. Studies of migrants from Japan to Hawaii show that the rates of breast cancer in migrants assume the rate in the host country within one or two generations, indicating that environmental factors are of greater importance than genetic factors (Brinton and Devesa., 1996).

In developing countries with limited resources, at least half of the women have advanced or metastatic breast cancer at the time of diagnosis. Because advanced breast cancer has the poorest survival rate and is the most resource intensive to treat, measures to reduce the stage at diagnosis are likely to have the greatest overall benefit in terms of both survival and costs (Anderson et al., 2003). Women should therefore have easier access to awareness, early detection, diagnosis and treatment irrespective of their socioeconomic status.

Institutions such as Mammocare-Ghana, Reach for Recovery-Ghana and others which have provided and facilitated easier access to combined awareness and screening programmes for a decade and half a decade respectively play a very significant role in helping to bring about early detection and improved survival.

1.1. PROBLEM STATEMENT

Breast cancer is emerging as a new health problem in Ghana. In spite of scanty data on breast cancer in Ghana; it is a common cause of hospital admissions and mortality among Ghanaian women (Biritwum and Amaning, 2000). Hospital data strongly suggests a doubling of its incidence over the period 1974 – 1994 (Wiredu, 1995). It is the leading cause of cancer deaths among women in Ghana. Low awareness levels, inadequate screening and lack of early detection services have led to late diagnosis. Many of the victims find themselves in a seemingly hopeless situation. The nationwide incidence level is not known in Ghana. Between 2004 and 2008, few centers in Ghana specialized in managing and treating breast cancer. Their numbers do did exceed five nationwide and most of them were found in the southern sector of the country i.e. Greater Accra and Ashanti Regions of Ghana. From 2004 to 2008, lack of routine reporting of ER/PR receptor and HER-2 as a component of histopathology reports was of great concern to surgeons who managed breast cancer cases in Ghana. The estimations of oestrogen/progesterone receptors and Her-2/neu protein became available at Komfo Anokye Teaching Hospital (KATH) in the middle of 2008 (Ohene-Yeboah and Adjei, 2012) In a few instances these data were acquired by sending specimen to South Africa for analysis at a great financial cost to the patient.

1.2. JUSTIFICATION

According to hospital records, breast cancer used to be the second commonest cancer after cancer of the cervix in Ghana. Its hospital incidence in Ghana has doubled over two decades; 11.18% in 1974 – 1977 as compared to 20.4% in 1991 – 1994 at the Korle Bu Teaching Hospital alone(Wiredu,1995; Wiredu and Armah,2006.).

Breast cancers begin to take their toll at an earlier age and are among the major causes of death for women at ages 35 to 54 years worldwide. On the average it kills 30 to 50% of those diagnosed with it (Swerdlow et al, 2001). In Ghana it kills more than 65% of those diagnosed (Darko, 1995; Wiredu and Armah, 2006.).

According to one study the average age at diagnosis for breast cancer was estimated at 46.29 years per woman in Ghana as compared to 60 years per woman in Caucasians and 56 years in African – American Black women (Agyei-Frempong et al, 2008., Olopade, 2005). Unfortunately, Black women have limited access to early detection and diagnostic facilities - , as is the case in Ghana (lack of adequate numbers of trained personnel and equipment nationwide). Breast cancer risk is related to the length of exposure of a woman's endogenous oestrogens. Delayed onset of menses, early pregnancy and early menopause / oophorectomy lowers the incidence of breast cancer (Li, 1983). Breast cancer types, which retain their oestrogen dependency, are better managed by manipulations of endogenous oestrogen levels. This can be achieved by using less toxic and less expensive hormonal therapy. However, in Ghana where breast cancer is one of the most common reported malignant neoplasms in women, oncologists report a low response to anti-oestrogen therapy. An attempt to:

- Establish nationwide prevalence estimates for neoplastic breast lesions, through nationwide awareness creation and screening as well as

- Sampling archival breast cancer specimen for biomarkers in cases seen in Ghana in this study; is justified.

They seem to be influencing rate of hospitalization and survival rates for breast cancer in Ghana.

1.3. AIMS AND OBJECTIVES:

AIMS

- To investigate the descriptive epidemiology of neoplastic breast lesions in Ghana.
- To study clinically important biomolecules of breast cancer in Ghana.

OBJECTIVES

The objectives of this study were to:

- Establish the prevalence of neoplastic breast lesions with a view of enhancing, early detection of breast cancer in Ghana.
- Profile and correlate clinical biomolecules such as, tumour proliferation index (Ki67), HER-2/neu oncogene over-expression, oestrogen receptor (ER) and progesterone receptor (PR) in breast cancer seen in Ghana.
- Demonstrate how treatment for breast cancer can be individualized, with, a novel semi-quantitative model for optimizing the benefits of chemotherapy for breast cancer.

1.4. RESEARCH HYPOTHESIS:

- The average age at detection/diagnosis for breast cancer in Ghana could be lowered significantly through effective nationwide breast cancer awareness and screening.

- Suspected aggressive tumour behaviour coupled with low ER positive rates; suggests there may be an inverse relationship between ER levels and HER-2 over expression in breast cancer seen in Ghana.

CHAPTER TWO

LITERATURE REVIEW

2.0. LITERATURE REVIEW

2.1. BENIGN BREAST DISEASES AND NEOPLASMS

The objective of screening women for breast lesions and neoplasms is to discover those among the apparently well who are in fact suffering from breast cancer. More often than not, the outcomes of breast screening programmes reveal, the presence of, a higher proportion of benign breast diseases and benign neoplastic breast lesions than breast cancer.

The most frequently seen benign lesions of the breast are summarized as;

- developmental abnormalities,
- inflammatory lesions,
- fibrocystic changes,
- Stromal lesions and neoplasms.

DEVELOPMENTAL ABNORMALITIES

The most common congenital abnormality of the breast is described as both supernumerary and aberrant breast tissue. Supernumerary breast tissue is seen mostly along the milk line; the most frequent sites are the chest wall, vulva, and axilla. It may vary in its components of nipple (polythelia), areola, and glandular tissue (polymastia) (Pfeifer et al., 1999). The accessory breast tissue responds in the same way as normal breast tissue to physiological influences. Accessory breast tissue and polymastia are more common among Asians, especially Japanese, than whites (Marshall et al., 1994). Recognition of ectopic breast tissue

is important because it can serve as a milieu for the development of a variety of benign and malignant lesions encountered in the normal breast. It has been reported that ectopic breast tissue is more prone to malignant change and that ectopic breast cancer occurs at an earlier age; however, malignancies in ectopic breasts are very rare (Markopoulos et al., 2001; Marshall et al., 1994; O'Hara and Page, 1985). Excessive breast growth (macromastia) can be seen in pregnancy as well as during adolescence. Congenital underdevelopment of the breast (hypoplasia) is usually associated with genetic disorders, such as ulnar-mammary syndrome (Schinzel, 1987), Poland's syndrome, Turner's syndrome, and congenital adrenal hyperplasia. Among these disorders, Poland's syndrome is the congenital anomaly that has been reported to be associated with breast cancer most often (Tamiolakis et al., 2004). There are some recent studies suggesting the association of ulnar-mammary syndrome and breast cancer; however, breast cancer has not been recorded in patients with Turner's syndrome (Fan et al., 2004; Swerdlow et al., 2001). Acquired hypoplasia, on the other hand, is usually iatrogenic, most commonly subsequent to trauma or radiotherapy. The complete absence of both breasts and nipple (amastia) or presence of only nipple without breast tissue (amazia) is rare (Rosen, 2001).

INFLAMMATORY LESIONS

Mastitis refers to inflammatory lesions of the breast. A variety of inflammatory and reactive changes can be seen in the breast. Some of these changes are a result of infectious agents; others do not have a well-understood aetiology and may represent local reaction to a systemic disease, or a localized antigen-antibody reaction, and are classified as idiopathic. Acute mastitis usually occurs during the first 3 months postpartum as a result of breast feeding. Also known as puerperal or lactation mastitis, this disorder is a cellulitis of the interlobular connective tissue within the mammary gland, which can result in abscess

formation and septicaemia. It is diagnosed based on clinical symptoms and signs indicating inflammation. Risk factors fall into two general categories:

- improper nursing technique, leading to milk stasis and cracks or fissures of the nipple, which may facilitate entrance of microorganisms through the skin; and
- Stress and sleep deprivation, which both lower the mother's immune status and inhibit milk flow, thus causing engorgement(Foxman et al., 2002; Michie et al., 2003).

Granulomatous mastitis results from granulomatous reactions resulting from the following;

- infectious aetiology,
- foreign material, or
- Systemic autoimmune diseases such as sarcoidosis and Wegener's granulomatosis

involving the breast.

Identification of the aetiology requires microbiologic and immunologic testing in addition to histopathologic evaluation. Many different types of organisms can cause granulomatous mastitis (Diesing et al., 2004; Erhan et al., 2000). Tuberculosis of the breast is a very rare disease and can be confused with breast cancer or pyogenic breast abscess by clinicians. Granulomatous breast lesions can occur without an identifiable cause. This condition may be referred to as idiopathic granulomatous mastitis (Azlina et al., 2003). A foreign body-type granulomatous reaction may occur in the breast. Foreign materials, such as silicone and paraffin, which are used for both breast augmentation and reconstruction after cancer surgery, are the usual cause. Fibrosis and contractions may lead to clinically apparent firm nodules that may be tender (Van Diest et al., 1998). Mammary duct ectasia is usually an asymptomatic lesion and is detected mammographically because of microcalcifications. It is also called periductal mastitis. It can mimic invasive carcinoma clinically because; it is a

disease of middle-aged to elderly parous women, who usually present with nipple discharge, a palpable sub areolar mass, noncyclical mastalgia, or nipple inversion or retraction.

Clinically, fat necrosis may mimic breast cancer if it appears as an ill-defined or spiculated dense mass, associated with skin retraction, ecchymosis, erythema, and skin thickness. It can occur secondary to accidental or surgical trauma, or it may be associated with carcinoma or any lesion that provokes suppurative or necrotic degeneration, such as mammary duct ectasia and, to a lesser extent, fibrocystic disease with large cyst formation (Kinoshita, 2002).

FIBROCYSTIC LESIONS

Fibrocystic changes (FCCs) may be multifocal or bilateral and constitute the most frequent benign disorder of the breast. It generally affects women between 20 and 50 years of age. Although the exact pathogenesis of the entity is not clear, hormonal imbalance, particularly oestrogen predominance over progesterone, seems to play an important role in its development (Vorherr, 1982). The most common presenting symptoms are breast pain and tender nodularities in breasts.

Cysts are found in as many as one third of women between 35 and 50 years. Cysts are fluid-filled, round or ovoid structures. They are derived from the terminal duct lobular unit. In most cysts, the epithelial lining is either flattened or totally absent. In only a small number of cysts, an apocrine epithelial lining is observed. Because gross cysts are not associated with an increased risk of carcinoma development, the current consensus on the management of gross cysts is routine follow-up of the patient, without further therapy (O'Malley and Bane, 2004).

STROMAL LESIONS AND NEOPLASMS

Adenosis of the breast is a proliferative lesion that is characterized by an increased number or size of glandular components, mostly involving the lobular units. Various types of adenosis have been described, of which sclerosing adenosis and microglandular adenosis merit detailed description (Lee et al., 1996). Defining features of Sclerosing adenosis of the breast are listed as follows:

- It is a benign lobulocentric lesion of disordered acinar, myoepithelial, and connective tissue elements.
- It can mimic infiltrating carcinoma both grossly and microscopically (Jensen et al., 1989).
- Sclerosing adenosis can manifest as a palpable mass or as a suspicious finding at mammography.
- It is strongly associated with various proliferative lesions, including epithelial hyperplasias, intraductal or sclerosing papilloma, complex sclerosing lesion, calcification, and apocrine changes.
- It can coexist with both invasive and in situ cancers (Gill et al., 2003).

Various studies have found sclerosing adenosis to be a risk factor for invasive breast cancer apart from its association with other proliferative lesions of the breast (Bodian et al., 1993, Jensen et al., 1989;). Microglandular adenosis of the breast is characterized by a proliferation of round, small glands distributed irregularly within dense fibrous and/or adipose tissue. Although microglandular adenosis is considered benign, there is some evidence of the potential of this lesion to become invasive carcinoma. Microglandular adenosis also has a tendency to recur if not completely excised (Acs et al., 2003).

Epithelial hyperplasia is the most common form of proliferative breast disease. Epithelial hyperplasia (ductal or lobular type) is one of the most challenging FCCs to diagnose properly. The term atypical ductal hyperplasia is defined as a type of a ductal hyperplasia that morphologically mimics low-grade ductal carcinoma in situ (DCIS). Characteristically, it has a uniform population of cells. Most lesions of atypical ductal hyperplasia are small and focal. The significance of this lesion comes from the fact that the patient has an increased risk for invasive breast cancer, which is about four to five times that of the general population, and reaching nearly a ten-fold risk if the patient has a first-degree relative with breast cancer (Ali-Fehmi et al., 2003; Hartman et al., 2005; Jacobs et al., 1999; Page et al., 1985; Pinder and Ellis., 2003; Tavassoli, ed., 1999; Webb et al., 2002). The risk for breast cancer is higher in the ipsilateral breast, but the contralateral breast is also at risk (Haj et al., 2004). Women with atypical ductal hyperplasia develop cancer usually within 10–15 years of the diagnosis. The risk for cancer declines after 15 years. The risk for breast cancer in women with atypical ductal hyperplasia is also related to the patient's menopausal status. Premenopausal women with atypical ductal hyperplasia have a substantially higher risk than postmenopausal women with that diagnosis (Collins et al., 2006; Dupont and Page, 1989; Page et al., 2003; Tavassoli and Norris., 1990)

2.2. MALIGNANT BREAST NEOPLASIA AND LESIONS (BREAST CANCER).

2.2.1. Prevalence and incidence

With 1 million new cases in the world each year, breast cancer is the commonest malignancy in women and comprises 18% of all female cancers. In the United Kingdom, where the age standardised incidence and mortality is the highest in the world, the incidence among women aged 50 approaches two per 1000 women per year, and the disease is the single commonest cause of death among women aged 40-50, accounting for

about a fifth of all deaths in this age group. There are more than 14 000 deaths each year, and the incidence is increasing particularly among women aged 50-64, probably because of breast screening in this age group. Of every 1000 women aged 50, two will recently have had breast cancer diagnosed and about 15 will have had a diagnosis made before the age of 50, giving a prevalence of breast cancer of nearly 2% (Brinton and Devesa. , 1996).

2.2.2. Neoplastic formations

Cancer arises from a loss of normal growth control. In normal tissues, the rates of new cell growth and old cell death are kept in balance. In cancer, this balance is disrupted. This disruption can result from uncontrolled cell growth or loss of a cell's ability to undergo cell suicide by a process called "apoptosis." Apoptosis, or "cell suicide," is the mechanism by which old or damaged cells normally self-destruct. This gradual increase in the number of dividing cells creates a growing mass of tissue called a "tumour" or "neoplasm." If the rate of cell division is relatively rapid, and no "suicide" signals are in place to trigger cell death, the tumour will grow quickly in size; if the cells divide more slowly, tumour growth will be slower. But regardless of the growth rate, tumours ultimately increase in size because new cells are being produced in greater numbers than needed. As more and more of these dividing cells accumulate, the normal organization of the tissue gradually becomes disrupted.

Breast cancer and other cancers are capable of spreading throughout the body by two mechanisms: invasion and metastasis. Invasion refers to the direct migration and penetration by cancer cells into neighboring tissues. Metastasis refers to the ability of cancer cells to penetrate into lymphatic and blood vessels, circulate through the bloodstream, and then invade normal tissues elsewhere in the body. Depending on whether or not they can spread by invasion and metastasis, tumours are classified as being either

benign or malignant. Benign tumours are tumours that cannot spread by invasion or metastasis; hence, they only grow locally. Malignant tumours are tumours that are capable of spreading by invasion and metastasis. By definition, the term “cancer” applies only to malignant tumours. A malignant tumour, a “cancer,” is a more serious health problem than a benign tumour because cancer cells can spread to distant parts of the body. For example, breast cancer cells can enter the bloodstream and spread to distant organs such as the liver or brain. Cancer cells in the liver would be called metastatic breast cancer, not liver cancer. Metastases share the name of the original (“primary”) tumour. Breast cancer cells growing in the brain or liver can disrupt the functions of these vital organs and so are potentially life threatening.

2.2.3. Early detection

Early detection of breast cancer and appropriate treatment can affect the outcome of the disease. When cancer is found, a doctor will determine what type it is and how fast it is growing. He or she will also determine whether cancer cells have invaded nearby healthy tissue or spread (metastasized) to other parts of the body. In some cases, finding cancer early may decrease a person’s risk of dying from the cancer. For this reason, improving our methods for early detection is currently a high priority for cancer researchers. Breast cancer can sometimes be detected in its early stages using a mammogram, an X-ray of the breast. Mammography is most beneficial for women as they age and undergo menopause. Mammography is a screening tool that can detect the possible presence of an abnormal tissue mass. By itself, it is not accurate enough to provide definitive proof of either the presence or the absence of breast cancer. If a mammogram indicates the presence of an abnormality, further tests must be done to determine whether breast cancer actually is present.

Cancer tissue has a distinctive appearance under the microscope. Among the traits the pathologist looks for are a large number of irregularly shaped dividing cells, variation in nuclear size and shape, variation in cell size and shape, loss of specialized cell features, loss of normal tissue organization, and a poorly defined tumour boundary. Microscopic examination also provides information regarding the likely behaviour of a tumour and its responsiveness to treatment. Cancers with highly abnormal cell appearance and large numbers of dividing cells tend to grow more quickly, spread to other organs more frequently, and be less responsive to therapy than cancers whose cells have a more normal appearance. Based on these differences in microscopic appearance, doctors assign a numerical "grade" to most cancers. In this grading system, a low number grade (grade I or II) refers to cancers with fewer cell abnormalities than those with higher numbers (grade III, IV). After a diagnosis has been made, doctors ask the following three questions to determine how far the disease has progressed:

1. How large is the tumour, and how deeply has it invaded surrounding tissues?
2. Have cancer cells spread to regional lymph nodes?
3. Has the cancer spread (metastasized) to other regions of the body?

Based on the answers to these questions, the cancer is assigned a "stage." A patient's chances for survival are better when cancer is detected at a lower stage.

2.2.4. Breast cancer risk

Breast cancer is often perceived as a disease that strikes for no apparent reason. While scientists don't yet know all the reasons, many of the causes of cancer have already been identified. Besides intrinsic factors such as heredity, diet, and hormones, scientific studies point to key extrinsic factors that contribute to the cancer's development: chemicals (e.g.,

smoking), radiation, and viruses or bacteria (Willett et al., 2000). One way of identifying the various causes of cancer is by studying populations and behaviours. This approach compares cancer rates among various groups of people exposed to different factors or exhibiting different behaviours. A striking finding to emerge from population studies is that cancers arise with different frequencies in different areas of the world. For example, stomach cancer is especially frequent in Japan, colon cancer is prominent in the United States, and skin cancer is common in Australia. The reasons for the prevalence of high rates of specific kinds of cancer in certain countries are quite unclear. In theory, differences in heredity or environmental risk factors might be responsible for the different cancer rates observed in different countries. Studies on people who have moved from one country to another suggest that exposure to risk factors for cancer varies by geographic location (Willett et al., 2000). For example, in Japan, the rate of colon cancer is lower, and the rate of stomach cancer is higher, than in the United States. But this difference has been found to gradually disappear in Japanese families that have moved to the United States. This suggests that the risk of developing the two kinds of cancer is not determined primarily by heredity. The change in risk for cancer for Japanese families could involve cultural, behavioral, or environmental factors predominant in one location and not in the other.

Increased rates of cancer also have been detected in people exposed to high-strength forms of radiation such as X-rays or radiation emitted from unstable atoms called radioisotopes. Because these two types of radiation are stronger than ultraviolet radiation, they can penetrate through clothing and skin into the body. Therefore, high-strength radiation can cause cancers of internal body tissues. Examples include cancer caused by nuclear fallout from atomic explosions and cancers caused by excessive exposure to radioactive chemicals.

Chemicals and radiation that are capable of triggering the development of cancer are called "carcinogens." Carcinogens act through a multistep process that initiates a series of genetic alterations ("mutations") and stimulates cells to proliferate. A prolonged period of time is usually required for these multiple steps. There can be a delay of several decades between exposure to a carcinogen and the onset of cancer. For example, young people exposed to carcinogens from smoking cigarettes generally do not develop cancer for 20 to 30 years. This period between exposure and onset of disease is the lag time. In addition to chemicals and radiation, a few viruses also can trigger the development of cancer location (Willett et al., 2000). In general, viruses are small infectious agents that cannot reproduce on their own, but instead enter into living cells and cause the infected cell to produce more copies of the virus. Like cells, viruses store their genetic instructions in nucleic acids. In the case of cancer viruses, some of the viral genetic information carried in these nucleic acids is inserted into the chromosomes of the infected cell, and this causes the cell to become malignant. This explains the strong association between Human Papilloma Virus (HPV) and cancer of the cervix, Epstien-Barr virus and Burkitt's lymphoma, and, Hepatitis B virus and liver cancer. Cancer is not considered an inherited illness because most cases of cancer, perhaps 80 to 90 percent, occur in people with no family history of the disease. However, a person's chances of developing cancer can be influenced by the inheritance of certain kinds of genetic alterations. These alterations tend to increase an individual's susceptibility to developing cancer in the future (Brinton et al, 1996., Moolgavkar et al, 1980). For example, about 5 percent of breast cancers are thought to be due to inheritance of particular form(s) of a "breast cancer susceptibility gene." Inherited mutations can influence a person's risk of developing many types of cancer in addition to breast cancer. For example, certain inherited mutations have been described that increase a person's risk of developing colon, kidney,

bone, skin or other specific forms of cancer. But these hereditary conditions are thought to be involved in only 10 percent or fewer of all cancer cases (DeMichele and Weber, 2000). Laboratory tests can determine whether a person carries some of the genetic alterations that can increase the risk of developing certain cancers. Women who inherit certain forms of a gene called BRCA1 or BRCA2 have an elevated risk of developing breast cancer. For women with a family history of breast cancer, taking such a test may relieve uncertainty about their future risk. However, the information obtained from genetic tests is often complex and difficult to interpret. Experts advice that, the decision to undergo genetic testing should therefore be a personal, voluntary one and should only be made in conjunction with appropriate genetic counseling (DeMichele and Weber, 2000). Because a number of mutations usually must occur for cancer to arise, the chances of developing cancer increase as a person gets older because more time has been available for mutations to accumulate. For example, a 75-year-old person is a hundred times more likely to develop colon cancer than a 25-year-old (DeMichele and Weber , 2000). Because life expectancy for men and women is higher today than 50 or 100 years ago, they have a longer exposure time to factors that may promote gene changes linked to cancer. Chemicals (e.g., from smoking in relation to lung cancer), radiation, viruses, and heredity all contribute to the development of cancer by triggering changes in a cell's genes. Chemicals and radiation act by damaging genes, viruses introduce their own genes into cells, and heredity passes on alterations in genes that make a person more susceptible to cancer (DeMichele and Weber , 2000).

2.2.5. Oncogenes

Genes are inherited instructions that reside within a person's chromosomes. Each gene instructs a cell how to build a specific product in most cases, a particular kind of protein. Genes are altered, or "mutated," in various ways as part of the mechanism by which cancer

arises. Genes can be mutated in several different ways. The simplest type of mutation involves a change in a single base along the base sequence of a particular gene much like a typographical error in a word that has been misspelled. In other cases, one or more bases may be added or deleted. And sometimes, large segments of a DNA molecule are accidentally repeated, deleted, or moved. Oncogenes are damaged genes. Oncogenes are genes whose presence in certain forms and/or over activity can stimulate the development of cancer (Isaacs et al., 2000). Characteristically, when oncogenes arise in normal cells, they can contribute to the development of cancer by instructing cells to make proteins that stimulate excessive cell growth and division (Bishop, 1991). Oncogenes are related to normal genes called proto-oncogenes that encode components of the cell's normal growth-control pathway. Some of these components are growth factors, receptors, signaling enzymes, and transcription factors. Growth factors bind to receptors on the cell surface, which activate signaling enzymes inside the cell that, in turn, activate special proteins called transcription factors inside the cell's nucleus. The activated transcription factors "turn on" the genes required for cell growth and proliferation. Oncogenes arise from the mutation of proto-oncogenes. They resemble proto-oncogenes in that they code for the production of proteins involved in growth control. However, oncogenes code for an altered version (or excessive quantities) of these growth-control proteins, thereby disrupting a cell's growth-signaling pathway. By producing abnormal versions or quantities of cellular growth-control proteins, oncogenes cause a cell's growth-signaling pathway to become hyperactive. To use a simple metaphor, the growth-control pathway is like the gas pedal of an automobile. The more active the pathway, the faster cells grow and divide. The presence of an oncogene is like having a gas pedal that is stuck to the floorboard, causing the cell to continually grow

and divide. A cancer cell may contain one or more oncogenes, which means that one or more components in this pathway will be abnormal (Bishop, 1991).

2.2.6. Tumour suppressor genes

A second group of genes implicated in cancer are the "tumour suppressor genes." Tumour suppressor genes are normal genes whose absence can lead to cancer. They act like the brake pedal of an automobile. In other words, if a pair of tumour suppressor genes are either lost from a cell or inactivated by mutation, their functional absence might allow cancer to develop. Individuals who inherit an increased risk of developing cancer often are born with one defective copy of a tumour suppressor gene. Often genes come in pairs (one inherited from each parent), an inherited defect in one copy will not lead to cancer because the other normal copy is still functional. However, if the second copy undergoes mutation, the person then may develop cancer because there no longer is any functional copy of the gene (Two - hits theory) (Moolgavkar et al, 1980).

One particular tumour suppressor gene codes for a protein called "p53" that can trigger cell suicide (apoptosis). In cells that have undergone DNA damage, the p53 protein acts like a brake pedal to halt cell growth and division. If the damage cannot be repaired, the p53 protein eventually initiates cell suicide, thereby preventing the genetically damaged cell from growing out of control and making several copies of damaged cells.

2.2.7. DNA repair genes

DNA repair genes are a third type of genes implicated in cancer. DNA repair genes code for proteins whose normal function is to correct errors that arise when cells duplicate their DNA prior to cell division. Mutations in DNA repair genes can lead to a failure in repair, which in turn allows subsequent mutations to accumulate. People with a condition called xeroderma pigmentosum have an inherited defect in a DNA repair gene. As a result, they cannot

effectively repair the DNA damage that normally occurs when skin cells are exposed to sunlight, and so they exhibit an abnormally high incidence of skin cancer. Certain forms of hereditary colon cancer also involve defects in DNA repair.

Mutations also are seen in the genes that activate and deactivate carcinogens, and in those that govern the cell cycle, cell senescence (or "aging"), cell suicide (apoptosis), cell signaling, and cell differentiation. And still other mutations develop that enable cancer to invade and metastasize to other parts of the body.

2.2.8. Growth molecules, signals and micro-environmental changes

In addition to all the molecular changes that occur within a cancer cell, the environment around the tumour changes dramatically as well. The cancer cell loses receptors that would normally respond to neighboring cells that call for growth to stop. Instead, tumours amplify their own supply of growth signals. They also flood their neighbours with other signals called cytokines and enzymes called proteases. This action destroys both the basement membrane and surrounding matrix, which lies between the tumour and its path to metastasis, which is a blood vessel or duct of the lymphatic system. Tumour related angiogenesis, occurs when new blood vessels form by sprouting off from existing vessels, to ensure additional blood supply required to sustain unusual growth bursts in cancers. Long-standing research shows that tumours cannot grow past about 1-2 mm without their own blood vessels. These findings have instigated research of angiogenesis inhibitors to treat cancer (Pohl et al, 2003). Pro-angiogenic factors are secreted by healthy tissues needing more blood flow and by tumour cells. Traditional therapies target proliferating cells and may affect rapidly dividing cells in normal epithelial tissues and cause many side effects (Pohl et al, 2003).

2.2.9. RISK FACTORS FOR BREAST CANCER

2.2.9. 1. Age

The incidence of breast cancer increases with age, doubling about every 10 years until the menopause, when the rate of increase slows dramatically. Compared with lung cancer, the incidence of breast cancer is lower at younger ages. In some countries there is a flattening of the age incidence curve after the menopause (Brinton and Devesa, 1996).

2.2.9.2. Geographical variation

Age adjusted incidence and mortality for breast cancer varies by up to a factor of five between countries. The difference between Far Eastern and Western countries is diminishing but is still about fivefold. Studies of migrants from Japan to Hawaii show that the rates of breast cancer in migrants assume the rate in the host country within one or two generations, indicating that environmental factors are of greater importance than genetic factors (Willet et al., 2000).

2.2.9.3. Age at menarche and menopause

Women who start menstruating early in life or who have a late menopause have an increased risk of developing breast cancer. Women who have a natural menopause after the age of 55 are twice as likely to develop breast cancer as women who experience menopause before the age of 45. At one extreme, women who undergo bilateral oophorectomy before the age of 35 have only 40% of the risk of breast cancer of women who have a natural menopause (Li, 1983).

2.2.9.4. Age at first pregnancy

Nulliparity and late age at first birth both increase the lifetime incidence of breast cancer. The risk of breast cancer in women who have their first child after the age of 30 is about twice that of women who have their first child before the age of 20 (Li, 1983). The highest risk group are those who have a first child after the age of 35; these women appear to be at even higher risk than nulliparous women. An early age at birth of a second child further reduces the risk of breast cancer (Li, 1983).

