ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF MEDICAL BIOCHEMISTRY

ASSESSMENT OF THE RELATIONSHIP BETWEEN SERUM ESTRADIOL, PROGESTERONE AND LIPID PROFILE LEVELS AMONG BREAST CANCER PATIENTS BEFORE AND AFTER HORMONAL THERAPY AT TIKUR ANBESSA SPECIALIZED HOSPITAL, ETHIOPIA.

ETAGEGN TADESSE (BSc)

A Research Thesis submitted to the School of Graduate Studies of Addis Ababa University, Department of Biochemistry in partial fulfillment of the requirements for Degree of Master of Science in Medical Biochemistry.

July, 2020

Addis Ababa, Ethiopia

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This is to certify that the dissertation prepared by Etagegen Tadesse entitled, **''assessment of the relationship between serum estradiol, progesterone and lipid profiles level among breast cancer patients before and after hormonal therapy at Tikur Anbessa Specialized Hospital, Ethiopia"** is submitted in partial fulfillment of the requirements for the degree "Master of Science in Medical Biochemistry" in the department of Biochemistry fulfill with regulations of the university and meets the accepted standards with respect to originality and quality.

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Acronyms and abbreviations

ANOVA	Analysis of Variance
DNA	Deoxy Ribonucleic Acid
ER	Estradiol Receptor
ER-	Estradiol Receptor Negative
ER+	Estradiol Receptor Positive
ER-α +	Estradiol Receptor Alpha Positive
ER-β +	Estradiol Receptor Beta Positive
PgR	Progesterone Receptor
PgR-	Progesterone Receptor Negative
PgR+	Progesterone Receptor Positive
SHBG	Sex Hormone Binding Globulin
TASH	Tikur Anbessa Specialized Hospital
TC	Total Cholesterol
TG	Triglyceride
LDL-C	Low Density Lipoprotein Cholesterol
HDL-C	High Density Lipoprotein Cholesterol
LPL	Lipoprotein Lipase
VLDL	Very Low-Density Lipoprotein
HTGL	Hepatic Triglyceride Lipase
HLPL	Hepatic Lipoprotein Lipase
BMI	Body Mass Index

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Abstract

Introduction: Most of breast cancers are induced by abnormal levels of estrogen, progesterone, and changes in lipid profiles. Cancer cells metabolize lipids unlike normal cells and affected by steroid hormones. Estrogen or estradiol controls metabolism of lipids and it regulates rate of progesterone metabolism. Progesterone regulates the effect of estradiol.Tamoxifen and armidex are drugs that block estrogen action on its receptors or decrease estrogen levels. Although, they change progesterone and lipid concentrations in breast cancer patients this may induce adverse effects like hyper /hypo estrogenic properties and lipidimea and other diseases as secondary complications. Thus, assessment of steroid hormones and lipid profile levels are necessary for breast cancer patients, but in Ethiopia, it has not yet been studied and used for treatment.

Objective: - To assess the relationship between serum estradiol, progesterone and lipid profiles level among breast cancer patients before and after hormonal therapy at Tikur Anbessa Specialized Hospital.

Methods: Cross-sectional study design was used to assess the relationships between serum estradiol, progesterone and lipid profile levels in breast cancer patients before and after hormonal therapy by comparing with healthy controls. Convenient sampling method was used to select 120 participants. Immunochemistry analyzer was used to measure steroid hormone levels and lipids were measured by chemistry analyzer. Data analyzes were done by independent sample t-test, Oneway ANOVA and Pearson‘s correlation coefficient. P-value < 0.05 was taken as statistically significant.

Results: In breast cancer patients without medication significantly lower mean serum progesterone, TC and HDL-C levels at ($p<0.05$) and insignificantly higher estradiol, TG and LDL-C levels at ($p>0.05$) were observed when compared with healthy controls. Significantly higher mean serum TC, LDL-C, TG, HDL-C levels ($p<0.05$) and insignificantly higher progesterone and lower estradiol levels were found in breast cancer patients with medication than without medication.

Conclusion: Synthesis of lower serum progesterone, HDL-C and higher serum estradiol levels were correlated in breast cancer patients. Armidex and Tamoxifen medications inhibit the levels of steroid hormones and lipid profiles through unclear mode of action. Thus, the change in these hormone and lipid levels may lead to other diseases as secondary complications.

