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ASSESSMENT OF SERUM PI3K LEVEL AS RISK OF BREAST CARCINOGENESIS IN WOMEN

BY

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DECLARATION

DECLARATION BY CANDIDATE

I declare that, aside from properly cited references to the work of other scholars, this thesis presents the results of independent research conducted by the author under academic supervision, in accordance with the regulations of the School of Graduate Studies at the University of Ghana. This work has not been submitted, in whole or in part, for the award of any other degree elsewhere.

Signature  Date: 20-10-2024

Waris Abubakari

DECLARATION BY SUPERVISORS

We officially affirm that the practical activities and the preparation of this thesis were carried out under our supervision, complying with the thesis supervision standards established by the University of Ghana

Principal supervisor

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Co-supervision

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Prof Joe-Nat Clegg-Lamptey

DEDICATION

I extend this dedication to my cherished wife, Ms. Sumaila Aliatu, and our children, Shammas Waris and Shahid Waris, whose steadfast love and support have been the foundation of my strength. I would also like to extend a heartfelt dedication to my parents, colleagues, and loved ones for their constant encouragement, wise counsel, and heartfelt support throughout this journey.

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LIST OF ABBREVIATIONS

ACS: American Cancer Society

ADH: Atypical ductal hyperplasia

AKT: Protein kinase B

ATGL: Adipose triglyceride lipase

BC: Breast cancer

BCRAT: Breast Cancer Risk Assessment Tool

BMI: Body mass index

BOADICEA: Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm Model

BRCA: Breast cancer-associated gene

BRCAPRO:

CBE: Clinical breast examination

CDC: Center for Disease Control

DCIS: Ductal carcinoma in situ

DMC: Dimethyl-celecoxib

ELISA: Enzyme-linked immunosorbent assay

ER: Estrogen receptor

FEA: Flat epithelial atypical

FOX01: Forkhead box 01

GLOBOCAN: Global cancer incidence, mortality, and prevalence

GSK3: Glycogen synthase kinase 3

HER2: Human epidermal growth factor receptor-2

IBIS: International Breast Cancer Intervention Study model

IDC: Invasive ductal carcinoma

IHC: Immunohistochemistry

IL: Interleukins

ILC: Invasive lobular carcinoma

KBTH: Korle-Bu Teaching Hospital

LCIS: Lobular carcinoma in situ

MMP: Matrix metalloproteinase

MRI: Magnetic resonance imaging

mTORC2: mammalian Target of Rapamycin Complex 2

PCNA: Proliferating cell nuclear antigen

PDK1: Phosphoinositide-dependent kinase 1

PI3K: Phosphoinositide 3-kinase

PIK3CA: Phosphoinositide 3-kinase cancer gene

PIP2: Phosphorylation of the lipid phosphatidylinositol 4, 5-bisphosphate

PIP3: Phosphatidylinositol 3,4,5-trisphosphate

PR: Progesterone receptor

PTEN: Phosphatase and tensin homolog

RTK: Receptor tyrosine kinase

SCAP: Sterol regulatory element binding protein cleavage-activating protein 1

SREBP: Sterol regulatory element-binding protein

TDLU: Terminal duct lobular unit

TSC2: Tuberous Sclerosis Complex 2

WHO: World Health Organisation

WHR: Waist-to-hip ratio

BS: Breast size

ABSTRACT

Background: Breast cancer is the leading diagnosed cancer in females and is the major cause of cancer-related mortalities in females global. Although advanced economies have a larger rate of breast cancer than economically poorer countries, breast cancer mortality rates are higher in economically poorer countries. In Ghana, mammography is unavailable in many district hospitals and health centers and when available the cost is prohibitive. This makes breast cancer screening services inaccessible and gives impetus to the search for other potential screening tools and methods. Obesity, a contributing factor to breast cancer development, is increasing in prevalence in Ghana.

The phosphoinositide 3-kinase (PI3K) protein/protein kinase B (AKT) (PI3K/AKT) pathway, engaged in cell replication and angiogenesis, is common to both obesity-related adipogenesis and breast carcinogenesis. Assessing serum PI3K levels may be helpful for screening the chance of breast cancer occurrence.

Aim: This study aimed to investigate serum PI3K concentration as a proxy for obesity-induced breast cancer in Ghanaian women

Methods: Thirty-two breast cancer patients, age-matched with 32 women without the disease, were enrolled for this study**.** For each consenting participant, body size and shape measurement, including height, weight (for body mass index), waist-to-hip ratio, breast size, and molecular subtype of breast cancer (for the cases) were recorded. Moreover, each participant provided 5 ml of peripheral blood for collection. PI3K antigen quantification was

performed using an ELISA kit on the serum of each participant. Data analysis using IBM SPSS 29 included Mann-Whitney U tests for PI3K levels and Spearman's correlation for anthropometric measurements. PI3K distributions across cancer subtypes were shown with box plots, and means were compared using one-way ANOVA. Statistical significance was established at a p-value less than 0.05, using a 95% confidence level.

Results: Age, BMI, and WHR were significant elements contributing to breast cancer risk, especially in women above 40 years. However, despite these risk factors, serum PI3K levels comparison between breast cancer patients and healthy controls did not reveal a statistically significant difference (p-value = 0.193, nor were there any significant differences in serum PI3K levels among different molecular subtypes of cancer (p-value 0.727)

Discussion: This study highlights that while age, BMI, and WHR are crucial elements influencing breast cancer susceptibility, especially in women over 40, serum PI3K levels do not show significant differences between breast cancer patients and healthy controls, nor among different cancer subtypes. Therefore, serum PI3K levels might not be a useful biomarker for breast cancer risk or classification.

Conclusion: Though commonly recognized predictors such as age, BMI, and waist-to-hip ratio are important for estimating the likelihood of breast cancer, serum PI3K levels do not appear to be a useful biomarker for differentiating between patients with breast cancer and healthy individuals or for distinguishing between cancer subtypes. Additional studies are required to discover more reliable biomarkers for assessing breast cancer risk and improving disease classification.

CHAPTER ONE

INTRODUCTION

1.1 Background

Breast cancer typically originates in cells of the epithelium that cover the ducts and lobules in the breast's glandular regions. At first, the malignant growth shows no symptoms and poses little danger of spreading beyond the duct or lobule where it began (Feng et al., 2018). Worldwide about 685,000 women died of cancer of the breast out of the 2.3 million diagnosed in 2020 (Bray et al., 2018). Breast cancer prevalence has increased by more than 34% from 2012 (1.7 million new cases) to 2020 (2.1 million cases), (Bray et al., 2018; Torre et al., 2015). The World Health Organization (WHO) reports that, during the previous five-year period, 7.8 million women currently living have received a breast cancer diagnosis. About 4482 new cases and 2055 fatalities of breast cancer were recorded in Ghana for the year 2020, this has caused breast cancer to be the common cancer diagnosis in Ghana (Ghana Source: Globocan Incidence, Mortality and Prevalence by Cancer Site, 2020).

The occurrence of breast cancer is more prevalent in affluent regions than in under-resource country. while the predominance of breast cancer is more in advanced nations, the number of deaths continues to increase in the under-developed nations (Bray et al., 2018). Breast cancer longtivity rates of five years following diagnosis are 40% in underdeveloped countries and over 90% in most high-income nations (Gonchen et al., 2015; Francies et al., 2020). In industrialised regions, the fatality rate from breast cancer has decreased because of the growing application of screening tools to detect the disease at an initial stage for better treatment and management, even though women of African

descent are more prone to experience death from the disease regardless of the size of their tumour (Maskarinec et al., 2011; Newman et al., 2006). Mammography, the recommended tool for breast cancer screening, is more widely available in developed nations, but developing nations like Ghana still have difficulty in the rural areas in accessing this tool due to the cost and technical difficulties involved in doing so, as well as the lack technical personnel in operating this machine (Black & Richmond, 2019; Corbex et al., 2012; Denny et al., 2017). Despite the definitive method label placed on mammography as a diagnostic tool, it gives false positives in highly dense breast women and younger women resulting in low sensitivity in these groups when used (Wang, 2017). Alternatively, biomarkers have been considered promising tools in early breast cancer detection and some studies have suggested identifying appropriate biomarkers for breast cancer prediction (Singh, 2019)

Biomarkers can be helpful for breast cancer prediction, progression, and prognosis (Wu & Chu, 2021). For earlier detection and better therapy of breast cancer, it is crucial to find several biomarkers that can assistance in diagnosis and prognosis (Weigel & Dowsett, 2010). Major advancements in genetic profiling and molecular signaling pathways have enabled the discovery of numerous tissue- and blood-based biomarkers (liquid biopsies). These biomarkers can help assess the danger of breast cancer progression or recurrence, influence treatment decisions, predict outcomes, and evaluate drug response and tolerance (Nalejska et al., 2014; Wu & Chu, 2021).

Breast cancer risk factors include obesity, alcohol, radiation, hormonal therapy, female gender, increasing age, genetics, menstrual cycle, and breast density (Momenimovahed & Salehiniya, 2019). Worldwide obesity rates are increasing, and demographic data connect obesity to an upsurge in the frequency of breast cancer. There is an epidemiologic shift from previously

recognised alterable contributors to breast cancer risk, such as smoking and alcohol use, toward obesity (Francies et al., 2020). Obesity is linked with a heightened risk of developing more violent forms of breast cancer and is linked to reduced survival rates.

The primary microenvironment for breast cancer is the adipose tissue. Adipose tissue undergoes structural, and functional alterations as a result of obesity, which is linked to inflammation and metabolic dysfunction (Ritter et al., 2022). While considering the landscape of breast cancer risk factors, attention must also be directed towards factors beyond traditional clinical metrics. Body Mass Index (BMI) and Waist-to-Hip Ratio (WHR), alongside other notable anthropometric measures, have emerged as crucial parameters of interest in understanding breast cancer risk (Renehan et al., 2008). Additionally, anatomical features, such as breast size, have garnered attention for their potential relevance to breast cancer risk.

Body Mass Index (BMI), a widely accepted metric for quantifying obesity, has emerged as a crucial parameter allied with breast cancer risk. Researches have revealed that a high BMI is connected with a rise in likelihood of breast cancer progress (Renehan et al., 2008). This connection is partly accredited to the role of adipose tissue in the production of estrogen, a hormone recognized to foster the occurrence of some types of breast cancer (Bhardwaj et al., 2019). High levels of estrogen in obese individuals, often facilitated by higher BMI values, contribute to the higher breast cancer risk observed in within this category (Picon-Ruiz et al., 2017). Waist-to-Hip Ratio (WHR) is another anthropometric measure that has garnered attention in breast cancer research. A high WHR, indicative of central obesity, connected to a heightened chance of breast cancer in women above 45 years (Renehan et al., 2008). Central obesity, characterized by excess fat accumulation around the abdomen, is linked to elevated levels of insulin and inflammation, both of which are connected in breast cancer progress

(AgursCollins et al., 2019). The link between WHR and breast cancer predisposition underscores the importance of considering not only overall obesity (as measured by BMI), in addition to the body's pattern of fat distribution. Breast size, a feature of actual relevance in the perspective of breast cancer, has also attracted the interest of researchers. Although research is still ongoing, some studies indicate that having larger breasts may be attributable to a faintly elevated chance of occurrence of cancer of the breast (Li et al., 2020).

The exact mechanisms behind this possible link remain unclear, though it may be influenced by differences in breast tissue structure and hormonal activity (Lundberg et al., 2022). According to Paplomata and O'regan (2014), angiogenesis and breast cancer are both affected by phosphoinositide 3-kinase (PI3K) protein. Mutations that lead to the loss of phosphatase and tensin homolog (PTEN) and PIK3CA, which most typically affect exons 9 and 20, are among the most prevalent abnormalities observed in the breast of an obese person with breast cancer (Wang et al. 2021, Beelen et al., 2014).

In individuals with normal physiology, the PI3K signaling pathway is activated due to certain growth factors, including estrogen, androgens, and progesterone. These hormones interact with receptor proteins that, in turn, stimulate PI3K activity. This process results to the phosphorylation of phosphatidylinositol 4,5-bisphosphate (PIP2), producing phosphatidylinositol 3,4,5-trisphosphate (PIP3). The catalytic p110 subunit, that functions in coordination with the regulatory p85 subunit, is responsible for facilitating this conversion. Other p110 isoforms may also participate in generating PIP3. Once produced, PIP3 accumulates at the inner surface of the plasma membrane and acts as a second messenger, triggering downstream signaling pathways. A key outcome of this process is the activation of AKT (Also called protein kinase B), facilitated by phosphoinositide-dependent kinase-1 (PDK1), which binds to PIP3, and by the

mammalian target of rapamycin complex 2 (mTORC2). This signaling cascade further activates the tuberous sclerosis complex 2 (TSC2), promoting cell growth and the growth of new vasculature (Lawrence et al., 2014). In various bodily tissues, including the breast, the PI3K/AKT pathway deteriorates in reaction to insulin resistance, which leads to the inception of obesity and cancer of the breast (Huang et al., 2018). The microenvironment of an obese woman's breasts is characterised by high levels of insulinlike growth factor 1 (IGF-1), oestrogen, hypercholesterolemia, pro-inflammatory cytokines and adipocyte-derived adipokines, and oxidative stress (Glassman et al., 2023). The primary substrate for all these hormones in the PI3K signaling cascade is IGF-1. IGF-1 is a strong mitogen that plays a vital role in the mammary gland. When IGF-1 binds to its corresponding receptor, IGF-1R, a signaling cascade that promotes proliferation and inhibits apoptosis is set off (Christopoulos et al., 2015). These molecular components disrupt the PI3K signaling pathway, which results in overactivation and increased cell proliferation. Additionally, breast cancer cells have IGF1 receptors, and when IGF1 binds to these receptors, it activates the phosphoinositide 3-kinase (PI3K) and MAPK pathways, increasing the evolution of cancer cells (Christopoulos et al., 2015).

1.2 Problem statement

Obesity is increasing among adults in Ghana with about 43% of adults in Ghana either obese or overweight (Ofori-Asenso et al., 2016). Breast cancer in female is the common diagnosed cancer in Ghana (Cumber et al., 2017) and the second primary origin of cancer mortality in Ghana (Shamar et al., 2022; Bray et al., 2018), and obesity in women is a recognised contributing factor for this disease.

In Ghana, mammography, the standard tool for assessing the chance of being diagnosed with breast cancer, is unavailable in many district hospitals and health centers, and when it is available, the cost is prohibitive, particularly in rural areas (Boamah et al., 2022). The financial and non-financial expenses of the screening itself, the radiation risk, discomfort, annoyance, and worry that may be associated with its usage are unfriendly for all age groups (Warner, 2011; Nat et al., 2016). Despite being a very sensitive test for postmenopausal women, mammography is less sensitive in younger women and those who have a genetic susceptibility to breast cancer (Liberman, 2004). This makes breast cancer screening services inaccessible, and uncomfortable (Boamah et al., 2022) and gives impetus to the search for screening tools and methods for checking the development of the disease to couple mammography even if available (Black & Richmond, 2019).