2.2.9.5. Family history

Up to 10% of breast cancer in Western countries is due to genetic predisposition. Breast cancer susceptibility is generally inherited as an autosomal dominant with limited penetrance. This means that it can be transmitted through either sex and that some family members may transmit the abnormal gene without developing cancer themselves. It is not yet known how many breast cancer genes there may be (Decker, 1993). Two breast cancer genes, BRCA1 and BRCA2, which are located on the long arms of chromosomes 17 and 13 respectively, have been identified and account for a substantial proportion of very high risk families—i.e. those with four or more breast cancers among close relatives. Both genes are very large and mutations can occur at almost any position, so that molecular screening to detect mutation for the first time in an affected individual or family is technically demanding. Certain mutations occur at high frequency in defined populations (Decker, 1993).

2.2.9.6. Familial breast cancer

The following categories identify women who have three or more times the population risk of developing breast cancer. A woman who has:

- One first degree relative with bilateral breast cancer or breast and ovarian cancer or
- One first degree relative with breast cancer diagnosed under the age of 40 years or
- One first degree male relative with breast cancer diagnosed at any age or
- Two first or second degree relatives with breast cancer diagnosed under the age of 60 years or ovarian cancer at any age on the same side of the family or
- Three first or second relatives with breast and ovarian cancer on the same side of the family.

First degree relative is mother, sister, or daughter. Second degree female relative is grandmother, granddaughter, aunt, or niece. Many families affected by breast cancer show an excess of ovarian, colon, prostatic, and other cancers attributable to the same inherited mutation. Patients with bilateral breast cancer, those who develop a combination of breast cancer and another epithelial cancer, and women who get the disease at an early age are most likely to be carrying a genetic mutation that has predisposed them to developing breast cancer (Isaacs et al., 2000). Most breast cancers that are due to a genetic mutation occur before the age of 65, and a woman with a strong family history of breast cancer of early onset who is still unaffected at 65 has probably not inherited the genetic mutation. A woman's risk of breast cancer is two or more times greater if she has a first degree relative (mother, sister, or daughter) who developed the disease before the age of 50, and the younger the relative when she developed breast cancer the greater the risk. For example, a woman whose sister developed breast cancer aged 30-39 has a cumulative risk of 10% of developing the disease herself by age 65, but that risk is only 5% (close to the population risk) if the sister was aged 50-54 at diagnosis. The risk increases by between four and six times if two first degree relatives develop the disease. For example, a woman with two

affected relatives, one who was aged under 50 at diagnosis, has a 25% chance of developing breast cancer by the age of 65 (Decker, 1993; Isaacs et al., 2000).

2.2.9.7. Previous benign breast disease

Women with severe atypical epithelial hyperplasia have a four to five times higher risk of developing breast cancer than women who do not have any proliferative changes in their breasts. Women with this change and a family history of breast cancer (first degree relative) have a nine fold increase in risk (Isaacs et al., 2000). Women with palpable cysts, complex fibroadenomas, duct papillomas, sclerosis adenosis, and moderate or florid epithelial hyperplasia have a slightly higher risk of breast cancer (1.5-3 times) than women without these changes, but this increase is not clinically important.

2.2.9.8. Radiation

A doubling of risk of breast cancer was observed among teenage girls exposed to radiation during the Second World War. Ionising radiation also increases risk later in life, particularly when exposure is during rapid breast formation. Mammographic screening is associated with a net decrease in mortality from breast cancer among women aged over 50(Willet et al, 2000).

2.2.9.10. Lifestyle

Although there is a close correlation between the incidence of breast cancer and dietary fat intake in populations, the true relation between fat intake and breast cancer does not appear to be particularly strong or consistent. Obesity is associated with a twofold increase in the risk of breast cancer in postmenopausal women whereas among premenopausal women it is associated with a reduced incidence (Moolgavkar et al, 1980).

Some studies have shown a link between alcohol consumption and incidence of breast cancer, but the relation is inconsistent and the association may be with other dietary factors rather than alcohol. Smoking is of no importance in the aetiology of breast cancer (Willet et al, 2000).

2.2.9.11. Oral contraceptive

When individuals take oral contraceptives for 10 years; after stopping these agents, there is a small increase in the relative risk of developing breast cancer. However, there is no significantly increased risk of having breast cancer diagnosed 10 or more years following cessation of the oral contraceptive agent. Cancers diagnosed in women taking the oral contraceptive are less likely to be advanced clinically than those diagnosed in women who have never used these agents, relative risk 0.88 (0.81-0.95). Duration of use, age at first use, dose and type of hormone within the contraceptives appear to have no significant effect on breast cancer risk. Women who begin use before the age of 20 appear to have a higher relative risk than women who begin oral contraceptive use at an older age. This higher relative risk applies at an age when the incidence of breast cancer is however very low (Collaborative Group on Hormonal Factors in Breast Cancer. , 1997).

2.2.9.12. Hormone replacement therapy

Among current users of HRT and those who have ceased use 1-4 years previously the relative risk of having breast cancer diagnosed increases by a factor of 1.023 (1.011-1.036) for each year of use. This increase is consistent with the effect of a delay in the menopause, because the relative risk of breast cancer increases in never users by a factor of 1.028 (1.021-1.034) for each year older at the menopause. The risk of breast cancer appears higher with combined oestrogen and progestogen combinations (Collaborative Group on

Hormonal Factors in Breast Cancer., 1997). HRT increases breast density and reduces the sensitivity and specificity of breast screening. Cancers diagnosed in women taking HRT tend to be less advanced clinically than those diagnosed in women who have not used HRT. Current evidence suggests that HRT does not increase breast cancer mortality (Collaborative Group on Hormonal Factors in Breast Cancer. , 1997).

2.2.10. Prevention of breast cancer

Screening as currently practiced can reduce mortality but not incidence, and then only in a particular age group. Advances in treatment have produced significant but modest survival benefits. A better appreciation of factors important in the aetiology of breast cancer would raise the possibility of disease prevention.

2.2.12. Other preventive agents

Retinoids affect the growth and differentiation of epithelial cells, and experiments suggest that they may have a role in preventing breast cancer. A clinical trial of fenretinoid has been reported. In a study of 2,972 women with breast cancer randomly allocated to fenretinoid or no treatment, no significant difference was seen in contralateral breast cancer between the two groups. There was a significant interaction with treatment and menopausal status with a beneficial effect being seen in premenopausal patients (adjusted hazard ratio 0.66, 95% CI, 0.14-1.07) and an opposite trend on, postmenopausal women. Selenium is another possible cancer preventing agent (Jordan et al., 1998)

2.3. EARLY DETECTION OF NEOPLASTIC BREAST LESIONS.

Conventionally, early detection of breast lesions is achieved by employing;

- Breast self-examination,
- Mammographic screening,

- Ultrasonography and
- Fine needle aspiration cytology; as tools.

With the exception of Breast self-examination all the other well known conventional early detection tools require specialized knowledge and are gadgets which are usually not easy to come by in depressed economies. They also require regular stable source of electric power which may be difficult to find in rural areas. A simple, handheld, radiationless, torch light called the breastlight has been evaluated as an adjunct to early detection of breast lesions in this study. The vast majority of women screened will have negative results. Therefore, the screening method should be,

- Quick,
- Safe,
- Readily available,
- Economical, and
- Acceptable to the women being screened.

Most importantly, the screening method should be able to detect breast cancer at a stage when it can be treated effectively. The rationale for screening for breast cancer is based on the patho-biological fact that; it is an insidious disease that may develop over many years without signs and symptoms. In summary, the entire duration of clinical signs and symptoms until death ensues is just one quarter of the entire life history of breast cancer in its host. Initial three quarters of breast cancer's life history in its host is insidious / silent. In my view breast cancer can be described as a clonal disease of genes and molecules which is manifest through over proliferation of renegade cells, whose deleterious effects takes a huge toll on its host and threatens to end its life. Scientifically, it has been established that,

it takes 6 to 8 years for breast cancer tumour to develop from a single cell till it attains a tumour volume of $1cm^3$.

There are some among the apparently well who are actually suffering from breast cancer and they happen to be the target of mass breast screening exercises. Mass breast screening exercises affords a better chance for early detection of breast cancer. Early detection of breast cancer affords a better chance for cure. Interest and dedication are essential ingredients for planning and implementing effective mass screening programmes

Transillumination is a practical aid in the differential diagnosis of pathological conditions in the breast. Different tissues display varying degrees of translucence. According to Max Cutler (Cutler, I929), fat is highly translucent. Fibrous tissue is less so. Epithelial and fibro-epithelial masses are opaque and blood is intensely opaque (Cutler, I929). Transillumination enables a more accurate estimate of the physical nature of a tumour than can be gained by inspection and palpation alone. This information correlated with a careful history and with the physical findings enables a more accurate judgment of the underlying pathological process than can be gained without the use of this method. The normal breast presents marked variations on transillumination depending upon the relative content of fat, fibrous tissue and epithelial elements. Three important technical details, to a large extent, determine the success or failure of this method. The room in which the examination is performed must be totally dark. When examining small lesions the intensity of the light must be reduced and the faintest shadow must be interpreted as positive. In examining certain breast lesions (Schimmelbush disease) the intensity of the light must be markedly increased.

Solid tumours are opaque to transillumination. The opacity lacks the intensity of the shadow cast by blood. The character of the opacity in itself does not permit of a differentiation between benign and malignant tumours.

Cysts containing clear fluid are translucent. This finding may be of considerable aid in differentiating between carcinoma and tense, deeply seated cysts which present the clinical features of solid masses. The intense opacity of blood is one of the most characteristic and important findings in the transillumination of different tissues.

Traumatic haematoma presents a specific and characteristic appearance on transillumination. The opacity is intense, uneven and irregular in outline. When the lesion is examined at repeated intervals the opacity diminishes in its extent and intensity and finally disappears as the blood pigments are absorbed. This finding may be of considerable importance in differentiating this lesion from carcinoma especially when traumatic haematoma is accompanied by skin adherence (Cutler, I929).

Intracystic and duct papilloma associated with a haemorrhagic discharge from the nipple, present a characteristic appearance on transillumination. The opacity is intense, uniform and sharply circumscribed.

Transillumination is especially helpful in cases presenting a haemorrhagic discharge from the nipple in which no tumour can be palpated in the underlying breast. In this group of cases transillumination may constitute the only available method of localizing the lesion and indicating the site for surgical removal.

A haemorrhagic discharge from the nipple may be associated with a single papilloma or with multiple papillomata. Multiple papillomata (with the exception of microscopical lesions) present multiple opacities. Transillumination is therefore of considerable aid in determining the extent of the disease in the underlying breast and constitutes the only non-operative

means of differentiating between single or multiple lesions. This determination is of special importance from a therapeutic standpoint in indicating the extent of the surgical procedure. The practical importance of differentiating between single and multiple papillomata is emphasized by those examples in which the local removal of a duct papilloma has been followed by further bleeding from the nipple. Subsequent transillumination in these cases has revealed the fact that only one of numerous papillomata had been removed (Cutler, I929).

Transillumination is a simple procedure and a valuable aid in the interpretation of pathological conditions in the mammary gland. Its use is recommended in the routine examination of the breast (Cutler, I929.)

The breastlight can also be adapted for use as an adjunct to BSE because of its simplicity. An optically based device, the Breastlight (Figure.1.) has been developed as an adjunct to self-examination and breast awareness by Dr. David Watmough, Chairman, CEO of Highland innovation centre, Inverness, Scotland. There is a need to evaluate the device among women in Ghana with breast symptoms or suspected symptoms prior to referral for X-ray mammographic examination and biopsy. The main objective of this evaluation is to test the suitability of a new device, Breastlight, for use as an adjunct to breast self-examination in Ghana (developing nation) where late detection and consequently low survival for breast cancer is prevalent.

2.4. BIOMOLECULES OF CLINICAL IMPORTANCE IN MANAGING BREAST CANCER PATIENTS.

2.4.1. PREDICTIVE MARKERS

The hallmarks of most anticancer therapies are their unpredictable efficacies and toxicities. Predictive markers fall under bio-molecules of clinical importance. Predictive markers are

factors that are associated with response or resistance to a particular therapy. A predictive marker can be defined as a factor that indicates sensitivity or resistance to a specific treatment. Predictive markers are important in oncology as different cancers vary widely in their response to particular therapies. Thus, for any specific type of cancer, only a proportion of patients will respond to a particular treatment. Most are likely to suffer from adverse side effects. Therefore, for optimum patient management, it is desirable to know in advance the likelihood of a tumour responding to the therapy before it is selected. The prototype predictive tests in oncology are the oestrogen receptor (ER) and progesterone receptor (PR), which are used to select patients with breast cancer likely to respond to hormone therapy.

A more recently introduced predictive marker is HER-2 for selecting patients with advanced breast cancer for treatment with the therapeutic antibody trastuzumab (Herceptin). In advanced breast cancer, overproduction of HER-2 may also indicate an enhanced sensitivity to high-dose anthracycline-based regimens. On the other hand, in both early and advanced breast cancer, high concentrations of HER-2 appear to correlate with a lower probability of response to hormone therapy. Although many different anticancer drugs appear to mediate tumour regression by inducing apoptosis, there is currently no consistent evidence that any of the molecules implicated in this process can be used as predictive markers. Currently, the only recommended predictive markers in oncology are ER and PR for selecting endocrine-sensitive breast cancers and HER-2 for identifying breast cancer patients with metastatic disease who may benefit from trastuzumab [appendix; FIGURE.**IV**]. For malignancies other than breast cancers, validated predictive markers do not exist at present. Some markers can have both prognostic and predictive utility. For example, the oestrogen receptor (ER) in breast cancer not only predicts response to endocrine therapy but also correlates with good

prognosis, at least in the short term. The use of markers for assessing prognosis has been widely discussed in recent years. Ki67 protein is a prognostic indicator. Prognostic factors provide information on outcome independent of systemic adjuvant therapy. Higher Ki67 expression by tumour cells correlates with relatively poor treatment outcome irrespective of the choice of therapy (Allred et al., 1998).

2.4.1.1. EXPRESSION OF OESTROGEN AND PROGESTERONE RECEPTORS IN BREAST CANCERS.

Oestrogens are a family of related molecules that stimulate the development and maintenance of female characteristics and sexual reproduction. The natural oestrogens produced by women are steroid molecules, which mean that they are derived from a particular type of molecular skeleton containing four rings of carbon atoms, giving the shape shown in appendix, Figure. I. The most prevalent forms of human oestrogens are oestradiol and estrone. Both are produced and secreted by the ovaries, although estrone is also made in the adrenal glands and other organs. Oestrogens are hormones, which means that they function as signaling molecules. A signaling molecule exerts its effects by traveling through the bloodstream and interacting with cells in a variety of target tissues. The breast and the uterus, which play central roles in sexual reproduction, are two of the main targets of oestrogen. In addition, oestrogen molecules act on the brain, bone, liver, and heart (appendix, Figure. I.).

Oestrogen receptors normally reside in the cell's nucleus, along with DNA molecules.

In the absence of oestrogen molecules, these oestrogen receptors are inactive and have no influence on DNA (which contains the cell's genes); but when an oestrogen molecule enters a cell and passes into the nucleus, the oestrogen binds to its receptor, thereby causing the shape of the receptor to change. This oestrogen-receptor complex then binds to specific

DNA sites, called oestrogen response elements, which are located near genes that are controlled by oestrogen (appendix, Figure. III.).

After it has become attached to oestrogen response elements in DNA, this oestrogen-receptor complex binds to co-activator proteins and more nearby genes become active. The activated genes produce molecules of messenger RNA, which guide the synthesis of specific proteins. These proteins can then influence cell behavior in different ways, depending on the cell type involved (DeFazio et al, 2000).

The oestrogen receptor (ER) is a regulator of cellular growth, proliferation, and differentiation. In addition to having prognostic value, ER is the most important biologic marker or bio-molecule of therapeutic response to antioestrogens (Tamoxifen) in breast cancer. Some level of measurable ER protein is expressed in 70–80% of human breast. Immunohistochemistry (IHC) is the current method of choice for ER assessment, and its predictive value has been shown to be superior to that of biochemically based assays. Although accurate ER protein assessment is critical for optimal treatment of patients with breast cancer, studies have demonstrated inter-laboratory variability in ER detection. False-negative results for tumours with low ER protein levels have been a subject of recent concern (Elledge et al, 2000). Lack of standardization for IHC between laboratories is thought to be the major reason for testing errors, although variability in scoring methods and reporting practices, which can affect results, also plays a role. Studies which address inter laboratory variability and recommend optimal testing techniques and reporting procedures for ER testing, with the goal of increasing interlaboratory standardization for ER analysis by IHC are of great importance. Recent biologic, molecular and gene expression profiling data related to ER in breast cancer have been highlighted (Allred et al., 1998).

Internal controls can be extremely useful for evaluating the quality of IHC. The two most widely used predictive factors in cancer are the ER and the progesterone receptor (PR). Both the ER and PR are ligand-activated transcription factors belonging to the family of nuclear hormone receptors. Nuclear hormone receptors have several common structural features. These include a central DNA-binding domain responsible for targeting the receptors to specific DNA sequences within regulatory regions of their target genes and a ligand-binding domain, located in the carboxyl-terminal half of the receptor that recognizes specific hormone and non-hormone ligands (Chang et al., 1999).

Both the ER and PR exist in two main forms (Chang et al., 1999) For the ER, these are known as ERα and ERβ. ERα and ERβ are the products of distinct genes but possess 95% and 60% homology in their DNA- and ligand-binding domains, respectively. Considerable divergence exists at the amino terminus with 25% homology. Both forms of receptor bind to the same DNA response elements and exhibit similar, but not identical, ligand-binding characteristics. In certain situations, ERβ can attenuate the actions of ERα (Hayashi et al., 2003). For clinical purposes, only ERα is currently measured.

The two forms of PR, termed PR-A and PR-B, are transcribed from a single gene under the control of separate promoters (Conneely and Lydon., 2000). The main structural difference between PR-A and PR-B is that the A form lacks the first 164 amino-terminal amino acids contained in PR-B. Both forms of PR bind progestins and interact with the PR-responsive element (Conneely and Lydon, 2000). A functional difference between PR-A and PR-B is that PR-A can act as a dominant repressor of both PR-B and ER in a promoter- and cell type-specific manner (Osborne et al., 1980; Allred et al., 1990).

Until the late 1990s, ER status was assessed in fresh (tumour) tissue using ligand binding assay. Recently, however, IHC has become the method of choice for determining ER status. Studies comparing IHC to the conventional biochemical assays have demonstrated that IHC is equivalent or superior to ligand binding assay for predicting response to hormonal therapy (Elledge et al., 2000; Hawkins et al., 1988; McGuire et al., 1978).

2.4.1.2. HER -2/NEU EXPRESSION IN BREAST CANCER TISSUE.

HER-2/neu over-expression has consistently been associated with higher grade and extensive forms of ductal carcinoma in situ (Bose et al., 1996; Moreno et al., 1997). HER-2/*neu* gene amplification occurs at a lower rate (less than 10%) and has been linked to an adverse outcome in invasive lobular carcinoma (Rosenthal et al., 2002). The frequency of HER-2/*neu* gene amplification appears to be strongly correlated with tumour grade and ductal versus lobular status. Only 1 of 73 grade I invasive ductal carcinomas and 1 of 67 classic lobular carcinomas showed amplification of the HER-2 gene (Rosenthal et al., 2002). HER-2/*neu* over-expression has been a consistent feature of both mammary and extra mammary Paget's disease (Fu et al., 2001; Hanna et al., 2003; Wolber et al., 1991). The majority of studies that have compared the HER-2/*neu* status in paired primary and metastatic tumour tissues have found an overwhelming consistency of the patient's status regardless of the method of testing (IHC versus FISH). In one study of node-positive tumours that were defined as biclonal by DNA ploidy profile, HER-2/neu status was determined by IHC in 17 primary tumours and their 82 axillary lymph node metastases (Symmans et al., 1995)**.** Despite this apparent heterogeneity of the predominant clone measured by ploidy status, in each metastatic site, the HER-2/neu status was consistent between primary tumours and their corresponding metastases (Bodian et al., 1993). HER-2/*neu* amplification and over-expression has been associated with adverse outcome in some studies of male

breast carcinoma, but not in others. Finally, low level HER-2/*neu* over-expression has been identified in benign breast disease biopsies and associated with an increased risk of subsequent invasive breast cancer (Rayson et al., 1998). To determine the score of HER2 expression the membrane staining pattern is estimated and scored on a scale of 0 to 3+. Tumours with scores of 2 or greater are considered to be positive for HER-2 over-expression.

2.4.1.3. Ki67 (TUMOUR PROLIFERATION INDEX) EXPRESSION IN BREAST CANCER TISSUE.

Expressed Ki67 antigen is found in the outer parts of the nucleolus, particularly in the granular components during late G1 (During the G1 phase, cells respond to extracellular signals by advancing towards another division or withdrawing from the cycle into a resting state (G0)). G2 (G2 is similar to G1) is an intermediate gap phase. It contains a checkpoint that responds to DNA damage and causes a delay to allow DNA repair before entry into mitosis and M (mitotic) phases. Other proliferation markers like proliferating cell nuclear antigen (PCNA) are detectable in G0 phase as well as G1, S, G2 and M (mitotic) phases. Determination of the proliferation index using Ki67 antigen gives a more accurate indication of proliferating cells than PCNA (Petit et al, 2004).

Uncontrolled proliferation is a common feature of malignant cells and the development of numerous molecules such as the retinoids has been aimed at this phenomenon. Proliferation correlates with a nuclear protein known as Ki-67, a molecule that accumulates from G1-phase to mitosis, where it is found at its highest content. Directly after mitosis the amount of the antigen decreases to a minimal level.

During interphase the Ki-67 protein is predominantly associated with the nucleoli, whereas during mitosis it shows a close association with the chromosomes. Detailed cell cycle

analysis revealed that the antigen is present in nuclei of proliferating (G1-, S-, G2-phase and mitosis) cells, but not in nuclei of quiescent or resting cells (G0-phase). Scoring criteria for Ki67 may be as follows (expressed as; proportion of nuclear staining = score): none = 0, <1/100 = 1, 1/100 – 1/10 = 2, 1/10 – 1/2 = 3, and > 1/2 = 4. Tumours with a score of 1 or greater for Ki67 are considered to be positive for Ki67 expression. Quantitative determination of the fraction of cells, which stain positive for the Ki-67 nuclear antigen, has been demonstrated to be a highly accurate way of assessing the fraction of proliferating cells within a given tissue. Ki-67 has been used as a marker to define the growth fraction in both benign and malignant human tissues including prostate, breast, and lymph tissues

Ki-67 expression is usually estimated as the percentage of tumour cells positively stained by the antibody, with nuclear staining being the most common criterion of positivity. Five out of six studies reporting the value of Ki-67 to predict response (clinical and/or pathological) to chemotherapy in early or locally advanced breast cancer found that higher Ki-67 was associated with better response but one found no association (Chang et al., 2000; Faneyte et al., 2003; Petit et al., 2004; Pohl et al., 2003;Shi et al., 1991).

2.5. THE CELL CYCLE

The cell division cycle is a carefully choreographed series of events that culminates in cell division. The cell division cycle seems to have a task to faithfully replicate DNA and to equally distribute identical chromosome copies to two daughter cells. Genetic defects affecting the cell cycle machinery contribute to uncontrolled cell division, the hallmark of cancer. Cancer cells;

- accumulate cell cycle alterations,
- abandon cell cycle control, and,

- tend to remain in cycle.

An appreciation of the molecules that regulate the cell cycle is central to our understanding of the fundamental cell division process and also pinpoints the mechanisms that lead to cancer.

Cell Cycle Phases

The fundamental cell cycle events of DNA replication and cell division occur during interphase and mitosis, respectively. Interphase is the longer phase and includes the sub-phases G1, S and G2.

G1 Phase

During the G1 phase, cells respond to extracellular signals by advancing towards another division or withdrawing from the cycle into a resting state (G0). The decision to divide occurs as cells pass a restriction point late in G1, after which they become refractory to extracellular growth regulatory signals and commit to division. Passage through the restriction point is controlled by cyclin dependent kinases (CDKs) that are sequentially regulated by cyclins D, E and A. As cells enter the cycle, D-type cyclins are induced in response to growth factor stimulation, and assemble with their catalytic partners, CDK4 and CDK6. Cyclin D-dependent kinases phosphorylate the retinoblastoma tumour suppressor protein, and this modification is required for G1 exit. While hypophosphorylated, pRb and its homologues (p107, p130) bind a family of transcriptional regulators, collectively termed the E2Fs, converting them into repressors that limit the expression of E2F target genes. Phosphorylation of pRb, initially by the cyclin D-dependent kinases and then followed by the cyclin E-CDK2 complex, releases these E2Fs, enabling them to transactivate the same genes. These E2F-regulated gene products are important for S phase entry. Specific inhibitors of

CDK4 and CDK6, the INK4 proteins, can directly block cyclin D-dependent kinase activity and cause cell cycle arrest in the G1 phase. The INK4 proteins ($p15^{INK4b}$, $p16^{INK4a}$, $p18^{INK4c}$ and $p19^{INK4d}$) bind and inhibit CDK4 and CDK6. Disruption of the retinoblastoma pathway, by $p16^{INK4a}$ inactivation, loss of pRb or overexpression of cyclin D1, is common in cancer.

S Phase

Once cells enter S phase, cyclin E and E2F activities are inactivated by ubiquitin-dependent proteolysis and cyclin A-CDK2 driven phosphorylation, respectively. Cyclin A-associated kinase activity is required for entry into S phase, completion of S phase and entry into mitosis. Cyclin A co-localises with sites of DNA replication, suggesting a role in DNA synthesis. Cyclin D-, E-, and A-dependent kinases are negatively regulated by a distinct family of CDK inhibitors that include at least three proteins, $p21^{Waf1}$, $p27^{Kip1}$ and $p57^{Kip2}$. The remarkable feature in relation to cancer is the inducibility of $p21^{Waf1}$ by the tumour suppressor, p53.

G2 Phase

Similar to G1, G2 is an intermediate gap phase. It contains a checkpoint that responds to DNA damage and causes a delay to allow DNA repair before entry into mitosis. Mitosis is regulated by CDK1 in association with cyclins A, B1 and B2. These cyclin-CDK1 complexes phosphorylate cytoskeletal proteins such as lamins, histone H1, and possibly components of the mitotic spindle. For cells to exit mitosis, cyclins A and B must be degraded (Chow, 2010).

CHAPTER THREE

MATERIALS AND METHODS

3.0. MATERIALS AND METHODS

3.1. STUDY DESIGN

The descriptive epidemiology and molecular characteristics of neoplastic breast lesions in Ghana, forms the core of this PhD thesis.

3.1.1. DESCRIPTIVE EPIDEMIOLOGY OF NEOPLASTIC BREAST LESIONS IN GHANA

ETHICAL CONSIDERATIONS

INFORMED CONSENT: All subjects were encouraged to go through the awareness seminars before consenting to be screened by the medical team.

ESTIMATION OF PREVALENCE FOR BREAST LESIONS.

The stated objective here is to; establish statistically reliable prevalence rates for neoplastic breast lesions in Ghana. **SAMPLE SIZE:** This part of the study was designed to expand a previous study (1999 – 2002) to capture prospectively, as many women as possible between 2004 and 2008, numbering 29,263 subjects. Using a sample size calculator; the required sample size for 95% confidence level, error margin of 1% and estimated prevalence of 5% (for neoplastic breast lesions) is 9,604 females.

Sample size, $N = [(1.96)^2 \times 0.5 \times (1-0.05)]/(0.01)^2 = 9,604$. The medical team screened 44,482 women in all. **INCLUSION AND EXCLUSION CRITERIA:** No rigid selection criteria for the subjects were employed. A cross-section of females (ranging from puberty to post-menopausal age) in Ghana were targeted prospectively in all 10 regions. Awareness creation, screening and early detection were used as vehicles to access information

nationwide. This was a 'prospective' cross-sectional survey. The screening and awareness team was made up of nurses, a biomedical scientist, and administrator, under the supervision of a breast pathologist / general surgeon. The team visited women's groups at their churches, mosques, work places and market and screened them manually after creating awareness. Rather than limiting the number of subjects in this survey using statistically predetermined numerical cut off points; time limits were used to enable the survey cover a wider area and larger numbers. Awareness creation seminars were used to reach subjects prospectively. In effect, the team went about prospecting for women with neoplastic breast lesions nationwide while creating awareness and encouraging early detection of neoplastic breast changes. After going through the awareness seminar, those consenting to be screened by the medical team were enrolled and their data captured. Capturing clinically obvious neoplastic breast lesions was the primary objective. In addition to that, data from the previous survey from 1999 to 2002 numbering 15,219 women were analyzed. This was used to demonstrate variations in yearly estimates in prevalence rates and also why it was desirable to collect prevalence data over several years and use coalesced (pooled) data to generate prevalence estimates (Agyei-Frempong et al, 2008). Data from a previous survey on nationwide awareness level for breast cancer also informed the decision to employ this mode of survey. It was expected to improve awareness level and capture a considerable proportion of clinically obvious breast lesions. In some instances, further investigations such as, mammography/ultrasonography and fine needle cytology were used to rule in or out clinically suspicious lesions by the medical team. In this study; a questionnaire was administered on each of 29,263 women as was done in the previous study; n= 15,219 (Agyei-Frempong et al, 2008) before being subjected to visual inspection

and manual palpation of the breast by the medical team. This was followed by further visual assessment of the breast with the novel device called the breastlight.