Key words: Breast cancer, estradiol, progesterone, lipid profile, Tamoxifen, Armidex

1. Introduction

1.1. Over View of Breast Cancer

Cancer is a group of diseases characterized by uncontrolled growth and spread of abnormal cells. If the spread is not controlled, which result in death (Abate *et al.*, 2016). About 18.1 million new cancer cases and 9.6 million cancer deaths were estimated in 2018 (Bray *et al.*, 2018). However, in 2012, new cases were only 14.1 million and 8.2 million cancer-related deaths had occured (Solomon and Mulugeta, 2019). In 2030, it is expected that the numbers of new cases probably will increase by 46% from the cases reported in 2018 (Egue *et al.*, 2019).

Breast cancer is the most commonly diagnosed cancer and the major cause of deaths worldwide, which account 2.1 million new cases and 626,679 deaths of the total cancer in 2018 (Bray *et al*.,2018). It is the most common malignancy among women throughout the industrialized world (Ali, 2014). In developed countries, the incidence rates of many cancers are generally two to three folds higher than developing countries. However, the differences between mortality rates are smaller. Because, in developing countries higher case of death are estimated for many cancer types (Bray *et al*., 2018), 60% of the deaths are estimated to occur in these countries. Because of the majority of these cases are diagnosed in later stages (Chauhan *et al*., 2016), due to these countries face resource and infrastructure limitation that challenges the goal of improving breast cancer outcomes by early detection, diagnosis, and treatment (Shah *et al.*, 2014).

In Africa, the number of new cases 1.06 million in 2018 (5.8% of the total) and the number of deaths 693,000 (7.3% of the total) were estimated (Bray *et al.*, 2018). Sub-Saharan Africa 94,000 women developed breast cancer and of which 48,000 were died in 2012 (Brinton *et al*., 2014). Ethiopia is one of the Sub-Saharan Africa countries,that the trend of breast cancer in the last 16 years; (1997-2012) were 3460 (prevalence=20.8%) new cases were registered among the total number of all new cancer cases registered in Tikur Anbessa Specialized Hospital (TASH) (Abate *et al.*, 2016). Based on recent report by Hadgu *et al.*, (2018), annual incidence and mortality rate of breast cancer accounted for 34% of female cancer cases, followed by cervical cancer at 16%.

Breast carcinomas are classified into five sub-types: - luminal A, luminal B, human epidermal growth factor receptor 2-enriched, triple-negative and normal-like based on molecular characterizations:

- Lumina A are included as estrogen receptor positive(ER+) and/or progesterone receptor positive (PgR+), human epidermal growth factor receptor 2-negative and low proliferation marker Ki67. The cancer is low-grade and tends to grow slowly.
- Luminal B also contains (ER+ and/or PgR+, either human epidermal growth factor receptor 2-positive or negative and with high levels Ki67).These two breast cancers originate in luminal cells lining the breast‘s milk ducts and proteins on the cell surfaces reach out and consume estrogen, which promotes the growth of the tumors (Hammond *et al*., 2010).
- Human epidermal growth factor receptor 2-enriched carcinoma cells can express either or both estrogen and progesterone receptors (ER and PgR). Amplified or overexpressed human epidermal growth factor receptor -2 gene amplification and account for 15% to 25% of all breast cancers. It is genomically diverse also usually aggressive disease with a poor prognosis and a shorter overall survival than other sub groups (Abramovitz *et al.*, 2016).
- Triple-negative breast cancer lacks the expression of ER, PgR, and absence of Human epidermal growth factor receptor 2-enriched overexpression or gene amplification. It has worst prognosis, tend to relapse early and more frequently and short progress free survival time (Mina *et al*.,2017; Jaglanian and Tsiani, 2020) , and
- Normal-like breast cancer is similar in profile to luminal A disease. But its prognosis is slightly worse than luminal A cancer (Breast Cancer Org., 2020).

Several available regional data sets in Africa determined that most breast cancers are ER+ (Hammond *et al*., 2010). Although, in Ethiopian Luminal B tumors of breast cancer patients exhibit highly proliferative and ER + tumors represent 65% of the cases (Hadgu *et al*., 2018).