Obesity, a potential driver of breast cancer cancer development, is involved in the PI3K/Akt signaling pathway. The PI3K/AKT pathway, involved in cellular proliferation and angiogenesis, is common to both obesity-related adipogenesis and breast carcinogenesis. Early detection of aberrations in this PI3K/AKT signaling pathway might be useful for risk assessment in overweight or obesity-related breast cancer development. There is an relationship between serum PI3K levels and breast carcinogenesis in women (Ulu et al., 2022). However, the cut-off points of serum PI3K level to enable monitoring of breast cancer progress has not yet been determined in women with and without breast cancer. This study hypothesises that serum PI3K levels in overweight or obese persons will predict the chance of being detected with breast cancer

1.3 Justification

In Ghana, breast cancer represents the most regularly diagnosed cancer in female and is the second major cause of cancer-related deaths. The rising rates of obesity, which affect approximately 43% of Ghanaian adults, contribute significantly to this public health issue. However, access to effective screening methods, such as mammography, is limited, especially in rural areas where cost and availability are major barriers. Additionally, the reduced effectiveness of mammography in younger women and those with a genetic susceptibility to breast cancer highlights the need for alternative screening approaches.

The PI3K/Akt signaling pathway, which is instrumental in both obesity-related adipogenesis and breast cancer development, represents a potential target for innovative screening methods. Emerging evidence links serum PI3K levels to breast carcinogenesis, suggesting that these levels could serve as a molecular marker used to assessing breast cancer predisposing, particularly in overweight or obese individuals. Despite this potential, there is currently no well-defined cut-off point for serum PI3K levels to effectively distinguish between cancerous and non-cancerous cases, limiting its clinical use.

This study sought to address this gap by investigating the affiliation between serum PI3K levels and breast cancer risk, with the goal of establishing a reliable biomarker that could be used in conjunction with, or as an alternative to, traditional screening methods. By doing so, this research aims to enhance early detection and improve outcomes for

individuals at risk of breast cancer, particularly in regions where conventional screening is not readily accessible

1.4 Aim

This study aimed to investigate serum PI3K concentration as a potential proxy for evaluating risk of obesity-induced breast cancer in Ghanaian women

1.5 Specific Objectives

- To determine serum PI3K levels among breast cancer and non-breast cancer females
- To relate serum PI3K levels with anthropometric parameters (BMI, WHR, and breast size) of participants
- To compare serum PI3K level and their relationship with molecular subtypes of breast cancer

1.6 Null Hypothesis

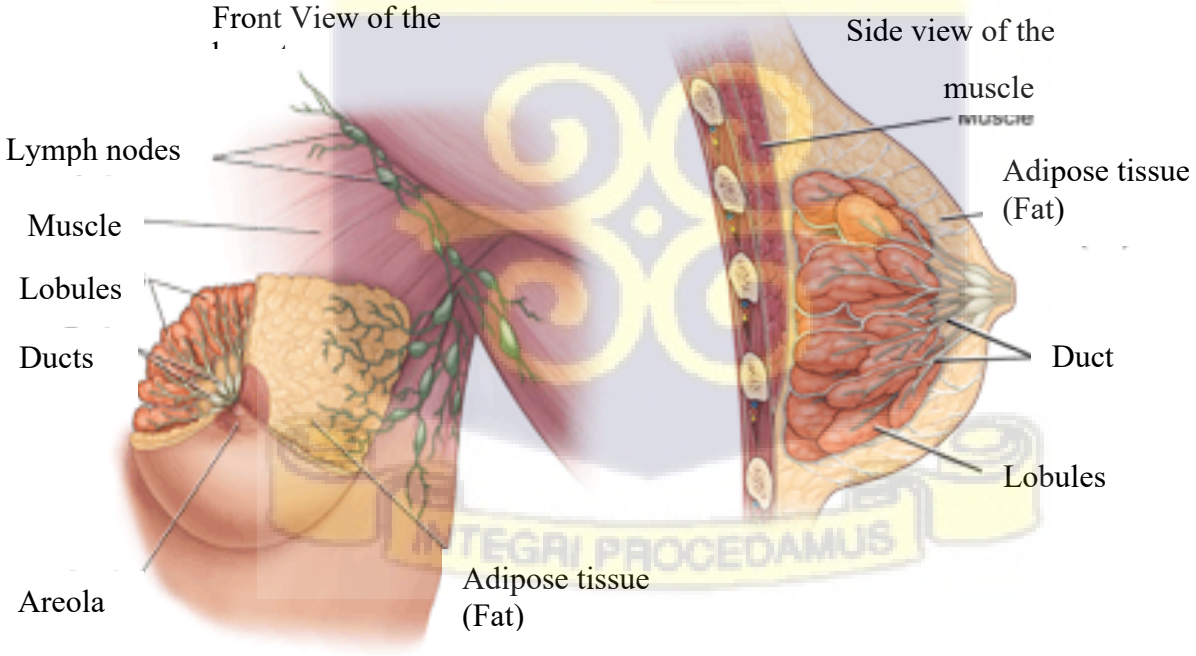
There is no distinction in serum Phosphoinositide 3-kinase level in breast cancer and non-breast cancer women

CHAPTER TWO

LITERATURE REVIEW

2.1 Anatomy of the breast

The breast is a glandular organ located on the chest of humans, primarily in females but also present in males, although typically less developed (Moore, 2018). In females, the breast serves an important function within the reproductive system, particularly in the framework of breastfeeding and the nurturing of infants. It is primarily composed of glandular tissue, adipose tissue, connective tissue, and an intricate network of blood vessels and lymphatic vessels (Moore, 2018) (Figure 1)



A B

Figure 1: The Anatomy of the post-pubertal breast. A represents the anterior view of the breast and B represents median view of the breast.
Adapted from Hopkinsmedicine.org

The breast is mainly made up of two tissue types: glandular and stromal. Glandular tissue encompasses the lobules and ducts responsible for milk production and transport, whereas stromal tissue is composed of fatty and fibrous connective tissue. The functional portion of the breast, known as the parenchyma, typically spans horizontally from the edge of the sternum to the anterior axillary line and vertically from around the second or third rib down to the inframammary fold, which generally lies near the sixth or seventh rib. An extension of the glandular tissue, referred to as the Tail of Spence, reaches into the axillary region. Several muscles-including the pectoralis major, serratus anterior, external oblique, and rectus abdominis-support the posterior surface of the breast (Mohamed Abdel Kader et al., 2015).

Histologically, the breast has a sophisticated and highly specialized architecture essential to its role. The breast is composed of glandular elements, adipose tissue, connective stroma, and a rich network of blood vessels and lymphatic channels (Gordon & Alsayouri, 2023). Its glandular component includes the milk-secreting alveoli along with the associated ductal system responsible for transporting milk (Biswas et al., 2022).

Alveoli are the physiological units of the mammary gland and are lined with secretory epithelial cells that produce milk during lactation (Watson et al., 2022). The breast contains a significant amount of adipose tissue, which surrounds and supports the glandular tissue. Adipocytes in this tissue store fat, and variations in adipose tissue content contribute to the differences in breast size and shape (Kothari et al., 2020). Connective tissue, including fibrous stroma, provides structural support to the breast. Suspensory ligaments within the stroma help maintain the breast's shape and position

(Rehnke, et al., 2018). The breast is well vascularized, with branches of the internal mammary and lateral thoracic arteries supplying oxygen and nutrients to the breast tissue The breast also has an extensive lymphatic network, which includes axillary lymph nodes located in the armpit. These nodes play a critical role in draining excess fluid from the breast tissue and removing waste products, and they are essential for immune function (Shahoud et al., 2023).

2.2 Prevalence and Risk factors in breast cancer

Among women across the world, breast cancer has the highest incidence rate, though its occurrence varies by geographic region (World Cancer Research Fund, 2020). According to the World Cancer Research Fund, breast cancer accounted for 11.7% of all new cancer cases in 2020 ("World Cancer Research Fund. Its prevalence is highest in North America, Europe, and Oceania, but it is also increasing in developing countries. Age and gender are significant factors in breast cancer prevalence. It primarily affects women, with most cases diagnosed in women aged 50 years and older (American Cancer Society, 2021). The age-standardized incidence rates provide a useful metric for comparing breast cancer prevalence across different populations (American Cancer Society, 2021). Worldwide, breast cancer accounts for 29.9% of cancer incidence and 22.1% of cancer-related mortality in Africa. In Ghana, breast cancer accounts for 31.8% of cancer prevalence (Global Cancer Observer, 2021).

East Asia has a breast cancer mortality occurance of 6 per 100,000 people, while Western Africa has a rate of 20 per 100,000 people (Momenimovahed, & Salehiniya, 2019). Racial and ethnic inequalities are evident in breast cancer prevalence. In the United States, non-Hispanic White women have a higher prevalence compared to non-Hispanic

Black, Hispanic, and Asian women (American Cancer Society, 2021). These disparities are influenced by various factors including genetics, socioeconomic status, and healthcare access (American Cancer Society, 2021).

Temporal trends in breast cancer prevalence are notable. Over the last few decades, there has been a growing rate of breast cancer cases, primarily due to factors such as lifestyle changes (diet, physical activity), delayed childbirth, and hormone replacement therapy (Osei-Afriyie, et al., 2021). A variety of factors are linked to an increased predisposition of cancer of breast, including a family history of the disease, inherited genetic mutations such as BRCA1 and BRCA2, hormonal influences like early onset of menstruation, late menopause, and hormone replacement therapy, as well as lifestyle-related factors such as obesity and alcohol consumption (American Cancer Society, 2021). Nonetheless, advancements in screening methods and increased public awareness have contributed to earlier diagnosis and improved survival outcomes (American Cancer Society, 2021). The implementation of mammography and breast self-examinations has played essential function in the prompt diagnosis of breast cancer. Early diagnosis is allied with higher survival rates and less aggressive treatment (Ginsburg et al., 2020). Efforts to increase awareness and access to screening programs have contributed to the prevalence of early-stage breast cancer cases (Ginsburg et al., 2020).

With respect to gender, breast cancer is more frequent in females than in males (Greif et al., 2012). Breast cancer is usually diagnosed at later phase in men likened to women because of its rare prevalence in men. Moreover, females with an AB blood type and Rh-negative status may be less susceptible to develop breast cancer than women with blood group A and Rh positive. The blood group is the most difficult determent for breast cancer (Momenimovahed, & Salehiniya, 2019). Additionally, Black women have a higher likelihood of being diagnosed with breast cancer before the age of 40 compared

to their White peers. However, the concept reversed after 40 years making white women more prone to breast cancer than black women (Williams et al., 2016).

Nulliparous women are greater likelihood of breast cancer as opposed to women who have had a full-term pregnancy (Zouré et al., 2016, Meo et al., 2017). The mammary gland cells undergo significant and long-lasting alterations during the first pregnancy. For instance, glandular cells have a protracted G1 phase (Gap 1 phase), which allows for DNA repair, and a delayed mitotic cycle. Therefore, the older the mother is when she becomes pregnant for the first time at term, the higher the chance that genetic alterations will occur (which will be passed with the proliferation of breast gland cells during pregnancy) (Davis & Lin, 2011). Furthermore, the chance of emerging breast cancer may be lowered by about 60% when two preventive factors, having two or more children and continuing to nurse for longer than 13 months, are combined. Breast cancer risk can be decreased by nursing; the longer the nursing, the more potent the protection remains (Abraham et al., 2013).

Elevated plasma levels of estrogen and progesterone in women above 45 years are linked to an increased predisposition cancer of the breast. Higher estrogen levels correlate with breast cancer development, while testosterone also plays a role because it can be converted into estrogen and has anabolic effects on breast cancer cells (Key et al., 2002). Use of hormone replacement therapy (HRT) has proven to raise both the risk of developing breast cancer and mortality rates; however, this risk declines after discontinuing HRT for at least five years (Beral et al., 2003). Furthermore, the degree of breast cancer risk varies based type of hormones used, with combined estrogen and progesterone therapies presenting a greater risk (Colditz, 2005).

Breast cancer risk is increased by genetic mutations in genes involved in DNA repair, oestrogen, progesterone, interferon alpha, and matrix metalloproteinase (MMP) receptors, enzymes that synthesise and break down hormones, and genes that code for these genes in families (Rojas et al., 2016; Cobain et al., 2016; Godet et al., 2017*)*. Other significant breast cancer risk factors include obesity after menopause, diabetes mellitus (most commonly type II), and high levels of IGF-1, especially when these risk factors are present together (Chen et al., 2016; Guo et al., 2018*)*. Insulin-like growth factor (IGF-1), prolactin, and long-term contraceptive use (over 10 years) have been known as possible risk factors for breast cancer. However, epidemiological studies continue to face challenges in conclusively establishing these associations (Satish et al., 2022).

2.3 Breast cancer pathology

Breast cancer is a complex disease with distinct pathological features that play crucial roles in its diagnosis, classification, and treatment. The pathology of breast cancer involves various aspects of the tumor's cellular characteristics, including its histological subtype, molecular profile, and staging (Makki, 2015*)*.

2.3.1 Histological Subtypes

Breast cancer is categorized into various histological subtypes, including ductal carcinoma in situ (DCIS), invasive ductal carcinoma (IDC), invasive lobular carcinoma (ILC), among others (Cserni, 2020). These subtypes are characterized by the appearance and behavior of cancer cells under a microscope

2.3.2 Ductal Carcinoma In Situ (DCIS)

Ductal carcinoma in situ is a non-invasive breast cancer subtype that originates in the milk ducts of the breast. Ductal carcinoma in situ (DCIS) represents about 20 to 25 percent of all breast cancer diagnoses (Allred, 2010).

2.3.3 Invasive Ductal Carcinoma (IDC)

Invasive ductal carcinoma (IDC) is the predominant histological subtype, accounting for approximately 70–80% of all cases of cancer of cancer (Allred, 2010). It arises from the milk ducts and subsequently spreads into the adjacent breast tissue.

2.3.4 Invasive Lobular Carcinoma (ILC)

Invasive lobular carcinoma is the second most common invasive breast cancer subtype, comprising approximately 10-15% of cases (Mouabbi et al., 2022). It originates in the lobules and can be challenging to diagnose.

2.3.5 Inflammatory Breast Cancer (IBC)

Inflammatory breast cancer is an infrequent yet aggressive form, making up less than 5% of breast cancer cases (Di Bonito et al., 2019). It is marked by symptoms such as redness, swelling, and warmth in the affected breast.

2.4 Molecular sub-type of luminal breast cancer

2.4.1 Luminal A

Luminal A breast cancer is defined by the expression of hormone receptors (ER+ and/or PR+), low Ki-67 expression, and absence of HER2 overexpression. It is one of the most prevalent subtypes and accounts for approximately 40-50% case of cancer of the breast (OrrantiaBorunda et al., 2022).