3.1.2. A VISUAL ASSESSMENT OF BREAST TUMOUR-RELATED ANGIOGENESIS AS A MODE OF ENHANCING EARLY DETECTION

The stated objective here was to; enhance early detection of breast lesions. Visual inspection of the breast was enhanced by, using a simple handheld torch device called the breastlight (Figure. 1) developed by Dr David J. Watmough (CEO of Highland Innovation Centre, Inverness, Scotland) to visualize vascularity and tumour-related angiogenesis in the breast. This was a novel intervention to improve breast awareness and early detection of neoplastic breast lesions in this study. Neoplastic lesions require angiogenesis (a process where new blood vessels are formed from existing ones with the sole aim of supporting, tissue repair, regeneration and growth) to be able to grow beyond 10mm in diameter. Angiogenesis facilitates the spread of malignant tumour cells to distant organs (metastasis). Therefore neoplastic lesions which showed enhanced vascularity when viewed with the breastlight demonstrate potential to grow and invade other organs if they ever become malignant. The breastlight emits light with wavelength 614 +/- 5nm, which is specifically absorbed by heam pigment of blood. Areas of high vascularity in the breast appear darker, when viewed in a darkened room (Watmough, 1982; Ghartey and Watmough, 2009). The breastlight was used as a novel adjunct to clinical inspection and palpation of the breast to; reduce the chances of missing a clinically obvious lesion.

3.1.3. CLINICALLY IMPORTANT BIOMOLECULES (BIOMOLECULES OF MALIGNANT NEOPLASTIC BREAST LESIONS).

Review of existing data on ER/PR from leading hospitals.

Existing data from several sources were reviewed to demonstrate:

1. Lack of uniformity in reporting of data for ER / PR in paraffin embedded breast cancer tissues.
2. The absence / presence of data on other clinically important molecules such as HER-2.

CLINICAL BIOMOLECULE ANALYSIS OF ARCHIVAL BREAST CANCER TISSUE SAMPLES; N=33.

The stated objective here was to; study the distribution of clinically important biomolecules in breast cancer. There is a paradigm shift towards more accurate selection and individualizing treatment for solid malignant tumours using tumour markers. There is some data to suggest the trend, where associations between some biomolecules occurring in malignant tumours are suggestive of poor or good prognosis (Konecny et al, 2003). In this study, clinically important biomolecules were profiled, analysed and summarised to describe molecular epidemiology of malignant lesions in Ghana, where a previous study suggested high prevalence of oestrogen independent breast cancer (Agyei-Frempong et al, 2008). Paraffin embedded samples obtained from archival breast neoplasms were used for analysis of tumour parameters within the scope of this study. For example; oestrogen receptors (ER), HER-2/neu oncogene over expression and tumour proliferative index (Ki67) were targeted. Tumour sizes and age at diagnosis were also looked at. Only those with histopathologically confirmed breast lesions and tumours were included in this study for tumour parameters mentioned above. ER, PR, HER-2 and Ki67 were assayed using IHC assay (N = 33). The sample size was limited by lack

of funds to procure commercial antibodies. Therefore reviewing existing data on IHC assay for ER/PR from leading hospitals was done for comparison (n = 228). The calculated sample size for 95% confidence level, with a 5% error margin and prevalence of ER+ rate of 70% as reported elsewhere was N =230. Biomolecules profiling and correlations between them were explored using the sample size n=33 archival specimen. This was intended to demons trate strong or weak associations between them and answer specific questions about breast cancer tumour behaviour which may influence clinical decisions. Are larger breast tumours more aggressive? Do older breast cancer patients usually have larger or smaller tumours? Are larger breast cancer tumours more likely to be oestrogen dependent or not? Does the p resence of a high HER-2 over-expression in breast cancer tumour indicate a very likely high Ki67 level or not? Are ER positive breast cancer tumours less likely to have lower HER-2 over-expression or not? Answers to these questions gave more credence to the need to expl ore ways to individualize treatment in a reproducible manner. The mathematical concepts and design for developing this novel model for individualizing treatment have been introduc ed in subsection 3.1.4. It incorporates tumour stage (TS) and Ki67 of the individual being dia gnosed and treated for breast cancer. Hypothetically generated data was used to demonstra te its potential.

3.1.4. A NOVEL MODEL TO DEMONSTRATE HOW TO INDIVIDUALIZE TREATMENT FOR BREAST CANCER.

A NOVEL, HYPOTHETICAL, SEMI-QUANTITATIVE MODEL FOR, OPTIMIZING THE BENEFITS OF CHEMOTHERAPY IN THE MANAGEMENT OF SOLID MALIGNANT BREAST LESIONS.

The stated objective here was to; demonstrate how treatment could be individualised for breast cancer with this novel model. This model is based on assembling the following data on breast cancer patients who have received treatment retrospectively;

- Overall survival period S_o ,
- Tumour Stage [TS],
- Number of cycles of chemotherapy administered [NCC], and
- Tumour proliferative index [Ki67].

This model is summarized by the equation;

$$S_c = S_o - S_i = \frac{K_c\,[NCC]}{[TS]\,[\,Ki67]} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Equation\ 3.$$

{[TS][Ki67]}$^{-1}$ is treated as the tumour biological coefficient

Equation 3 could be used to predetermine the number of cycles of chemotherapy required to improve survival by a certain number of years. Tumour stage [TS] ranges from 1 – 4 on an arbitrary scale. Higher TS values correlate with more advanced disease and hence lower survival. Tumour proliferation index [Ki67] ranges from 1 – 4. Higher Ki67 values correlate with higher tumour proliferation rates and hence lower survival. These two values can be obtained from the histopathological and immunohistochemical analysis of the patient's solid malignant breast lesion / breast cancer tissue sample.

Equation 3; is further simplified to obtain;

$$S_c = K^c\,[NCC] \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots Equation\ 3'.$$

Equation 3', could be used to compute patient and chemotherapy specific improvement in survival; before it is administered.

For a particular patient,

$$K^c = \frac{K_c}{[TS]\,[Ki67]}$$

K^c is a constant which indicates her unique response to treatment. It represents the calculated patient specific improved survival per cycle of chemotherapy. This constant is specific to a particular patient and specific combination/type of chemotherapy to be administered on her. If K^c is high it implies the combination/type chemotherapy to be administered is more likely to be beneficial and vice versa.

3.2. METHODS

3.2.1. DESCRIPTIVE EPIDEMIOLOGY AND PREVALENCE ESTIMATES FOR NEOPLASTIC BREAST LESIONS IN GHANA.

In all 29,263 women (and a few girls, less than 5%, with neoplastic breast lesions) between 10 years and 99 years of age were offered breast cancer awareness and screening nationwide from 2004 - 2008. A questionnaire was administered on each of the 29,263 women (as was done in a previous study; n= 15,210) to collect;

- Demographic data (age, sex,),
- Reproductive data (parity, menarche, menopausal status, age at first full-term pregnancy),
- Anthropometric data (bust, waist, hips, waist/hip ratio),
- History of previous breast disease,

– Family history of breast cancer, and

– Clinical Data (Breast lesion size and site etc.).

This data was statistically analysed using histograms to highlight descriptive statistics. These were discussed and summarized as the descriptive epidemiology of neoplastic breast lesions in this study. The prevalence of breast lesions was also established.

SOCIAL AND ETHICAL ISSUES: The outcome of screening raised important ethical issues. Those who were found to have pathological lesions which they were previously not aware of were counseled and reassured of the support of the medical team. They were assigned a trained healthcare person to navigate them through further investigations and finally referred for appropriate treatment by the medical team. From time to time the medical team communicated with them. They were assured their identities would be concealed during data analysis. However, most of subjects were not bothered if revealing their identities publicly would encourage other women to participate. Another important issue was the extremely high cost of follow up investigations and treatment. This was thoroughly discussed during prescreening awareness sessions. There was a continued periodic screening programme currently on going beyond the period 2004-2008 to sustain gains during the study period and correct shortcomings of the study.

3.2.2. A VISUAL ASSESSMENT OF BREAST TUMOUR-RELATED ANGIOGENESIS

All subjects who were manually screened for breast lesions in this study from 2007 – 2008 were examined with the breastlight as well (Figure.1.). The breastlight is a radiation-less device which emits light of the wavelength around 614 nm. It functions on the principle of light penetration of tissues of varied density and opacity to light. It is able to detect

impalpable lesions with additional blood supply as well. Heamoglobin, the red pigment in whole blood absorbs light around the wavelength of 614 nm, hence blood vessels appear dark when the breastlight was placed on the inferior surface of the breast and viewed from the superior surface in a totally darkened room. Lesions with an additional capillary network due to angiogenesis appeared as, dark patches or shadows when viewed with, the breastlight.

Those with lesions picked up with the breastlight as dark shadows were referred for further investigations. A Nikon D70 camera (provided by Dr. David Watmough) was used to take photographs of the lesions. The results of follow up mammography/ ultrasonography and Fine needle cytology of the breast, ordered by the surgeon were matched with the photographs and clinical data.

3.2.3. ANALYSIS OF TISSUE CHARACTERISTICS AND CLINICALLY IMPORTANT BIOMOLECULES OF ARCHIVAL BREAST CANCER SPECIMEN.

3.2.3.1. A Review of existing data on ER / PR content of 228 breast cancer patients

A sample of, existing data on ER / PR content of 228 patients treated at leading hospitals was reviewed. Sixteen (16) tissue blocks had no residual cancers and were set aside. Thirty – three (33) out of the remaining (212 tissue blocks) were selected at random and re-analysed for ER, HER2, and Ki67 biomolecules in this study, using immunohistochemistry.

CLINICAL BIOMOLECULE ANALYSIS OF ARCHIVAL BREAST CANCER TISSUE SAMPLES; N=33.

Immunohistochemistry (IHC) is a method for demonstrating the presence and location of proteins in tissues sections. It enables the visualization of bio-molecules in intact tissue. It is especially useful for assessing the progression and treatment of diseases such as cancer. When IHC is combined with microscopy it provides a “big picture” that can help make sense

of data obtained using other methods. Immunohistochemical staining is accomplished with antibodies that recognize the target antigen protein or bio-molecule.

PRINCIPLE OF TEST: Under controlled conditions antibodies are highly specific and will bind only to the complementary antigen protein of interest in the tissue section. The antibody-antigen interaction is then visualized using either chromogenic detection, in which an enzyme conjugated to the antibody cleaves a substrate to produce a coloured precipitate at the location of the protein, or fluorescent detection, in which a fluorophore is conjugated to the antibody and can be visualized using fluorescence microscopy. Usually, neutral buffered formalin is used for fixing the tissue to be analysed and then embedded in paraffin before being sectioned. The basic steps of the IHC protocol are as follows:

1. Fixing and embedding the tissue: Proper fixation is key for the success of immunohistochemistry.10% neutral buffered formalin (NBF) was the fixative of choice used in this study. Other fixatives such as paraformaldehyde (PFA) or Bouin solution (formalin/picric acid) are used less frequently. The ideal fixation time depended on the size of the tissue block and the type of tissue, but fixation between 18-24hours seems to be ideal for most applications. Under-fixation can lead to edge staining, with strong signal on the edges of the section and no signal in the middle; over-fixation can mask the epitope. Antigen retrieval can help overcome this masking, but if the tissue has been fixed for a long period of time (i.e. over a weekend); there may be no signal even after antigen retrieval.

2. Cutting and mounting the sections: 33 archival breast cancer specimen blocks already embedded in paraffin, were cut in a microtome to the desired thickness; approximately 4 - 5 microns was ideal for IHC, and affixed onto the slides in quadruplicates (4 slides) per tissue block making a total of 132 slides. Slides were carefully labeled. Each slide had an ID number

and primary antibody label representing a specific BIOMOLECULE. Since four BIOMOLECULEs were assayed in each case, each ID number had four corresponding BIOMOLECULE labels namely; ER, PR, HER2 and Ki67.Tissue sections were best mounted on positively charged or APES (amino-propyl-tri-ethoxy-silane) coated slides. Once mounted, the slides were dried to remove any water that was trapped under the section. This was done by leaving the slide at room temperature overnight. If there was a problem with the section adhering to the slide, they were also incubated at 60ºC for two hours.

Positive control slides: endometrial carcinoma tissue sections known to be ER positive mounted on 4 slides were put through all the steps till positive staining achieved

Negative control slides: endometrial carcinoma tissue known to be ER negative were mounted on 4 slides were treated similarly till negative staining was achieved. This was done to make sure optimum conditions for optimum staining were achieved before putting the sample slides through the entire procedure.

3. Deparaffinizing and rehydrating the section: Before proceeding with the staining protocol, the slides were deparaffinized and dehydrated. Incomplete removal of paraffin can cause poor antibody binding/staining of the section.

Materials and reagents

• Xylene

• 100% ethanol

• 95% ethanol

Method

The slides were placed in a rack, and the following washes performed:

a. Xylene: 2 x 3 minutes

b. Xylene 1:1 with 100% ethanol: 3 minutes

c. 100% ethanol: 2 x 3 minutes

d. 95% ethanol: 3 minutes

e. 70 % ethanol: 3 minutes

f. 50 % ethanol: 3 minutes

g. Running cold tap water was used to rinse

The slides were kept in the tap water until ready to perform antigen retrieval. At no time from this point onwards were the slides allowed to dry. Drying out will cause non-specific antibody binding and therefore high background staining.

4. Antigen retrieval: Most formalin-fixed tissues require an antigen retrieval step before immunohistochemical staining can proceed. This is due to the formation of methylene bridges during fixation, which cross-link proteins and therefore mask antigenic sites. The two methods of antigen retrieval are heat-mediated (also known as heat-induced epitope retrieval, or HIER) and enzymatic. Both antigen retrieval methods serve to break the methylene bridges and expose the antigenic sites in order to allow the antibodies to bind. Some antigens prefer enzymatic to heat mediated antigen retrieval and vice versa .Enzymatic retrieval can sometimes damage the morphology of the section, so the concentration and treatment time need to be tested. Antigen retrieval with Tris/EDTA pH 9.0 buffer is suitable for most antigens. Sodium citrate pH 6.0 was also used. Heat-induced epitope retrieval was achieved in this study by using a pressure cooker.

A microwave, or a vegetable steamer can also be used. Additionally, some laboratories use a water bath set to 60°C and incubate the slides in retrieval solution overnight. Unless the antigen retrieval method is stated on the antibody datasheet, the optimal method for each antigen must be found experimentally.

Buffer solution for heat-induced epitope retrieval (HIER)

In the absence of suggestions from other researchers for a particular antibody, choice of retrieval buffer is best accomplished by experiment. Sodium Citrate Buffer (10mM Sodium Citrate, 0.05% Tween 20, pH 6.0) was chosen for HIER. Sodium Citrate Buffer was prepared by weighing 2.94 g Tri-sodium citrate (dihydrate) and dissolving it in Distilled water to a final volume of 1litre. It was mixed well to dissolve. The pH was adjusted to 6.0 with 1N HCl. Then 0.5 ml of Tween 20 was added and mixed well. This buffer was stored at room temperature for 3 months or at 4^0C for longer storage.

Heat-induced epitope retrieval method using a Pressure cooker

The mounted slides were placed in a metal rack for this procedure.

Materials and reagents

- Domestic stainless steel pressure cooker
- Hot plate
- Vessel with slide rack to hold approximately 400-500 ml
- Antigen retrieval buffer (i.e. Tris/EDTA pH 9.0, sodium citrate pH 6.0)

Method

The citrate antigen retrieval buffer was added to the pressure cooker. The pressure cooker was placed on the hotplate and turned on to full power. The lid of the pressure cooker was not secured at this point. It was simply rested on top of it. While waiting for the pressure cooker to come to a boil, the sections were deparaffinized and rehydrated as above. Once boiling, the slides were transferred from the tap water to the pressure cooker. CARE WAS USED WITH HOTSOLUTION – FORCEPS were used. The pressure cooker lid was secured as in the manufacturer's instructions. As soon as the cooker

reached full pressure, it was timed for 3 minutes. When 3 minutes had elapsed, the hotplate was turned off and the pressure cooker placed in an empty sink.

The pressure release valve was activated (according to the manufacturer's instructions) and cold water was run over the cooker. Once de-pressurized, the lid was opened and cold water was run into the cooker for 10 minutes. CARE WAS USED WITH HOT SOLUTION!

5. Immunohistochemical staining: The immunohistochemical staining protocol was continued as follows:

- All slides were submerged in peroxidase quenching solution and rinsed with PBS.
- A serum blocking solution (Invitrogen) was applied to reduce background staining.
- A primary antibody (ER/PR/Her2/Ki67) was added to each sample slide and incubated for 30-60 minutes at room temperature; and rinsed with PBS.
- A secondary antibody was added to each sample slide and incubated for 10 minutes at room temperature; and rinsed with PBS.
- The enzyme conjugate (streptavidin-horseradish peroxidase, from invitrogen) was added to each sample slide and incubated for 10 minutes at room temperature; and rinsed with PBS.
- The chromogen DAB was added to each sample slide and incubated for 5-10 minutes at room temperature; and rinsed with PBS.

Primary antibodies used:

- Mouse anti-Ki-67, Clone 7B11**,** For In Vitro Diagnostic Use**.** 08-1192 6.0 mL
- Mouse anti-ER,
- Mouse anti-PR,

- Mouse anti-HER-2,
- Mouse anti-Ki67,

Secondary Antibody used.

- *2nd Gen*Predilute Antibody, Ready-To-Use from Invitrogen.

D22187: Diaminobenzidine (DAB) Histochemistry Kit #3 with streptavidin.HRP

The use of horseradish peroxidase (HRP) for enzyme-mediated immunodetection, commonly referred to as immunoperoxidase labeling, is a well-established histochemical technique. The most widely used HRP substrate for these applications is diaminobenzidine (DAB), which generates a brown-coloured, polymeric oxidation product. The DAB reaction product is discretely localized at HRP-labeled sites, providing high resolution images of Sub-cellular antigen distribution. DAB staining can be visualized directly by bright-field light microscopy or, following osmication, by electron microscopy. Molecular Probes offers DAB Histochemistry Kits for detection of mouse IgG primary antibodies(D22185) and biotinylated antibodies or tracers (D22187).

Reagents and Materials

- 3,3'-Diaminobenzidine, tetrahydrochloride, dehydrate (DAB; Component A), 50 mg.
- HRP–conjugated secondary antibody or streptavidin conjugate (Component B), 100 µg
- Blocking Reagent (Component C), 4 g
- Staining Buffer (Component D), 55 mL
- Hydrogen peroxide (H_2O_2, Component E), 300 µL of a 30% stabilized solution

Each kit provided sufficient materials to stain 200 slide preparations.

Storage; upon receipt and prior to use, the kits were stored at –20ºC, desiccated and protected from light.

Applications Protocols

Preparations;

1.1 Prepared phosphate-buffered saline (PBS) (not provided) according to standard laboratory protocols.

1.2 Prepared a 10 mg/mL DAB stock solution by dissolving the50 mg of solid material provided (Component A) in 5 mL of Staining Buffer (Component D). Mixed by swirling or gentle vortexing until the DAB powder had gone into solution. I filtered the DAB solution through a 0.2 µm syringe filter. I divided the filtered DAB stock solution into small aliquots and stored frozen at–20ºC.

1.3 Prepared a 500 µg/mL stock solution of the HRP conjugate stock solution by reconstituting the material provided (Component B) in 200 µL of PBS. This solution may be stored at 4°Cfor up to 3 months if required. Optionally, 0.02% thimerosal could be added as a preservative. Please Note that sodium azide must NOT be used for this purpose.

1.4 Prepared a 1% (10 mg/mL) solution of Blocking Reagent in PBS. I prepared only as much as was needed for immediate use. However, unused solution was stored frozen at –20°C for 1 month if necessary.

Peroxidase Labelling

2.1 Cell or tissue specimens were fixed following customary procedures.

2.2 When necessary, I quenched endogenous peroxidase activity by incubating the specimen in 1–3% H_2O_2 (diluted into PBS from the 30% solution provided; Component E) for 1 hour.

2.3 Incubated the specimen with 1% Blocking Reagent solution for 60 minutes at room temperature or 37°C.

2.4 Labeled the specimen with the primary antibody diluted in 1%Blocking Reagent for 60 minutes at room temperature.

2.5 Rinsed the specimen three times with PBS.

2.6 Prepared a 1 µg/mL working solution of the HRP conjugate by diluting the stock solution (prepared in step 1.3) 1:500 in 1%Blocking Reagent.

2.7 Applied 250 µL of the HRP conjugate working solution to the specimen and incubated for 30–60 minutes at room temperature.

2.8 Rinsed the specimen three times with PBS.

Staining Tissue Sections

3.1 I Diluted the DAB stock solution (prepared in step 1.2) 1:10 in PBS to a final working concentration of 1 mg/mL. When diluting aliquots of the DAB stock solution that had been stored frozen, it was necessary to redissolve precipitated DAB by either vortexing or pipeting up and down.

3.2 For slide-mounted sections: I added H_2O_2 to the DAB working solution to a final concentration of 0.03% (1:1000 dilution from the 30% solution provided); Immediately applied about 250 ml of DAB/H_2O_2 working solution to the surface of the slide. I incubated the slide horizontally and visually inspected the section at low magnification under a microscope against a white background to assess the degree of colour development. The time required for completion varied from 10 seconds to 5 minutes.

3.3. When the DAB staining reaction was complete, as indicated by visual inspection, DAB solution was washed from the slide or staining dish into a hazardous waste disposal container.

3.4. The tissue sections were washed extensively with PBS to remove residual DAB; and transferred used wash buffer to a hazardous waste disposal container.

4.1. Sections were mounted out of PBS onto gelatinized slides; air dried, dehydrated in an ascending ethanol series, cleared in xylene and mounted in DPX under a coverslip.

Mounting and Storing Slides

Sections can be mounted in an aqueous mountant, such as Aqua-Poly/Mount (Poly sciences Inc., Warrington, PA) or 90%glycerol in PBS, directly after the final buffer wash (step 3.5). Alternatively, sections can be dehydrated in an ascending ethanol series and then cleared in xylene before being mounted under a cover slip in an organic mounting medium such as Permount®(Fisher Scientific, Pittsburgh, PA) or DPX. Allow slides to dry thoroughly before viewing or storing. Slides can be stored horizontally or vertically in a slide box at room temperature.

Visualization by Light Microscopy

Photographic contrast of DAB reaction products can reportedly be increased by inserting a blue-violet band pass filter (e.g., Schott BG12) in the transmitted light path. Colour intensification of DAB staining by addition of nickel or cobalt ions to the staining solution has been widely reported and practiced. Nickel enhancement also changes the colour of the DAB reaction product from brown to gray–black. Another reported intensification method involves incubating DAB-stained tissue with nitro blue tetrazolium (NBT, N6495), followed by a brief period of illumination under the microscope.7

Notes

[**A**] DAB is a hazardous chemical — harmful if swallowed, inhaled or placed in contact with the skin; an irritant to the eyes, skin and respiratory system; and a suspected carcinogen. DAB should be handled with appropriate precautions — if necessary, consult your

institution's chemical safety officer or laboratory safety manuals for guidance. Dispose of DAB in accordance with local, state and federal regulations.

[**B**] Optimal dilutions for primary antibodies should be determined empirically or from specifications provided by the supplier.

[**C**] In general, dilutions of the HRP conjugate between 1:50 and 1:200 can be used, depending on the abundance of the target primary antibody

3.2.4. A NOVEL, HYPOTHETICAL, SEMI-QUANTITATIVE MODEL FOR, OPTIMIZING THE BENEFITS OF CHEMOTHERAPY IN THE MANAGEMENT OF SOLID MALIGNANT BREAST LESIONS.

DETRMINATION OF K_C

K_C is a proportionality constant which is specific for the type or combination of chemotherapeutic drugs used for treatment. K_C must be predetermined from previous records available at a particular treatment centre. Archival records on patients treated at the centre with the most effective type or combination of chemotherapeutic drugs could be used to obtain the following data.

- S_o Overall survival period (years)
- [TS] tumour stage can be obtained from patient records.
- [NCC], the number of cycles of chemotherapy administered
- [Ki67] can be obtained by analyzing archival paraffin-embedded tissue samples of each patient treated.

Such data was unavailable at the time of this study, therefore carefully generated, hypothetical data was used to demonstrate its future potential.

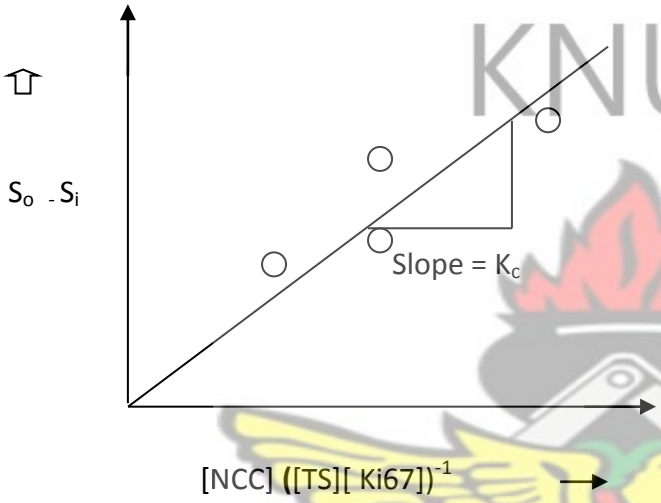
A graphical representation of equation 3,

$S_c = S_o - S_i = K^c$ [NCC] ……Equation 3'.

with S_o - S_i on the y - axis

And,

$\frac{[NCC]}{[TS]\ [\ Ki67]}$ on the x - axis will give a gradient that represents K_c.



K_c is measured in number of years of survival per cycle of chemotherapy, and could be called the **survival index** for the type or combination of chemotherapeutic drugs.

$\{[TS][Ki67]\}^{-1}$ is treated as the tumour biological coefficient

Statistical Analysis of Data:

Descriptive and molecular epidemiology data handling:

Demographic data, reproductive data, prevalence, average age at diagnosis and confidence intervals, were analysed and computed with Microsoft excel computer software. Significance testing of differences in oestrogen receptor (ER) status versus menopausal status, histograms, correlation analysis and tables were done with SPSS and prism statistical

software. Data handling and coding was done under supervision of a Biostatistician from Institute of Social Statistics and Economic Research, (ISSER) Legon.

CHAPTER FOUR

RESULTS

4.0. RESULTS

4.1. EPIDEMIOLOGICAL FINDINGS AND PREVALENCE OF BREAST LESIONS IN GHANA.

4.1.1a. EPIDEMIOLOGICAL FINDINGS [1999 – 2002].

The average age of female breast cancer patients was 46.29. Their ages ranged from 18.0 to 80.0 years when a total of 15,219 women nationwide were screened for breast lesions from 1999 to 2002. The ninety-five per cent confidence interval was 34.79 – 57.79 years/woman. Among pre-menopausal Ghanaian women the modal (peak) age at diagnosis was 39.0 years. The modal age at diagnosis among post-menopausal Ghanaian women was 54.0 years. In th at study, the average age at first menses (menarche) was 14.91 years. By the age of 16 years , 88.91% of them had experienced their first menses. The average age at menopause was es timated from the data to be 47.77 years for Ghanaian women. More than ninety per cent of them had menopause between the ages 40.75 to 54.79 years. Approximately 44.29% of the m had menopause between 40.75 years to 47.77 years. A few had menopause beyond 55 years (2.86%).

4.1.1b. EPIDEMIOLOGICAL FINDINGS [2004 -2008];

For wider coverage, an extension of the breast awareness study (1999 – 2002) was carried out. Based on outcome and lessons learned from the study in 1999 -2002, an additional 29,263 women were screened for neoplastic breast lesions and educated on breast awareness nationwide from 2004 to 2008. According to the survey from 1999 to 2002, prevalence rate for breast cancer in Ghana ranged from 0.41% – 1.11% (95%confidence interval) among females aged 18 to 80 years in Ghana (black Africans), whiles prevalence of

benign breast lumps ranges from 0.69% – 6.89% (95%confidence interval) (Table 1). Both data sets were coalesced / pooled to enhance reproducibility and reliability of statistical estimates. In all (29,263 + 15,219) 44,482subjects were involved. The occurrence of breast cancer among first degree relatives (mother and sister) increases the risk for breast cancer among females. In this study 0.7% of the respondents reported their mother was a breast cancer victim, 0.7% reported their sister was a breast cancer victim. Other responses were; maternal aunt 1.0%, paternal aunt 0.3%, maternal grandmother 0.5%, Paternal grandmother 0.1% and none or nil of note 96.7% (Table.2., Figure.18). The pooled data estimates show prevalence for breast cancer as 0.40% and prevalence for benign neoplastic breast lesions (breast lumps and fibroadenomas) as 2.9%. In all, the prevalence of neoplastic breast lesions was 3.7% (Table 3a.). The youngest female screened was 10 years old and the oldest was 99 years old. 25% percent of the participants in this screening exercise were below the age of 29 years. 75% of them were below 47 years old (Table 3b.). The median age was 38.00, mean was 38.47 and the modal age was 39.0 years. The lowest age at menarche recorded was 8.0 years, the highest was 25 years. 25% of the participants in this survey had menarche at or below age 14.0 years, 75% of them had menarche at or below 16.0 years of age. The median menarche was 15.0 years, the mean was 15.20 years and the modal menarche was 15.0 years (Table 3b, Figure.12.). Nulliparity (no childbirth) is associated with an increased risk for breast cancer in women. 25% of the participants had parity of 0 or 1.0. 75% had parity of 4.0 or below, and the median parity was 2.0. The mode for parity was 0.0 (Table 3b, Figure.13.). The age at first full-term pregnancy recorded in this survey ranged from 12.0 years to 55.0 years. 25th percentile was 20.0 years, median was 22.0 years, 75th percentile was 26.0 years, the mean was 23.0 years and mode was 20.0 years (Table 3b, Figure. 14). Android obesity in women is associated with increased risk for

breast cancer and cardiovascular disease. Hip and waist measurements were recorded for the participants in this survey. Hip measurements ranged from 21.0 inches to 66.0 inches. 25th percentile was 38.0 inches, median was 40.0 inches, 75th percentile was 44.0 inches and the mode was 40 inches (Table 3b, Figure.15). Waist measurements ranged from 19.0 inches to 60.0 inches. 25th percentile was 29.0 inches, median was 33.0 inches, 75th percentile was 36.0 inches and the mean was 33.14 inches. The mode was 34.0 inches (Table 3b, Figure.16.). Hip / waist ratios were computed to assess whether a participant in this survey was obese or not. Hip / waist ratios ranged from 0.5769 to 2.2632. 25th percentile was 0.7609, median was 0.8095, 75th percentile was 0.8605, mean was 0.8129 and the mode was 0.8000 (Table 3b, Figure.17.). Average age of breast cancer cases detected through screening was 46.29 years as compared to average age of breast cancer cases reporting for surgical treatment being 51.84 years (n = 228).(Table 4).