Higher levels of estradiol and lower levels of progesterone induce its mitogenic effects in breast cells. As a consequence, they induce breast cancer cells, which contain large number of hormonal receptors that are sensitive to imbalanced hormone levels. This refers to as hormonal sensitive breast cancer/hormonal receptor positive breast cancer (NCI, 2017). The growth of many breast tumors is estrogen-dependent and approximately 70-80% of all breast cancers are hormone sensitive (Komen, 2019; GSN, 2016; Buijs *et al.*, 2008). It is classified into ER+ and/or PR+, in breast tissue ER and PgR) are broadly studied markers (Kome, 2017). The hormonal receptor status allows to distinguish four subgroups of breast cancers such as: 65% are ER+PgR+, 13% are ER +PgR-,2% are ER-PgR+, and ER-PgR- (Breast Cancer Org, 2020; Effi *et al*., 2017).

Although, malignant cells are metabolize lipids in different way from the normal cells and the change of lipid profile levels may be the result of abnormal lipid metabolism, which associated with tumor pathogenesis and tumor host interactions (Shah *et al*., 2008). Mammary tissue is rich in lipids **(**Nayak *et al*., 2016). The enhancement of estrogen activity can derive from change of lipid profile. This indicated that lipids are closely linked to steroid hormones (Pecks *et al*., 2016). Alghough, estradiol regulates the rate of progesterone metabolism that arises from cholesterol (Miller and Mclean, 1987). Low HDL-C and sex hormone levels may stimulate growth of epithelial and stromal tissues, as a result may increase cellular proliferation in the breast tissue (Flote *et al.*, 2015). The influence of the progestogens on the estrogen effects on the lipid profile is dependent on the progestogen androgenicity (Callejon *et al*., 2009). Hormonal therapies substantially reduce morbidity and mortality in patients with breast cancer patients that express ER and/or PR (Hammond *et al.*, 2010; Althuis *et al*., 2004). In addition, the changeof plasma lipid profile levels in breast cancer patients are regressed by effective treatment of the tumor (Shah *et al*., 2008). Stopping the action of estrogen in the breast is a known therapeutic approach for preventing recurrence (ASCO, 2019). Tamoxifen is a drug which blocks ER in breast cancer cell via stopping estrogen from binding to the cancer cells. Armidex is a drug which decrease estrogen levels and deactivate the aromatase complex, (Breast Cancer Org., 2020; NBCF, 2016; Coscia *et al*., 2017).

1.2. Literature Review

1.2.1. Serum Estrogen Hormone and Breast Cancer Risk

Estrogens are major natural steroid hormones, which is important in sexual development and others body function (Hussain *et al.,* 2013). It regulates the growth and differentiation of mammary cells and promotes the development of breast cancer (Jamall *et al.,* 2010; YU *et al.,* 2003). Its regulation mediates by estrogen receptor (ER) in nuclear and extranuclear compartments (M´arquez *et al.,* 2006),

1.2.1.1. Forms of Estrogen Hormone

There are several forms of estrogen that can attach to the ER such as estrone, estretrol, estriol, estradiol, which are with different amount of time it stays bound to receptors, affinity and strength of the response (Fuentes & Silveyra, 2019). The more intoxicating/potent of the estrogen, the longer the binding time, a weak potency of estrogens are providing a low and safe to stimulate the cell. However, strong estrogens have more effect to stimulate the receptor in the cell (Taylor and BellTaylor, 2009). Estrone is a type of estrogen formed primarily in adipose tissue and found in the body after menopause. Estriol and estretrol are the weakest estrogen, found in the body primarily during pregnancy (Kolan, 2014). However, the word estrogen is commonly used to refer as estradiol, due to its physiological relevance and predominance during reproductive years (Samavat & Kurzer, 2015)

1.2.1.2. Serum Estradiol and Breast Cancer Risk

Estradiol is one of the most potent forms from others estrogens and plays an important role in maintaining the health of tissue in the body (ZRT Laboratory, 2014; Yaghjyan, 2011). Serum levels and estrogenic activity of estradiol is higher and strongest. Thus, estradiol stimulates ER (Taylor and Bell-Taylor 2009), which affects the targeted tissues in the breast through interacting with two nuclear receptors such as ER-α and ER-β (Palmisano *et al*, 2018; GSN, 2016). Although, it's exerted a rapid action through membrane-localization of ERα and ERβ occur in tumor environments. In cancer cells,

membrane initiated ERα signaling mediates the proliferative effects of estradiol (Acconcia and Marino, 2011; Platet *et al.*, 2004).