2.4.2 Luminal B

Luminal B breast cancer is also characterized by hormone receptor expression but typically has higher Ki-67 expression and may show HER2 overexpression. It comprises approximately 1020% of case of cancer of the breast (Cheang et al., 2009).

2.4.3 HER2-Enriched

HER2-enriched breast cancer is defined by the overexpression or amplification of the HER2 gene and accounts for about 15 to 20% of breast cancer cases (Cheang et al., 2009).

2.4.4 Basal-like/Triple-Negative

Basal-like, also known as triple-negative breast cancer, is defined by the absence of estrogen and progesterone receptors (ER-, PR-) and the absence of HER2 overexpression. This subtype represents about 10 to 15% of breast cancer cases (Cheang et al., 2009).

2.4.5 Claudin-Low

Claudin-low breast cancer is a less common subtype, representing around 5-10% of cases, and is known by low expression of tight junction proteins and immune cell infiltration (Dias et al., 2017).

2.4.6 Normal-like

The normal-like subtype is relatively rare and accounts for only a small percentage of cases of cancer of breast. It shares similarities with the luminal A subtype but lacks the distinctive features of other subtypes (Prat et al., 2010).

2.5 Genes involved in breast cancer

Breast cancer is linked to a variety of genes, with mutations and abnormal activations of oncogenes and tumor suppressor genes functioning as critical factors in oncogenesis and metastasis

2.5.1 HER2

Human epidermal growth factor receptor 2, also known as c-erbB-2, is situated on the long arm of chromosome 17 in humans (17q12). Neu, which was first recognised in 3-methylcholanthrene-induced rat neuroblastoma cells, has a mouse homolog called neu (Iqbal, & Iqbal, 2014). The manifestation of the HER2 gene is primarily induced by two mechanisms: gene amplification and gene rearrangement. Epidermal growth factor receptor 2 (HER2) forms heterodimers with other ligand-bound members of the EGFR family, such as HER3 and HER4, which then activate downstream signaling pathways (Hsu & Hung, 2016). Knocking down HER2 disrupts mammary duct formation, even though it is produced at low levels in normal epithelial cells at all stages of life (Press et al., 1990). Overexpression of HER2, which is existing in 20% of major breast cancers, is a predictor of poor clinical outcomes (Gutierrez, & Schiff, 2011), as is the presence of PTEN/Akt/mTORC1 signaling, which bolsters the development of cancer stem cells.

2.5.2 BRCA1/2

Both breast cancer-associated genes 1 and 2 (BRCA1 and BRCA2) are well recognised as important protective factors against the emergence of breast cancer. The BRCA genes are located on chromosomes 13q12 and 17q21 of the human genome. They both serve as genetic blueprints for anti-cancer proteins. Apoptosis, genomic instability, and a malfunctioning cell cycle checkpoint are all results of BRCA1 deficiency (Dine & Deng, 2013). The expression of BRCA1 is silenced by "pocket proteins, " such as p107 and p130, and the retinoblastoma protein in an E2F-dependent manner. It known that the

BRCA1 gene's promoter interacts with its introns and terminator to regulate gene expression, forming a loop between these three regions (Sun et al. 1017). The BRCA2 protein regulates the recombinational repair of DNA double-strand breaks by communicating with the RAD51 and DMC proteins. The most common genetic abnormalities in breast cancer are mutations in the BRCA1 and BRCA2 genes, which result in almost half in every case of inherited breast cancer. Approximately 4590% of breast cancer cases in families with apparent autosomal dominant breast cancer transmission are attributable to mutations in the BRCA1 gene.

2.5.3 Tumor Protein p53(TP53) Gene

TP53 is a tumor suppressor gene, and mutations in this gene are related with a higher incidence of breast cancer. TP53 mutations are predicted to occur in about 20-30% of all breast cancers (Shahbandi et. al., 2020).

2.5.4 PIK3CA (Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic SubunitnAlpha) Gene

Mutations in the PIK3CA gene are common in breast cancer and are implicated in the activation of the PI3K signaling pathway. PIK3CA mutations are found in nearly 30-40% of cases of cancer of the breast (Samuels et al., 2004).

2.5.5 PTEN (Phosphatase and Tensin Homolog) Gene (PTEN)

PTEN is a tumor suppressor gene that adversely controls the PI3K pathway. Mutations or loss of PTEN function are associated with breast cancer, although the prevalence of PTEN mutations can vary (Georgescu 2010).

2.6 Tools used in the assessment of breast cancer risk

In standard clinical practice, risk-assessment techniques are used to determine which women are at an elevated predisposition of advancing cancer of the breast and to advise counseling concerning lifestyle modifications, genetic testing, screening timing or modality, and suitability for risk-reducing medications or surgery (Kim, & Bahl, 2021). Some of the risk assessment methods for breast cancer that are now available are the Gail Model, Breast Cancer Risk Assessment Tool (BCRAT), and Tyrer-Cuzick Model. All three tools overpredict the probability of breast cancer incidence (Tery et al., 2019*);* though, BCRAT performs the best in predicting breast cancer
risk.

2.6.1 Gail Model

The Gail Model, developed by Mitchell Gail and his colleagues, is one of the earliest risk assessment tools for breast cancer. The model considers several risks factors-such as age, ethnicity, family history, age at first menstruation, age at first childbirth, and the number of prior breast biopsies-to estimate a person's likelihood of emerging invasive cancer of the breast over the next five years and throughout their lifetime. It has been extensively applied used for risk assessment and is available online, making it easily accessible to both health care providers and individuals (Bener et al., 2019).

2.6.2 Breast Cancer Risk Assessment Tool (BCRAT)

Also termed "National Cancer Institute (NCI) Breast Cancer Risk Assessment Tool," the BCRAT is also a broadly utilized used tool for assessing breast cancer risk. Using data such as age, race, family medical history, age at first menstruation, age at first live birth, and the number of breast biopsies, the model calculates an individual's predisposition of developing breast cancer over five years and across their life time. The NCI provides an online version of this tool, making it an indispensable aid for medical care providers and

those aiming to gain insight into their breast cancer susceptibility (National Cancer Institute, 2021).

2.6.3 Tyrer-Cuzick Model

The Tyrer-Cuzick Model, also known as the International Breast Cancer Intervention Study (IBIS) model, factor in various risk factors, including hormonal and genetic factors such as the presence of BRCA1/BRCA2 mutations. This model offers a more thorough evaluation of breast cancer risk by considering a wider range of variables. The Tyrer-Cuzick Model allows for a personalized threat evaluation, particularly among those with a significant family history of breast cancer (Valero et al., 2020)

2.7 Screening tools for breast cancer

Women exclusive of any symptoms or warning signs may undergo breast cancer screening to enable early detection and improve treatment outcomes (Bevers, 2009). The prime goal of screening is to discovery cancer of the breast at an initial phase, which facilitates more effective management and helps lower mortality rates. A comprehensive breast cancer screening may involve a physical examination, risk evaluation, mammography, and magnetic resonance imaging (MRI). The choice of screening methods often depends on factors such as the woman's age, medical and family history, and her level of breast health awareness (Brakohiapa et al., 2013).

2.7.1 Mammography

Two radiographic images (views) of each breast are routinely taken during screening mammography: one craniocaudal and the other mediolateral oblique (Humphrey et al., 2002*)*. All other forms of screening must be done in addition to mammography because it is the only screening approach that has exhibited a capacity to lower death from breast cancer to date. For women aged 39 to 49, mammography screening lowers breast cancer

fatality by 15% (Nelson et al., 2009). Mammography has several concerns, including false-positive and false-negative readings, overdiagnosis, and radiation-induced malignancies, aside from the discomfort that many women endure from the breast compression required for a technically perfect mammogram. Overdiagnosis estimates range from 1% to 10%. Younger women receive fewer biopsies but more imaging and false-positive mammography results than older women (Warner, 2011).

2.7.2 Magnetic resonance imaging

For young women at increased predisposition of breast cancer, mammography alone often has limited sensitivity, so breast magnetic resonance imaging (MRI) has been endorsed as a supplementary screening tool. While MRI tends to have lower specificity than mammography and a greater chance of false-positive findings, it is more effective at detecting breast cancer. Numerous studies have demonstrated the benefits of MRI screening for women with a hereditary risk of cancer of the breast (Kuhl et al., 2005; Lehman & Smith, 2009). Based on guidelines from the American Cancer Society (ACS), breast MRI is advised as an addition to mammography for screening high-risk women (Saslow et al., 2007). Although some research reports no significant increase in false positives from MRI screening, other studies have noted higher rates. For instance, one study involving high-risk women found that MRI screening led to three times more benign biopsies compared to mammography. This group primarily included younger women with dense breast tissue, categorized as high-risk or having raised hereditary predisposition of breast cancer (Gao et al., 2021).

2.7.3 Clinical breast examination

A thorough medical history is followed by clinical breast examination (CBE) as the first step in the process of identifying anomalies and screening for breast cancer. Patients should be examined while supine and upright to check the breasts. Patients may also be positioned to detect any minute changes in the breast's shape or contour. CBE should include a thorough palpation of the whole breast in both the supine and upright positions, as well as the axilla and any nodal basins (axillary, supraclavicular, and internal mammary) that affect the breasts. Symptoms or abnormal findings on physical examination may include a detectable lump or mass, uneven thickening or nodules, nipple discharge without an associated lump, and skin changes such as peau d'orange, redness, nipple irritation, flaking, or dermatitis (Parthasarathy & Rathnam, 2012).

2.8 PI3K signaling pathway in cells

To support normal cell growth and division, the PI3K signaling pathway responds to external stimuli by engaging G-protein-coupled receptors (GPCRs) and receptor tyrosine kinases (RTKs) (Law et al., 2016) (see Figure 2). This pathway includes a heterodimeric enzyme complex called PI3K-alpha, which consists of a catalytic subunit (p110) encoded by the *PIK3CA* gene and a regulatory subunit (p85) encoded by the *PIK3R1* gene. Once activated, PI3K catalyzes the conversion of phosphatidylinositol 4,5-bisphosphate (PIP2) into phosphatidylinositol 3,4,5-trisphosphate (PIP3) through phosphorylation of the lipid molecule. (PIP3) is carried out by the regulatory component p110, which binds to and is blocked by p85 (PIP3), (Mazloumi et al., 2018). This route can be used by other p110 isoforms such as p110 to signal and generate PIP3.

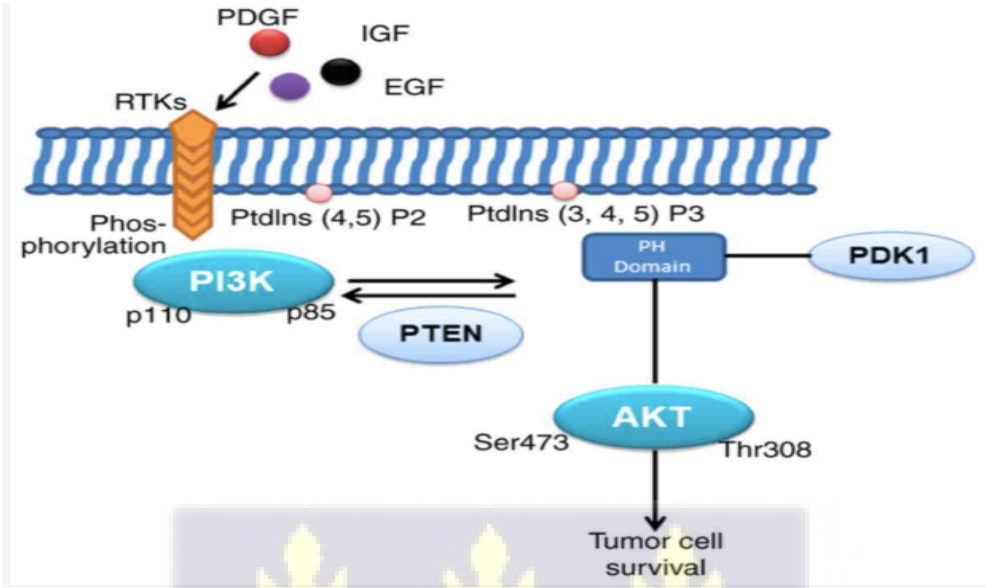


Figure 2: Normal cell growth in the PI3K/Akt pathway. In the extracellular environment of a cell are growth factor receptors such as human epidermal receptor 2 (HER2), insulin growth factor 1 receptor (IGF1R), and fibroblast growth factor receptor 1 (FGRF1) which are attached to the growth factors which lead to the phosphorylation of PIP2 to PIP3 which phosphorylate tyrosine kinase A (AKT) via Phosphoinositol -dependent kinase (PDK). AKT phosphorylate Mammalian target of rapamycin complex 2 (mTORC2) through the activation of tuberous sclerosis 2 which leads to gene transcription for cell proliferation and growth
(Adapted from Vasan et al., 2019)

The build-up of PIP3 at the inner surface of the plasma membrane acts as a crucial second messenger, triggering a downstream signaling cascade. This cascade includes the activation of AKT, which is mediated by phosphoinositide-dependent kinase 1 (PDK1)—a protein that binds to PIP3—and the mammalian target of rapamycin complex 2 (mTORC2). Once activated, AKT phosphorylates and inhibits tuberous sclerosis complex 2 (TSC2), a suppressor of mTOR activity, thereby promoting further mitogenic signaling downstream AKT. For PI3K to be fully activated, it must connect to both Ras and membrane-bound RTKs.

On the other hand, the lipid phosphatase and tensin homolog can dephosphorylate PIP3 to PIP2 (PTEN). RTK overexpression, PIK3CA and AKT oncogene mutation, PTEN tumour suppressor loss of function, and others all contribute to cellular and tumour PI3K pathway reliance (Liu et al., 2009)

2.9 Physiological role of PI3K

The phosphoinositide 3-kinase (PI3K) pathway plays a critical role in various physiological processes in the body (Arcaro et al., 2007). PI3K phosphorylates phosphatidylinositol lipids in the cell membrane, resulting in the production of secondary messengers such as phosphatidylinositol 3,4,5-trisphosphate (PIP3) (Katan & Cockcroft, 2020). These secondary messengers activate downstream signaling pathways, primarily through the protein kinase Akt (also known as protein kinase B or PKB) (Manning & Cantley, 2007). Some of the key physiological roles of the PI3K pathway are as follows:

2.9.1 Cell Growth and Survival

The PI3K pathway plays a key role in regulating cell growth and survival (Engelman et al., 2006). It encourages cell proliferation while preventing apoptosis, or programmed cell death (Manning & Cantley, 2007). When this pathway becomes dysregulated, it is often linked to cancer, as it can result in unchecked cell growth and resistance to cell death (Jan et al., 2019).

2.9.2 Metabolism

PI3K is involved in regulating glucose uptake and metabolism (Fontana, et al., 2024). Activation of the pathway promotes glucose transport into cells, especially in response

to insulin (Petersen & Shulman, (2018). This has implications for metabolic disorders like diabetes (Saltiel & Kahn, 2001).