4.1.2. VISUAL ASSESSMENT OF BREAST TUMOUR-RELATED ANGIOGENESIS

Between July 2007 and December 2008 a total of 6,085 healthy women were examined with the breastlight after going through visual inspection and manual palpation of the breasts. Of these 475 were found to have symptoms or suspected symptoms and among these 49 have had breast cancer confirmed. The number with benign breast lesions was 426. It was found that 47 of the 49 with confirmed breast cancer cases were positive [96 %] using the Breastlight, 2 [4%] breast cancer cases were missed by the breastlight. In all the above 47 cases the women themselves were able to see the ' shadow ' associated with the lesion. Among 60 cases of benign lesions re-examined using the Breastlight 20 [33 %] showed no shadow or other indication of abnormality. Examples of images positive for cancer are shown in figures 1, 2, 3, 4 and 5.

4.2. TISSUE CHARACTERISTICS AND CLINICAL BIOMOLECULE ANALYSIS OF ARCHIVAL BREAST CANCER SPECIMEN. N=33.

4.2.1. TISSUE CHARACTERISTICSOF ARCHIVAL BREAST CANCER SPECIMEN. N=33.

Pre / peri - menopausal breast cancer cases made up 54.84%, post-menopausal breast cance r cases made up 45.16%. After analysing cross-sectional data on mastectomy / lumpectomy / biopsy specimen size and weight at presentation for tissue diagnosis, it became obvious th at a majority of breast cancer patients in Ghana had bulky breast tumours at presentation fo r treatment. The average weight of these mastectomy / lumpectomy / biopsy specimen was 916.91 grams. The smallest lumpectomy specimen weighed 18 grams and the largest weigh ed 1780 grams. The average specimen size was 12.58cm by 10.59cm. The smallest specimen size was 2cm by 1.3cm and the largest was 23cm by 16cm. Invasive Ductal Carcinoma of the breast (IDC) was the most predominant tissue characteristic among the malignant neoplastic lesions (7/10) treated in leading hospitals in Ghana.

4.2.2. A Review of, existing data on ER / PR content of 228 patients

Existing data on ER / PR content of 228 patients treated at leading hospitals in Ghana revealed the following. One hundred and fifty (150) of them were reported as either negative or positive by different pathologists without any scores. Sixty two (62) of them were scored by other pathologists. Sixteen (16) of these specimen were reported to have no residual tumours present as a result of effective preoperative chemotherapy therapy administered on them hence no scoring. The average age for all of them (n=228) was 51.84 years and the mode was 50 years. The minimum age was 28.00 years and the maximum age was 86.00 years (Table 4, Figure.19). Sixty-two (62) of them were given scores (0, 1, 2, 3) by the pathologists (Figure.23, and Figure 24.),

Among those reported as either negative or positive; ER positive tumours were just 17.92 %

of the total (n =. 150) PR positive tumours were 19.81 % of the total (Table 5, Figure.20, and Figure.21. respectively.) Among the scored data on a subset of 62 paraffin embedded breast cancer tissue, the minimum age of the subjects was 28.0 years, highest age was 79.0 years and mean was 47.9 (Figure. 22) The lowest score for ER and PR was 0 and the highest was 3. A higher receptor score indicates higher ER and PR content. **(**Table 6., Figure.23. and Figure. 24**)**. Frequencies for ER scores were; ER score of 0 was seen in 64.52% of the specimen, ER score of 1 in 14.52% of the specimen, ER score of 2 in 8.06% of the specimen and ER score of 3 in 12.90%. (Table 7., Figure.23) In this subset, 35.48% were ER positive (n=62). Similarly, frequencies for PR scores were; PR score of 0 was seen in 61.29% of the specimen, PR score of 1 in 12.90% of the specimen, PR score of 2 in 9.68% of the specimen and PR score of 3 in 16.13% of them (Table 7., Figure.24). ER+/PR+ represents 27.42% of the specimen analysed, ER+/PR- represents 8.06%, ER-/PR+ represents 11.29% and ER-/PR- representing 53.23%. In all PR+ tumours (oestrogen receptor positive tumours) represented 38.77% (27.42% + 11.29%) in this subset of 62 samples (Table 8.). The overall (pooled) ER+ rate for all 212 (n = 150 + 62) samples was calculated as 22.17%.

Finally, a subset of these tumours numbering 33 were randomly selected from 212 blocks of histologically confirmed breast cancer tumours, where there was enough tumour tissue and re-analysed for tumour size / diameter, ER, PR, HER 2 and Ki67 (biomolecules of clinical importance in managing breast cancer). The average age of this subset was 49.73 years, minimum age was 20.00 years and the maximum was 70.00 years. The modal age was 55.00 years (Table 9, Table 10, Figure.25) Tumour diameter (cm) was used as a proxy measure of tumour size. The smallest tumour measured 0.5 cm and the maximum was 12.00 cm. The mean was 4.04 and the mode was 3.00 cm (Table 10, Figure.26). ER / PR scores range

d from 0 to 3, the mode was 0, this implies most of the tumours were hormone receptor negative, although 18.18% were ER positive. HER-2 scores ranged from 1 to 3 in this data set, no specimen had HER-2 score of 0, the cut off point for HER-2 positive was 2, 45.45% had a score of 3. Another 27.27% had a score of 1 (HER-2 negative) and 27.27% had a scor e of 2. Cut off point for Ki67 positive was 1. Ki67 scores ranged from 0 to 4. 12.12% had a score of 0, 39.39 % had a score of 1. 27.27 % had a score of 2. 12.12% had a score of 3 an d 9.09 % had a score of 4 (Table 10).

CLINICAL BIOMOLECULE PROFILES OF ARCHIVAL BREAST CANCER SPECIMEN. N=33

ER positives (18.18%): ER+/HER-2-/Ki67+ (1) = 3.03%, ER+/HER-2+/Ki67+ (2) = 6.06%, ER+ /HER-2-/Ki67- (3) = 9.09%

ER negatives (81.82%): ER-/HER-2+/Ki67+ (22) = 66.67%, ER-/HER-2+/Ki67- (0) = 0%, ER-/HER-2-/Ki67+ (4) = 12.12% **triple negative**, ER-/HER-2-/Ki67- (1) = 3.03% **triple negati ve**.

HER-2 positives (72.73%): ER-/HER-2+/Ki67+ (22) = 66.67%, ER-/HER-2+/Ki67- (0) = 0%, E R+/HER-2+/Ki67+ (2) = 6.06%, ER+/HER-2+/Ki67-(0) = 0.0%.

HER-2 negatives: (27.27%): ER-/HER-2-/Ki67+ (4) = 12.12%, ER+/HER-2-/Ki67- (3) = 9.09%, ER+/HER-2-/Ki67+ (1) = 3.03%, ER-/HER-2-/Ki67- (1) = 3.03%.

Ki67 positives (87.98%): ER-/HER-2+/Ki67+ (22) = 66.67%, ER-/HER-2-/Ki67+ (4) = 12.12% ER+/HER-2+/Ki67+ (2) = 6.06%, ER+/HER-2-/Ki67+ (1) = 3.03%.

Ki67 negatives (12.02%): ER-/HER-2-/Ki67- (1) = 3.03%, ER-/HER-2+/Ki67- (0) = 0%, ER+/HER-2+/Ki67- (0) = 0.0%, ER+/HER-2-/Ki67- (3) = 9.09%.

Triple negative Breast cancers: they occurred at a rate of 15.15%.

I. ER-/HER-2-/Ki67+ this biochemical sub-type of triple negative breast cancer occurred at a rate of 12.12%, in these tissue samples. Both ER and HER-2 over-expression were absent.

II. ER-/HER-2-/Ki67- this biochemical sub-type of triple negative breast cancer occurred at a rate of 3.03%.

CORRELATIONS BETWEEN CLINICAL BIOMOLECULES AND TISSUE CHARACTERISTICS (PEARSON'S CORRELATION, r)

Results of bio-molecule correlation analysis of ER, HER-2 and Ki67 in breast cancer tumours (n=33) were summarised as follows: There was a weak negative non-significant correlation between Age and ER score. Younger breast cancer patients in this series tend to have higher ER scores. Pearson r is -0.3043, p=0.0851 and alpha=0.05 (Table 11.). There was a weak correlation between age and HER-2 scores. Pearson r was 0.019 (Table 11.). There was a highly significant, negative, and moderate correlation between HER-2 and ER. Pearson r = -0.4805, p^{**} (two tailed) = 0.0046., alpha=0.05. (Table 11.). There was a significant, negative, and moderate correlation between Ki67 and ER scores. Pearson r = -0.4159, p* (two tailed) = 0.0161., alpha = 0.05 (Table 11.). There was a moderate, significant negative correlation between breast cancer tumour size and age of the patient. Pearson r is -0.3999, p^{*} = 0.0211 (two tailed) and (alpha=0.05). The younger the patient the larger the breast cancer tumour presented for treatment (Table.11, Figure.27).There was a highly significant, positive and moderate correlation between HER-2 and Ki67. Increasing HER-2 scores were highly significantly correlated with increasing Ki67 scores in breast cancer tumours. Pearson r = 0.4550, p^{**} (two tailed) = 0.0078 and alpha = 0.05 (Table.11, Figure.28). There was positive, moderate and significant correlation between age and Ki67 score. Increasin

g age correlated with increasing Ki67 scores. Pearson r = 0.3564, p^* (two tailed) = 0.0418, and alpha=0.05 (Table 11, Figure.29.).

4.2.3. A MODEL TO DEMONSTRATE HOW TO INDIVIDUALIZE TREATMENT FOR BREAST CANCER

A NOVEL, HYPOTHETICAL, SEMI-QUANTITATIVE MODEL FOR, OPTIMIZING THE BENEFITS OF CHEMOTHERAPY IN THE MANAGEMENT OF SOLID MALIGNANT BREAST LESIONS.

Hypothetically,

Patient A was admitted with tumour stage3disease initially ($[TS]_{initially}$.) Equation 3 was used to predetermine the number of cycles of chemotherapy that could theoretically improve her survival ($S_o - S_i$) by 5 years as 5 cycles ($[NCC]_{initially}$.) Her oncological surgeon administers 2 cycles preoperatively to patient A and realises her disease had improved to tumour stage 2 finally ($[TS]_{finally}$.).

Equation 3 was used to redetermine the number of cycles of chemotherapy now required if Ki67 had also decreased (or remained unchanged). This may (or may not) indicate a downward adjustment of the remaing number of cycles from say 3 cycles to 1 cycle. Cummulatively **patient A** may now received 3 ($[NCC]_{cummulatively}$.) instead of 5 cycles ($[NCC]_{initially}$.) that was predetermined for her initial stage 3 disease on admission. Furthermore, this model demonstrates why patients with same stage tumours have different survival and may reqiure different intensities of the same treatment. Finally, it helps to identify potentially aggressive tumours with low stage (early stage), therefore, some lower stage tumours may require more intense treatment than some higher stage tumours. Also, some lower stage tumours will have lower survival than some higher stage tumours (Table 13.).

TABLES

Table1. Yearly prevalence of breast conditions seen in Ghana (1999 – 2002)

	Year						
	Number of cases						
Breast condition	**1999**	**2000**	**2001**	**2002**	**Total**	**Standard deviation**	**Average**
Breast cancer	23	36	21	22	102		
Yearly Prevalence (%)	1.22	0.57	0.67	0.57		0.31	0.76
95% confidence interval: = 0.76 +/-0.35, median = 0.62							
Benign breast mass	147	199	48	104	498		
Yearly Prevalence (%)	7.81	3.16	1.52	2.68		2.77	3.79
95% confidence interval: = 3.79 +/-3.10, median = 2.92							
Aberrations of normal development and involution (ANDI) **/ No abnormality detected (NAD)**	1,712	6,072	3,084	3,753	14,621		
Total	1,882	6,307	3,153	3,877	15,219		

Table 2.Prevalence of Family history of breast cancer (Pooled data from 1999 to 2008; 29,263+15,219)

Relationship to respondent	Frequency	Percent
Mother	321	0.7
Sister	301	0.7
Maternal Aunt	467	1.0
Paternal Aunt	128	0.3
Maternal Grandmother	226	0.5
Paternal Grandmother	39	0.1
Sub Total	1482	3.3
Nil of note	43000	96.7
Total	44482	100.0

Table 3a. Prevalence of breast conditions n=44,482
(Pooled data from 1999 to 2008; 29,263+15,219)

Breast condition	Frequency	Percent	Remarks
Breast Lump/Breast Mass	921	2.1	Neoplastic breast lesions
Breast Cancer/CA Breast	177	0.4	
Axillary Breast Mass/Lymph Nodes	198	0.4	
Fibroadenomata	361	0.8	
Total	**1657**	**3.7**	
Dense Nodular Breasts	1532	3.4	Aberrations of Normal Development and Involution (ANDI)
Bilateral Nodularity/Lumpy Breast/Pain	3027	6.8	
Breast Abscess/Infection/Mastitis	123	0.3	
Post-Partum Breast Abscess	39	0.1	
Focal Nodularity	47	0.1	
Bloody Nipple Discharge	59	0.1	
Macromastia/Hypertrophy	600	1.3	
Galactorrhea/Milky Discharge	74	0.2	
Lactating/Breastfeeding & tenderness	711	1.6	
Total	**6212**	**13.9**	
Kelloids	60	0.1	Superficial Scars
Post-Lumpectomy Scar	150	0.3	
No abnormality detected(NAD)	**36403**	**81.84**	NAD
Grand Total	**44482**	**100.0**	

Table 3b. Demographic, Reproductive and Anthropometric data on women screened for neoplastic breast lesions = 44,482. Pooled data from 1999 to 2008; (15,219+29,263)

	Number of values	minimum	25% percentile	Median	Mean	Mode	75% percentile	maximum
Age(years)	44,230	10.0	29.0	38.0	38.48	40.0	47.0	99.0
Menarche (years)	36,759	8.0	14.0	15.0	15.20	15.0	16.0	25.0
Parity	40,207	0.0	1.0	2.0	2.69	0.0	4.0	11.0
Age at first full term pregnancy (years)	24,380	12.0	20.0	22.0	22.96	20.0	26.0	51.0
Hip circumference (inches)	34,506	21.0	38.0	40.0	40.75	40.0	44.0	66.0
Waist circumference (inches)	34,506	19.0	29.0	33.0	33.14	34.0	36.0	60.0
Waist/Hip ratio	34,619	0.58	0.76	0.81	0.81	0.80	0.86	2.26

Table 4. Average Age of breast cancer cases detected through awareness and screening versus Average Age of breast cancer victims reporting for surgical treatment. N=228

	AGE
Number of values	227
Minimum	20.00
25% Percentile	44.00
Median	50.00
75% Percentile	59.00
Maximum	86.00
Mean	51.84
Std. Deviation	12.04
Std. Error	0.7991
Lower 95% CI of mean	50.27
Upper 95% CI of mean	53.42
One sample t test	
Mean (breast cancer victims detected through breast screening)	46.29
Mean (breast cancer patients reporting for treatment)	51.84
Discrepancy	-5.551
95% CI of discrepancy	3.985 to 7.118
t, df	t=6.947 df=226
P value (two tailed)	P<0.0001
Significant (alpha=0.05)?	Yes

Table.5. IHC DETERMINED ER ANDPR STATUS OF HISTOLOGICALLY CONFIRMED BREAST CANCER CASES (reviewed data). N=150

ER Status	**Frequency %**
0.0 (Negative)	82.07547
1.0 (Positive)	17.92453
PR Status	**Frequency %**
0.0 (Negative)	80.18868
1.0 (Positive)	19.81132

Table 6.Age and Hormone receptor scores of breast cancer patients (reviewed data) n=62

	AGE	ER SCORE	PR SCORE
Number of values	62	62	62
Minimum	28.00	0.0	0.0
25% Percentile	41.00	0.0	0.0
Median	49.50	0.0	0.0
75% Percentile	58.00	1.000	2.000
Maximum	79.00	3.000	3.000
Mean	49.79		
Std. Deviation	10.85		
Std. Error	1.378		
Lower 95% CI of mean	47.03		
Upper 95% CI of mean	52.55		

Table.7.Frequency Distribution of ER and PR Scores for breast cancer Patients (IHC) (reviewed data) n=62

ER SCORE	FREQUENCY (%)
0.	64.51613
1.	14.51613
2.	8.064516
3.	12.90323
PR SCORE	**FREQUENCY (%)**
0.	61.29032
1.	12.90323
2.	9.67742
3.	16.12903

Table 8.DISTRIBUTION OF ER /PR STATUS IN BREAST CANCER SEEN IN GHANA (IHC) (reviewed data) n=62

	ER+/PR+	ER+ / PR-	ER- / PR+	ER- / PR-
FREQUENCY	17	5	7	33
FREQUENCY (%)	27.42	8.06	11.29	53.23
N = 62				

Table 9. Tumour characteristics and biomolecule scores of histologically confirmed breast cancer specimen. N=33

IDC = Invasive Ductal Carcinoma of the breast.

Specimen ID.	Age	Tumour size (cm)	ER SCORE	PR SCORE	HER-2 SCORE	KI67 SCORE	Tissue Diagnosis
82	41.0	3.0	2	2	1	0	IDC
87	47.0	8.0	0	0	1	1	IDC
693	36.0	6.0	0	0	2	1	IDC
815	52.0	6.0	0	0	2	2	IDC
961	56.0	1.5	0	0	3	1	IDC
1148	69.0	2.0	0	0	3	4	IDC
1345	41.0	0.5	0	0	2	1	IDC
2181	41.0	4.0	3	3	1	0	IDC
2255	47.0	2.5	3	3	1	0	IDC
3621	44.0	10.0	0	0	1	0	IDC
3966	59.0	2.5	0	0	2	1	IDC
4051	45.0	3.0	0	0	2	4	IDC
4201	49.0	2.0	0	0	3	1	IDC
4212	53.0	3.5	0	0	3	3	IDC
4214	55.0	10.0	0	0	3	2	IDC
4261	54.0	5.5	1	2	3	4	IDC
4343	70.0	1.5	0	0	2	2	IDC
4350	42.0	2.5	0	0	3	1	IDC
4353	48.0	5.0	0	0	1	2	IDC
4383	54.0	3.0	0	0	1	2	IDC
4417	49.0	2.5	0	0	1	1	IDC
4429	55.0	6.0	0	0	3	3	IDC
4529	34.0	5.5	1	2	2	1	IDC
4533	37.0	2.0	0	0	3	2	IDC
4638	67.0	1.5	0	0	3	1	IDC
4708	56.0	1.0	0	0	3	2	IDC
4868	68.0	4.0	0	0	2	3	IDC
4901	56.0	8.0	0	0	2	2	IDC
4932	61.0	2.5	0	0	3	1	IDC
5533	35.0	12.0	0	0	3	3	IDC
5816	69.0	2.5	0	0	3	2	IDC
5989	20.0	11.0	0	1	3	1	IDC
7428	31.0	3.0	2	2	1	1	IDC

Table 10. Clinically important bio-molecule analysis. N = 33

	Age	Tumour size(cm)	ER score	PR score	HER-2 score	Ki67 score
Number of values	33	33	33	33	33	33
Minimum	20.00	0.5000	0.0	0.0	1.000	0.0
25% Percentile	41.00	2.250	0.0	0.0	1.000	1.000
Median	49.00	3.000	0.0	0.0	2.000	1.000
75% Percentile	56.00	6.000	0.0	0.0	3.000	2.000
Maximum	70.00	12.00	3.000	3.000	3.000	4.000
Mean	49.73	4.348	0.3636	0.4545	2.182	1.667
Std. Deviation	12.04	3.063	0.8594	0.9384	0.8461	1.137
Std. Error	2.095	0.5332	0.1496	0.1634	0.1473	0.1978
Lower 95% CI of mean	45.46	3.262	0.05889	0.1218	1.882	1.264
Upper 95% CI of mean	54.00	5.435	0.6684	0.7873	2.482	2.070
Sum	1641	143.5	12.00	15.00	72.00	55.00

Table 11. SUMMARISED CORRELATION ANALYSIS OF BREAST CANCER TUMOUR BIOMOLECULES. N=33

	ER	Ki67	HER-2	Tumour size
AGE	r = - 0.3043, P = 0.0851,w,ns	r = 0.3564. p* = 0.0418,m,s	r = - 0.01918 p= 0.9156,w,ns	r = - 0.3999. p*= 0.0211,m,s
TUMOUR SIZE	r = - 0.1149, p = 0.5242,w,ns	r = 0.05685. P = 0.7533, w, ns	r = 0.00792 p = 0.9366,w,ns	
HER-2	r = - 0.4805 p ** = 0.0046,m,hs	r = 0.4550. p** = 0.0078, m, hs		r = 0.00792 p = 0.9366,w,ns
Ki67	r = - 0.4159 p*= 0.0161,m,s		r = 0.4550. p** = 0.0078, m, hs	r = 0. 05685. P = 0.7533, w, ns

KEY

w – weak,

m – moderate,

s – significant,

ns – not significant,

hs – highly significant.

Table12. SUMMARY OF SIGNIFICANT CORRELATIONS

SUMMARY OF SIGNIFICANT CORRELATIONS (CLINICALLY IMPORTANT BIO-MOLECULES FOR BREAST CANCER (N=33)		
ER(X) positive (18.18%) ER+/HER-2-/Ki67- (3) = 9.09%	HER-2(Y) Negative (27.27%) ER+/HER-2-Ki67- (3) = 9.09%	Increasing ER scores correlated significantly with decreasing HER-2 scores (vice versa). Pearson r = - 0.4805, p**
ER(X) negative (81.82%) ER-/HER-2+/Ki67+ (22) = 66.67%	HER-2(Y) positive (72.73%) ER-/HER-2+/Ki67+ (22) = 66.67%.	
Ki67(X) positive (87.98%) ER-/HER-2+/Ki67+ (22) = 66.67%	HER-2(Y) positive (72.73%) ER-/HER-2+/Ki67+ (22) = 66.67%	Increasing Ki67 scores correlated significantly with increasing HER-2 scores (vice versa). Pearson r = 0.4550, p**
Ki67(X) negative (12.02%).ER+/HER-2-Ki67- (3) = 9.09%, ER-/HER-2-/Ki67- (1) = 3.03%	HER-2(Y) negative (27.27%).ER+/HER-2-/Ki67- (3) = 9.09%, ER-/HER-2-/Ki67- (1) = 3.03%	
Age(X) Higher age at diagnosis for breast cancer tumour.	Tumour size(Y) Smaller breast cancer tumour sizes.	Increasing age correlated well with decreasing tumour sizes. This correlation was stronger among post-menopausal breast cancer tumours (0.4267) as compared to (0.3866) in premenopausal tumours. Average tumour size for pre-menopausal tumours was 4.38 cm, compared to 3.36 cm in post -menopausal ones. Pearson r = - 0.3999, p*
Age(X) Higher age at diagnosis for breast cancer tumour.	Ki67(Y) positive (87.98%)	Increasing age correlated well with increasing Ki67 scores. This correlation is stronger among premenopausal ages < 54 years (0.1536) as against post-menopausal ages (0.0547). Overall Pearson r = 0.3564, p*
Age(X) Lower age at diagnosis for breast cancer tumour.	Ki67(Y) negative (12.02%)	

Table13. INDIVIDUALIZING TREATMENT FOR BREAST CANCER

A NOVEL, HYPOTHETICAL, SEMI-QUANTITATIVE MODEL FOR, OPTIMIZING THE BENEFITS OF CHEMOTHERAPY IN THE MANAGEMENT OF SOLID MALIGNANT BREAST LESIONS.

ID	ALL TUMOURS						K_C	K^C
	S	TS	Ki67	NCC	[TS][Ki67]	S[TS][Ki67]	S[TS][Ki67]/NCC	S/NCC
A	5	1	1	4	1	5	1.25	1.25
B	4	2	2	6	4	16	2.67	0.67
C	3	3	3	8	9	27	3.38	0.38
D	2	4	4	10	16	32	3.20	0.20
	STAGE ONE TUMOURS						K_C	K^C
	S	TS	Ki67	NCC	[TS][Ki67]	S[TS][Ki67]	S[TS][Ki67]/NCC	S/NCC
A	5	1	1	4	1	5	1.25	1.25
B	4	1	2	6	2	8	1.33	0.67
C	3	1	3	8	3	9	1.13	0.38
D	2	1	4	10	4	8	0.80	0.20
	STAGE FOUR TUMOURS						K_C	K^C
	S	TS	Ki67	NCC	[TS][Ki67]	S[TS][Ki67]	S[TS][Ki67]/NCC	S/NCC
A	5	4	1	4	4	20	5.00	1.25
B	4	4	2	6	8	32	5.33	0.67
C	3	4	3	8	12	36	4.50	0.38
D	2	4	4	10	16	32	3.20	0.20
	FIVE YEAR SURVIVAL						K_C	K^C
	S	TS	Ki67	NCC	[TS][Ki67]	S[TS][Ki67]	S[TS][Ki67]/NCC	S/NCC
A	5	1	1	4	1	5	1.25	1.25
B	5	2	2	6	4	20	3.33	0.83
C	5	3	3	8	9	45	5.63	0.63
D	5	4	4	10	16	80	8.00	0.50

KEY:

- Overall survival period S_o (years) ,
- Tumour Stage [TS],
- Number of cycles of chemotherapy administered [NCC], and
- Tumour proliferative index [Ki67].

Table 14. Biomolecule Profiles of 33 human breast cancer samples

ER positives (18.18%): ER+/HER-2-/Ki67+ (1) = 3.03%, ER+/HER-2+/Ki67+ (2) = 6.06%, ER+/HER-2-/Ki67- (3) = 9.09%. **ER negatives (81.82%):** ER-/HER-2+/Ki67+ (22) = 66.67%, ER-/HER-2+/Ki67- (0) = 0%, ER-/HER-2-/Ki67+ (4) = 12.12%, ER-/HER-2-/Ki67- (1) = 3.03%. **HER-2 positives (72.73%):** ER-/HER-2+/Ki67+ (22) = 66.67%, ER-/HER-2+/Ki67- (0) = 0%, ER+/HER-2+/Ki67+ (2) = 6.06%, ER+/HER-2+/Ki67- (0) = 0.0%. **HER-2 negatives: (27.27%):** ER-/HER-2-/Ki67+ (4) = 12.12%, ER+/HER-2-/Ki67- (3) = 9.09%,ER+/HER-2-/Ki67+ (1) = 3.03%, ER-/HER-2-/Ki67- (1) = 3.03%. **Ki67 positives (87.98%):** ER-/HER-2+/Ki67+ (22) = 66.67%, ER-/HER-2-/Ki67+ (4) = 12.12% ER+/HER-2+/Ki67+ (2) = 6.06%, ER+/HER-2-/Ki67+ (1) = 3.03%.
Triple negative breast cancers (TNBC) occurred at a rate of 15.15%. **Two biochemical sub-types of TNBC;** [ER-(PR-)/HER-2-/Ki67+] occurred at a rate of 12.12%. [ER-(PR-)/HER-2-/Ki67-] this biochemical sub-type of triple negative breast cancers (which could be referred to as 'quadruple negative') made up to 3.03% of the cases.

FIGURES

Figure.1.Breastlight. [This is breastlight 171007.]

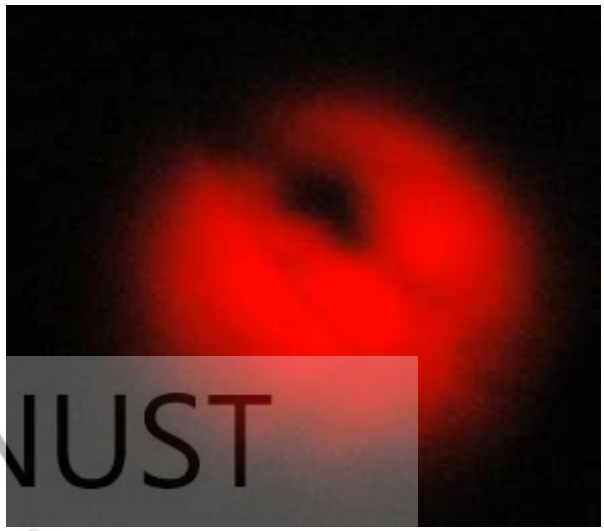


Figure.2.Breast 589 lt jpg. Ghana 250808x mass is 1.5 cm size in a 61 year old lady.