There are enzymatic pathways responsible for deactivation and removal of estradiol from the body. Deactivation of estradiol including biotransformation to less reactive estrogen such as estrone, estriol, and 2-hydroxyestrone. Single nucleotide polymorphism gene is responsible for encoding the enzyme necessary to convert estradiol to less active estrogen metabolites. This gene can change gene expression and enzyme function and also can compromise these biochemical processes and increased breast cancer risk (GSN, 2016; Taylor and Bell-Taylor, 2009). Estradiol associated with the development and progression of breast cancer (Travis and Key, 2003). Althgough, It is plays significant role in maintaining health, but when certain catechol metabolites are forming in excess and not eliminated properly it can also have the opposite effect (ZRT Laboratory, 2014). It is increase proliferation of breast epithelium and stroma cells, increase the chances of mutation in rapidly proliferating epithelium and the delicate hormonal balance of breast tissue cells (Velloso *et al.*, 2017; Atoum *et al.*, 2014; Yaghjyan, 2011). Higher exposure of estrogen concentration is more susceptible in the formation of breast cancer (Ali, 2014).

1.2.1.3. Estrogen Metabolism and Breast Cancer

Estrogen metabolism takes place primarily in the ovaries especially membrana granulose and luteinized granulosa cells (Hussain *et al.*, 2013). High amount of estrogens (estradiol and estrone) are producing by ovaries in cyclical monthly patterns response to the pituitary-derived luteinizing and follicle stimulating hormones in premenopausal women (figure.1) (Yaghjyan, 2011; Hammond *et al.*, 2010; Buijs *et al*, 2008; Travis and Key, 2003; Feigelson and Henderson, 1996).

However, in postmenopausal women estrogen metabolism takes place in peripheral tissues (figure.1). The conversion of estrone is the major source of estradiol. It is produced mostly through the peripheral conversion of androgen precursors, predominantly androstenedione, in extraglandular tissue such as adipose tissue (figure.2). Adipose tissue is the main source of estrogen (Hussain *et al.*, 2013). In these women, the

ovaries produce few or no estrogenic compounds and homeostatically estrogen production is not controlling by pituitary gonadotrophins (Travis and Key, 2003). However, androgen (testosterone and androstenedione) are precursors of estrogen, which produced by the adrenal gland and converted to estradiol and estrone by an enzyme that catalyzes the rate-limiting step in estrogen biosynthesis called cytochrome P450 aromatase (aromatase) in peripheral tissues through aromatization. Because, the gene cytochrome P450cl9α are accumulates in the peripheral tissues (figure.1 and 2) (Dixon, 2014; Hammond *et al.*, 2010; Santen *et al*., 1999). These process is the most important source of estrogens in the breast tissue (Yaghjyan, 2011). The physiologic origins adrenal or ovarian of the circulating androgens in women are an important risk factor for breast cancer (figure.1) (Kaaks *et al.*, 2005).