2.9.3 Immune Response

PI3K signaling is integral to the immune response (Okkenhaug, 2013). It influences immune cell activation, migration, and function (Andrews et al., 2007). Abnormal PI3K activity can lead to immune dysfunction, autoimmune diseases, or increased susceptibility to infections (Fruman & Rommel, 2014).

2.9.4 Cardiovascular Function

The PI3K pathway plays a role in the regulation of vascular function (Zhao et al., 2021), influencing blood vessel dilation and constriction. It is crucial for maintaining normal blood pressure and overall cardiovascular health (Chaudhry et al., 20220).

2.9.5 Neurological Function

In the nervous system, PI3K is involved in various processes, including neuronal survival, synaptic plasticity, and neurite outgrowth (Sánchez-Alegría et al., 2018). Dysregulation can contribute to neurological disorders (Costa et al., 2016).

2.9.6 Inflammation

The PI3K pathway plays a important role in the regulation of inflammatory responses (Fruman & Rommel, 2014). It controls the activation and migration of immune cells to sites of inflammation (Okkenhaug, 2013).

2.9.7 Cell Migration

PI3K is active in cell motility and migration (Vadas et al., 2011), which are important for processes such as wound healing and embryonic development (Andrews et al., 2007).

2.9.8 Hormone Signaling

Hormones like insulin and growth factors can activate the PI3K pathway (Manning & Cantley, 2007). This is critical for hormonal control of various physiological functions (Engelman et al., 2006).

2.9.9 Reproduction

The PI3K pathway is involved in processes related to reproduction, such as oocyte maturation and embryo development (Suh et al., 2015)

2.10 PI3K/Akt pathway in adipogenesis

Lipid production is stimulated and lipolysis is prevented via the PI3K/AKT signalling pathway (Figure 3). Fatty acid synthase and genes associated with cholesterol are managed by the substrate sterol regulatory element-binding protein (SREBP) (Huang et al., 2018).

In addition to regulating cholesterol and lipid homeostasis, sterol regulatory element binding proteins (SREBPs) regulate the expression of many enzymes involved in the production of endogenous fatty acids (FA), triacylglycerols, and phospholipids.

The three isoforms of SREBP—SREBP-1a, SREBP-1c, and SREBP-2—each have divergent roles in lipid metabolism. SREBP-1c is primarily concerned in fatty acid synthesis and insulin-regulated glucose metabolism, particularly in lipogenesis, while

SREBP-2 is more specifically associated with cholesterol synthesis, as demonstrated by studies using transgenic and knockout mice. SREBP-1a appears to participate in both processes. These SREBP transcription factors are initially produced as inactive precursors anchored to the endoplasmic reticulum (ER) membrane. Upon activation, a two-step cleavage process releases the NH2-terminal active domain, which then translocates to the nucleus to exert its function (Eberlé et al., 2004).

The key substrate for AKT-mediated lipid metabolism is forkhead box 01(FOXO1), which regulates the production of a lipolytic enzyme called adipose triglyceride lipase (ATGL) (Sparks, & Dong, 2009).

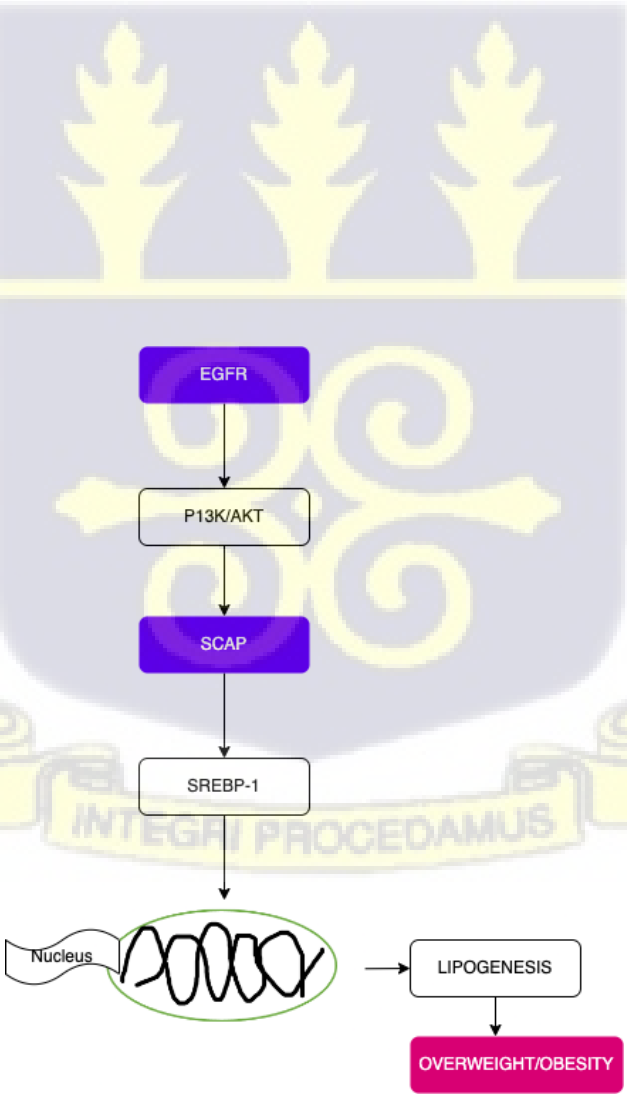


Figure 3: PI3K signaling pathway in lipogenesis. The binding of epidermal growth factor receptor (EGFR) to epidermal growth factor leads to the dimerisation of EGFR to phosphorylate phosphoinositol 4, 5 biphosphate (PIP2) to phosphoinositol 3, 4, 5 triphosphate (PIP3), which then phosphorylates terosine kinase A (AKT). AKT activates sterol regulatory element-binding protein cleavage-activating protein 1 (SCAP), which leads to the release of sterol regulatory element-binding proteins to the nucleus, which transcribe genes to initiate lipogenesis. Accumulating of lipid leads to overweight or obesity. (Adapted from Huang et al., 2018)

Several systemic alterations, such as changes in the levels of insulin, insulin-like growth factor1, leptin, adiponectin, steroid hormones, and cytokines, are brought on by the positive energy balance associated with obesity (Figure 4). Each of these factors can alter the nutritional environment and potentially foster the initiation and spread of tumours (Hopkin et al., 2016). The onset cancer of the breast in obese women has been linked to the excessive presence of proinflammatory cytokines, insulin resistance, heightened activity of insulin-like growth factor (IGF) pathways, adipokine release from adipocytes, high cholesterol levels, and increased oxidative stress (Glassma et al., 2023)

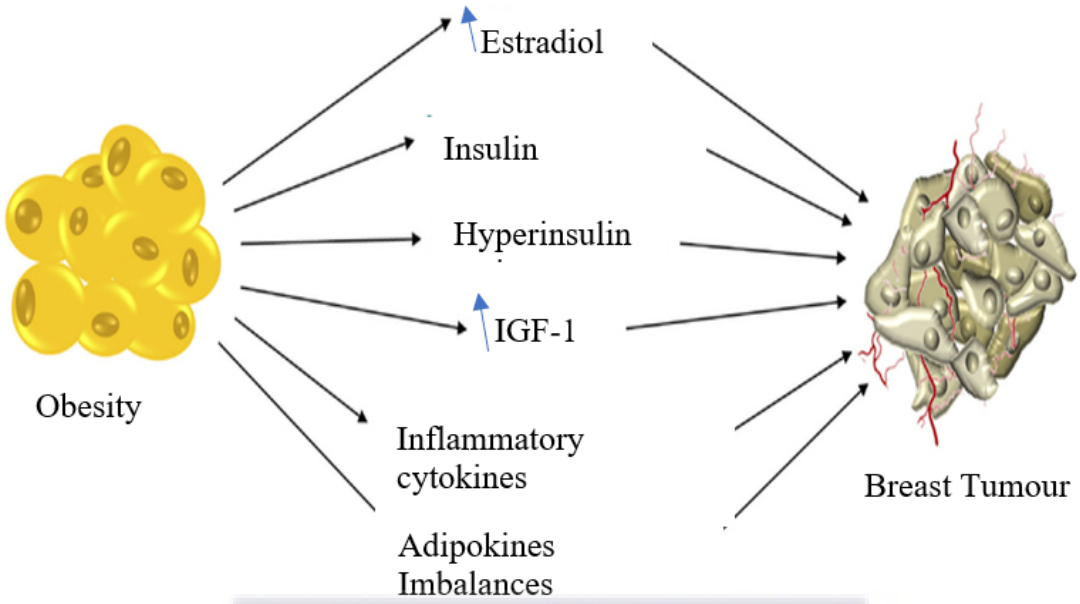


Figure 4: How obesity contributes to the onset, development, and spread of breast cancer and the key processes underlying this association. (Friedman et al., 2017)

The PI3K/AKT pathway is often overactivated in a variety of human cancers. Phosphoinositide-3-kinase (PI3K) pathway activity has been linked to many different types of cancer. This pathway affects a wide variety of downstream target proteins that play roles in cancer cell carcinogenesis, proliferation, invasion, and metastasis. Obese women have the highest incidence of phosphoinositide 3-kinase 1 alpha activating mutations in the PIK3CA gene (PI3K1 alpha), which leads to cancer of the breast(Figure 5) (Yang et al., 2019).

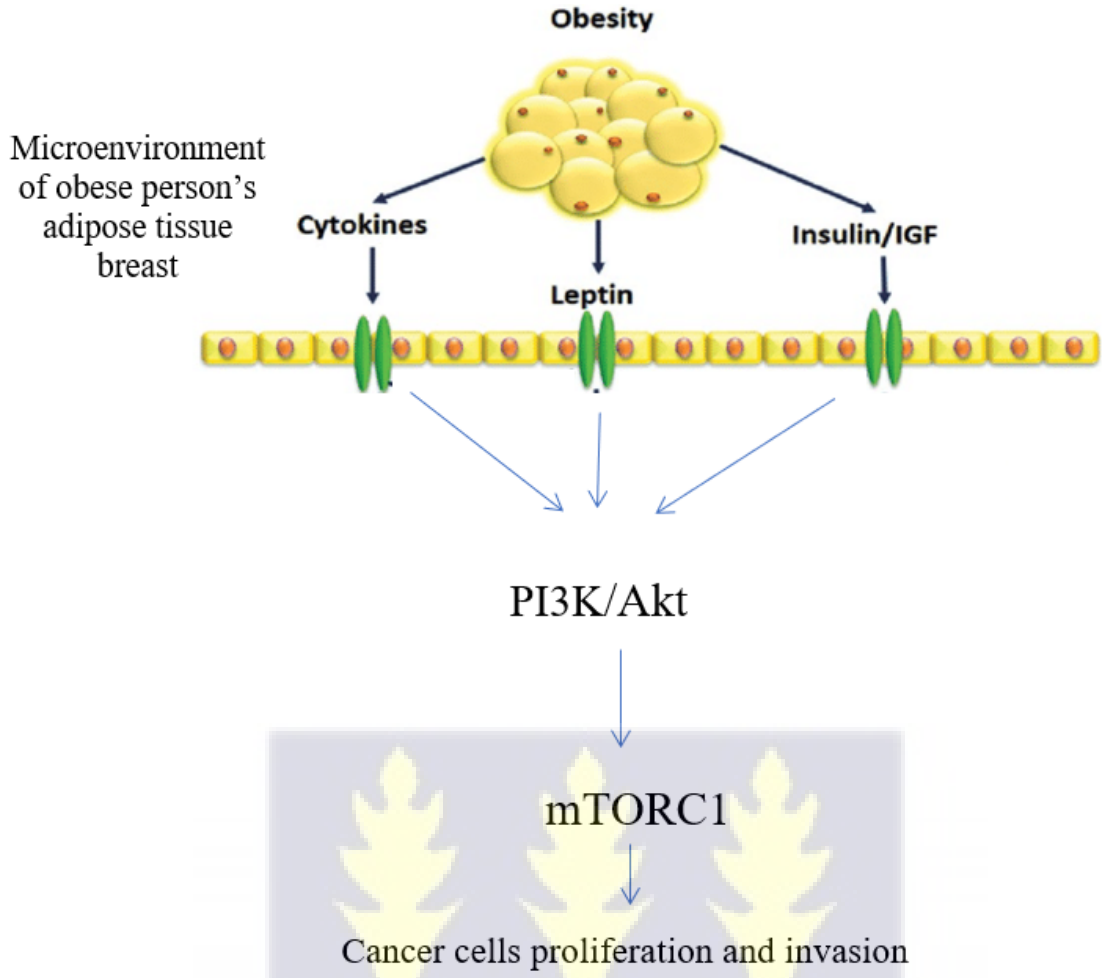


Figure 5: PI3K/Akt pathway in breast cancer initiation and invasion with the microenvironment of the adipose tissue. In the microenvironment of obese person is an increase level of insulin like growth factor 1, leptin and inflammatory cytokines that triger hyperactivation of the PI3k/AKT signaling pathway that initiate abnormal cellular proliferation by mTORC1 which lead to an cancer cell initiation and metastasis. (Adapted from Yang et al., 2019)

2.12 PI3K mutations in breast cancer

The PI3K/Akt pathway is altered in nearly 70% of cancerous breast, which results in hyperactivation of the pathway (Frenel et al., 2020). AKT mutation, PI3K mutation (catalytic domain or helical), loss of PTEN function (deletion or loss of expression, epigenetics), or the regulatory function of TSC1/TSC2 proteins can all lead to hyperactivation of the PI3K pathway (tuberous sclerosis complex). Exons 9 and 20 of

the protein's helical (PIK3CA) domain are hotspots where the majority of mutations occur (Glaviano et al., 2023*)*. Nearly 40% of cases involving advanced hormone receptor-positive breast cancer exhibit an abnormal PIK3CA mutation, which is a common feature of the disease (Frenel et al., 2020). Approximately >70% of HR+ breast tumours have PI3K/Akt/PTEN/mTOR pathway activation due to AKT1 mutation, PTEN loss, or PI3K activator mutation (Miller et al., 2011). With respect to breast cancer subtype, the prevalence of PIK3CA mutations varies, with the exception of androgen receptor-positive, triple-negative breast cancers. PIK3CA mutations are less frequent in ERnegative breast cancers, accounting for 30–50% of advanced ER+ HER2 breast cancers (Samuels & Velculescu, 2004).

By directly or indirectly interacting with RTK through adaptor proteins, PI3K is stimulated. The RTK with the best understanding of breast cancer carcinogenesis is gene-amplified HER2. The HER2/HER3 heterodimer is regarded to are the most potent in stimulating cell division and transformation within the four forms of dimers that HER2 can theoretically create with HER1, HER2, HER3, or HER4 (Mukohara, 2015). Two distinctive traits set HER3 apart from other HER family members: it lacks tyrosine-kinase activity on its own and has at least six docking domains for PI3K p85 (Gala, & Chandarlapaty, 2014) Due to these characteristics, HER3 can effectively act as a scaffold protein to activate the PI3K pathway. A study found that breast cancer cell lines that express both HER2 and HER3 seem to have more phosphorylated AKT (Takagi, et al., 2014). Approximately 1.4% to 8% of breast tumours have been discovered to contain an AKT1 mutation (E17K). AKT1 mutations were found only in tumours that expressed both ER and PgR, according to a large-scale genotyping effort that included 547 breast tumours and 41 breast cancer cell lines (Malanga et al., 2022).