Figure.3.Breast 2675 jpg lt. An impalpable lesion in a 62 year old lady at 2 o'clock.

Figure.4.Breast 3233 jpg Ghana 150708c mass is 1.5 cm size situated at 12 o'clock. in a 61 year old lady.

Figure.5.Bloody nipple discharge in a 60yr old lady (Ghartey F.N., August 2009).

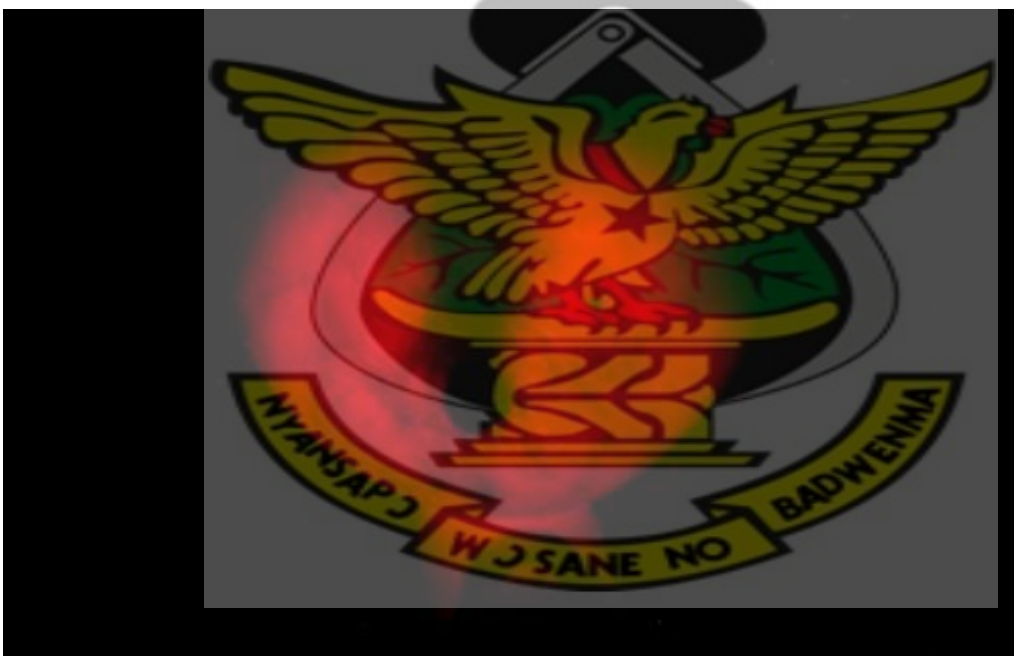


Figure.6.Bloody nipple discharge in a 60 year old lady detected with the new breastlight (Ghartey F.N., August 2009)

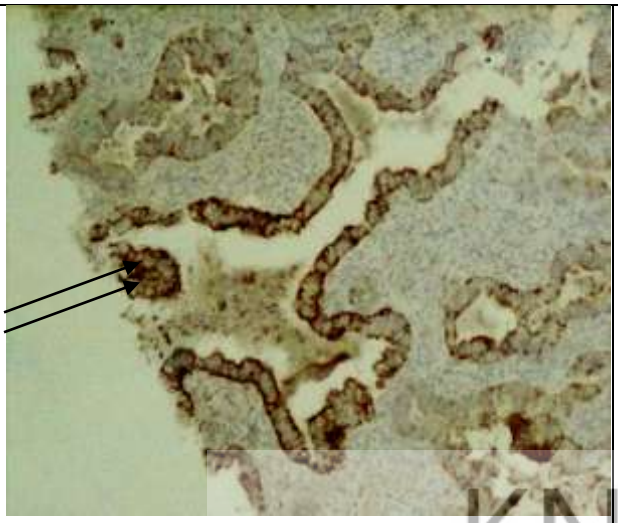


Figure.7. Positive control for ER. Demonstrating the presence of ER in Endometrial carcinoma using IHC method on paraffin embedded tissue ER score is 2. Nuclear Staining (brown) with DAB as Chromogen.

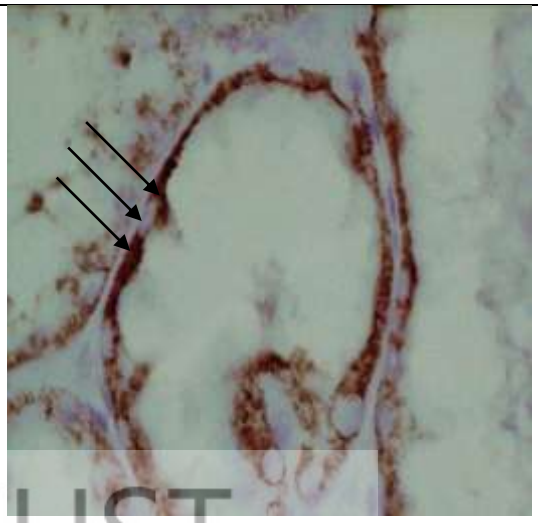


Figure.8. Positive control for ER. Demonstrating the presence of ER in Endometrial carcinoma using IHC method on paraffin embedded tissue ER score is 3. Nuclear Staining (brown) with DAB as Chromogen.

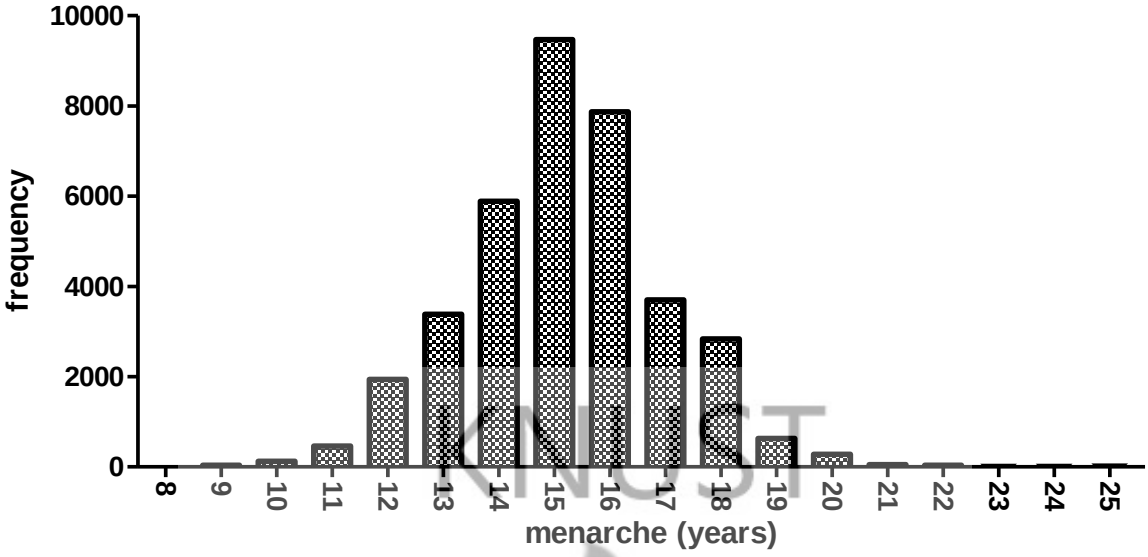
Figure.9. Positive control for ER. Demonstrating the presence of ER in Endometrial carcinoma using IHC method on paraffin embedded tissue ER score is 1. Nuclear Staining (brown) with DAB as Chromogen.

Figure.10. Negative control. ER (–) demonstrated in endometrial carcinoma. ER score = 0. No brown stain (nuclear staining)

Figure.11."Age distribution of females captured in a Nationwide Breast Awareness and Screening." Data from 1999 to 2008, Ghana.

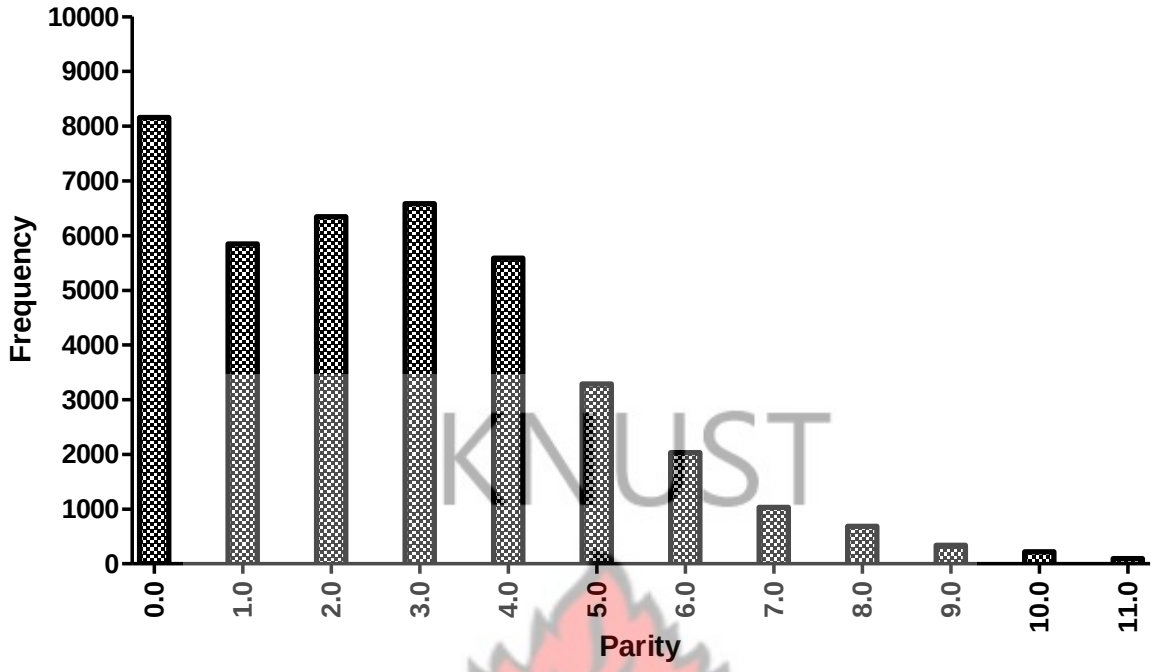
Total number of values	44230
Number of excluded values	0
Number of binned values	44230
Minimum	10.0
25% Percentile	29.0
Median	38.0
75% Percentile	47.0
Maximum	99.0
Mean	38.4745
Std. Deviation	12.2478
Std. Error	0.058237
Lower 95% CI of mean	38.3604
Upper 95% CI of mean	38.5887

Figure.12. "Frequency distribution of Menarche." Data from 1999 to 2008, Ghana.



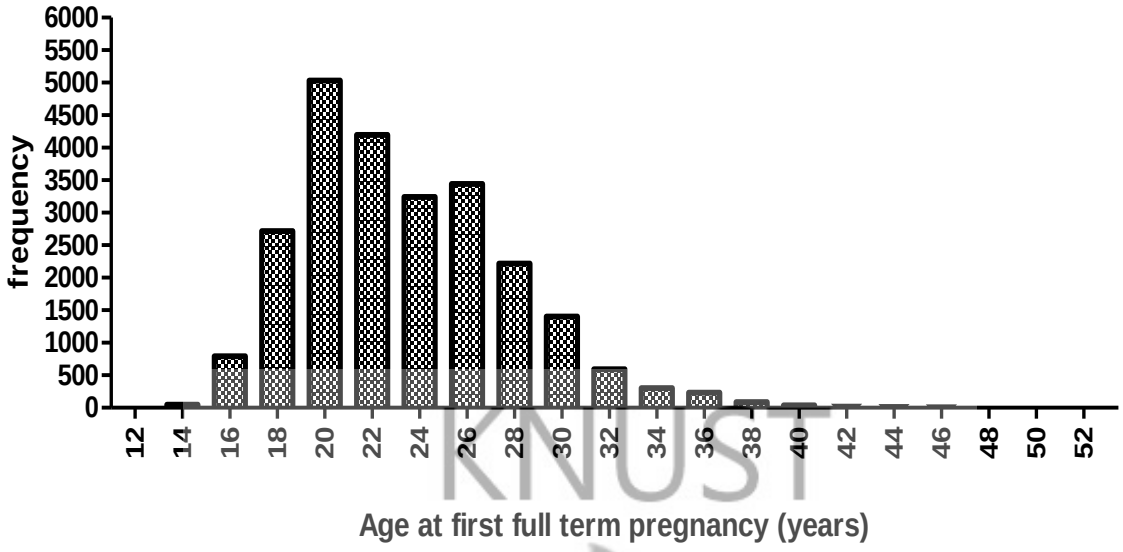
Total number of values	36759
Number of excluded values	0
Number of binned values	36759
Minimum	8.0
25% Percentile	14.0
Median	15.0
75% Percentile	16.0
Maximum	25.0
Mean	15.2016
Std. Deviation	1.80444
Std. Error	0.00941152
Lower 95% CI of mean	15.1831
Upper 95% CI of mean	15.22

Figure.13. "Frequency distribution of Parity." Data from 1999 to 2008, Ghana.



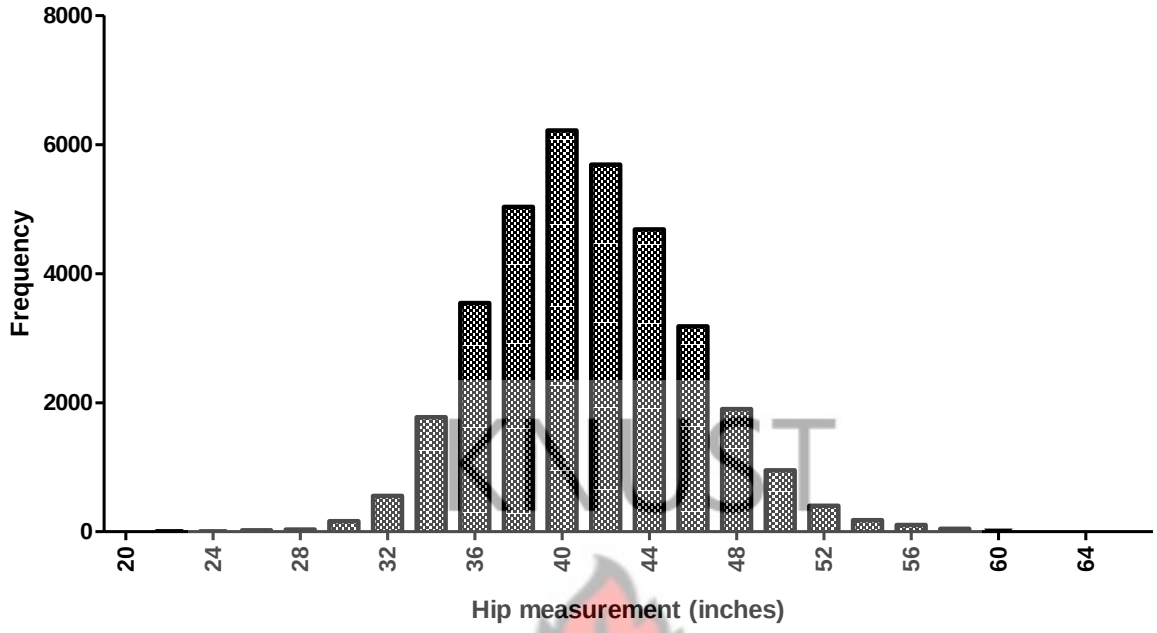
Total number of values	40207
Number of excluded values	0
Number of binned values	40207
Minimum	0.0
25% Percentile	1.0
Median	2.0
75% Percentile	4.0
Maximum	11.0
Mean	2.69157
Std. Deviation	2.22377
Std. Error	0.0110902
Lower 95% CI of mean	2.66983
Upper 95% CI of mean	2.71331

Figure.14. " Frequency distribution of Age at First full-Term Pregnancy (years)"



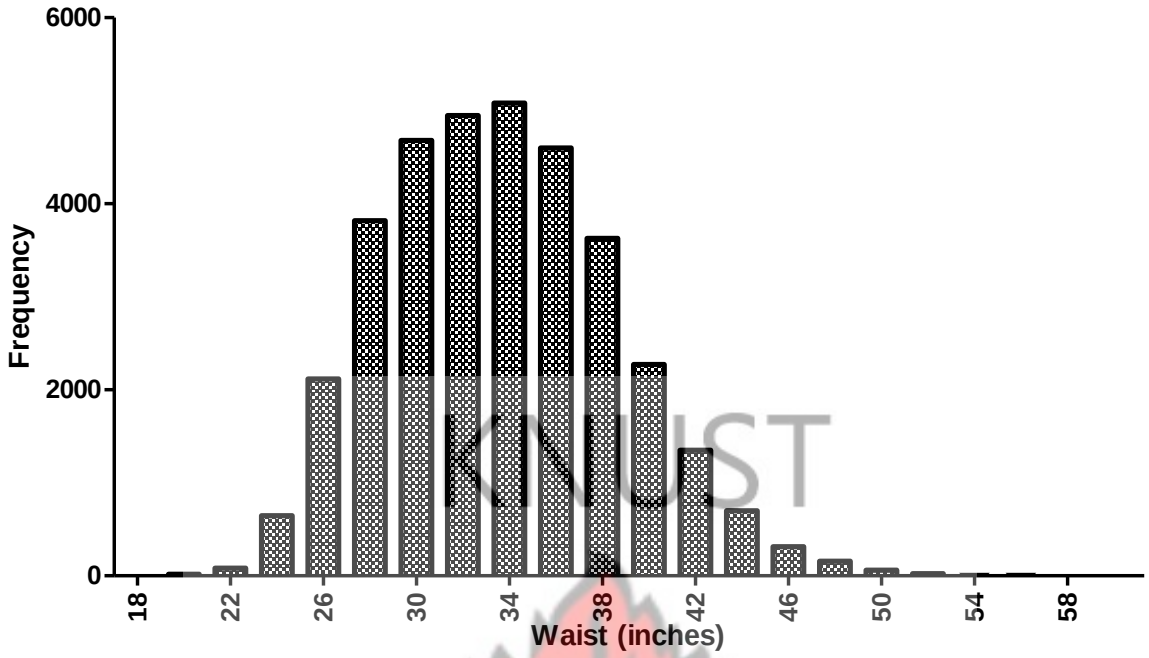
Total number of values	24380
Number of excluded values	0
Number of binned values	24380
Minimum	12.0
25% Percentile	20.0
Median	22.0
75% Percentile	26.0
Maximum	51.0
Mean	22.9693
Std. Deviation	4.40438
Std. Error	0.0282077
Lower 95% CI of mean	22.914
Upper 95% CI of mean	23.0246

Figure.15. "Frequency distribution of hip measurements (inches)"



Total number of values	34506
Number of excluded values	0
Number of binned values	34506
Minimum	21.0
25% Percentile	38.0
Median	40.0
75% Percentile	44.0
Maximum	66.0
Mean	40.7518
Std. Deviation	4.56841
Std. Error	0.0245934
Lower 95% CI of mean	40.7036
Upper 95% CI of mean	40.8

Figure.16."Frequency distribution of waist measurements (inches)."



Total number of values	34506
Number of excluded values	0
Number of binned values	34506
Minimum	19.0
25% Percentile	29.0
Median	33.0
75% Percentile	36.0
Maximum	60.0
Mean	33.1353
Std. Deviation	4.96592
Std. Error	0.0267333
Lower 95% CI of mean	33.0829
Upper 95% CI of mean	33.1877

Figure.17. Frequency distribution of waist: hip ratios

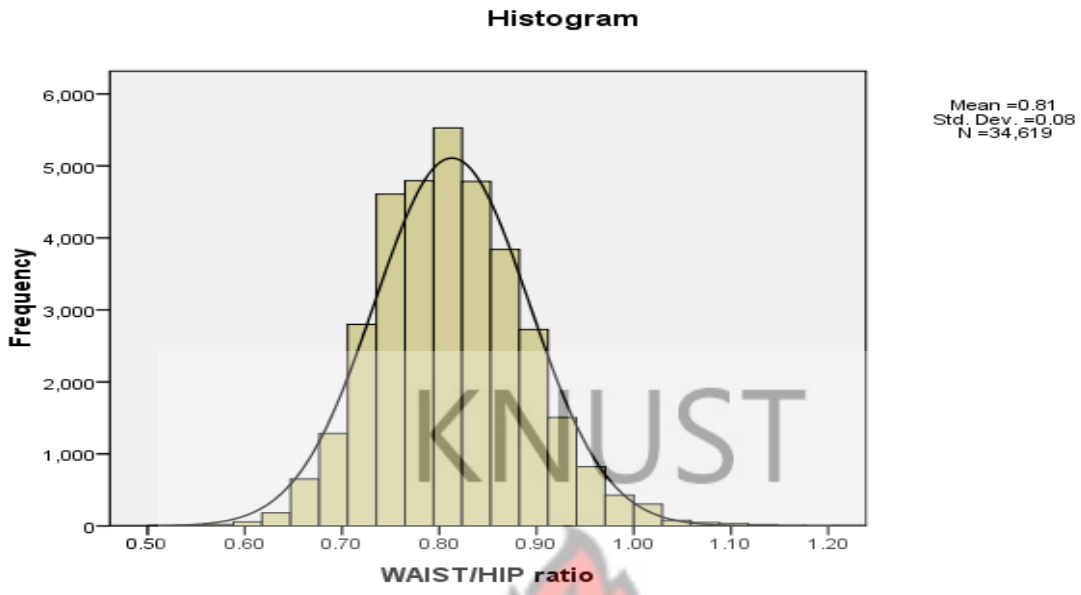
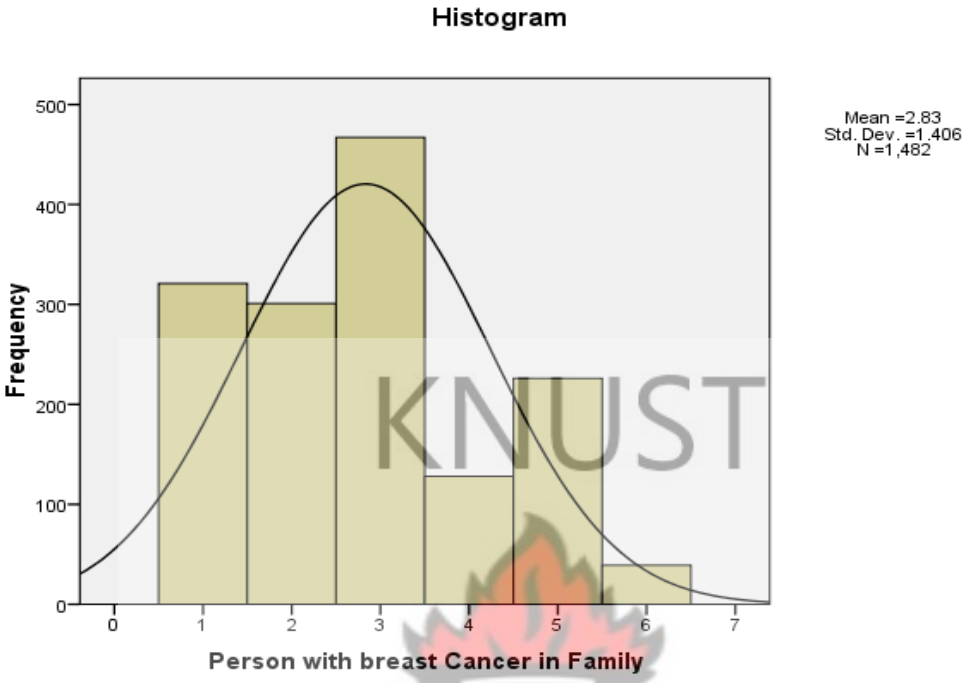


Figure.18. Distribution of Family history of breast cancer



Relationship to respondent.

1. Mother
2. Sister
3. Maternal Aunt
4. Paternal Aunt
5. Maternal Grandmother
6. Paternal Grandmother

Figure.19."Frequency distribution: Ages of patients with histopathologically confirmed breast cancer patients. N=228"

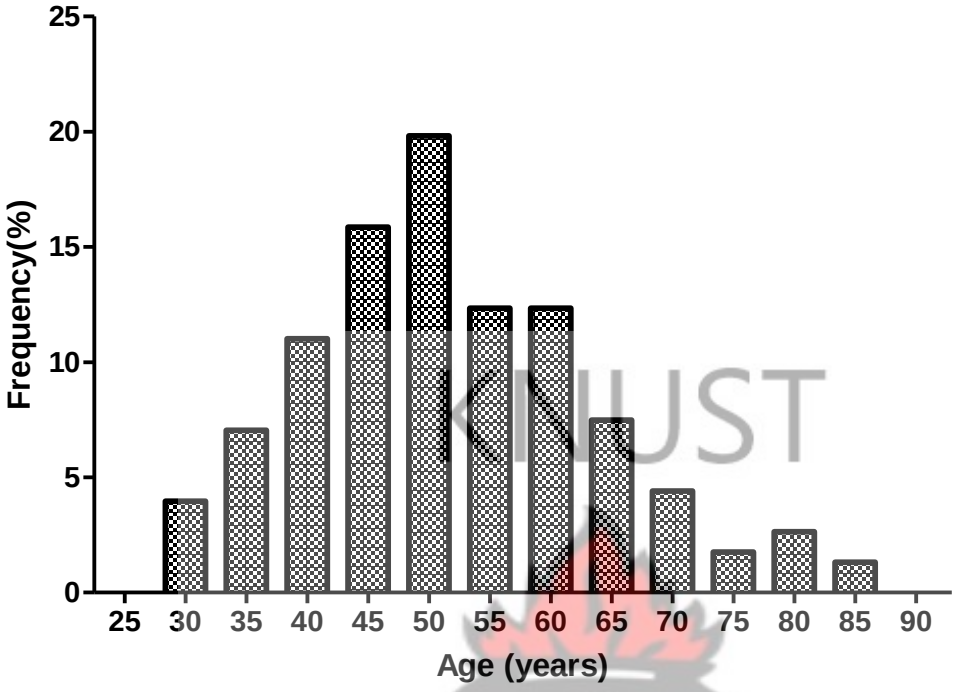


Figure.20. "ER Status in histologically confirmed paraffin embedded breast cancer tissues." N=150

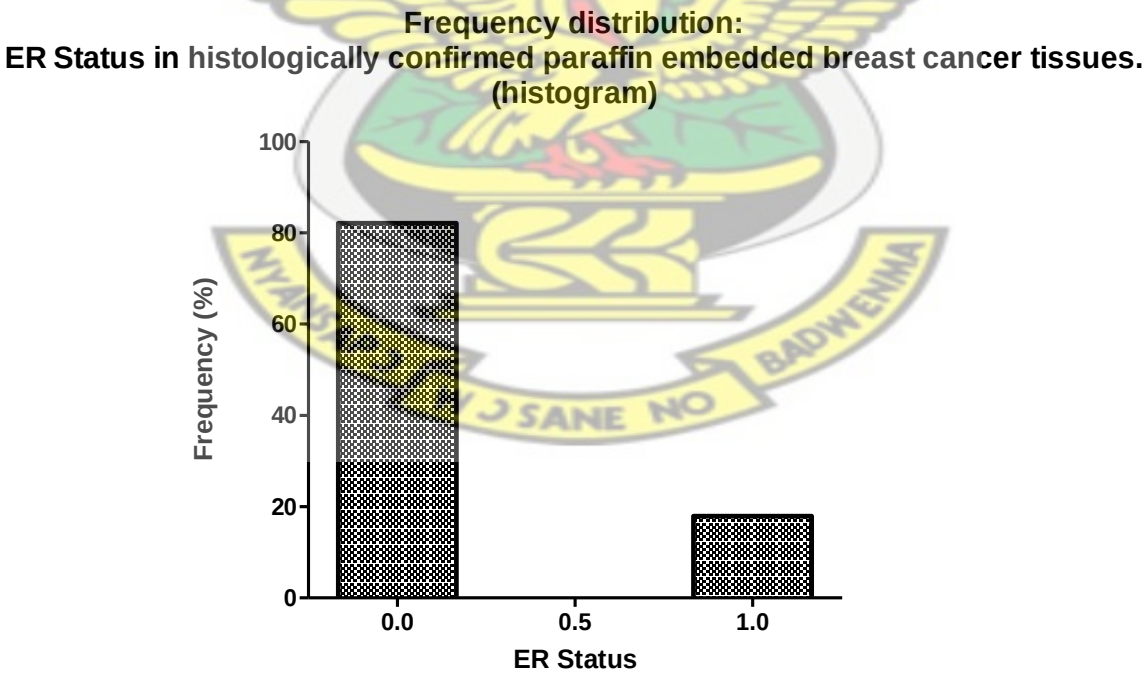


Figure.21. "PR Status in histologically confirmed paraffin embedded breast cancer tissues." N=150

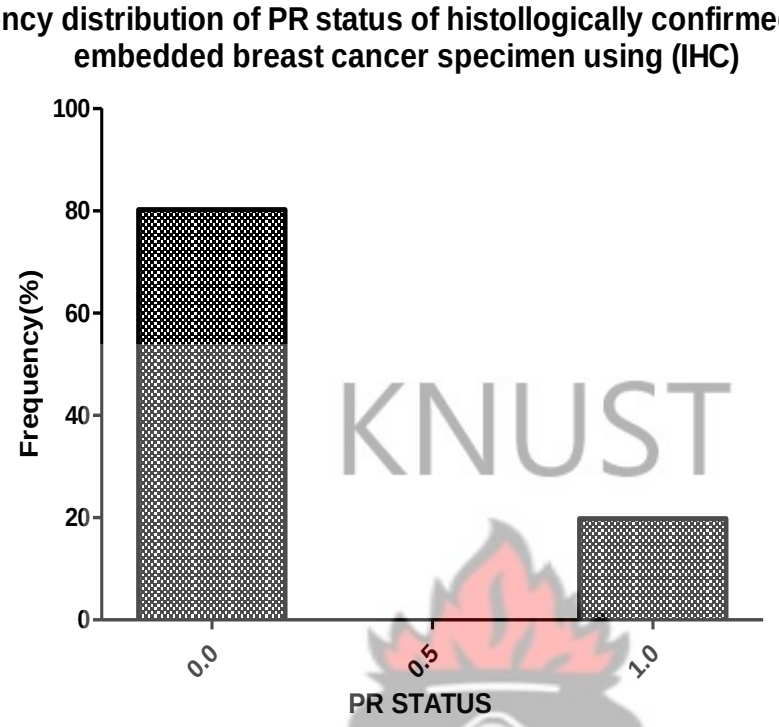


Figure .22. "Frequency distribution of Age of breast cancer patients analysed for ER and PR." n=62