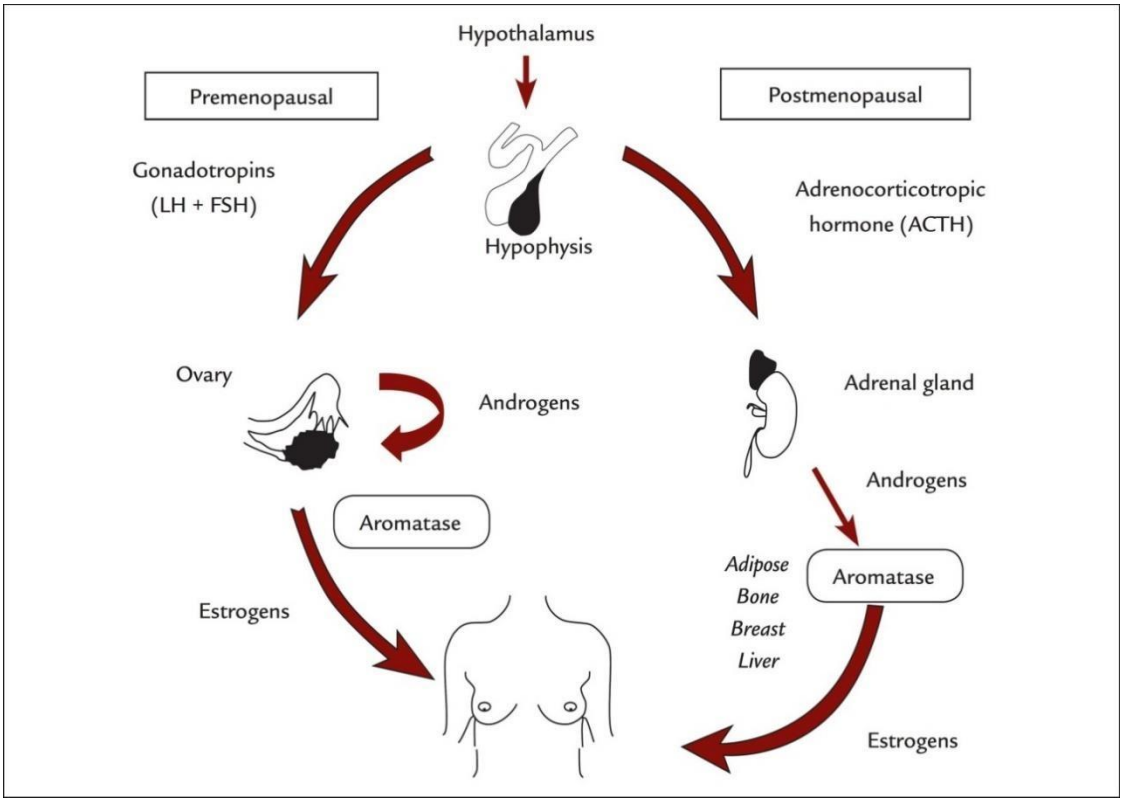

Figure 1: premenopausal and postmenopausal synthesis of estradiol from different organs and pathway. Adopted from (Barros-Oliveira et al., 2017). In premenopausal breast cancer patients estradiol is produced by ovaries in monthly patterns through the pituitary-derived luteinizing and follicle stimulating hormones aromatization of androgen. In postmenopausal women estrogen metabolism is take place in peripheral tissues through aromatization of androgen (testosterone and androstenedione). Androgen is produced through adrenal gland and converted by an aromatase in to estradiol and estrone.

Breast tissue can make estradiol from epithelial cells, stroma, and macrophages, which infiltrate normal tissue (Santen *et al.*, 1999). Stromal cells in breast carcinomas are the major source of estrogens in the tumor. The local estrogen production by stromal fibroblasts is in early stages of breast carcinogenesis drives epithelial expansion later replaced or additionally supplied by epithelial cells themselves (Yaghjyan, 2011). Liver metabolized estrogen through three different pathways. Depending on the pathway, estrogen can be converted in to low risk (2α-hydroxy) or higher risk (16α-hydroxy and 4α-hydroxy) metabolites for breast cancer (figure.2) (Folk, 2019).