2.13 PI3KCA mutation and clinicopathology factors

In-depth research has been conducted on the connection between PIK3CA mutations and hormone-receptor status. Various researchers discovered a strong bond between PIK3CA mutations and the expressions of ER and PgR (Pang et al., 2014). Early research proposed a relationship between HER2 overexpression and PIK3CA mutation in terms of HER2 status (Kim et al., 2023). However, later research found no connection between PIK3CA and HER2 status (Castaneda et al., 2014) or a correlation with HER2-negativity (Pestrin et al., 2009). Additionally, some research discovered links between PIK3CA mutations and positive prognostic indicators, including lower histological grades, non-triple-negative subtypes, luminal A subtypes, smaller tumour sizes, and lower levels of Ki67 (Azim et al., 2016)

Preclinical investigations revealed that hot-spot PIK3CA mutations were gain-of-function mutations that increased the phosphorylation of downstream signalling molecules on the PI3K pathway (Zardavas et al., 2014). Several researchers used tumours obtained from patients to investigate the clinical significance of this increased phosphorylation. The most often used biomarker of PI3K-pathway activity, phosphorylated AKT (Ser473 or Thr308), and PIK3CA mutations are correlated, but the conclusions of this correlation are debatable, with some studies suggesting a positive link (Hung et al., 2022), and some no correlation (Alqahtani et al., 2019). In a study employing reverse-phase protein array technology for complete protein profiling, no significant differences were observed in the phosphorylation levels of AKT, GSK3, mTOR, or p70S6K between tumors with PIK3CA mutations and those with wild-type

PIK3CA. However, tumors with low PTEN expression exhibited significantly higher phosphorylation of AKT, mTOR, and p70S6K likened to those with high PTEN levels (Stemke-Hale et al., 2008). These inconsistent observations imply that the relationship between PIK3CA mutations and activation of downstream proteins remains unclear, at least in clinical samples. The discrepancies may stem from variability in methods used to detect phosphorylated proteins and fluctuations in phosphorylation levels depending on the condition of the samples (Mukohara, 2015).

2.14 Anthropometric measurement and breast cancer

Body size Body size is typically classified as normal, underweight, overweight, or obese based on body mass index (BMI), which is computed as weight in kilogrammes divided by height in meters squared (Zierle-Ghosh & Jan 2023). Height has a positive correlation with breast cancer advance in premenopausal women compared to excess adiposity in adulthood (His et al., 2020). Normal BMI with central obesity (waist circumference) greater than 88 cm increases the predisposition of breast cancer development equated to abnormal BMI with less central obesity. Women with elevated WHR (>0.83) has been linked to a greater chance of developing breast cancer, while women with a trunk-to-height ratio greater than 34 exhibit high predisposition for breast cancer (Park et al., 2017).

Being overweight and obese are associated risk factors in breast carcinogenesis in women (Sun et al., 2017). Breast cancer predisposition in premenopausal women Rises with increasing height but declines as weight or body mass index increases, and there is no correlation with central obesity. Height, weight, body mass index, waist-hip ratio,

waist circumference, and weight gain are all anthropometric features connected with a high risk of breast cancer in postmenopausal women. Weight lowers risk, especially if it takes place in advanced age. Breast size may be a risk factor for breast cancer (Friedenreich, 2001). The PI3KCA gene is the second-most often mutated gene among patients with obesity and breast cancer when comparing gene mutations in individuals (Madsen et al., 2018*)*. Mechanistic studies provide substantial evidence for the idea that excess body weight and DNA damage are related (Włodarczyk, & Nowicka, 2019)

CHAPTER THREE

METHODOLOGY

3.1 Study design

A cross-sectional study was undertaken at the Breast Unit of Korle-Bu Teaching Hospital (KBTH).

3.2 Study site

The study was conducted in the Breast Unit of the Department of Surgery, Public Health Unit, and Gynaecological Unit at the Korle-Bu Teaching Hospital. The hospital is affiliated with the University of Ghana Medical School and is the leading and largest referral and teaching hospital in Ghana. It has a core of excellent healthcare practitioners and facilities for treating cancer. Any woman suspected of having breast cancer is

referred to the Breast Unit at the Surgical Department. The Breast Unit receives about five newly diagnosed breast cancer patients per week. Additionally, the Public Health Unit and Gynaecological Unit receive about 50 women per day for medical check-ups or reviews other than breast cancer. The units served as sites for recruitment of non-breast cancer individuals as controls for the study.

3.3 Study participants

Women clinically diagnosed with cancer in the breast were recruited from the Breast Unit of the Department of Surgery at KBTH. This department was chosen because all suspected or diagnosed breast cancer patients are referred there for further investigation and management. Healthy age-matched women without any signs of malignancy or chronic conditions, such as diabetes, were also enrolled as controls. These control participants were recruited from the gynaecological and public health units at KBTH. Each consenting healthy control participant was screened by a midwife using standard breast examination to rule out breast tumors. Additionally, they were screened with a glucometer to rule out other chronic conditions, such as diabetes. Patient records were also reviewed to exclude any other forms of cancer.

3.4 Inclusion criteria

The inclusion criteria were as follows.

- Women 18 years and above who were clinically diagnosed with breast cancer.

- Newly diagnosed breast cancer patients who were yet to start treatment.

3.5 Exclusion criteria

The exclusion criteria were as follows**.**

- Breast cancer patients who were receiving any form of cancer treatment.
- Patients with breast cancer who had been diagnosed with another cancer.

3.6 Sample size

The formula below was used to determine the sample size (Charan, & Biswas, 2013)

$$N = 2SD2\frac{(z\alpha + z\beta)2}{\Delta 2}$$

From the formula above,

α = 0.05 for the significant level

Zα = The 1.96 standardised standard deviation number corresponds to an equivalent level of statistical significance.

β = 0.20

Zβ = 0.84 probability of detection or power (80%)

N= number of participants

Standard Deviation (SD) = 1 from previous studies

Δ = 0.8 effect size for small, moderate, and large is (0.2, 0.5, and 0.7 for two groups in comparison) as a guide by Jacob Cohen- Statistical Power Analysis for the Behavioral Sciences.

$$N = 2SD2\frac{(Z\alpha + Z\beta)2}{\Delta 2} \qquad (2)$$

N= $2(1)^2(1.93 + 0.84)^2/ (0.7)^2$

N = $2(2.8)^2/0.49$

N = 2(7.84)/0.49

N = 15.68/0.49

N = 32 per group.

Therefore, 32 breast cancer women and 32 non-breast cancer women

3.7 Participant selection

A purposive selection method was used to select 32 patients with breast cancer who were detected with the disease and had been referred to the Breast Unit during breast clinic days before the start of therapy, and who were willing to participate in the study at the Breast Unit of the Department of Surgery at KBTH between June and August 2023. Each potential breast cancer participant's medical record was checked to rule out any form of cancer before participating in the study. Thirty-two healthy age-matched controls were recruited after seeking their consent in the gynaecological and public health units after each person had undergone a normal breast examination by a midwife in the KBTH blood glucose test and medical records to exclude diabetes and any diagnosed cancer. All participants were recruited after they completed the consent form (Appendix I).

3.8 Anthropometric measurements

3.8.1 Waist-to-hip ratio (WHR)

The waist-to-hip ratio is a dimensionless ratio of the circumference of the waist to that of the hip. In measuring the waist circumference, a calibrated tape measure in centimeters was carefully placed between the lower ribs and the iliac crest passing through the umbilicus anteriorly to measure the approximate midpoint in a horizontal plane. Participants were asked to stand with their feet close together for an even distribution of the weight across their feet before taking the measurement. Hip circumference was also measured with a calibrated tape by snugging it around the two greater trochanters and the pubic symphysis of the pelvic bone at the greatest protuberance of the buttocks, while the participants stood with their feet closer to each other. The participants were asked to remove any heavy clothing, and the waist and hip were measured with the assistance of a trained female nurse.

The waist measurement in centimeters was divided by the hip measurement in centimeters to obtain the WHR.

$$WHR = \frac{Waist\ maeasured\ in\ cm}{Hips\ measured\ in\ cm}$$

3.8.2 Body mass index (BMI)

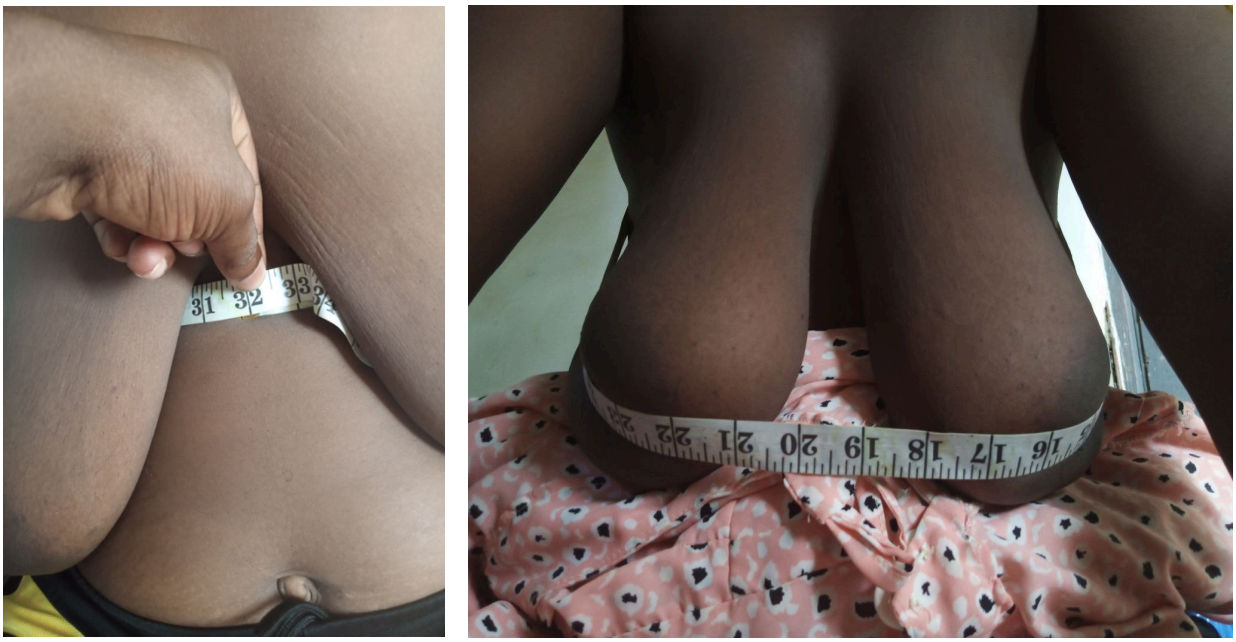
The Body Mass Index (BMI) estimates body fat by dividing a person's weight in kilogrammes by the square of their height in metres. On a flat surface, the zero point of

a calibrated scale was established. Both in patients with breast cancer and the healthy controls were instructed to remove their shoes and any other bulky items before standing erect on the calibrated scale. In addition, participants were instructed to stand barefoot next to a stadiometer with their feet together. The head was positioned so that the external auditory meatus aligned horizontally with an imaginary line drawn from the lower edge of the eye socket. When measuring height, participants were instructed to stand as tall as possible. BMI was computed as weight in kilograms divided by height in meters squared.

3.8.3 Breast size

Bust and thorax circumference were used to calculate the breast size. Thorax circumference was measured by snugging a tape measure around the thorax posterior to the breast. The approximate midpoint was recorded as the thorax circumference Figure 6 A. To estimate the bust, participants were made to sit and tilted anteriorly for the suspension of the breast, and a calibrated tape was snugged obliquely around the thorax, through the lateral sides of the two breasts, and posterior to the nipple to the body of the scapular posteriorly, the approximate midpoint was recorded as the bust Figure 6 B. The bust was subtracted from the thorax circumference to obtain the approximate breast size. The measurement was taken by an assistance-trained female nurse at the KBTH

$$Bust(cm) - thorax(cm) = Breast\ size \quad (cm)$$



A **B**

Figure 6: Image showing how breast size was measured using a calibrated tape measure. A tape measure was placed around the thorax, passing posterior to the right and left breasts, and the approximate midpoint was recorded as the thorax circumference **(A).** The participants were also tilted forward for both breasts to suspend for the Bust to be recorded (**B).**
Source: Unpublish

3.9 Clinicopathological parameters

The molecular subtypes of breast cancer, such as luminal A, luminal B, HER2+ enriched-like, and triple-negative for each case were obtained from the patient's hospital folder during clinic days after the patient had done immunohistochemistry**.**

3.10 PI3K expression and estimation

3.10.1 Material and reagents

Serum Separator Tube, Centrifuge, Sterile Pipette Tips, Pipettes, PI3K ELISA Kits (96 well Plate), Distilled Water, Eppendorf Tubes, Phosphate Balance Saline (PBS), Ficoll-Paque solution, and Vortex

3.10.2 Sample preparation for PI3K quantification

About 4-5 mls whole venous blood sample was drawn from each of the 64 participants into a serum separator tube and left undisturbed for 45 minutes to clot. Red blood cells were separated via centrifugation at 4000 rpm for 15 minutes, and serum was aliquoted.

3.10.3 Quantifying PI3K protein

PI3K enzyme-linked immunosorbent assay (ELISA) kits from Sunlong Biotech (Protocol number SL1388Hu) were used to quantify PI3K protein levels in the serum samples. This assay involves the use of specific antigens to generate a standard protein curve. Initially, PI3Kspecific antibodies were introduced into the serum samples, allowing for the formation of antigen-antibody complexes. These complexes were then applied to the wells of the microtiter plates.

The wells were then washed thoroughly to remove any unbound antibodies. A secondary enzyme-conjugated antibody, which binds to the primary antibodies, was added to facilitate detection. The number of primary antibodies present in each well was determined by measuring the optical density, which correlated with the concentration of PI3K protein.

A standard curve was created by plotting the optical densities of known concentrations of PI3K against their respective concentrations on a logarithmic scale. The optical density values for the samples were used to determine their PI3K level by comparing them to the standard curve. The concentration of PI3K in each serum sample was calculated using an equation derived from the standard curve.

3.11 Statistical analysis

Demographic and clinical data were initially recorded using Microsoft Excel for validation purposes. Statistical analyses were carried out using IBM SPSS Statistics version 29. Descriptive statistics, including the mean, median, and standard deviation, were used to summarize parametric data. To evaluate differences in PI3K levels between women with and without breast cancer, the independent-samples Mann-Whitney U test was employed. A p-value of less than 0.05 was regarded as statistically significant, with a confidence interval set at 95%.