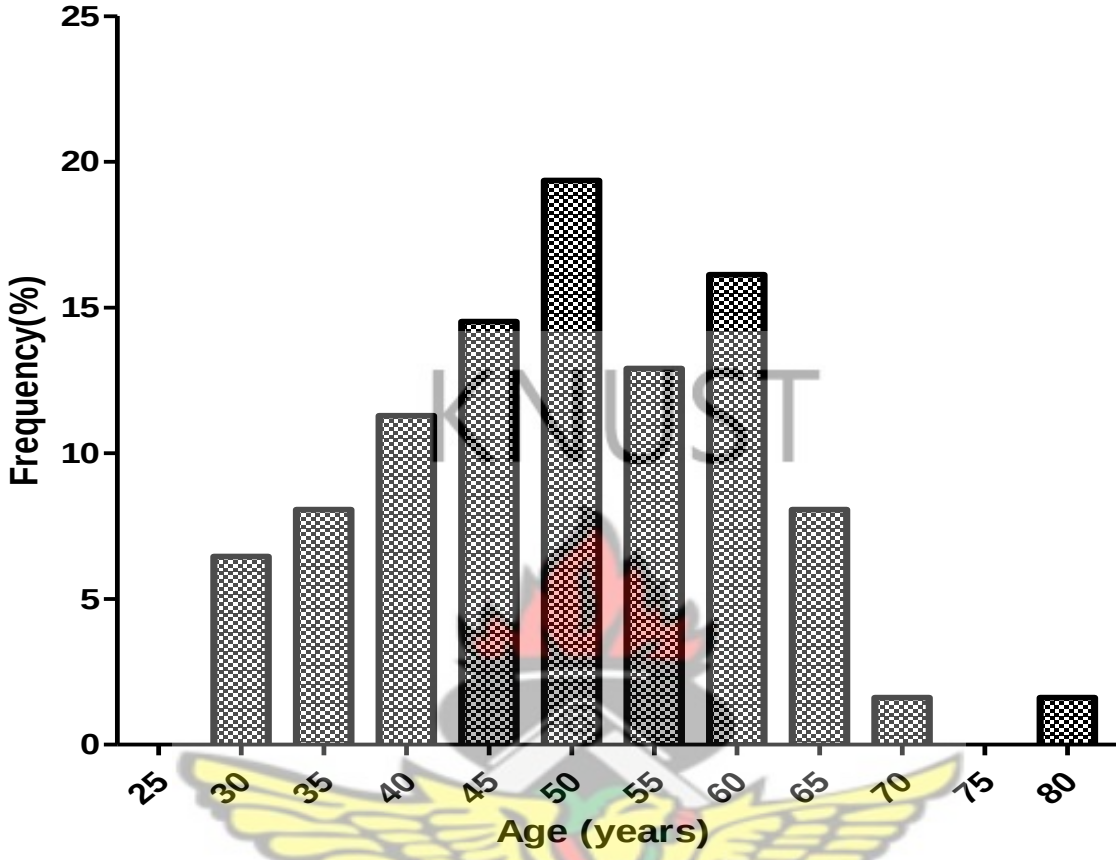


Figure.23."Frequency distribution of ER Scores for breast cancer tissues. (Histogram)" n=62

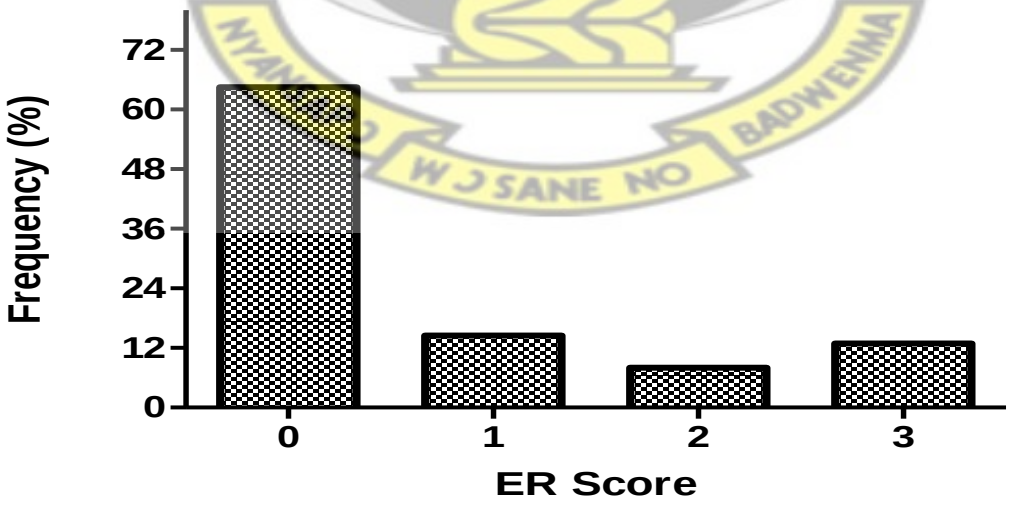


Figure.24."Frequency distribution of PR Scores in breast cancer patients." N=62.

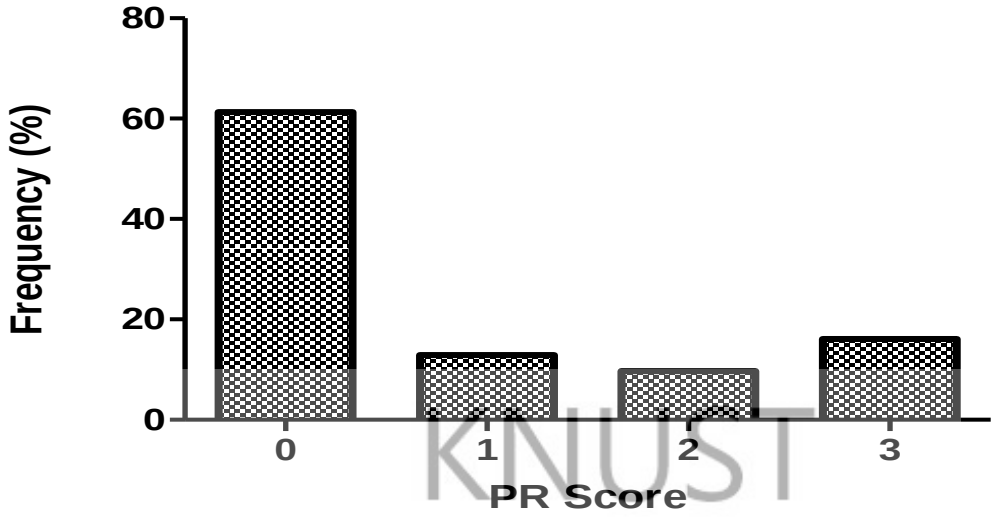


Figure.25."Clinically important BIOMOLECULE analysis: Frequency distribution of Age. n=33"

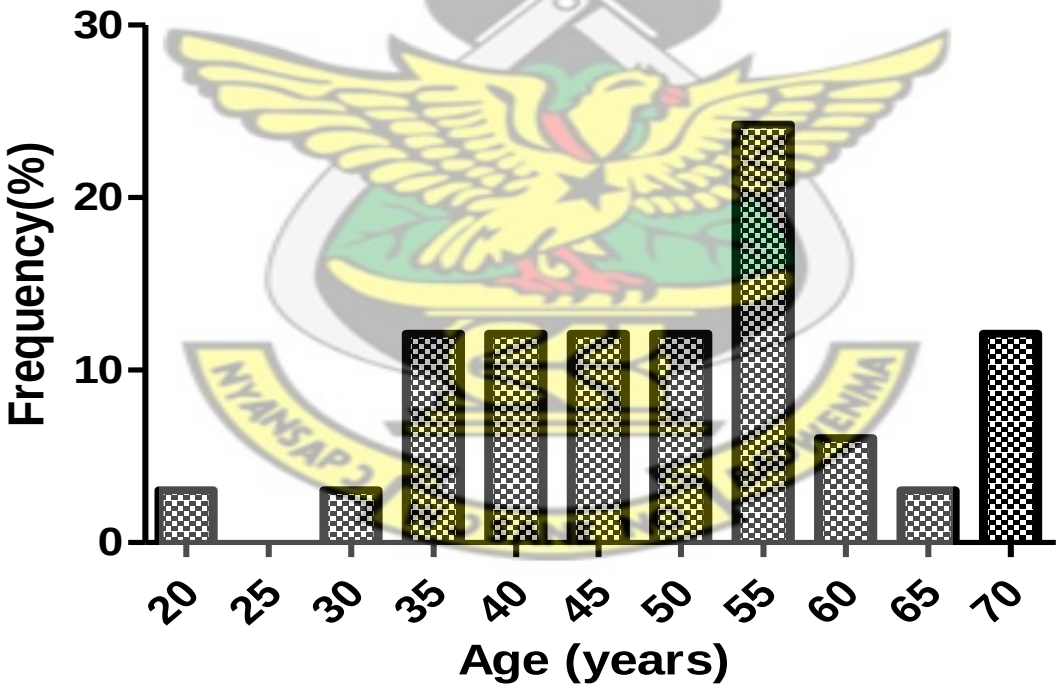


Figure.26."Clinically important biomolecule analysis: frequency distribution of Tumour size (cm). n=33"

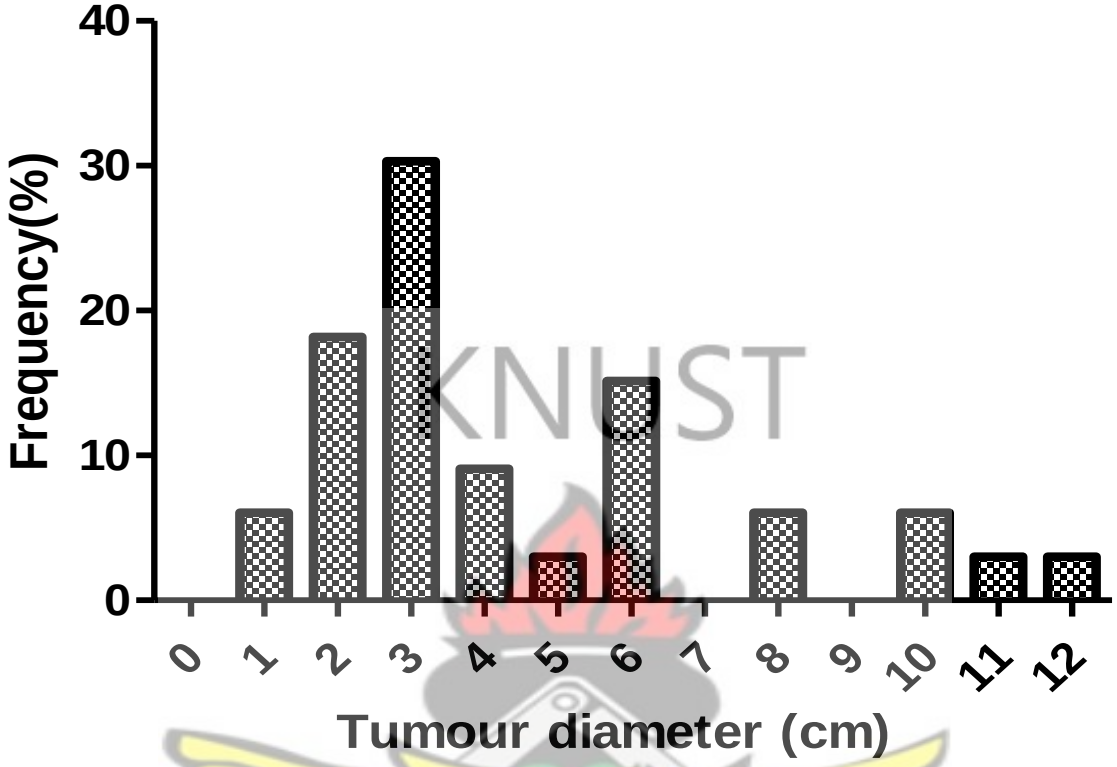


Figure.27." Clinically important BIOMOLECULE analysis: Correlation between Tumour size (cm) and Age. n=33"r = - 0.3999.p*= 0.0211.

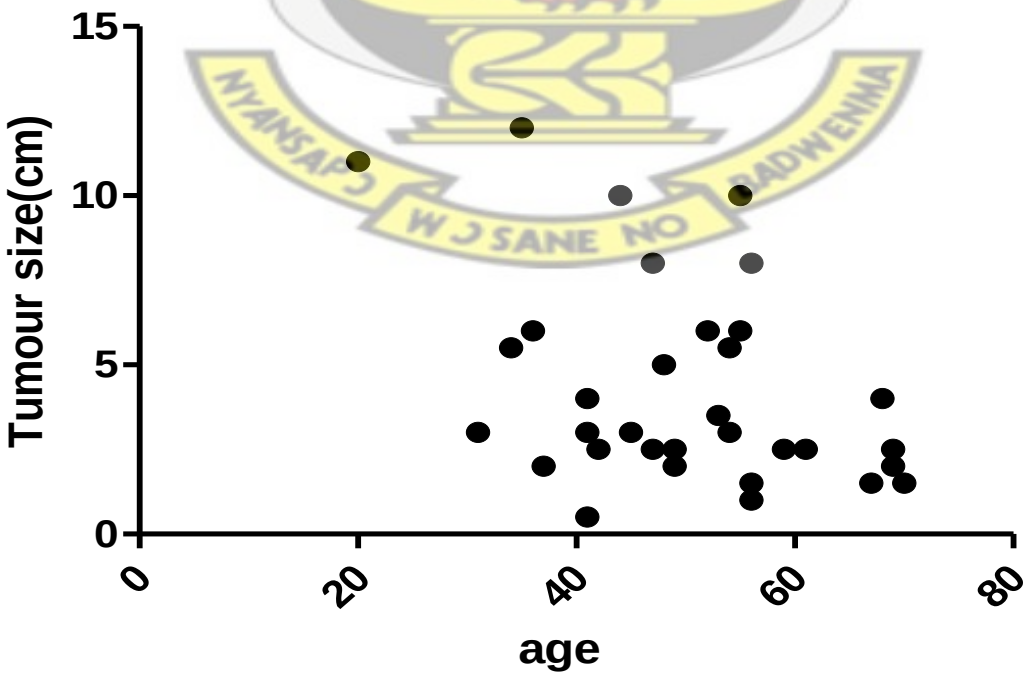


Figure.28.Clinically important BIOMOLECULE analysis: correlation between HER-2 and Ki67.r = 0.4550. p = 0.0078,**

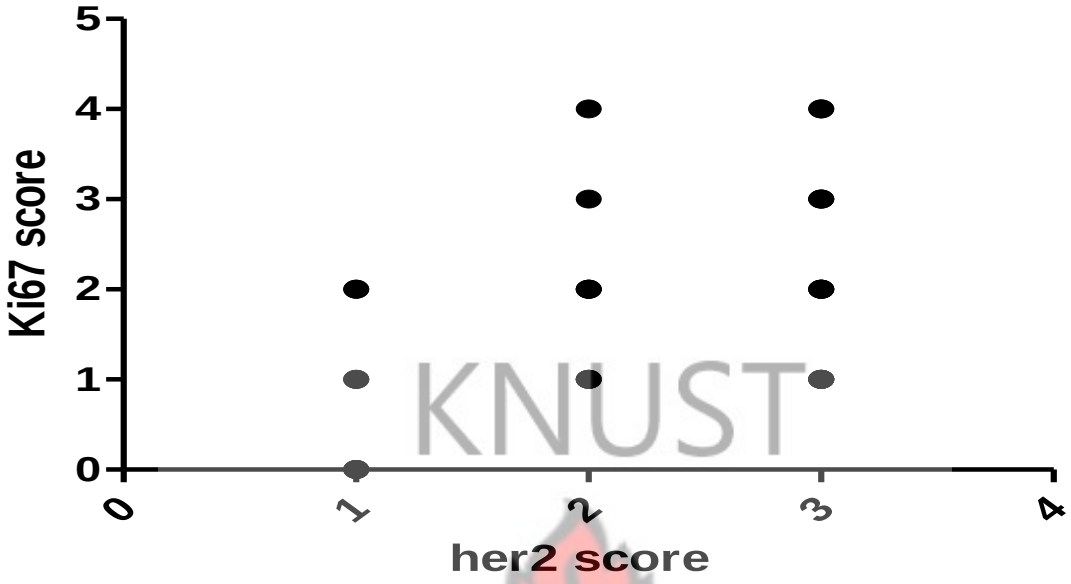


Figure.29.Clinically important BIOMOLECULE analysis: correlation between Age and Ki67.r = 0.3564.p* = 0.0418

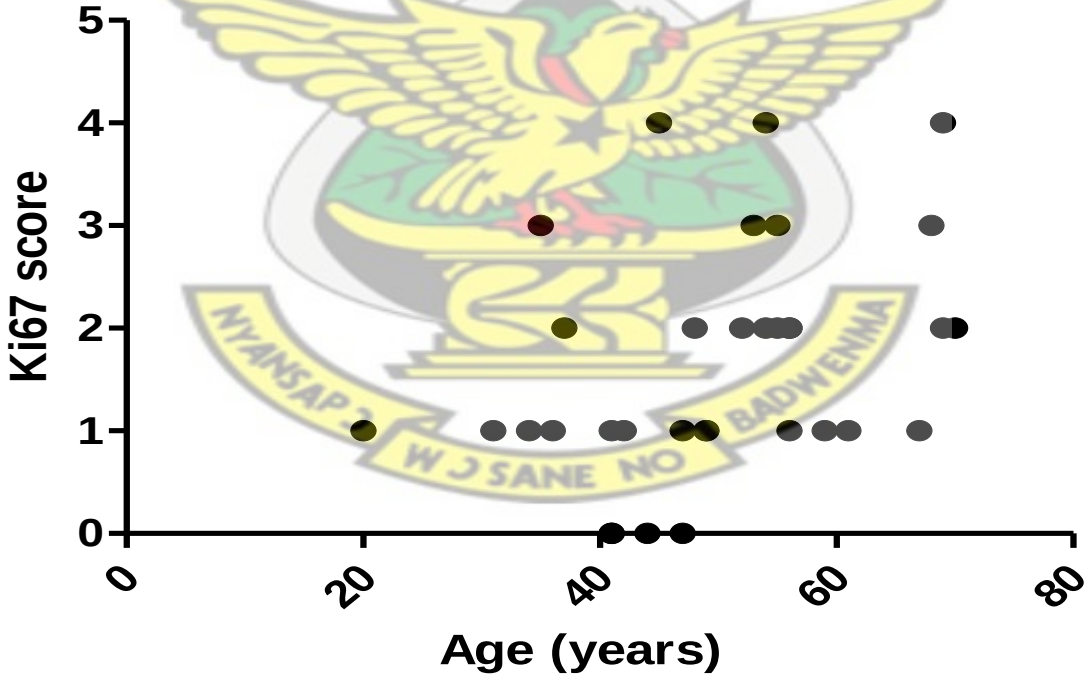


Figure.30.Clinically important BIOMOLECULE analysis: correlation between Tumour size and ER scores = - 0.1149, p = 0.5242

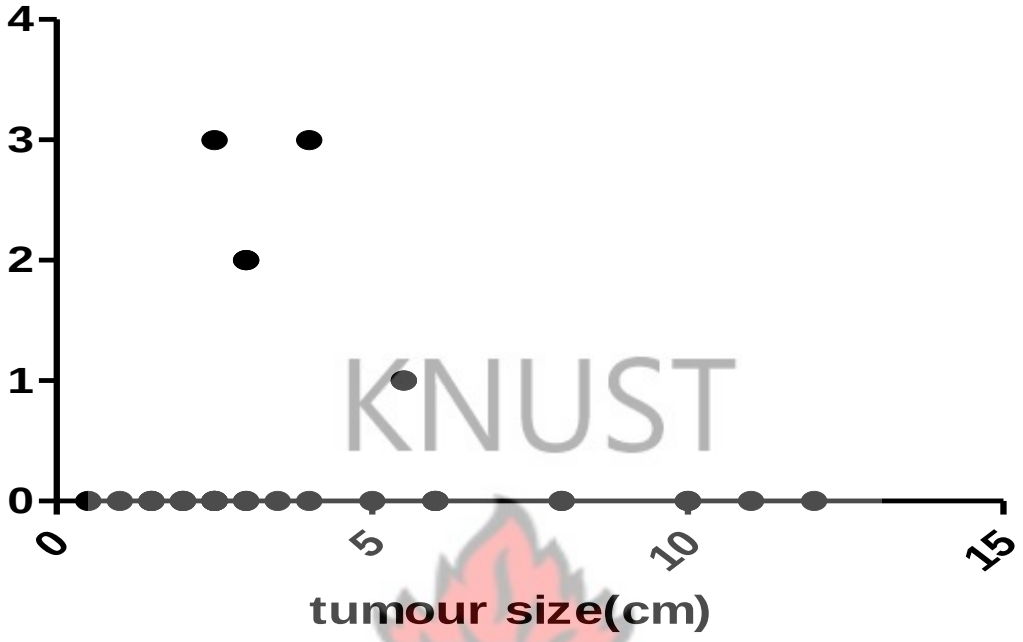
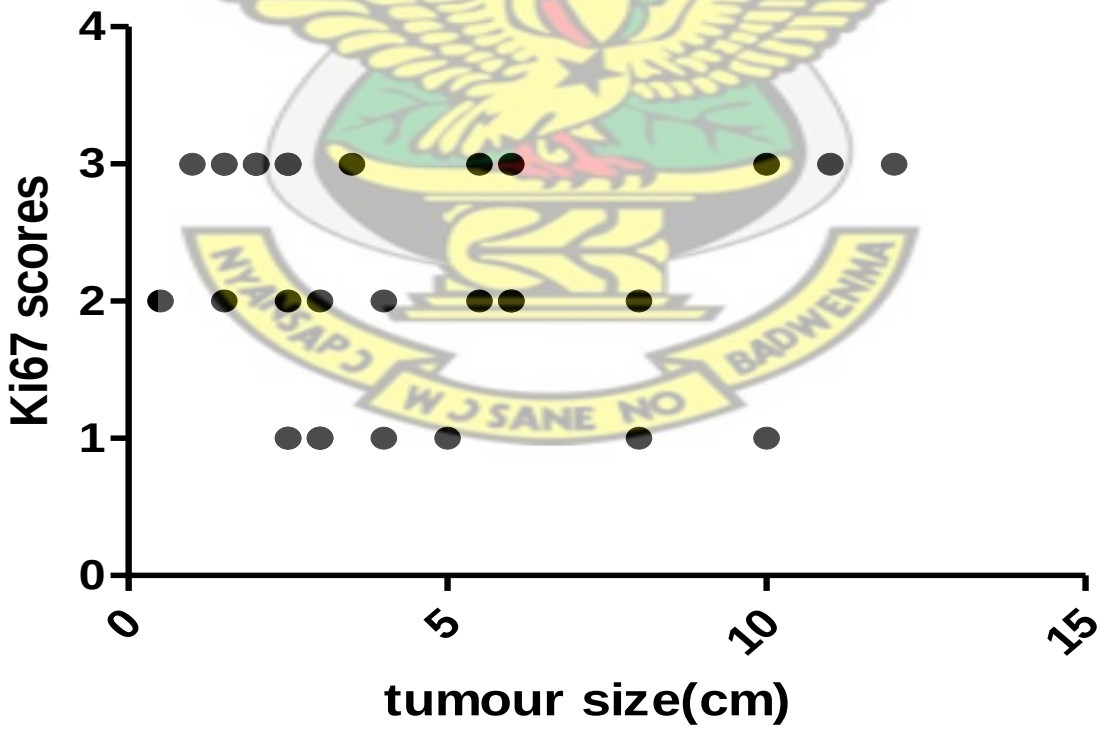


Figure.31.Clinically important BIOMOLECULE analysis: correlation between Tumour size and Ki67.



CHAPTER FIVE

DISCUSSION OF RESULTS

5.0. DISCUSSION OF RESULTS

5.1. Descriptive epidemiology of neoplastic breast lesions in Ghana

Availability of statistical estimates of prevalence rates may be helpful to fund managers and policy makers of our infant national health insurance scheme (NHIS), which covers some treatment for benign and malignant breast conditions. Prevalence rate determined in this study for, breast cancer in Ghana ranged from 0.41% – 1.11% (95% confidence interval) among females aged 18 to 80 years in Ghana (black Africans), whiles prevalence of benign breast lumps ranged from 0.69% – 6.89% (95% confidence interval) (Table 1). This is quite significant and requires a concerted national approach to tackle it. Average Age of breast cancer cases detected through awareness and screening was significantly lower than Average Age of breast cancer victims reporting for surgical treatment; $P < 0.0001$ (Table 4,). This finding gives credence to the need for nationwide breast screening.

Neoplastic breast lesions seem to affect females from puberty to post-menopausal age data in this study does not suggest otherwise. It is widely known that, the incidence of breast cancer increases with age, doubling about every 10 years until menopause, when the rate of increase slows down dramatically (Trichopoulos et al., 1972) Compared with lung cancer, the incidence of breast cancer is higher at younger ages. In some countries there was a flattening of the age - incidence curve after menopause. Increased breast cancer risk is associated with age at first menstrual cycle (menarche) (Bilimoria and Morrow, 1995). By the age of 16 years, three quarters of those who could recall their menarche had their first menses (Figure.12.). Polish girls who started menstrua

ting before the age 16 years had a risk for breast cancer, which was twice that for those whose menarche was later than 16 years (Pike et al., 1983). More than nine out of ten women in Ghana had menopause between the ages 40.75 to 54.79 years. Approximately four out of ten women studied had menopause between 40.75 years to 47.77 years. 3% had menopause beyond 55 years (Agyei-Frempong et al., 2008). Trichopoulus and others reported doubling of the risk for women who continued menstruating beyond the age of 55 years compared with those whose natural menopause was before the age of 45 years (Trichopoulos et al., 1972). The average age at diagnosis for a cross-section of breast cancer patients in this study was 46.29 years (range; 18 to 80 years). It may theref ore be inferred that Ghanaian women may develop breast cancer 10 - 15 years earlier than white women (Agyei-Frempong et al., 2008, Olopade et al, 2005). Breast cancer patients can be classified as post-menopausal or pre-menopausal depending on whether they are still capable of having menstrual periods or not. Approximately 54.84% of breast cancer patients in this study were pre-menopausal. Among pre-menopausal Ghanaian wo men the most common age at diagnosis was 39 years. On the other hand, 45.16% represe nts breast cancer patients who are post-menopausal. Among post-menopausal subjects the most common age at diagnosis was 54 years. Clearly, breast cancer among Ghanaian women may not necessarily be a disease that is more common in post-menopausal women when compared to western Countries (Caucasian – whites) (Agyei-Frempong et al., 2008).

Females who start menstruating early in life (before age 12) or who have a late menopause have an increased risk of developing breast cancer. Women who have a natural menopause after the age of 55 are twice as likely to develop breast cancer as women who experience the menopause before the age of 45. At one extreme, women

who undergo surgically induced menopause before the age of 35 have only 40% of the risk of breast cancer of women who have a natural menopause (Feinleib, 1968). Increased breast cancer risk is associated with age at first menstrual cycle (menarche). Uninterrupted menstrual cycling due to lack of pregnancy and breastfeeding is known to increase a woman's risk for breast cancer (Kelsey and Gammon; 1990., McTiernan and Thomas; 1986). Nulliparity (no childbirth) is associated with an increased risk for breast cancer in women. 25% of females in this study were nulliparous or mono-parous and 50% were multi-parous. Nulliparity was most common representing 20% (Figure.13.). Late childbearing is known to be associated with increased risk for breast cancer in women (Moolgavkar et al; 1980). Approximately, 89% of the subjects had their first full-term pregnancies before 32 years of age (Figure.14.). First full-term pregnancy at the age of 32 or more is associated with increased breast cancer risk in women. Nulliparity and late age at first birth both increase the lifetime incidence of breast cancer. The risk of breast cancer in women who have their first child after the age of 30 was reported to be about twice that of women who have their first child before the age of 20 (Bilimoria and Morrow, 1995). The highest risk group are those who have a first child after the age of 35; these women appear to be at even higher risk than nulliparous women. An early age at birth of a second child further reduces the risk of breast cancer (Bilimoria and Morrow, 1995). Android obesity in women is associated with increased risk for breast cancer and cardiovascular disease. Hip and waist measurements were recorded for the participants in this survey. Waist / hip ratios were computed to assess whether a participant in this survey was obese or not. Waist / hip ratios ranged from 0.5769 to 2.2632 (Figure.17.). When waist / hip ratio exceeds 0.86, one is said to be obese. 25% of women in Ghana were classified obese in this study. Comparatively, the first study of waist / hip ratios and

breast cancer risk in an indigenous African population, conducted in Nigeria, as well as other studies has shown a positive association between obesity and breast cancer risk among postmenopausal women (Adebamowo et al, 2002). Obesity was also associated with significant hormonal changes such as increased serum oestradiol, decreased sex hormone binding globulin (SHBG) levels, increased peripheral fat conversion of oestrogens to progesterone and increased serum testosterone levels that may be associated with an increased risk of breast cancer (Pollack., 2000). Obesity is reported to be associated with a twofold increase in the risk of breast cancer in postmenopausal women whereas among premenopausal women it is associated with a reduced incidence (Pollack. 2000).