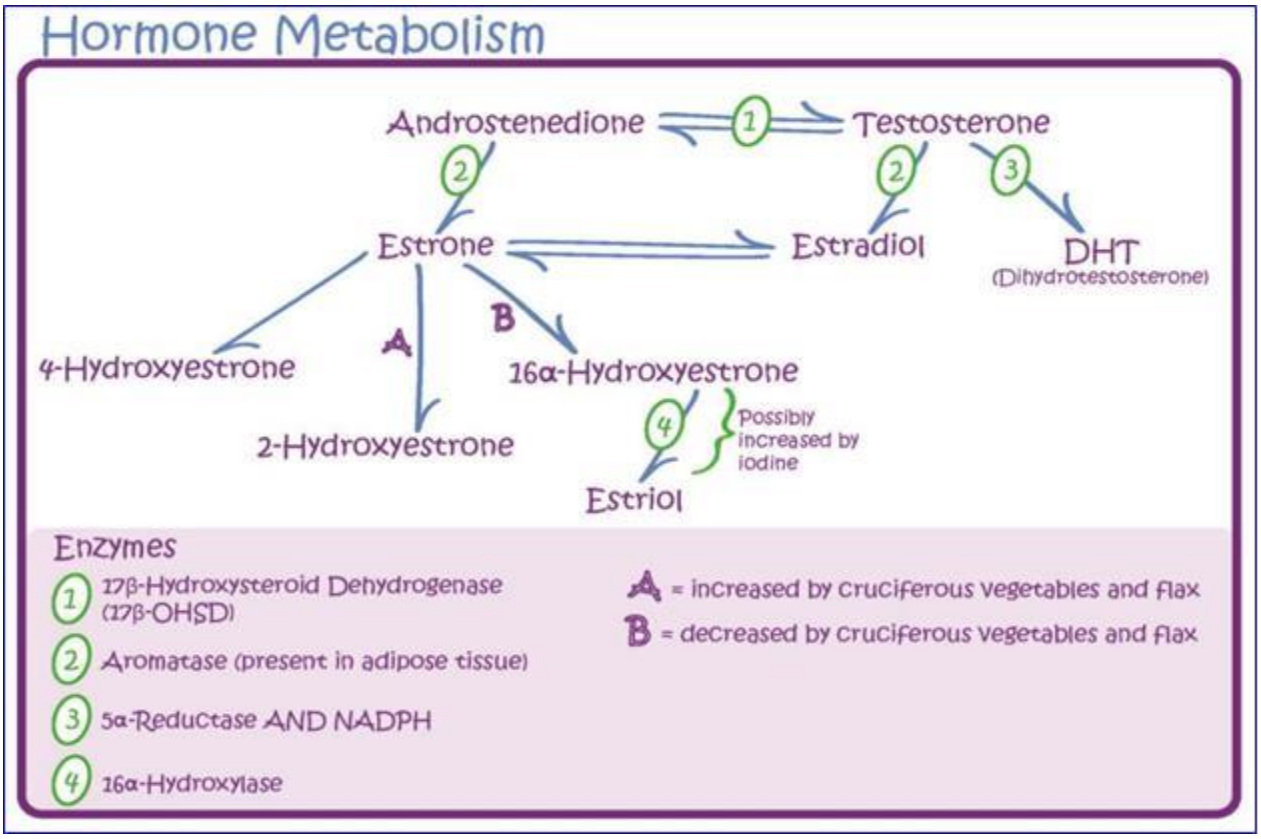

Figure 1: Estrogen metabolism. Conversion of androgen: testosterone and androstenedione in to estrone and estradiol. Adopted from (Kolan, 2014). Estrone is intermediate substance, which further converted in to estradiol and three different metabolites (2α-, 4α-, and 16α-Hydroxyestrone) in liver.

It is widely accepted that cancer causation is the result of the combined influence of genetic susceptibility and environmental exposures (including endogenous hormone exposure). The primary risk factors for breast cancer can be understood as regulators of the lifetime endogenous estrogen exposure (Manni, 1999).

1.2.1.4. Mechanism and Action of Estrogen in Breast Cancer Formation

The precise mechanism action of estrogen in breast cancer is unknown and controversial in women (Santen *et al.,* 20015 and 1999). However, estrogen exerts their effect in breast cancer through two alternative complex mechanisms: (classical) genomic and nongenomic (non-classical) mechanism (Fuentes and Silveyra, 2019).

Genomic or nuclear-initiated estrogen signaling phathway is involed when estradiol bind to ER (ERα) in the cytoplasm, after which the receptors dimerizes translocates into the nucleus, then bind to specific Deoxy Ribo nucleic Acid (DNA) sites in estrogen dependent tissues called Hormone Responsive Elements and promotes target genes expression (Owiredu *et al.,* 2009; Bjo¨ rnstro¨m and Sjo¨ berg 2005). After estrogen response elements bind ligand bound ER dimers, modulation of transcription occurs via interaction with coactivators or corepressors (Platet *et al*., 2004). Morevere, ERs are ligand-dependent transcription factors that regulate genes, which are involved in cell proliferation, differentiation, apoptosis, and cell migration.