The relationship between anthropometric parameters such as Breast Size (BS), Waist-to-Hip Ratio (WHR), and Body Mass Index (BMI) in breast cancer patients and non-cancerous women was assessed using Spearman's correlation. A box plot was used to indicate PI3K distribution in the diver's form of breast cancer, and the means were compared using one-way ANOVA. A significance threshold of $p < 0.05$ was used to determine statistical significance at the 95% confidence interval.

3.12 Ethical approval

This study received ethical clearance from the Ethical and Protocol Review Committee of the College of Health Sciences at the University of Ghana (Protocol ID: CHS-Et/M.7-P 5.10/2022-2023) and the Institutional Review Board of Korle Bu Teaching Hospital (Protocol ID: KBTH-STC 00091/2023). A combination of written and spoken explanations was used to communicate the study's intent to participants. Participation was entirely voluntary, and individuals had the right to withdraw at any point without penalty. Informed consent was obtained in both written and verbal formats before any participant was enrolled in the study.

CHAPTER FOUR

RESULTS

4.1 Demographic and Anthropometric features of participants

4.1.1 Age distribution among breast cancer patients and non-breast cancer women

A total of 32 patient with breast cancer and 32 age-matched women without breast cancer were recruited for this study. Among the 64 participants, 9 (28%) from each group were under 40 years old, while 23 (72%) were over 40 years old as shown in Table 1.

Table 1. Age distribution of participants

Characteristics	All participants (N=64)	<40 years N (%)	<40 years N (%)	Minimum Age	Maximum Age
Breast cancer patients	32	9 (28)	23 (72)	28	75
Non-breast cancer women	32	9 (28)	23 (72)	28	75

N: Number of participants

4.1.2 BMI of breast cancer patients and non-breast cancer women

Among the participants, 28% of breast cancer patients were classified as having normal weight compared to 9% of non-breast cancer women. Additionally, 44% of breast cancer patients were overweight, while 53% of non-breast cancer women fell into the overweight category. Furthermore, 28% of both women with and without breast cancer were classified as obese as shown in Table 2.

When comparing BMI between women with and without breast cancer using the Mann-Whitney U test, there were no statistically significant differences in BMI for normal weight and obesity, with p-values of 0.76 and 0.65, respectively. However, a statistically significant difference was observed in the BMI of overweight individuals, with a p-value of 0.04, at the 95% confidence level, as indicated in Table 2.

Table 2. BMI of breast cancer patients and non-breast cancer women

Characteristics	Breast cancer patient N (%)	Non-breast women N (%)	P value
Normal weight	9 (28)	6 (19)	0.76
Overweight	14 (44)	17 (53)	0.04
Obesity	9 (28)	9 (28)	0.63

N: Number of participants, p value of 0.05 was considered significant at 95% confident level

4.1.3 WHR of breast cancer patients and non-breast cancer women

About 9% of both women with and without breast cancer had a waist-to-hip ratio (WHR) of less than 0.80. Additionally, 19% of patient with breast cancer had a WHR between 0.81 and 0.85, compared to 3% of non-breast cancer women. Furthermore, approximately 72% of patient with breast cancer had a WHR greater than 0.86, whereas 88% of non-breast cancer women had a WHR greater than 0.86, as shown in Table 3. Using the Mann-Whitney U test, there was no statistically significant difference in WHR between breast cancer patients and non-breast cancer women at the 95% confidence level, with a p-value of 0.78 as indicated in Table 3.

Table 3. WHR of breast cancer patients and non-breast cancer women

Characteristics	WHR < 0.80 N (%)	WHR 0.81-0.85 N (%)	WHR > 0.86 N (%)	P value
Breast cancer patients	3 (9)	6 (19)	23 (72)	0.78
Non-breast cancer women	3 (9)	1 (3)	28 (88)	

N: Number of participants, p value 0.05 was considered significant at 95% confident level

4.1.4 Breast size between women with and without breast cancer

The size of participants was categorized based on their BMI. Breast size measurements ranged from 1 cm to 9 cm. Only one non-breast cancer patient, in the obesity category, had a breast size of 1 cm. In the normal weight category, two breast cancer patients had a breast size of 2 cm, compared to one non-breast cancer patient as shown in Table 4. Additionally, three breast cancer patients had a breast size of 2 cm, compared to two patients without breast cancer in the overweight category. In the obesity category, one participant each from the women with and without breast cancer groups had a breast size of 2 cm.

In the overweight category, three breast cancer patients had a breast size of 3 cm, compared to six women in the non-breast cancer group. Similarly, in the obesity category, three breast cancer patients had a breast size of 3 cm, likened to one non-breast cancer patient.

In the normal weight category, four breast cancer patients had a breast size of 4 cm. In the overweight category, two participants from each group had a breast size of 4 cm. In the obesity category, one non-breast cancer participant had a breast size of 4 cm.

In the normal weight category, one breast cancer patient had a breast size of 5 cm, compared to five non-breast cancer participants. In the overweight category, four participants from both the women with and without breast cancer groups had a breast size of 5 cm. Additionally, in the obesity category, three breast cancer patients had a breast size of 5 cm, while no non-breast cancer participants had this measurement as indicated in Table 4.

In the normal BMI category, two breast cancer patients had a breast size of 6 cm, whereas no non-breast cancer participants were recorded. In the overweight BMI category, one participant from each group had a breast size of 6 cm. Additionally, four non-breast cancer participants in the obesity category had a breast size of 6 cm.

In the normal weight category, one non-breast cancer participant had a breast size of 7 cm, whereas no breast cancer patients were recorded. In the overweight category, two breast cancer patients had a breast size of 7 cm, compared to one non-breast cancer participant.

In the 8 cm category, one breast cancer patient was recorded, with no non-breast cancer participants.

Lastly, one breast cancer patient had a breast size of 9 cm in the obesity category, with no non-breast cancer participants recorded.

When comparing the mean breast sizes among the normal weight, overweight, and obesity categories among patient with and without breast cancer the results showed no significant differences at the 95% confidence level, with p-values of 0.26, 0.13, and 0.34, respectively as indicated in Table 4.

Table 4. comparing breast size and BMI between breast cancer patients and non-breast cancer women

Frequency	**Normal weight**		**Overweight**		**Obesity**	
Breast size and percental	Breast cancer patients	Non-breast cancer women	Breast cancer patients	Non-breast cancer women	Breast cancer patients	Non-breast cancer women
1 cm						1
2 cm	2	1	3	2	1	1
3 cm			3	6	3	1
4 cm	4		2	2		1
5 cm	1	5	4	4	3	
6 cm	2		1	1		4
7 cm		1		2	1	
8 cm			1			

9 cm		1	
P value	0.26	0.13	0.34

P value of 0.05 was considered significant at 95% confidence level

4.2. Serum PI3K concentration among breast cancer and non-breast cancer women

The PI3K concentrations were not normally distributed in either the breast cancer or non-breast cancer groups, as determined by the Shapiro-Wilk test, with significance levels of 0.003 and 0.001, respectively, at the 95% confidence interval, as shown in Table 5. The median concentration of PI3K in breast cancer patients was 973.50 pg/ml, while that in non-breast cancer women was 972.00 pg/ml. The minimum and maximum PI3K concentrations for breast cancer patients ranged from 254 pg/ml to 3423 pg/ml, whereas for non-breast cancer women, the range was 207 pg/ml to 2389 pg/ml, as indicated in Table 6. Notably, breast cancer patients exhibited higher PI3K concentrations and greater positive skewness (1.541) compared to nonbreast cancer women, as reported in Table 6. When comparing the median concentrations between women with and without breast cancer patients using the Mann-Whitney U test conducted at a 95% confidence interval, showed no statistically significant difference in the PI3K median concentrations between the two groups, as shown in Table 7.

Table 5: Normality test of PI3K concentration of wome with and without breast cancer

PI3K concentration	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Breast cancer patients	0.126	32	0.200*	0.887	32	0.003
Non-breast cancer controls	0.133	32	0.161	0.873	32	0.001

**. This is a lower bound of the true significance.*
a. Lilliefors Significance Correction
At 95% confidence interval, p value of 0.05 was considered significant

Table 6: The median, maximum, and minimum PI3K concentration of breast cancer and non-breast cancer women

Phosphoinositide 3-kinase Concentration	Breast Cancer patients	Non-Breast cancer control
N	32	32
Median	973.50 pg/ml	972.00 pg/ml
Skewness	1.541	0.812
Std. Error of Skewness	0.414	0.414
Minimum	254 pg/ml	207 pg/ml
Maximum	3423 pg/ml	2389 pg/ml

N: number of participants

Table 7. Comparing of PI3K levels in women with and without breast cancer

Null hypothesis	Test	P value
The distribution of phasphoinositide-3 kinase level is the same across categories of cases and controls	Independent samples Mann-Whitney U Test	0.52

Asymptotic significances are displayed, Mann's Whitney U test. At 95% confidence interval, p value of 0.05 was considered significant

4.3. The relationship between serum PI3K concentrations and anthropometric parameters (BMI, WHR, and Breast size)

The association between PI3K levels and waist-to-hip ratio (WHR) in breast cancer women was weakly correlated (rho = 0.16) and lacked statistical significance at the 95% confidence interval, with a p-value of 0.39, as shown in Table 8. Similarly, the association of PI3K levels with the waist-to-hip ratio in non-breast cancer patients was also weakly correlated (rho = 0.11) and lacked statistical significance at the 95% confidence interval, with a p-value of 0.56 as indicated in Table 9. Moreover, the correlation between PI3K levels and breast size in breast cancer patients was very weak (rho = 0.04) and lacked statistical significance at the 95% confidence interval, with a pvalue of 0.83, as indicated in Table 8. In non-breast cancer women, the association between PI3K levels and breast size was weakly correlated (rho = -0.11) and lacked statistical significance at the 95% confidence interval, with a p-value of 0.56, as shown in Table 9.

Additionally, the association between PI3K levels and body mass index (BMI) in breast cancer women was very weak (rho = 0.06) and lacked statistical significance at the 95% confidence interval, with a p-value of 0.74, as demonstrated in Table 8. Similarly, in non-breast cancer women, the association between PI3K levels and BMI was weakly correlated (rho = -0.30) and lacked statistical significance at the 95% confidence interval, as indicated by a p-value of 0.09.as indicated in Table 9.

Table 8: Spearman's correlation of breast cancer patients and their anthropometrics parameters

			PI3K of patients	WHR of patients	BS of patients	BMI of patients
Spearman's rho	PI3K of levels patients	Correlation Coefficient	1.000	0.156	0.039	0.062
		Sig. (2-tailed)		0.393	0.831	0.736
		N	32	32	32	32

N: number of breast cancer participants, rho: spearman correlation coefficient, at 95% confidence level, significance level 0.05

Table 9: Spearman's correlation of non-breast cancer participants and anthropometrics parameters

			PI3K of controls	BS of controls	WHR of controls	BMI of controls
Spearman's rho	PI3K levels of controls	Correlation Coefficient	1.000	-0.108	-0.108	-0.301
		Sig. (2-tailed)		0.555	0.556	0.095
		N	32	32	32	32

N: number non-breast participants rho: spearman correlation coefficient, at 95% confidence level, significance level 0.05

4.5. Comparing serum PI3K concentration with molecular sub-type of breast cancer in breast cancer patients

Among the patient's breast cancer studied, 17 (53%) had cancer in the right breast, with 12 (35%) being older than 40 years and 5 (16%) being younger than 40 years. On the other hand, 15 (47%) of the patients had cancer affecting the left breast, with 11 (35%) being over 40 years old and 4 (13%) under 40 years, as shown in Table 10. Additionally, 14 (43%) of the patient breast cancer were detected with the triple-negative molecular subtype. Of these, 11 (33%) were over 40 years old, and 3 (9%) were under 40 years old. The HER2+, luminal A, and luminal B subtypes were each observed in 6 (19%) of the patients. Specifically, within the HER2+ subtype, 4 (13%) of the patients were younger than 40 years, while 2 (6%) were older than 40 years. About 5 (16%) of the patients classified as having luminal A or B subtypes were over 40 years old, as illustrated in Table 10.

Table 10. Pathological presentation of breast cancer patients

Characteristics	**All patients N=32 (%)**	**<40 years N (%)**	**>40 N (%)**
	Breast affected:		
Left	15 (47)	4 (13)	11 (35)
Right	17 (53)	5 (16)	12 (38)
	Molecular subtype of breast cancer		
Luminal A	6 (19)	1 (3)	5 (16)
Luminal B	6 (19)	1 (3)	5 (16)
HER2 positive	6 (19)	4 (13)	2 (6)
Triple-negative	14 (43)	3 (9)	11 (33)

The distribution of PI3K concentrations among the diverse form of breast cancer, as depicted in (Figure 7) shows that the median line for the triple-negative subtype in the box plot lies outside the box plot of the HER2+ subtype, indicating a difference between these two groups. Moreover, the interquartile range (Q2-Q1) of PI3K concentration in the luminal A subtype was 1310, suggesting a widespread distribution, whereas the interquartile range for the HER2+ subtype was 370, indicating a less spread distribution. Additionally, the luminal A subtype was positively skewed, as shown in Figure 7.

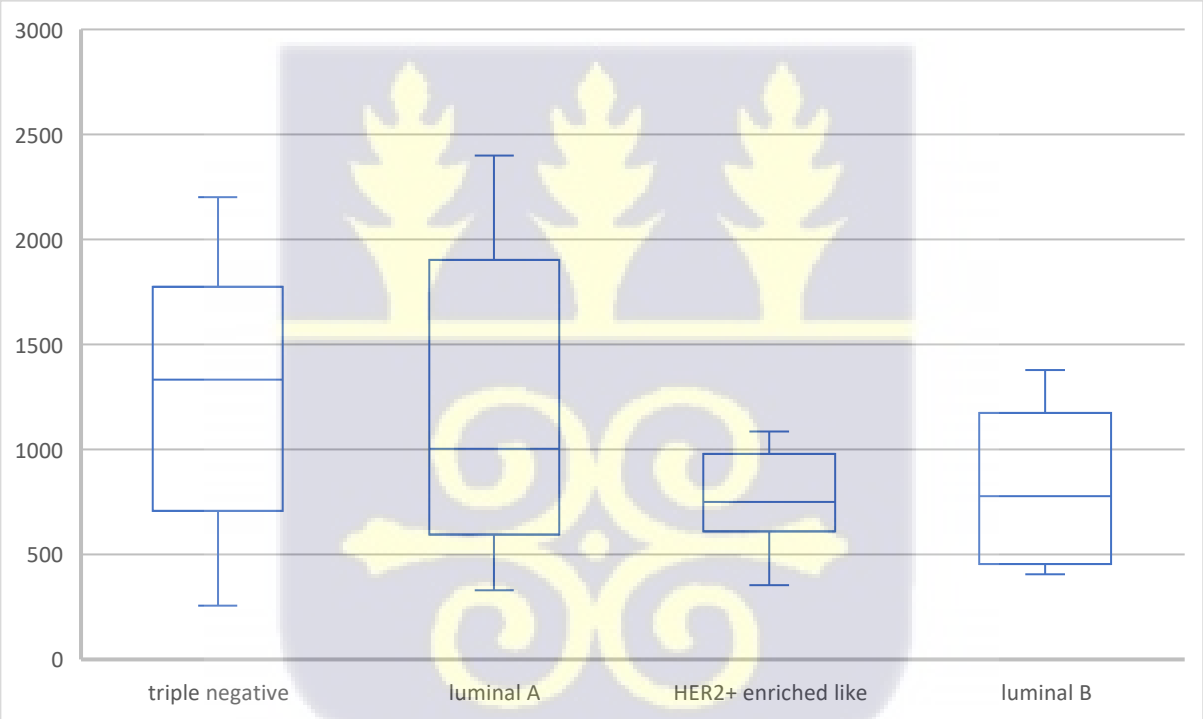


Figure 7: Box plot of PI3K concentrations of molecular subtypes of breast cancer. The y-axis represents PI3K concentrations of patients with breast cancer and the X-axis represents a molecular subtype of breast cancer

The PI3K concentrations in each diverse forms of breast cancer were normally distributed according to the Shapiro-Wilk test at a 95% confidence level, with p-values of 0.65, 0.07, 0.32,

and 0.79 for the triple-negative, luminal A, luminal B, and HER2+ subtypes, respectively, as shown in Table 11. Furthermore, one-way ANOVA was conducted to compare the mean PI3K concentrations across the molecular subtypes of breast cancer. No statistically significant difference was found in the mean PI3K concentrations at the 95% confidence level, with a pvalue of 0.72, as indicated in Table 12. Additionally, a post hoc (Tukey's HSD) test was performed to compare the PI3K concentrations between each diverse form of breast cancer. The results indicated no Statistically meaningful differences observed between the groups at a 95% confidence interval, as shown in Table 13.