The occurrence of breast cancer among first degree relatives (mother and sister) increases the risk for breast cancer among females. In this study 0.7% of the respondents reported their mothers were breast cancer victims, 0.7% reported their sisters were breast cancer victims. Therefore, 1.4 % of women screened for breast disease reported knowledge of positive family history of breast cancer. This means that it can be transmitted through either sex and that some family members may transmit the abnormal gene without developing cancer themselves. It is not yet known how many breast cancer genes there may be. Two breast cancer genes, BRCA1 and BRCA2, which are located on the long arms of chromosomes 17 and 13 respectively, have been identified and account for a substantial proportion of very high risk families—i.e. those with four or more breast cancers among close relatives. Tumour size was used as a proxy-measure fore late stage of presentation and therefore tissue characteristics were studied as well.

Ignorance and poverty have caused low access to early detection, diagnosis and treatment services nation-wide, and have contributed largely to late presentation of neoplastic breast lesions for treatment in Ghana. At least three-quarters of the mastectomy and or lumpectomy specimen analyzed for oestrogen-receptors had dimensions 5.5cm x 2.5cm or more. This feature is an indication of late stage presentation and contributes to low survival in Ghana. Prevalence of neoplastic breast lesions among females as reported in this study was quite significant. Clinically obvious breast cancer represents 10.81% of all neoplastic breast lesions seen in Ghana (Table 3a). This represents the fact that one out of 10 breast masses (neoplasia) are likely to be malignant as is usually reported in breast awareness literature. Breast tissue density is known to affect quality and accuracy of mammography in detecting breast lesions. Alternatively, ultrasonography of the breast was less expensive but was also largely unavailable. Therefore, the option to explore other methods of breast examination was very compelling. Hence, a new hand held torch, which was introduced into the study to, transilluminate breast tissue and visually demonstrate angiogenesis, was evaluated; to serve as an adjunct to early detection.

5.2. Visual assessment of tumour-related angiogenesis to enhance early detection of breast lesions.

The breastlight was easy to use; and may identify only the dangerous cancers - those with angiogenesis mainly. Light can demonstrate a cancer because of associated angiogenesis (Watmough, 1982) which greatly increases light absorption at 618 nm the wavelength employed in Breastlight. The device is not diagnostic since some benign tumours and blood filled cysts gave rise to shadows reminiscent of cancer. The Breastlight was used co rrectly, hence, 47 of the 49 with confirmed breast cancers were positive [96 %] using the

Breastlight, 2 [4%] were missed. Women in this study presented with advanced disease (i.e. harbouring relatively large tumours) as presented in the data. A surprisingly high positive rate for cancer of 96 % was found. The breastlight had some noticeable advantages that were noteworthy.

Advantages of the breastlight;

First of all it has no radiation exposure. It detected angiogenesis, which is a hallmark of cancer in exponential tumour growth phase and spread; therefore by implication it may detect dangerous breast lumps with angiogenesis accurately. It may detect interval cancers / breast lesions as well since it can be used several times without harmful effects. No breast compression was required; it caused no pain to the user and subjects. It could demonstrate suspicious breast lesions in younger women with high risk for breast cancer. It may be a useful adjunct to breast screening where mammography is scarce. The breastlight may be ideal for populations where breast cancer occurs at an earlier age, below 50 yrs. It is Ideal for examining ectopic breast tissue. Serial images obtained with the breastlight can be used to monitor/measure effectiveness of chemotherapy if breast images are documented and digitized. The breast light has not been studied in any large study over a prolonged period. It does not differentiate benign from malignant breast lesions and also shines through non-bloody cysts, fibroadenomas (20%), glandular tissue and fatty lumps.

5.3. Molecular characteristics of breast cancer seen in Ghana

5.3.1. Bio-molecules of clinical importance for managing breast cancer

The quantitative determination of oestrogen-receptors in breast cancer tissue samples is used to predict their response to anti-oestrogen therapy (McGuire et al., 1975). Patients with ER-negative tumours have a higher recurrence rate within the first 20 months follow

ing mastectomy (McGuire et al., 1975). These patients have low response rates to hormonal manipulations when they develop metastatic cancer (less than 10%). Such patients are better managed with cytotoxic chemotherapy, possibly including adjuvant chemotherapy, even in the absence of tumour in the axillary lymph nodes (Knight et al., 1980). Therefore, receptor data are helpful in deciding whether to employ hormonal therapy for metastatic disease and delaying the generally more toxic chemotherapeutic approach. ER has complex relationships with other bio-molecules relevant in breast cancer. The majority of cancers express ER and HER-2 in an inverse manner, and a subset of tumours (approximately 10%) express both (deFazio et al., 2000; Konecny et al., 2003). Although individual luminal cells of the normal breast rarely co-express ER and the prolife ration marker Ki-67, a substantial proportion of breast cancer cells show this co-expression (Clarke et al., 1997). The interactions of ER with growth factors and signal transduction molecules appear to be important in the development of resistance to endocrine therapy (Nicholson et al., 1999). Oestrogen sends signals through the receptors that tell breast cancer cells to grow. Cells with oestrogen receptors grow and multiply wh en oestrogen attaches to the receptors. After a breast cancer is removed, the cancer cells are tested to see if they have hormone receptors. If either oestrogen or progesterone receptors are present, a response to hormonal therapy is very possible. The more oestrogen or progesterone receptors present on those cells, the more likely that hormonal therapy will work against the particular cancer. If high levels of both oestrogen and progesterone receptors are present, an even greater response to hormonal therapy is likely. The goal of therapy is to starve the breast cancer cells of the hormone they thrive on, which is oestrogen.ER data on a subset of 62 human breast cancers reported in this work follows the trend of results seen in other laboratories, although the overall prop

ortion of ER+ breast cancers appears remarkably lower, 35.48% compared to 75.00% (poo led data from many laboratories). The overall ER/PR negative tumours was remarkably higher, 53.23% compared to 25.00% (pooled data from many laboratories) (Osborne et al, 1980., Elledge et al, 2000). HER-2 scores range from 1 to 3 in this data set, no one had a HER-2 score of 0, 45.45% had a score of 3. Another 27.27% had a score of 1 and 27.27% had a score of 2. These were all potentially metastatic breast cancers and were likely to respond to immunotherapy based drug called trastuzumab or Herceptin. HER-2 is express ed in all breast epithelial cells. IHC staining for HER-2 has been the predominant method utilized. Unlike most Immunohistochemical (IHC) assays, the assessment of HER-2/neu status is quantitative rather than qualitative, since HER-2/neu is expressed in all breast epithelial cells. Previous studies have established a relationship between the number of HER-2/neu receptors on a cell's surface and the distribution and intensity of the immune-stain. In this study (n=33), the correlation between ER and HER-2 established and proven to be highly significant. Pearson r = -0.4805, p^{**} (two-tailed) = 0.0046 and alpha = 0.05 (Table11.). Quantification of the proportion of cells with nuclear Ki-67 antigen expression is a measure of growth fraction and hence biological aggressiveness in malignancy. This IHC detected marker has shown potential as a marker for prognosis in many malignancies including breast cancer. Many studies have demonstrated that rapidly proliferating tumours with high Ki67 scores have poor outcome irrespective of the type of treatment. A reduction in Ki-67 index has been demonstrated after chemotherapy, tamoxifen therapy, and chemo-endocrine therapy by many researchers. Higher Ki67 scores are associated with higher tumour proliferation rates and poor prognosis with or without biochemical treatment (Dardes et al., 2000). 87.98% of the tumours, studied for biomolecules of breast cancer tumours (n = 33) had a score of 1 or more hence the

generally low survival after treatment for breast cancer patients in Ghana. This may be a feature of late presentation for treatment and offering ample opportunity for the breast cancer tumours to undergo further malignant transformations and become more aggressive. Statistical measurement of the relationship between biomolecules were estab lished. There was a significant moderate negative correlation between breast cancer tumour size and age of the patient. Pearson r was -0.3999, p^* = 0.0211 (two tailed) and (alpha=0.05). Hence, the younger the patient the larger the breast cancer tumour present ed for treatment (Table 11, Figure.27.). Increasing age correlated well with decreasing tumour sizes. This correlation was stronger among post-menopausal breast cancer tumours (0.4267) as compared to (0.3866) in premenopausal tumours. Average tumour size for pre-menopausal tumours was 4.38 cm, compared to 3.36 cm in post -menopausal ones. Pearson r = - 0.3999, p^*. By deduction younger breast cancer patients in Ghana tend to have more aggressive breast cancers. By the same reasoning, it is expected that; younger breast cancer tumours will have higher Ki67 scores. Surprisingly, this data indicates an opposite trend overall. Correlation between age and ki67 was positive, and significant. Pearson r = 0.3565, p^* = 0.0418 and alpha = 0.05. This correlation was stronger among premenopausal ages < 54 years (0.1536) as against post-menopausal ages (0.0547). Overall Pearson r = 0.3564, p^*. This may be attributable to the frequent use of preoperative chemotherapy which is more destructive to dividing cell (S-phase) and also, smaller tumours have a higher propensity to divide, Ki67 protein is found only in cells at the S phase. Hence may not be available at the time of tissue analysis. Cancer cells over expressing HER-2 protein are in a relatively perpetual state of proliferation and express more Ki67 molecules. HER-2 belongs to a family of growth factor receptors which when over expressed puts cells in a perpetual proliferation mode as seen in cancers.

Correlation between HER-2 and Ki67 was highly significant in this study and supports aggressive nature of such tumours (Table 11, Figure28). This was expected, due to the fact that Ki67 bio-molecule is a by-product of cell proliferation. Low ER scores and high HER-2 scores were likely to occur in advanced or late breast cancer at presentation for treatment (Konecny et al; 2003). Correlation between Ki67 and ER was moderate and negative but significant (Table 11.). By deduction high Ki67 scores were also more likely to be found in breast cancer tumours with low ER content. These is another feature of aggressive breast cancers which have a relatively poor outcome of treatment, irrespective of the kind of treatment selected (Allred et al, 1998).

Researchers at all three Mayo Clinic sites namely; Jacksonville, Scottsdale - Arizona and Rochester - Minnesotta, USA., participated in a study, which examined 401 women who were treated for breast cancer between 2001 and 2005 at the breast cancer clinics in Jack sonville and Scottsdale. Their results indicated that:

- The vast majority (87%, or 350 women) had tumours that were classified as ER/PR positive and HER-2 negative (in short, HER-2 negative/ER/PR+). Juxtaposed with ER+/HER-2-/Ki67+ (1) = 3.03%, and ER+/HER-2-/Ki67- (3) = 9.09%. in this study representing 12.12%
- Twenty-seven women (6.7 %) had tumours that were HER-2+ and 24 patients (5.9 %) were diagnosed with triple negative cancer — that is, ER/PR negative and HER-2 negative. Juxtaposed with 72.73% HER-2+ and 15.15% triple negative breast cancer in this study. ER/PR+ breast cancer was considered the least aggressive of the three categories. Generally, studies have shown that 15 to 20 % of breast cancers are HER-2+ and about 10 to 15 percent are triple negative. In the study at Mayo clinic, patients were followed up for an average of almost three

years, and so far researchers have data on all patients with HER-2+ and triple negative cancers and on 219 women with HER-2 negative/ER/PR+ cancer. These researchers found that:

- Breast Cancer came back more frequently in HER-2+ tumours (7.4 % of patients relapsed) and triple negative cancers (12.5 % relapsed), compared to HER-2 negative/ER/PR+ cancer (1.3 % relapsed).
- The death rate was higher in triple negative breast cancer: there was one death in the 24 patients with triple negative tumours, none in the HER2+ group of 27 women, and one death related to relapse in 219 women with HER-2 negative/ER/ PR+ cancer.

Their findings suggest that women with HER-2+ and triple negative tumours should receive as much treatment as possible in order to prevent cancer relapse. In addition, their study high-lighted the fact that HER-2 positive tumours, even if very small, may warrant more aggressive therapy.

According to data in this current study, low ER scores were more likely to be found in breast cancers that had high HER-2 and Ki67 scores and hence were more aggressive and less likely to respond to hormonal therapy. However, high HER-2 scores, high Ki67 scores and smaller breast cancer tumour sizes were more likely to be seen in older women at presentation. It can be inferred that, older women were more likely to report earlier for diagnosis and treatment for breast lesions in Ghana. In spite of the high HER-2 and Ki67 scores seen in some older women with breast cancer, their smaller tumour sizes at presentation may indicate a lower clinical stage of disease at presentation. Their lower clinical stage at presentation may lead to under treatment if the clinician does not as well base his treatment on the levels of these bio-molecules. Therefore, some older breast

cancer patients with clinical stage one or two breast cancer may in fact need more intensive treatment than some younger breast cancer patients with similar or higher clinical stage disease at presentation. At best two out of three (ER+ were 35.48%, by IHC) breast cancers seen in this study were ER negative. Among the ER negative breast cancers HER-2 and Ki67 scores were of prime importance in determining how intense treatment must be and whether immunotherapy (the use of herceptin / trastuzumab) was an option. Among the ER positive breast cancers, those with higher ER scores were likely to benefit the most from hormonal therapy (Tamoxifen, Aromatase inhibitors, etc.) and hence could be spared more intense, expensive and toxic chemotherapy for a while. Some experts have emphasized the importance of high ER scores (in breast cancer) in determining better outcome of treatment of any form. Generally, much has not been said about PR in this study because, its presence indicates the ER response pathway is intact. These explanations highlight the clinical importance of bio-molecules such as ER/PR, HER-2 and Ki67 in managing breast cancer.

5.3.2. Clinical implications of Biochemical sub-types of breast cancer treated in Ghana (n=33)

ER positive breast cancers (18.18%): biochemical sub-types;

ER+/HER-2-/Ki67+ biochemical sub-types of breast cancer occurred at a rate of 3.03% in these tissue samples. Hence, they would usually proliferate when oestrogen is bio-availab le. Cell cycle specific chemotherapy is effective when cells are in a proliferative cycle, hence they (ER+/HER-2-/Ki67+) would respond favourably to chemotherapy as well as hormonal therapy. They are expected to have a better prognostic outcome than ER+/HER -2+/ki67+ (Allred et al, 1998; Knight et al, 1980; Konecny et al, 2003; McGuire et al, 1975).

ER+/HER-2+/ki67+ biochemical sub-types of breast cancer occurred at a rate of 6.06% in

these tissue samples. By inference, the cells were proliferating and the predominant cause of cell proliferation was due to the presence of tumour cell populations that were oestrogen dependent and others which could proliferate independently of oestrogen and were over-expressing HER-2. Cell cycle specific chemotherapy is effective when cells are in a proliferative cycle, hence they will respond favourably to such chemotherapy initially, however, if HER-2 over-expression is the more dominant cause of cell proliferation then, the prognostic outcome will be relatively worse. If oestrogen stimulation is the predominant cause of cell proliferation the prognosis will be better relatively. Both scenar ios are likely because the correlation between ER and HER-2 was negative, moderately strong and highly significant (Allred et al, 1998; Knight et al, 1980; Konecny et al, 2003; McGuire et al, 1975). Pearson r = - 0.4805, p**.

ER+/HER-2-/Ki67- biochemical sub-types of breast cancer occurred at a rate of 9.09% in these tissue samples (Table 14). By inference, the cells were not proliferating. The predo minant tumour cell populations were oestrogen dependent. There was no evidence of HER-2 over expression (HER-2-), no evidence of cell proliferation (Ki67-). Their prognosis is expected to be better than ER+/HER-2-/Ki67+ and ER+/HER-2+/ki67+ (Allred et al, 1998; Knight et al, 1980; Konecny et al, 2003; McGuire et al, 1975).

ER negatives (81.82%): Biochemical sub-types;

ER-/HER-2+/Ki67- biochemical sub-types did not appear in breast cancer tissue samples. This is strong evidence to support the fact that Ki67 indicates evidence of cell proliferation caused by either ER positive tumours or HER-2 over-expression.

ER-/HER-2+/Ki67+ biochemical sub-types of breast cancer occurred at a rate of 66.67%, in these tissue samples. It was the most common biochemical sub-type in these samples. They showed over expression of HER-2 as the only / predominant cause of cell

proliferation which was demonstrated by a concomitant Ki67 staining in the tissues. Their prognosis is worse than ER+/HER-2-/Ki67+, ER+/HER-2+/Ki67+, ER+/HER-2-/Ki67-, and ER-/HER-2-/Ki67+ (Allred et al, 1998; Knight et al, 1980; Konecny et al, 2003; McGuire et al, 1975). Triple negative bio-chemical subtypes of breast cancer (TNBC) occurred at a rate of 15.15% (Table14). ER-(PR-)/HER-2-/Ki67+ triple negative biochemical sub-type of breast cancer occurred at a rate of 12.12%, in these tissue samples. Both ER and HER-2 over-expression were absent. Therefore cell proliferation demonstrated by Ki67 staining was caused by other bio-molecules or mechanisms not captured in this work. They are not likely to respond to hormonal and immunotherapy. They are expected to respond well to cell cycle specific chemotherapy. Their prognosis is worse than ER+/HER-2-/Ki67+, ER+/HER-2+/Ki67+ and ER+/HER-2-/Ki67- (Knight et al; 1980, Konecny et al; 2003, McGuire et al; 1975).

ER-(PR-)/HER-2-/Ki67- Triple negative breast cancer (TNBC) occurred at a rate of 3.03%. The cells were not proliferating. They did not express ER, there was no HER-2 over-expression hence, no Ki67 staining. Triple-negative breast cancers are known to have a more aggressive clinical course than other forms of breast cancer. While some patients with TNBC initially respond well to standard chemotherapy, these tumours are more likely to recur after treatment and have a poorer prognosis (less than 30 per cent of women with metastatic TNBC – which has spread outside the breast – survive 5 years). "It's a pretty significant health problem from the standpoint that 11 per cent of Caucasians, 17 % of Hispanics, and 25 per cent of African-Americans have this type of breast cancer," Pietenpol said (Konecny et al, 2003). Knowing the specific subtype could help physicians determine which therapies would work best in patients with TNBC and also inform the discovery and development of new drugs to treat this aggressive form of breast cancer.

The difficulty in treating these tumours stems from what they lack. The term "triple-negat ive breast cancer," is just a definition of what the cancer isn't in terms these clinically imp ortant biomolecules ER, PR and HER2.(Konecny et al, 2003).TNBC tumours lack the oestro gen receptors (ER) and progesterone receptors (PR) that drive the majority (about 60 %) of breast cancers. They also show no amplification of another receptor, called HER-2, which drives about 20 per cent to 30 per cent of breast cancers. The absence of these receptors means that the tumours are unlikely to respond to hormone therapies like tam oxifen and to therapies targeted to HER-2 like trastuzumab (Herceptin). Overall, the most predominant cause of cell proliferation in breast cancer tissue samples studied (n=33), wa s HER-2 over-expression (66.67% + 6.06% = 72.73%), compared to oestrogen dependent c ell proliferation (18.18%). Correlation between HER-2 and Ki67 revealed; Pearson r = 0.45 50, p^{**} (two tailed) = 0.0078 and alpha = 0.05. It was moderately strong, positive and highl y significant, hence (HER-2+/Ki67+/.....) occurred and was predominant. On the other ha nd, correlation between Ki67 and ER; Pearson r = -0.4159, p^{*} (two tailed) = 0.0161., alpha = 0.05., was moderate, negative and significant, hence (ER+/Ki67-/.....) but was not pred ominant. This explains the relatively more frequent more aggressive nature of breast can cers seen in our study. "Ki67 scores demonstrate the extent of cell proliferation caused by ER mediated cell proliferation and HER-2 over-expression to a very large extent". If this s tatement is true, then; the sum of frequencies of ER positive tumours and HER-2 positive tumours should closely equal the frequency of Ki67 positive tumours;

[{HER-2+} 72.73% $_{observed}$ + {ER+} 18.18% $_{observed}$ = [{HER-2+} + {ER+}] 90.91%

[{HER-2+} + {ER+}] 90.91%]≠ [{Ki67} 87.98% $_{observed}$]. Some ER+ tumours did not express Ki67 ([ER+/..... /Ki67-]). The paradigm shift towards more accurate selecting and individ ualizing of treatment for solid malignant tumours using biomolecules provided

motivation to explore and develop new concepts in this study, as presented in subsection 5.4.

5.4. A NOVEL WAY TO INDIVIDUALISE TREATMENT FOR BREAST CANCER

"A NOVEL, HYPOTHETICAL, SEMI-QUANTITATIVE MODEL FOR, OPTIMIZING THE BENEFITS OF CHEMOTHERAPY IN THE MANAGEMENT OF SOLID MALIGNANT BREAST LESIONS."

Consider the following parameters;

Let survival be represented by S.

- S_o represents optimum survival,
- S_i represents intrinsic survival attributable to surgical treatment.
- S_c represents additional survival attributable to adjuvant chemotherapy.

Hypothetically, $S_o = S_i + S_c$……Equation 1

It has already been established that, with or without treatment, survival for breast cancer is greatly dependent on the rate of cell division, that is, tumour proliferation. This may be measured as the tumour proliferative index [Ki67], and so,

$$S \propto \frac{1}{Tumour\ proliferation\ index[Ki67]}$$

Thus, the smaller the extent of tumour proliferation or, the lower the tumour proliferative index [Ki67]; the better/greater the survival of the cancer victim and vice versa.

Also, with/without treatment, survival for breast cancer is greatly dependent on the extent of viable metastasis and tumour stage [TS], so that,

$$S \propto \frac{1}{Tumour\ stage[TS]}$$

Thus, the lower the tumour stage at treatment, the better/greater the survival of the cancer victim and vice versa.

Within limits of toxicity with regards to effective adjuvant chemotherapy, improved survival for breast cancer could be proportional to the number of cycles of chemotherapy [NCC] administered.

And so,

$$S\ \alpha\ number\ of\ cycles\ of\ chemotherapy\ [NCC],$$

where α is a proportionality symbol.

If K is the proportionality constant, then, Ki represent proportionality constant for Si, and K_c represents proportionality constant for S_c.

Therefore,

$$S_i = \frac{K_i}{[TS]\ [\ Ki67]}$$

and

$$S_c = \frac{K_c\ [NCC]}{[TS]\ [\ Ki67]}$$

Then,

$S_o = S_i + S_c$ ……Equation 1 becomes

$$S_o = \frac{K_c\ [NCC]}{[TS]\ [\ Ki67]} + \frac{K_i}{[TS]\ [\ Ki67]}$$

And so,

$$S_o = \frac{K_c\ [NCC]}{[TS]\ [\ Ki67]} + S_i \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{Equation 2.}$$

Therefore,

$$S_o - S_i = \frac{K_c\ [NCC]}{[TS]\ [\ Ki67]} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{Equation 3.}$$

{[TS][Ki67]}$^{-1}$ is treated as the tumour biological coefficient and therefore; is a measure of some property or characteristic of the tumour.

Generally, patients with higher stage tumours have a lower long-term survival. However, at the extremes, there are a few exceptions. According to breast cancer facts and figures handouts; the five year survival rates for breast cancer are;

- Stage I; 85%
- Stage II; 75%
- Stage III; 55% and
- Stage IV; 14%.

In this model, using hypothetical data and computations, higher staged tumours showed higher initial response to chemotherapy represented by K_C. On the other hand, lower staged tumours showed higher long-term response / survival represented by K^C (Table. 12.1.). These features are in tandem with what occurs in reality (Allred; 1998). The initial response to chemotherapy was probably influenced by Ki67 levels, which is a measure of cells in the proliferative phase. Once these cells are effectively wiped out by chemotherapy, the residual disease may undergo further deleterious genetic transformations, become less responsive to chemotherapy and bounce back into proliferation. These events are more likely to occur in higher staged tumours, hence their lower long-term survival rates (K^c). Furthermore, same stage tumours may have different Ki67 levels, hence different proliferation rates and therefore, showed different survival represented by K^C (Table. 12.1.). The individual's tumour biological character and behaviour, such as tumour stage (TS) and Ki67 level could be used to compute long-term survival rates per cycle of chemotherapy (K^c). The individual's tumour character and

behaviour are summarized by; **{[TS][Ki67]}$^{-1}$ the tumour biological coefficient, as proposed.**

Potential benefits:

- This method may help to select the most effective chemotherapeutic agent or combinations in terms of years of survival per cycle of chemotherapy administered (K_c).
- This method may help to reduce the risk of over treating or under treating breast cancer patients with chemotherapy.
- This method may help tailor treatment to each breast cancer patient, since every breast cancer patient is unique.
- Within safety limits, those who require more cycles of chemotherapy can be distinguished from those who require less and should be spared some toxic side effects and high costs.
- This method may simultaneously, help optimize survival and minimize toxic side effects of chemotherapy, in the management of solid malignant breast lesions.

CHAPTER SIX

CONCLUSIONS

6.0 CONCLUSIONS

The descriptive epidemiology of neoplastic breast lesions in Ghana has been investigated. In spite of increased efforts at combating breast cancer in Ghana, the awareness level is still low and as a result most victims or families of victims still suffer heavy losses due to low survival rates prevalent in Ghana. The five year survival rate estimate for breast cancer, could be less than 45% in Ghana.

Prevalence rate for breast cancer in Ghana ranged from 0.41% – 1.11% (95% confidence interval) among females aged 18 to 80 years in Ghana (black Africans), whiles prevalence of benign breast lumps ranged from 0.69% – 6.89% (95% confidence interval), n = 15,219 (Agyei-Frempong et al,2008). This awareness and screening programme was expanded to cover 44,482women nationwide between 2004 and 2008. These figures were confirmed by the expanded study from 2004 - 2008, as 0.4% and 2.9% respectively, where n = 44,482 (15,219 + 29,263).

The Average Age at diagnosis/detection for breast cancer was significantly lowered through awareness and screening nationwide; $P < 0.0001$ (Table 4).

Among the study population; the average age was 38.47years (range; 10 years to 99.00 years). The average age at menarche was 15.20 years (range; 8 years to 25 years). Average parity was 2.69 children/woman (range; 0 to 11). Average age at first full-term pregnancy was 22.97 years (range; 12.00 years to 51 years). Approximately, 30% were classified as obese with a waist to hip ratio of 0.85 or more.

54.84% of breast cancer patients in Ghana are pre-menopausal. Breast cancer among Ghanaian women may not necessarily be a disease that is more common in post-menopausal women when compared to western Countries (Caucasian - whites).

In this study the average age at diagnosis for breast cancer was 46.29 +/ – 11.498 years/woman; ninety-five confidence range of 23.75 – 68.86 years/woman, as compared to average age at diagnosis of 60 years/woman in Caucasians.

There were more premenopausal breast cancer victims in this study, most of them had dense breast tissue with mammography. Mammography and ultrasonography of the breast were largely unavailable and unaffordable to women in Ghana.

A new device, the breastlight, though not diagnostic has proved a useful adjunct to early detection methods and breast self-examination in Ghana. There is no doubt that, breast cancer is emerging as a new health problem in Ghana. The tendency of finding a relatively low proportion of oestrogen receptor positive breast cancers in Ghana (35.48%, n = 62) and for that matter black African women has been demonstrated. Ghanaian women diagnosed with breast cancer were known to have a low response rate to anti-oestrogen therapy. Ghanaian women (black Africans) were likely to develop breast cancer some 10 to 15 years earlier than Caucasians; and were less likely to respond to anti-oestrogen therapy.

ER and PR assay in breast cancer had dominated the study of breast cancer tumour bio-molecules of clinical importance. HER-2 and Ki67 Bio-molecules analysed in this work shows they were relevant in clinical decision making, although, no scientific study had assayed HER-2 and Ki67 previously in Ghana.

In the study of 33 malignant neoplastic breast lesions treated in Ghana;18.18% of breast cancer tumours analysed (n=33) were ER positive (ER+), compared to 87.00% in Mayo clinic study, USA. 81.82% of breast cancer tumours analysed were ER negative (ER-), 72.73% of them were HER-2 positive, compared to 6.70% in Mayo clinic study, USA. 27.27% of breast cancer tumours analysed were HER-2 negative. 87.98% of breast cancer tumours analysed were Ki67 positive, whiles, 12.02% were Ki67 negative. 15.15% of breast cancer tumours analysed were triple negative, compared to 5.9% in Mayo clinic study, USA.

The correlation between ER and HER-2 over-expression was moderate, negative and highly significant. Correlation between age at diagnosis and ki67 was positive, moderate and significant. Correlation between age and Ki67 score was positive, moderate and significant. According to this data increasing age at diagnosis was associated with increasing Ki67 scores in breast cancer tumours. Correlation between HER-2 and Ki67 was highly significant, moderate and strong. Increasing HER-2 scores were moderately correlated with increasing Ki67 scores in breast cancer tumours. Decreasing ER scores were more likely to be associated with increasing HER-2 and Ki67 scores in breast cancer tumours; and are pointers of late and aggressive breast cancers which have a relatively poor outcome of treatment.

- Ki67 scores demonstrated the extent of cell proliferation caused by ER mediated cell proliferation and HER-2 over-expression, in breast cancers.
- Overall, the most predominant cause of cell proliferation in these breast cancer tissue samples studied (n=33) was HER-2 over-expression (66.67% + 6.06% = 72.73%), (this is also a well-documented feature of late, aggressive breast cancers) compared to less aggressive oestrogen dependent cell proliferation (18.18%).