Nongenomic or membrane-initiated estrogen signaling are mediated by a G protein-coupled receptor 30 /GPR30 and ER-α36 that start the activation of a variety of cytoplasmic signal transduction pathways(Zhou *et al.*, 2014) , and induced intracellular signaling events through second messengers. Also, interaction with others membrane receptors such as insulin like growth factor-1-receptor and epidermal growth factor receptor, and stimulate effector molecules such as tyrosine kinase receptors and phosphatidyl inositol 3 kinase, serine/ threonine protein kinase, and mitogen-activated protein kinase (Song *et al*., 2005), that enable the activation of other transcription factors associated to proliferation, apoptosis, and differentiation (Tecalco-Cruz *et al*., 2017). That mean, it have important physiological consequences leading to DNA synthesis, cell proliferation and apoptotic inhibition (Song *et al*., 2005), cell migration, metastasis and hormone dependent drug resistance (Rosen, *et al*., 2006).

Therefore, the high expression of ER and the activation of its genomic and nongenomic pathways have effects on breast cancer development and drug resistance (Tecalco-Cruz, 2017). This also may be related with the levels of estrogen in blood and tissues are

associated with breast cancer carcinogenesis (Zhou *et al.,* 2014). Because, cellular pathways deregulation has been associated with breast cancer, leading to alterations in cell proliferation, apoptosis, and the indirect hormonal balance of breast tissue cells (Velloso *et al.*, 2017).

In other ways, studies classifed the mechanism of estrogen induce breast cancer in two ways as a genotoxin and mitogen (Ali, 2014). Estrogens are substances, which can directly produce the stimulation of mitotic activity in the tissues of the female genital tract. The initiation of mitotic effect by estrogens is considered as a multistage process which is susceptible to disruption at many stages, such as ER binding, transcription, macromolecular synthesis, cell proliferation, and clinical consequences (Starek, 2003).. The mitotic rate of human breast epithelial cells is greatest during luteal phase of the menstrual cycle. Estradiol is the major factor of the mitotic rate of human breast epithelial cells and increased serum concentrations of estradiol in both premenopausal and postmenopausal women. High estradiol concentrations are related to an increased risk for breast cancer (Key, 1999). Because, estrogen exposure can influence estrogen activity based on its receptor. The more estrogen in the body the more ERs are in the body. This phenomenon may play a role in the development of cancer in women. (Taylor and Bell-Taylor, 2009) (figure 3).

Estrogen metabolites can exert genotoxic effects, which contribute to the development of breast cancer (Yue *et al.,* 2010). The levels of estrogen in the breast increase when aromatase is overexpressed. Sufficient amounts of aromatase in the breast tissue and enough estradiol as substrate available for formation of substantial amounts of genotoxic metabolites. When epithelial, stroma, and macrophages cells overexpress aromatase and provide sufficient amounts of estradiol locally to allow conversion to genotoxic quinine metabolites (Santen *et al*., 2015). Estrogens stimulate proliferation of breast cells and increase the chances for genetic mutations that could result in cancer. Estrogen metabolism generates oxygen-free radicals and quinones which produce both stable and unstable DNA adducts. Both result in genetic mutations which accumulate and could ultimately cause cancer (Santen *et al*., 1999). The ER independent, carcinogenic effects of estrogens are occurring through the actions of estrogen metabolites. The presence of

the aromatic A-ring, oxidative metabolism of estradiol results in the formation of 2, 3- and 3, 4-catechols (quinones) catalyzed by cytochrome P450 enzymes present in human breast tissue. These quinones can also reduced to semiquinones by cytochrome P450 reductase. This process can establish a redox cycle to produce reactive oxygen species that cause oxidative DNA damage (Santen *et al.*, 20015). Additional, 2, 3- and 3, 4-estradiol-quinones, which form covalent adenine and guanine DNA adducts. Adducts of the 4-hydroxylated products of adenine and guanine are unstable. Thus, cleaved from DNA with formation of depurinated sites. Mutation at these DNA sites can occur through error prone DNA repair and end results are formation of mutations. Accumulation of these mutations then contribute to the development of breast cancer) (figure 3). Breast cancer are expected in women with combinations of mutations of estrogen metabolizing enzymes (Yue *et al*., 2010).