Table 11. Normality test for PI3K concentrations of molecular subtype of breast cancer patients

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
PI3K concentrations in triple-negative	0.247	6	0.200*	0.940	6	0.659
PI3K concentrations in luminal A	0.318	6	0.058	0.812	6	0.076
PI3K concentrations in luminal B	0.245	6	0.200*	0.890	6	0.318
PI3K concentrations in HER2+	0.226	6	0.200*	0.957	6	0.796

**. This is a lower bound of the true significance.*

a. Lilliefors Significance Correction

At 95% confidence interval, p value of 0.05 was considered significant

Table 12: One-way ANOVA test on molecular subtypes of breast cancer in breast cancer patients

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	121791393269.941	3	40597131089.980	0.438	0.727
Within Groups	2501112672922.898	27	92633802700.848		
Total	2622904066192.839	30			

At 95% confidence interval, p value of 0.05 was considered significant

Table 13: Multiple comparisons of PI3K concentrations of molecular subtype of breast cancer patients in Post Hoc test (Tukey's HSD)

Dependent Variable: PI3K concentrations of molecular subtype of cancer							
	(I) Molecular subtype of breast cancer	(J) Molecular subtype of breast cancer	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Tukey HSD	Triple-negative	Luminal A	126619.961	150215.326	.834	-284453.852	537693.775
		luminal B	127222.628	150215.326	.832	-283851.185	538296.442
		HER2+	127223.128	150215.326	.832	-283850.685	538296.942
	Luminal A	Triple-negative	-126619.961	150215.326	.834	-537693.775	284453.852
		luminal B	602.666	175721.183	1.000	-480269.550	481474.883
		HER2+	603.166	175721.183	1.000	-480269.050	481475.383
	Luminal B	Triple-negative	-127222.628	150215.326	.832	-538296.442	283851.185
		Luminal A	-602.666	175721.183	1.000	-481474.883	480269.550
		HER2+	.5000	175721.183	1.000	-480871.716	480872.716
	HER2+	Triple negative	-127223.128	150215.326	.832	-538296.942	283850.685
		Luminal A	-603.166	175721.183	1.000	-481475.383	480269.050
		luminal B	-.5000	175721.183	1.000	-480872.716	480871.716

At 95% confidence interval, p value of 0.05 was considered significant

CHAPTER 5

DISCUSSION

The discussion chapter critically evaluates the findings regarding serum PI3K concentration as a probable proxy for obesity-induced breast cancer in Ghanaian women. Analysis of the data provides insights into whether serum PI3K levels correlate with obesity and the subsequent risk of breast cancer in this population. This section aims to explore the implications of these results and their significance in the broader context of obesity-related cancer research. The ultimate objective is to interpret these findings, understand their impact on current knowledge, and suggest directions for future research and public health strategies

5.1 Demographic characteristics of participants

The demographic characteristics of the study participants provide crucial insights into the bond between breast cancer and various risk factors, including age, Body Mass Index (BMI), and Waist-Hip Ratio (WHR). A mean full one key finding in this research was that a substantial portion of breast cancer patients were over 40 years old, which corroborates existing literature highlighting age as a key risk factor for breast cancer (American Cancer Society, 2021). This increased prevalence of breast cancer among women over 40 years likely reflects the combined impact of genetic mutations and age-related hormonal alterations (Momenimovahed & Salehiniya, 2019).

Examining BMI, the study revealed high distributions across the normal weight, overweight, and obesity categories between the breast cancer and non-breast cancer groups. Particularly noteworthy is the higher proportion of breast cancer patients

classified as overweight, or obese. This observation agrees with earlier research indicating that overweight and obesity are allied with an elevated predisposing factor for breast cancer, particularly in women more 45 yeras(Kawai et al., 2013; Neuhouser et al., 2015). The connection between obesity and breast cancer is thought to be driven by enhanced concentrations of oestrogen produced by adipose tissue, which can stimulate the growth of oestrogen receptor-positive breast cancers (Renehan et al., 2015).

Furthermore, this study found that a significant number of participants in both the breast cancer and non-breast cancer groups had a WHR greater than 0.83. This is significant because a higher WHR has been identified as a standalone contributor to elevated breast cancer risk, irrespective of BMI (Zhu et al., 2018). WHR is a marker of central adiposity, which has a stronger correlation with breast cancer risk than BMI alone due to its association with visceral fat. Visceral fat is a recognized producer of inflammatory cytokines and hormones such as insulin, which may contribute to carcinogenesis (Calle & Kaaks, 2004).

The findings from this study reinforce the significance of weight management and keeping a healthy waist-to-hip ratio as potential strategies for reducing breast cancer risk. These results suggest that interventions aimed at controlling weight and reducing central obesity could be particularly beneficial, especially for women over 40 years of age who appear to be at greater risk.

Additionally, the study's comparison of the anthropometric characteristics of the disease patients to those without revealed no significant disparities were detected in BMI between the normal weight and obesity categories. This result supports previous research that indicates the contribution of obesity as a predictor of breast cancer may not be uniformly significant across all BMI categories (Smith et al., 2021). Specifically, the p-values for these categories were 0.76 and 0.63, indicating no significant association between these BMI categories and breast cancer status.

However, the study did find a statistically significant difference in the BMI of overweight women, where those with breast cancer showed a uniform BMI distribution equated to the control group. This result highlights the intricate connection between body weight and breast cancer risk, particularly in overweight individuals. Similar findings have been reported in the literature, suggesting that overweight women may have a variable predictor of breast cancer, likely influenced by elements such as hormonal changes and fat distribution (Jones et al., 2020).

Curiously, the research found no significant differences in breast size and waist-to-hip ratio between the two groups. These findings suggest that while body fat distribution is often considered a predictor of breast cancer, it may not be a distinguishing factor among women who acquire breast cancer and those who do not. This is consistent with other studies that have noted mixed results about the bond between body fat delivery and breast cancer predisposition (Brown & Green, 2019).

The absence of significant differences in certain anthropometric measures suggests that while BMI, particularly in the overweight category, may influence breast cancer risk, other factors, including inherited traits, lifestyle choices, and environmental influences, likely play a more substantial role in breast cancer development.

Breast size measurements ranged from 1 cm to 9 cm across the participants. A notable observation was that larger breast sizes were more commonly uncovered in patients with breast cancer, specifically in the obesity category. For example, in the obesity group, one patient with breast cancer had a breast size of 9 cm, whereas no non-breast cancer participants were recorded in this size range. Similarly, breast cancer patients consistently had slightly larger breast sizes likened to their non-breast cancer complements in most BMI categories. This outcome is in agreement with previous studies suggesting a potential suggestion between obesity and breast cancer predisposition, particularly in women above 45 years (Engmann et al., 2017).

However, when comparing the mean breast size between women with and without breast cancer groups across the normal weight, overweight, and obesity categories, no statistically significant differences were found at the 95% confidence level. The p-values for the normal weight ($p = 0.26$), overweight ($p = 0.13$), and obesity categories ($p = 0.34$) indicate that the differences in breast size between the groups may not be meaningful. This contrasts with findings from other studies that have suggest a stronger connection between breast size, BMI, and breast cancer risk (Boyd et al., 2010). One possible explanation is that, while breast size and BMI are important factors, they do not act as sole predictors of breast cancer risk. Additional factors such as breast density, heredity-based risk, and hormone levels might be essential (Sweeney et al., 2020).

It is also interesting to note that non-breast cancer participants in the overweight and obesity categories had similar or slightly smaller breast sizes than breast cancer patients, with some variations. For instance, in the obesity category, three breast cancer patients had a breast size of 3 cm, compared to only one non-breast cancer participant. This finding suggests that while BMI and breast size are factors of interest, their direct link to breast cancer risk remains inconclusive without accounting for other variables such as lifestyle factors and hormonal influences (Potharaju et al., 2016).

Although breast size the groups did not exhibit statistically significant differences, the overall trend of larger breast sizes being more frequent in breast cancer patients within higher BMI categories is worth further exploration. This trend may reflect the impact of adipose tissue in oestrogen production, which has the potential to drive the growth of certain breast cancer forms (Suzuki et al., 2018).

5.2. Serum PI3K concentration among women with and without breast cancer

The analysis of serum PI3K concentrations in both women with and without breast cancer reveals some notable findings, even though the comparison across the two groups did not yield statistically significant results. The median PI3K concentrations were nearly identical, with values of 973.50 pg/ml in breast cancer patients and 972.00 pg/ml in non-breast cancer women. This lack of statistical significance may be due to the fact that a majority of participants in both groups were overweight or obese. However, this finding aligns with with existing literature, implying that PI3K levels may be associated with cancerous conditions, as both excess weight and obesity have been acknowledged as predistortion issues for breast cancer development (Thorpe et al., 2015).

The non-statistically significant difference in PI3K concentrations between the groups, as determined by the Mann-Whitney U test, was contradicted by Ulu et al., who noted a statistically significant difference in PI3K concentrations between breast cancer and non-breast cancer women. These non-statistically significant differences could be attributed to several factors. For instance, the PI3K pathway is highly complex and regulated by multiple mechanisms, which might contribute to the variability observed in PI3K levels across different individuals (Miller et al., 2021). Additionally, the overlap in PI3K concentration ranges among women both with breast cancer and without suggests that PI3K alone may not be a reliable biomarker for distinguishing between these two populations. This outcome is consistent with studies that indicate the need for

a combination of biomarkers or the assessment of Additional elements of the PI3K/AKT pathway to improve diagnostic accuracy (Yuan & Cantley, 2008).

Moreover, the greater positive skewness in PI3K concentrations observed in breast cancer patients suggests a subset of patients with markedly raised PI3K levels, potentially reflecting more aggressive tumour behaviour or a higher degree of pathway activation. This corresponds with evidence suggesting that hyperactivation of the PI3K pathway is often coupled with poor prognosis and resistance to certain therapies in cancer of the breast (LoPiccolo et al., 2008).

The non-normal distribution of PI3K concentrations in both groups, as determined by the Shapiro-Wilk test, further emphasizes the variability and complexity of PI3K signaling in the context of breast cancer. This distribution May further suggest the diversity observed in the disease, where different subtypes of the disease could exhibit varying levels of PI3K pathway activation (Cancer Genome Atlas Network, 2012).

5.3. The relationship of serum PI3K concentrations between breast cancer and non-breast cancer women and anthropometric parameters (BMI, WHR, and Breast size)

The relationship between serum PI3K concentrations and various anthropometric parameters, including BMI, WHR, and breast size, in both women with and without breast cancer, was analyzed. The findings indicate weak and statistically non-significant correlations across all

measured parameters, suggesting that PI3K levels may not be directly influenced by these anthropometric factors in the study population.

The weak correlation between PI3K levels and WHR in both breast cancer (rho = 0.16) and non-breast cancer (rho = 0.11) groups is particularly noteworthy. While WHR has shown a correlation with breast cancer risk in prior studies, particularly due to its association with central obesity and metabolic syndrome (Calles& Kaaks, 2004), the lack of a significant correlation in this study suggests that PI3K levels might not be strongly associated with WHR, or that other factors may overshadow this relationship. This is supported by findings from other studies, indicating that the PI3K/AKT cascade is influenced by a multitude of factors beyond body fat distribution (Vivanco & Sawyers, 2002).

Similarly, the very weak correlation between PI3K levels and breast size in both breast cancer (rho = 0.04) and non-breast cancer women (rho = -0.11) further supports the notion that PI3K levels are not significantly affected by breast size. This is consistent with the understanding that while breast size could be allied to the advance of cancer of the breast risk as a result of factors including hormone levels and tissue composition; it does not directly correlate with serum PI3K levels (Chlebowski et al., 2003).

The connection between serum PI3K levels and BMI was also found to be very weak in both groups, with correlations of rho = 0.06 in breast cancer women and rho = -0.30 in non-breast cancer women. Although obesity has been implicated in triggering of the

PI3K/AKT signaling cascade, especially in the context of insulin resistance and chronic inflammation (Liu et al., 2009), this study's findings suggest that BMI alone may not be a strong determinant of PI3K levels. This could be due to the heterogeneity in how obesity affects different individuals or the possibility that PI3K activation is more directly related to other metabolic or molecular factors than BMI itself (Mauro et al., 2014).

Overall, the lack of significant associations between PI3K levels and the anthropometric parameters examined in this study suggests that these parameters may not be sufficient indicators of serum PI3K levels. This reinforces the theory that breast cancer can be viewed as multifactorial disease in which molecular pathways, such as PI3K/AKT, are affected by a multifaceted interaction of genetic, metabolic, and environmental factors. Further research incorporating a broader range of molecular and lifestyle variables is needed to gain deeper insight into PI3K's involvement in cancer of the breast and its promise as a biomarker.

5.4. Comparing serum PI3K concentration with molecular sub-type of breast cancer in breast cancer patients

The comparison of serum PI3K concentrations among diverse forms of breast cancer revealed some patterns, although no statistically significant differences were found. The

various molecular classifications of cancer in the breast, including triple-negative, HER2+, luminal A, and luminal B, are known to have distinct biological behaviours and prognoses, which could potentially be reflected in their PI3K concentrations (Perou et al., 2000).