- Two main causes of Ki67 activity demonstrated in breast cancer were presence of ER and HER-2 over-expression. There was a highly significant measurable inverse relationship between ER scores and HER-2 scores. This inverse relationship was seen in 78.79% of breast cancer samples studied in Ghana (83.70%. in Mayo clinic study, USA) 66.67% were ER-/HER-2+/....., and 12.12% were ER+/HER-2-/.....
- Only 6.06% expressed both [ER+/HER-2+/Ki67+]. These were 'triple positive' biochemical subtypes of breast cancer. Biochemically, they appeared aggressive but may be responsive to all forms of treatment (hormonal therapy, immunotherapy and chemotherapy).

Clinical implications / importance of ER, HER-2 and Ki67: were demonstrated by defining biochemical sub-types of breast cancers listed as follows;

- [ER+/HER-2+/Ki67+]
- [ER+/HER-2-/Ki67+],
- [ER+/HER-2-/Ki67-]
- [ER-/HER-2+/Ki67+]

Triple negative breast cancers occurred at a rate of 15.15%.

There were two biochemical sub-types;

[ER-(PR-)/HER-2-/Ki67+]biochemical sub-type of triple negative breast cancer tumours may be chemosensitive but are not expected to respond to tamoxifen or Herceptin and occurred at a rate of 12.12%. Their prognostic outcome is predictably relatively worse than ER+/HER-2-/Ki67-, ER+/HER-2-/Ki67+, ER+/HER-2+/Ki67+, and ER-/HER-2+/Ki67+ irrespective of therapy.

[ER-(PR-)/HER-2-/Ki67-] this biochemical sub-type of triple negative breast cancers (This may be referred to as 'quadruple negative') made up 3.03% of the cases. This category of breast cancers, are known not to respond well to any of the treatments (hormonal and immunotherapy) above. Their prognostic outcome is predictably relatively worse than ER+/HER-2-/Ki67-, ER+/HER-2-/Ki67+,ER+/HER-2+/Ki67+, ER-/HER-2+/Ki67+ and ER-/HER-2-/Ki67+ subtypes, irrespective of therapy.

Women with small tumours showing high Ki67+ scores should be given full treatment, especially chemotherapy. Their prognostic outcome would be relatively worse irrespective of therapy (Allred DC et al., 1998).

Theoretically, it may be possible to predict the number of cycles of chemotherapy required to achieve improved survival, by testing and validating the hypothetical, novel, semi-quantitative model proposed in this work. This model incorporates tumour stage (TS) and Ki67 expression in predicting the outcome of treatment. It may help minimize risk of over treating or under treating breast cancer patients.

Ki67 in effect may help to identify chemosensitive patients; the role of other biomolecules, such as; ER, PR and HER-2 could be complementary. Ki67 activity demonstrated in these breast cancer tumours represents proliferative activity mediated by ER and HER-2 over-expression in 8 out of 10 cases treated in Ghana ({[...../...../Ki67+]**87.98%} – {**[ER-/HER-2-/Ki67+]**12.12%} = 75.86%)**. Some breast cancer tumours expressed both (ER+ and HER-2 over-expression) or none, majority expressed one or the other. All breast cancer tumours over-expressing HER-2 did show Ki67 activity. On the other hand, 50% of ER+ breast cancer tumours did not show any Ki67 activity. 87.98% of these breast cancer tumours showed Ki67 activity; hence, they are expected to

have a bad prognosis irrespective of treatment. According to some experts, a high Ki67 expression could define a subset of chemosensitive tumours within ER-positive breast cancers. Other biomolecules are complementary to Ki67 expression in the identification of chemosensitive ER-positive tumours (Paik et al, 2006). 12.02% showed no Ki67 activity hence they are expected to have a good prognosis if treatment was timely and adequate with the drug of choice. This work has attempted to throw some light on the descriptive epidemiology and biochemical nature of a cross-section of breast cancers treated in Ghana. Other retrospective studies elsewhere, have sought to provide a rationale for performing a prospective trial that would explore indications for choice of adjuvant chemotherapy according to ER, HER-2, and Ki67 immunostaining (Berry et al, 2006; Conforti et al, 2007).

The novel, hypothetical, semi-quantitative model, introduced and described in this study, demonstrates clearly how treatment for breast cancer and other solid malignant tumours should be individualised.

Recommendations

The following recommendations were teased out of the findings in this study:

* A special awareness screening package should be developed with Ghanaian women in mind.
* A breast disease awareness and screening service/center should be set up to organize and coordinate awareness and screening programmes in a more organized and consistent manner.
* A national advocacy body or association for breast cancer patients and their

relatives must be set up.

* A breast cancer awareness week should be celebrated every year probably in October.
* A visual assessment of Tumour-related angiogenesis should be explored further as an adjunct to early detection of potentially dangerous breast lesions.
* The novel mathematical model introduced here must be evaluated in a real life setting.

Limitations of study

The high cost of commercial antibodies for immunostaining studies on archival tissue samples posed a limitation on sample size. However, the importance of these clinically relevant biomolecules was demonstrated. Data for evaluating the proposed model for optimizing benefits of chemotherapy was unavailable during the study period.

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APPENDIX

Figures I – IV (source; National Cancer Institute, USA.)

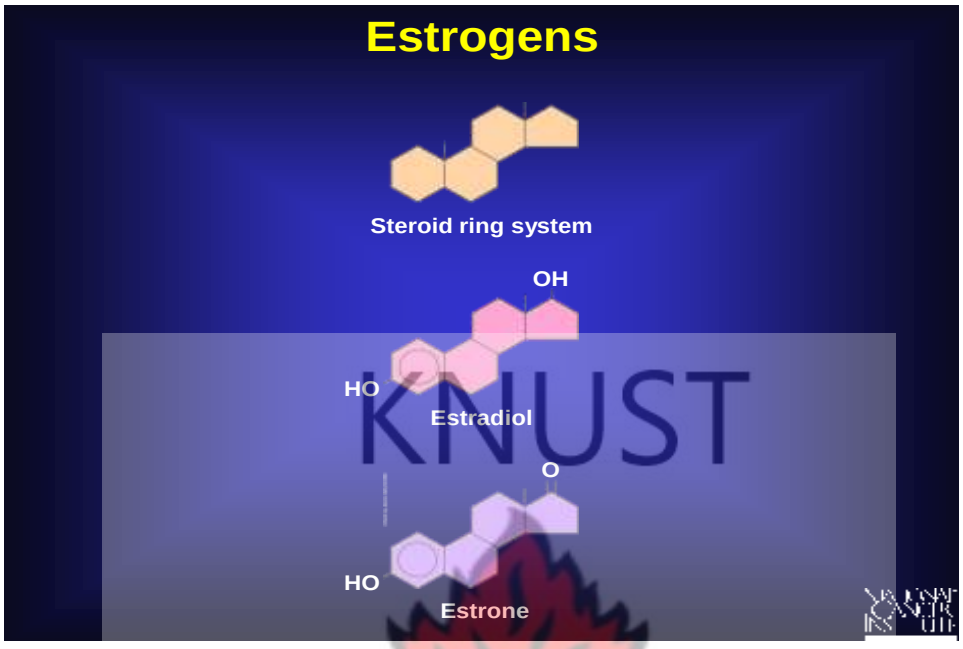


Figure I.OESTROGENS

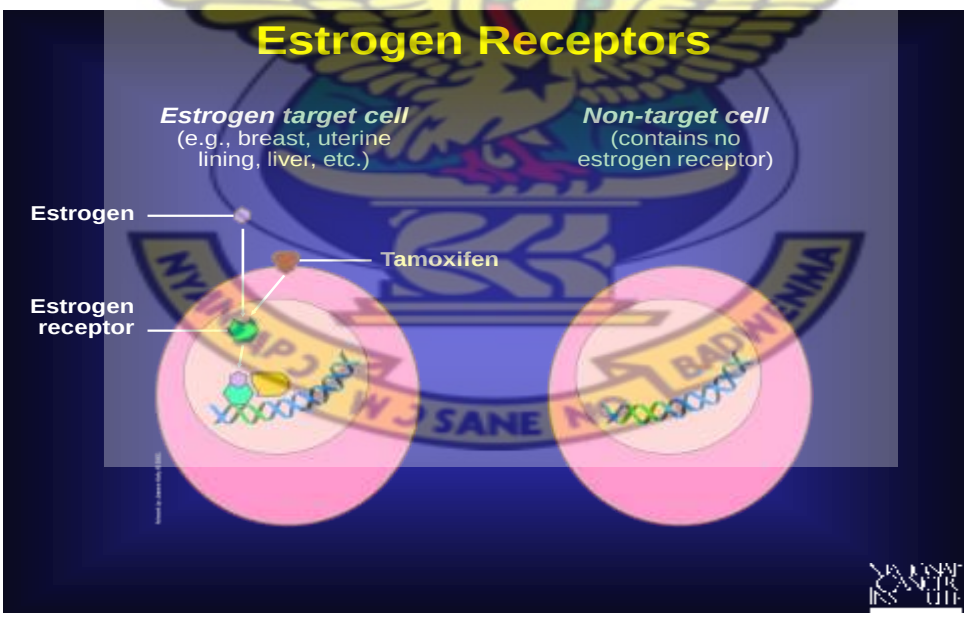


Figure II. OESTROGEN RECEPTOR ACTION MECHANISM

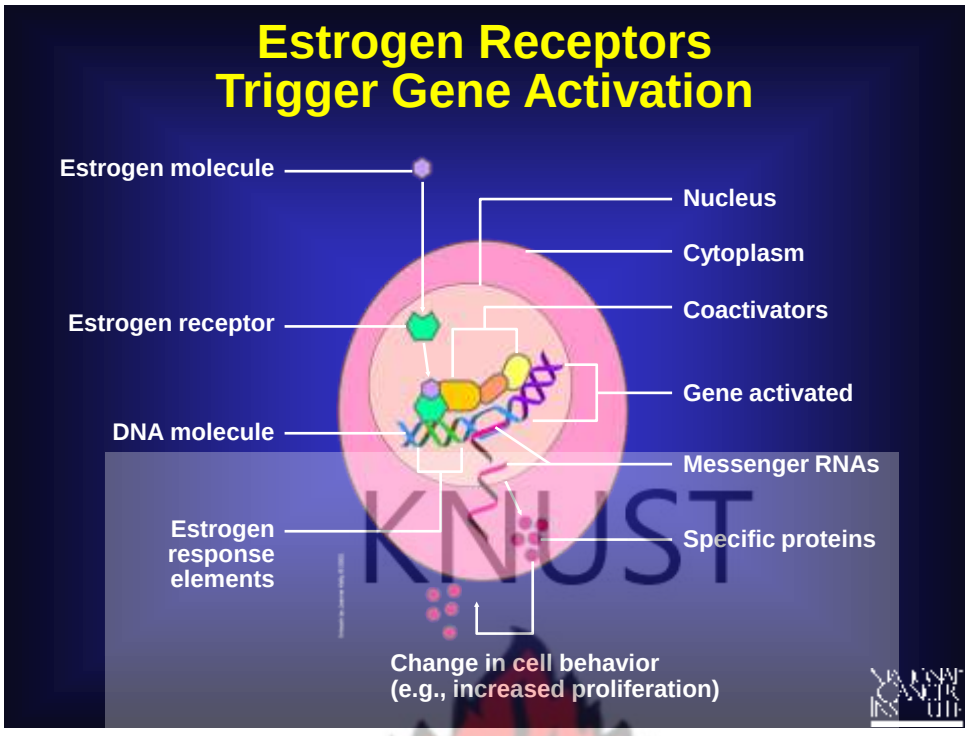


Figure III. OESTROGEN RECEPTORS TRIGGER GENE ACTION

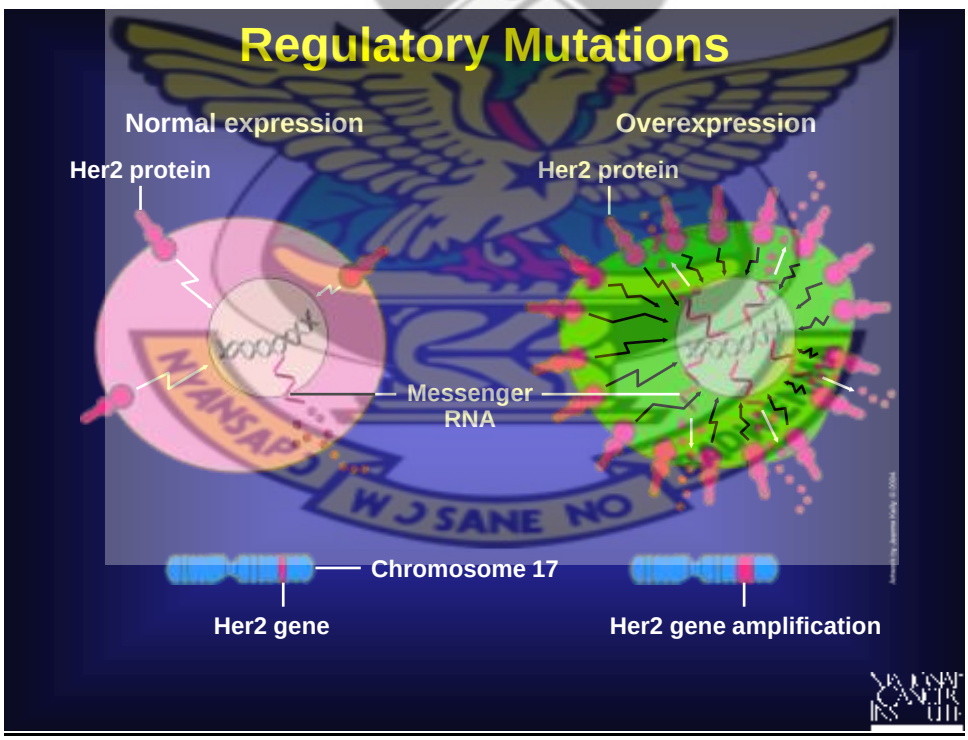
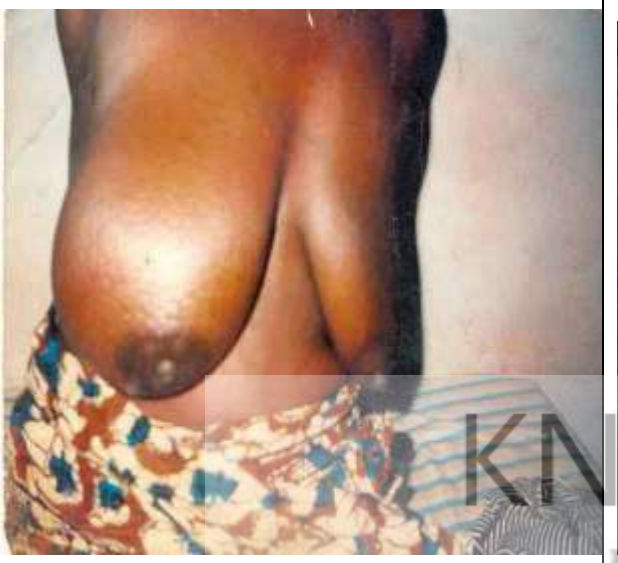


Figure IV. HER-2 OVER-EXPRESSION MECHANISM

Figure v. Clinically obvious neoplastic breast lesions seen in Ghana 1999 - 2008



A 35 year old female with inflammatory carcinoma of the right breast.

A 25 year old female with a large benign left breast tumour

A 70 year old female with a malignant right breast mass

A cross sectional view of Oestrogen Receptors in 32 Human Breast Cancer Tissues in Ghana.

Research Paper

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A Cross Sectional View of Estrogen Receptors in 32 Human Breast Cancer Tissues in Ghana

M.T. Agyei-Frempong, F.N. Ghartey, S. Asamie-Poku and B. Wiafe-Addai

The aim of this research was to determine the prevalence and proportion of estrogen-dependent, breast cancers in Ghana. Thirty two breast cancer tissues were obtained at random from patients seen at Komfo Anokye and Korle Bu Teaching Hospitals. Estrogen receptors were identified and quantitated using the cytosol-based dextran-coated charcoal assay. This was followed-up with a nationwide awareness and screening exercise from 1999 to 2002 to help improve awareness, encourage earlier detection and determine the prevalence of breast cancer in Ghana. Six out of 32 (18.75%, p>0.05) breast tumors were estrogen-dependent with receptor contents ranging from 12.75 to 101.31 (mean: 50.59±28.83) f mol mg^{-1} of cytosol protein. The mean tumor size was 12.58 × 2.59 cm. Prevalence rate for breast cancer in Ghana ranged from 0.41-1.11% with a mean of 0.76±0.35% (at 95% confidence interval) among females aged 18 to 80 years. The mean age at diagnosis for breast cancer was 46.29 (±11.50) years/woman (confidence interval of ±22.54 years). Ghanaian women develop breast cancer some 10 to 15 years earlier than Caucasians and are less likely to respond to anti-estrogen therapy due to low proportion of estrogen receptor positive breast cancers.

Key words: Breast neoplasm, anti-estrogen therapy, prevalence, breast screening, menopause

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INTRODUCTION

Breast cancer is the most common malignant neoplasm in women (Maaroufi *et al*., 2000). Breast cancer risk is related to the length of exposure of a woman to endogenous estrogens. Delayed onset of menses, early pregnancy and early menopause/oophorectomy lowers the incidence of breast cancer (Clavel-Chapelon and E3N-EPIC Group, 2002). Since breasts are estrogen dependent organs, they undergo changes throughout a woman's life. These changes are generally classified as physiological, aberrations of normal development and involution (ANDI) or malignant.

For reasons that are not fully understood the mammary epithelial cells behave in an aberrant manner. The cells of the breasts become disturbed and local control mechanisms fail to operate. This results in an increase in breast cell types, followed by derangement of both structure and function (Mommer *et al*., 1999).

Breast cancer types, which retain their estrogen dependency, are better managed by manipulations of endogenous estrogen levels. This can be achieved by using less toxic and less expensive hormonal therapy. However, in Ghana where breast cancer is the second most common malignant neoplasm in women, oncologists report a low response to anti-estrogen therapy. This is probably because the estrogen receptor status in breast cancers in Ghanaians has not been elucidated. Despite this, almost all cancer patients have to go through hormonal therapy which is a waste of patient's time and resources and also puts the patient at risk to some extent.

The objective of this study was to determine the proportion of estrogen dependent breast cancers and the prevalence of breast neoplasms, in Ghana.

MATERIALS AND METHODS

Estrogen receptor study

The study population: Specimens for estrogen receptor analysis were obtained at random from 32 breast cancer patients seen at Komfo Anokye and Korle Bu Teaching Hospitals (KATH and KTH) from January to October 1999. Patients' examination, specimen collection and subsequent submission of specimen for analysis were carried out by competent surgeons and analysis performed by research scientists at KATH and KTH with patients' consent in accordance with the Helsinki Declaration. Physical examination, with careful description of tumor size, weight, location, fixation, nodal status and contours were performed on each breast cancer patient by surgeons at KATH and KTH. In some cases mammograms were used to assess the extent and size of multiple tumors and invasions of skin and chest wall. Excision biopsy (with or without mastectomy) and axillary nodal removal was advised for all but the most advanced (T4 or N3) lesions.

Estrogen receptor assay: The cytosol-based dextran-coated charcoal assay (McGuire *et al*., 1978) was used to identify and quantitate the estrogen receptor in breast cancer. Cytosolic preparations from breast cancer tissue (histologically confirmed) were incubated with various concentrations of ^{3}H-estrogens (0.5-20.0 nM) at 4°C for 16 h. The free ligand was removed with dextran-coated charcoal suspension and ligand-receptor complex quantitated using Scatchard analysis.

Follow-up breast cancer awareness and screening programme: Women aged 18 to 80 years nationwide were included in this exercise. The screening and awareness team was made up of nurses, a biomedical scientist and administrator, under the supervision of a breast pathologist/general surgeon. The team visited women's groups at their churches, mosques, work places and market and screened them manually after creating awareness.

A total of 15,221 women nationwide were involved from 1999 to 2002.

Statistical analysis: Estrogen receptor values, the mean age at diagnosis, prevalence and confidence intervals were computed with Microsoft excel computer software.

RESULTS AND DISCUSSION

The quantitative determination of estrogen-receptors in breast cancer tissue samples is used to predict their response to anti-estrogen therapy (Maaroufi *et al*., 2000). Patients with ER-negative tumors have a higher recurrence rate within the first 20 months following mastectomy (Crowe *et al*., 1991). These patients have low response rates to hormonal manipulations when they develop metastatic cancer (less than 10%). Such patients are better managed with cytotoxic chemotherapy, possibly including adjuvant chemotherapy, even in the absence of tumor in the axillary lymph nodes (Fitzgibbons *et al*., 2000). Therefore, receptor data are helpful in deciding whether to employ hormonal therapy for metastatic disease and delaying the generally more toxic chemotherapeutic approach. Estrogen binding studies performed on 32 human breast cancers (one male and 31 female patients) identified six (18.75%) estrogen-dependent tumors which is not significant [$\chi^2 = 0.07018$, $p>0.05$] (Table 1). This is in conformity with the results obtained by Olopade (2005) and Ikpatt and Ndoma-Egba (2003), who reported that only 23 and 24%, respectively of

Table 1: Menopausal status versus estrogen receptor status of 31 female breast cancer patients

Menopausal status	Estrogen receptor positive (%)	Estrogen receptor negative (%)	Total (%)	p-value
Pre/peri	3 (50)	14 (56.00)	17 (54.84)	p>0.05
Post	3 (50)	11 (44.00)	14 (45.16)	
Total	6 (100)	25 (100.00)	31 (100.00)	

At 95% confidence level ($\chi^2 = 0.07018$, p>0.05)

Table 2: Mean estrogen-receptor contents (results of scatchard analysis) related to menopausal status of patients

Menopausal status	Mean±ER (fmol mg^{-1})
Pre/peri	72.12±26.94
Post	29.06±19.41
Total (pre/peri/post)	50.59±28.83

Range (ER) (fmoles mg^{-1} protein) = 12.75 (in a postmenopausal woman) to 101.31 (in a pre-menopausal woman). (ER) = Estrogen receptor concentration

African breast cancer tumors are estrogen-dependent as compared to 80% in Caucasians. The only male breast cancer specimen was estrogen receptor (ER) negative.

Breast cancer tumor weight and size are good proxy measures of late diagnosis and treatment. After analyzing cross-sectional data on breast cancer tumor weight and size, it became obvious that the majority of breast cancer patients in Ghana had bulky breast cancer tumors at presentation for treatment. The mean weight of these mastectomy/lumpectomy/biopsy specimens was 916.91 g. The smallest lumpectomy specimen weighed 18.00 g and the largest weighed 1780.00 g. More than three-quarters of the breast masses analyzed for estrogen-receptors had dimensions of 12.5×2.5 cm or more. The mean specimen size was 12.58×10.59 cm. The smallest specimen size was 2.0×1.3 cm and the largest was 23×16 cm. One study has reported a mean primary tumor diameter of 10 cm in 129 Nigerian women (Hassan *et al*., 1992). This feature may point to late presentation of breast cancer for treatment leading to low survival in Ghana. In fact, a number of retrospective studies have reported that African women present with stage 3 or 4 disease (Hassan *et al*., 1992; Amir *et al*., 1984, 1997; Muguti, 1993).

The estrogen-dependent (estrogen receptor positive) tumors had receptor contents ranging from 12.75 (in a post-menopausal woman) to 101.31 (in a pre-menopausal woman) femtomoles mg^{-1} (fmoles mg^{-1}) of cytosol protein (Table 2). The cut-off point for ER positive status was 10.0 f moles mg^{-1} protein. The lower limit for estrogen-dependency in most specialized laboratories worldwide corresponds to [ER] of 10.00 femtomoles mg^{-1} of cytosol protein. Prior to this study, which established [ER] in Ghana for the first time, many surgeons/oncologists in Ghana reported a low response rate to anti-estrogen therapy among their patients (unpublished data).

The mean age at diagnosis for breast cancer in this study was 46.29±11.50 years/woman, with a confidence interval of ±22.54 years. This is in concordance with the results in other African countries and confirms that African women may develop breast cancer at an earlier age (approximately 10-15 years earlier) as compared to Caucasians whose mean age at diagnoses is 60 years (Olopade, 2005; Adebamowo *et al*., 2003; Yawitch *et al*., 2000; Ihekwaba, 1992; Ikpatt *et al*., 2002). Out of the 31 breast cancer women, 54.84% were of pre/peri-menopausal status whiles 45.16% were of post-menopausal status (Table 1). This confirms the report of Chiedozi (1995), who has indicated that breast cancer in Nigeria is a disease of pre-menopausal and peri-menopausal females. Thus, breast cancer among Ghanaian women and for that matter black women may not necessarily be more common in post-menopausal women in contrast to that in the Caucasian women. The modal (peak) age at diagnosis for the pre/peri-menopausal women was 39 years while that for the post-menopausal women was 54 years.

Estrogen receptor positive versus menopausal status: Menopausal and estrogen receptor status were analyzed in all the 31 women studied. Three (50%) of the six estrogen receptor positive (ER+) breast cancers were observed in post-menopausal women and three (50%) in pre/peri-menopausal women. (Table 1). The mean estrogen receptor concentration for the post-menopausal women was 29.06±19.41 fmoles mg^{-1} protein as compared to 72.12±26.94 fmoles mg^{-1} protein in pre/peri-menopausal women as a group (Table 2).

Estrogen receptor negative versus menopausal status: Fourteen (56.00%) out of twenty-five estrogen receptor negative (ER-) breast cancers were observed in pre/peri-menopausal women, whiles, eleven estrogen receptor negative (ER-) breast cancers (44%) were seen in post-menopausal women (Table 1).

Nationwide breast cancer awareness and screening programme: The earlier observations were confirmed in a larger follow-up awareness and screening programme involving 15,221 women from the period 1999 to 2002 (Table 3). The programme is still ongoing and has captured over 44,777 women nationwide till date.

In this study, the mean age at first menses (menarche) determined for 500 Ghanaian women selected at random from the total (15,221) countrywide was 14.91 years. By the age of 16 years, 88.91% of them had had their first menses.

Table 3: Data from follow-up breast cancer awareness and screening programme for the period 1999-2002

	Year						
Breast condition	1999	2000	2001	2002	Total No. of cases	SD	Mean
Breast cancer[a]	23	36	21	22	102		
Yearly prevalence[a] (%)	1.22	0.57	0.67	0.57		0.31	0.76
Benign breast mass[b]	147	199	48	104	498		
Yearly prevalence[b] (%)	7.81	3.16	1.52	2.68		2.77	3.79
AND/NAD	1,712	6,072	3,084	3,753	14,621		
Total	1,882	6,307	3,153	3879	15,221		

[a]95% confidence interval = 0.76 ± 0.35, median = 0.62, [b]95% confidence interval = 3.79 ± 3.10, median = 2.92, NAD = No abnormality detected

Polish girls, who started menstruating before the age 16 years had a risk for breast cancer, which was twice that for those whose menarche was later than 16 years (Osborne, 1998). This indicates that as far as menarche is concerned about 11% of the Ghanaian female population may have about twice as much risk for developing breast cancer than the others.

The mean age at menopause was estimated from the data in this study to be 47.77 years for Ghanaian women. More than 90% of them had menopause between the ages 40.75 and 54.79 years.

Approximately 44.29% of them had menopause between 40.75 years and 47.77 years. A few had menopause beyond 55 years (2.86%). Some researchers have reported doubling of the risk for women who continued menstruating beyond the age of 55 years compared with those whose natural menopause was before the age of 45 years (Osborne, 1998).

Prevalence rate for breast cancer ranged from 0.41-1.11% with a mean of 0.76±0.35% (95% confidence interval) among females aged 18 to 80 years in Ghana. The prevalence of benign breast lumps ranged from 0.69-6.89% with a mean of 3.79±3.10% (95% confidence interval) (Table 3).

For the first time, the prevalence of breast cancer and benign breast conditions have been established in Ghana, through data captured nationwide as presented in this study (Table 3). The low prevalence rate and the early age of onset of breast cancers in Ghanaian women as observed in this study have been confirmed by Olopade (2005), who has reported that breast cancer strikes fewer women in Africa, but, it hits earlier and harder.

Availability of such statistical estimates of prevalence rates is crucial and timely for fund managers and policy makers of our infant National Health Insurance scheme (NHIS), which covers treatment for benign and malignant breast conditions.

CONCLUSIONS

Ghanaian women diagnosed with breast cancer have been known to have a low response rate to anti-estrogen therapy. The reason for this has clearly been shown in this study; a low proportion of estrogen receptor positive breast cancers in Ghana (18.75%, $p>0.05$) and for that matter black African women has been demonstrated.

The low estrogen receptor status in Ghanaians could be attributed to the fact that they are late cancers that have lost their estrogen dependency through malignant transformation, since most cancer patients report late at the hospital.

Breast cancer among Ghanaian women may not necessarily be a disease that is more common in post-menopausal women. In this study the mean age at diagnosis for breast cancer (46.29±11.498) indicates that Ghanaian women develop breast cancer 10-15 years earlier than Caucasians (60 years).

Currently, public concern about breast cancer and its destructive consequences on women in their reproductive ages is rising in Ghana, but the awareness level is still low and as a result most victims or families of victims still suffer heavy losses due to low survival rates prevalent in Ghana, cumulating from late detection. It is therefore suggested that awareness programmes are stepped-up and the survival rate for breast cancer during the first five years after treatment is estimated.

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