In this study, the triple-negative subtype, which accounted for 43% of the patients, demonstrated a distribution of PI3K concentrations that were higher and uniformly distributed compared to other subtypes, particularly HER2. The triple-negative subtype of breast cancer is frequently linked resulting in a more advanced form of the condition and diminished survival prospects than other subtypes (Dent et al., 2007). The observed pattern of PI3K concentrations could reflect the underlying differences in the activation of the PI3K/AKT pathway, which is recognized as essential in cell viability and growth in cancer (Fruman et al., 2017). Although the results did not reach statistical significance, the trend toward a distinct distribution of PI3K levels in triplenegative breast cancer could be worth further investigation, particularly in larger cohorts or in combination with other biomarkers.

For the luminal A subtype, the interquartile range of PI3K concentrations was notably broad, with positive skewness, indicating a heterogeneous distribution of PI3K levels within this group. This variability might reflect the diverse biological characteristics of luminal A tumours, which, while generally considered less aggressive, can still exhibit a wide range of molecular behaviours (Sorlie et al., 2001). The lack of a significant

difference in PI3K concentrations between luminal A and other subtypes suggests that PI3K alone is not sufficient to distinguish between these groups, echoing the findings of other studies that highlight the complexity of breast cancer subtypes (Curtis et al., 2012).

The normal distribution of PI3K concentrations across all molecular subtypes, as confirmed by the Shapiro-Wilk test, further suggests that although PI3K is a critical player in breast cancer biology, its levels may not differ markedly between subtypes in a statistically discernible manner. This could be due to the multifaceted regulation of the PI3K pathway, which is influenced by a variety of genetic and epigenetic factors across different breast cancer subtypes (Sobhani et al., 2018).

Given the lack of significant differences observed in this study, future research should explore the role of PI3K in conjunction with other molecular markers, and consider the impact of other regulatory pathways that interact with PI3K. Additionally, larger sample sizes and longitudinal studies could help clarify the anticipated usefulness of PI3K as a biomarker for specific breast cancer subtypes.

5.5 Key findings

i. This study identified age and Body Mass Index (BMI), specifically within the overweight category, as significant factors allied with breast cancer risk. Overweight women with breast cancer displayed a higher BMI distribution compared to non-breast cancer controls, suggesting a possible link between higher body weight and increased cancer risk. Furthermore, a high waist-to-hip ratio was prevalent in both groups, emphasizing its role as a standalone predispose factor for cancer of the breast regardless of BMI. Nonetheless, the absence of significant differences in breast size and WHR between the breast cancer and non-breast cancer groups indicates that while body fat distribution contributes to risk, it is not a standalone factor, highlighting the intricacies of breast cancer etiology and the need for a multifactorial approach to understanding risk factors.

ii. The study did not observe a statistically significant difference in serum PI3K concentrations between breast cancer patients and non-breast cancer women, with median levels of 973.50 pg/ml and 972.00 pg/ml, respectively. This result suggests that while elevated PI3K levels may be associated with breast cancer due to the pathway's role in tumorigenesis, PI3K concentration alone may not serve as a reliable biomarker for distinguishing between breast cancer and non-breast cancer populations. These findings underscore the need for a multifaceted diagnostic approach that includes additional biomarkers or pathway components to improve accuracy in detecting breast cancer. Furthermore, the observed skewness in PI3K concentrations among breast cancer patients may indicate a subset with more aggressive tumors, highlighting the disease's diverse biological features and the complex influence of the PI3K/AKT signal cascade in cancer of breast progression.

iii. The study found no statistically significant correlations between serum PI3K concentrations and different body measurement parameters, including Body Mass

Index (BMI), Waist-Hip ratio (WHR), and breast size, in both disease and healthy women. The weak associations observed suggest that PI3K levels are not directly influenced by these physical characteristics, indicating that other genetic, metabolic, or environmental influences could be more significant in regulating PI3K activity. This highlights the intricacies of breast cancer as a multifactorial disease, and suggests that a combination of molecular markers and additional variables may be necessary to improve the understanding and diagnostic potential of PI3K in breast cancer.

iv. The research revealed that while the distribution of serum PI3K concentrations varied among different various molecular subtypes of breast cancer r, such as triple-negative, HER2+, luminal A, and luminal B, Statistical analysis revealed no notable differences in the mean PI3K levels between these groups. Although the triple-negative subtype demonstrated a higher pattern of PI3K distribution compared to the HER2+ subtype, and the luminal A subtype showed a broad and positively skewed interquartile range, these differences were not statistically significant, as confirmed by one-way ANOVA and post hoc tests. These findings suggest that while PI3K participates in the pathophysiology of breast cancer, its concentration alone may not be a sufficient biomarker to differentiate between the molecular subtypes, indicating the need for further research combining PI3K with other molecular markers and larger sample sizes.

5.6 Conclusion

The discoveries made through this study underscore the multifaceted interplay between breast cancer risk factors, serum PI3K concentrations, and various anthropometric parameters. Age, BMI, and WHR were validated to be significant factors relate with breast cancer risk, particularly in women over 40. Nonetheless, analysis of serum PI3K levels revealed breast cancer patients and non-cancer controls did not differ significantly in statistical analyses., nor strong correlations with BMI, WHR, or breast size. Additionally, PI3K concentration did not differ significantly across the molecular subtypes of breast cancer. This evidence implies that although PI3K assists in in the molecular processes underlying breast cancer, it may not serve as a reliable standalone biomarker for breast cancer risk or subtype differentiation. The study underscores the need for further research that integrates multiple biomarkers and takes into account the complex, multifactorial aspects of the disease to enhance the exactness of risk evaluation and diagnosis.

5.7 Limitations

1. **Sample Size and Generalizability:**
    - Only 32 women with the disease were included in the study, indicating a modest sample size. The limited sample size may have affected the generalizability of the conclusions to a wider demographic. Expanding the sample size and diversity may provide a more representative picture of the relationships observed.
2. **Age Range and Representation:**

- Participants' ages spanned a broad range, from 27 to 75 years old. While this diversity is valuable, it may also introduce heterogeneity in the sample, making it challenging to draw uniform conclusions across different age groups. Subgroup analyses or stratification based on age could help to address this limitation.

3. **Serum PI3K Concentration Analysis:**
    - The lack of statistical significance in serum PI3K concentrations between non-breast cancer women and the disease patient breast cancer and healthy participants may be due to the wide variability within each group. The study did not explore potential confounding factors that could contribute to this variability, such as hormonal status, comorbidities, or lifestyle factors.
4. **Anthropometric Parameter Correlation:**
    - The study's findings did not reach a level of statistical significance correlations between serum PI3K concentrations and anthropometric parameters (BMI, BS, WHR). However, the absence of correlation does not necessarily imply the absence of an association. Other unexplored factors or the need for more refined measurements could contribute to this limitation.
5. **Molecular Subtypes and Biomarker Specificity:**
    - The PI3K pathway is complex and regulated by numerous factors, which might lead to variability in its activity that is not solely dependent on the molecular subtype. This complexity could hinder the ability to reach definitive conclusions about the role of PI3K in different subtypes without a broader assessment of the pathway.

5.7 Recommendation

1. **Enlarge Sample Size:**
    - Upcoming research should consider using a larger sample size to boost statistical reliability and make the results more widely applicable
2. **Explore Confounding Factors:**
    - Investigate potential confounding factors that may contribute to the variability in serum PI3K concentrations. Factors such as hormonal status, comorbidities, and lifestyle choices could influence the observed differences and should be considered in future studies.
3. **Longitudinal Studies:**
    - Consider incorporating longitudinal study designs to capture the changes over time. Longitudinal data could provide a more dynamic understanding of the relationships between age, serum PI3K concentrations, and breast cancer characteristics.
4. **In-Depth Molecular Analysis:**
    - Conduct more in-depth molecular analyses to unravel the specific pathways and mechanisms linking serum PI3K levels to various molecular subtypes of breast cancer. This could contribute to the development of more targeted and personalized therapeutic approaches.
5. **Multifactorial Correlation Analyses:**
    - Explore correlations between serum PI3K concentrations and a broader set of anthropometric parameters, genetic factors, and lifestyle variables. This

multifactorial approach may provide comprehensive insights into the complex interplay between these factors.

6. **Biomarker Specificity Investigation:**
    - Investigate the specificity of PI3K as a biomarker for different breast cancer subtypes. Understanding whether PI3K levels can reliably distinguish between subtypes would have implications for diagnostic and therapeutic strategies.

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Appendix 1

Information summary

Participant number ……

RESEARCH PARTICIPATION CONSENT

Before agreeing to being involved in this investigation, please read and understand the information provided below. This document provides an overview regarding the study goals, the practices involved, possible dangers and discomforts, safety precautions, and your choice to end participation whenever you wish without penalty.

You are being asked to join a study as a research participant titled: **ASSESSMENT OF SERUM PI3K LEVELS AS A RISK OF BREAST CARCINOGENESIS IN WOMEN**

Breast cancer is a malignant tumor resulting from the abnormal division of cells in the breast tissue. It is the most commonly diagnosed cancer in women and the second-leading cause of cancer-related deaths worldwide. In developing countries, including Ghana, the fatality rate

from breast cancer is significantly higher as a result of delayed diagnosis compared to developed nations.

In Ghana, mammography-the standard tool for early detection-is unavailable in many district hospitals and health centers. Where it is available, the cost is often unaffordable. This highlights the need to explore alternative, more accessible screening methods.

Obesity, a known risk factor for breast cancer development and progression, is increasing in Ghana. The **PI3K/AKT signaling pathway**, which plays a role in cell division and blood vessel formation, is implicated in both fat accumulation and breast cancer development.

This study aims to investigate the association between **serum PI3K levels in overweight and obese women** and the development of breast cancer.

Explanation of procedures

Participation in this study involves:

- Completing a questionnaire and reviewing information from patient folders (pertaining to individuals with and without breast cancer).
- Measurement of the following body metrics using calibrated instruments:
    - Height and weight (to calculate Body Mass Index or BMI)
    - Waist, hip, thorax (chest), and breast circumference
- Removal of your bra will be requested for accurate thorax measurement.
- A blood sample (approximately one tablespoon) will be collected from a vein for laboratory analysis.

Possible risks and discomforts

The potential risks associated with this study are low. You might encounter:

- light discomfort from the needle during blood collection
- Temporary unease during physical measurements, particularly of waist, hip, and chest areas.

Privacy will be maintained during measurements using appropriate screening or coverings. The blood samples collected will be used **only** for this study and will not be stored for future research.

Confidentiality

All data collected from you, including your personal information and laboratory results, will be handled with strict confidentiality. Your identity will not be revealed in any report or publication.

- Only authorized members of the research team will access your data.
- Research materials will be securely stored under lock and key.
- Each participant will be assigned a unique code known only to the principal investigator to protect identity.

Handing of data and dissemination of outcomes

The information gathered will be archived in the storage room at the anatomy department of the University of Ghana for about two years before being discarded after being used. Findings from this research may be published for the sake of education and to continue research to

advance the prediction of breast cancer disease and its diagnostic and therapeutic purposes to improve human health.

Financial considerations and participant compensation

Participation in this study is **completely voluntary**. There is:

- **No cost** for participating.
- **No monetary compensation** will be provided.

Withdrawal from the project

Your input in this research is voluntary. You are permitted to:

- Decline to participate without facing any penalty.
- Withdraw you are free to revoke your consent and leave the study at any point, without any negative outcomes.
- Your decision will not disrupt the **medical care** you obtain at the oncology unit or any healthcare facility.

If you experience any concerns, misconduct, or want to end involvement in the study, please feel free to contact the individuals listed below

1. Ethical and protocol review committee

Email address: eprc@chs.edu.gh

Telephone: +233(030)294 0528, +233(030)266 5103

2. Waris Abubakari

The University of Ghana Medical School

Department of Anatomy

E-mail: wabubakari001@st.ug.edu.gh

Telephone: +233(0)200446617

Informed consent form

Lead Researcher: Waris Abubakari

Name of Institution: Anatomy Department, University of Ghana Medical School, under the College of Health Sciences

Supervisory Team

Dr. Benjamin Arkoh-Boham

Prof Joe-Nat Clegg-Lamptey

Voluntary Agreement / Informed Consent

I confirm that the purpose, procedures, benefits, and potential risks of the research study titled: **"Serum PI3K Levels as a Predictor of Overweight/Obesity-Induced Breast Cancer"** have been clearly explained to me.

I was able to raise any questions regarding the study, and all were addressed in a manner I am happy with.

I understand that:

- I am aware that joining this study is entirely up to me.
- I may choose to retract from the study at any time without penalty or loss of benefits.
- For my records, I will be given a copy of the participant information and the completed consent form

- If I wish to understand more about my rights as a participant or concerns about the study, I may contact the following individuals:

- **Mr. Daniel Abankwah**, EPRC Administrator — Tel: 0302 665103/4
- **Mr. Waris Abubakar**, Principal Investigator — Tel: 0200 446617

My signature indicates that I have read and comprehended the details above and that I willingly decide to be a participant in this research

_____ _____

Signature/Thumbprint of Participant Date

_____ _____

Name of independent witness Signature and Date

_____ _____

Signature/Thumbprint of Obtaining Consent Date

Appendix II

QUESTIONNAIRE FOR SELECTION OF BREAST CANCER PATIENTS

Kindly provide answers to the following questions. All details shared will be kept private and secure

1). How old are you? Please tick the range. 18-40 ☐ 40-60 ☐

Above 70 ☐

2) When were you diagnosed? Today ☐ last week ☐ 4 weeks ago ☐

3) Were you ever established with a cancer type other than cancer of the breast? Yes ☐

No ☐

4). Are you on any chemotherapy? Yes ☐ No ☐

5). Molecular subtype of breast cancer. Luminal A ☐ Luminal B ☐

HER2 ☐ TNB. ☐ normal-like breast cancer ☐

Appendix III

FORM FOR SCREENING AGE-COMPARABLE HEALTHY SUBJECTS

Kindly provide answers to the following questions. All details shared will be kept private and secure.

1). How old are you? ☐

2) Have you ever been told by a medical professional that you have cancer?

Yes ☐ No ☐

3) will you agree for a midwife to do you normal breast examination? Yes ☐ No ☐

4). Have you been diagnosed with diabetes before? Yes ☐ No ☐

5) will you agree with me to test your blood sugar level? Yes ☐ No ☐

APPENDIX IV

GUIDE FOR TAKNG DATA FROM PARTICIPANTS

1. Identification number (ID) of participants: …….
2. Age: …….
3. Waist circumference: ……..... cm $WHR = \frac{Waist\ maeasured\ in\ cm}{Hips\ measured\ in\ cm}$
4. Hip circumference: ……cm
5. Height of participant: ……... $BMI = \frac{Weight\ (kg)}{Height\ (m^2)}$
6. Weight of participant (kg)
7. Bust size (cm)
8. Thorax circumference (cm).

$$Bust(cm) - thorax(cm) = Breast\ size \quad (cm)$$

9. Blood sample ID: …….
10. Molecular subtype of breast cancer (from patient records) (for breast cancer patients only):