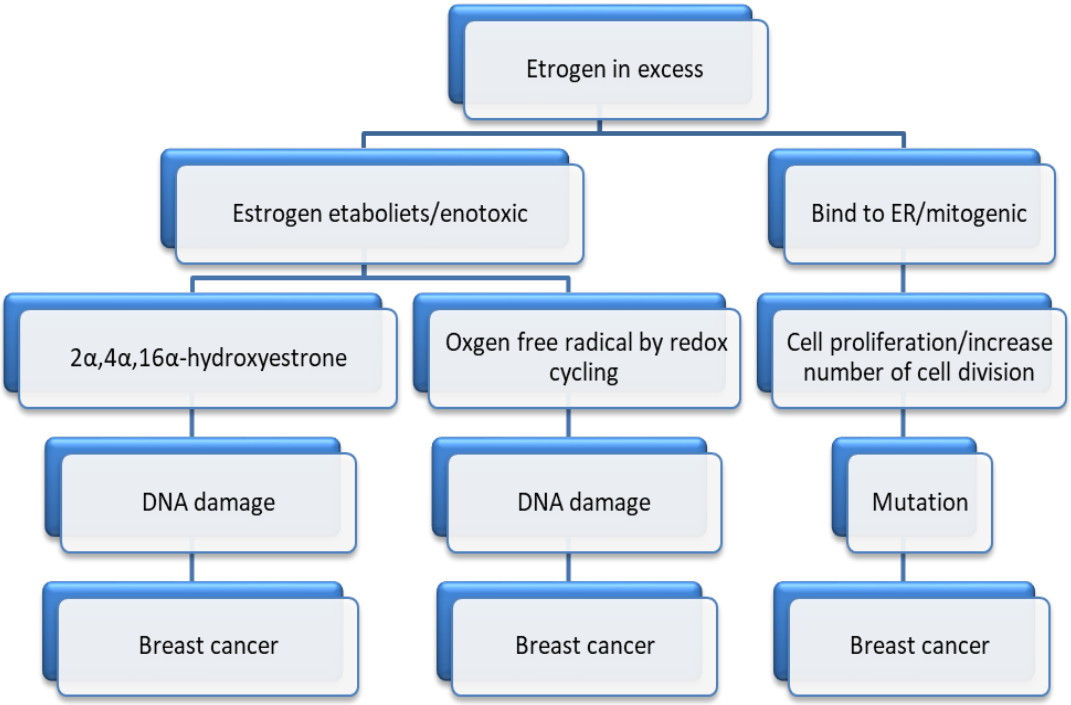

Figure 2: Mechanism and action of estrogen in breast cancer formation depending on mitogen and genotoxic effects of estradiol. Adopted from (Santen et al., 2015).

Estradiol induced breast cancer through over activation of transcriptional process stimulate cell proliferation and increase the chances for genetic mutations. Although, estrogen metabolism generates oxygen-free radicals and quinones which produce unstable DNA adducts. That means the 2, 3- and 3, 4-quinones can be reduced to semiquinones by cytochrome P450 reductase, and this process can establish a redox cycle to produce reactive oxygen species that cause oxidative DNA damage.

1.2.1.5. The Factors Influencing Estrogen Activity

Factors influencing estrogen activity such as estrogen binding to sex hormone binding globulin (SHBG), estrogen production, estrogen exposure, estrogen metabolism and elimination. SHBG is a protein that carries the majority of estrogen in the bloodstream. Estrogen is a fat-soluble and it must attach to SHBG, which is a water-soluble protein that transported to target tissues (Taylor and Bell-Taylor, 2009). The availability of estrogen in tissues is determined not only by the production of the hormone and concentrations in circulation but also by the extent to which it is bound to a binding protein, SHBG. Thus, high concentrations of SHBG decrease the proportion of estradiol that is able to leave the circulation and enter the cells (Travis and Key, 2003). Thus, when the concentration of SHBG decrease and the free estrogen is excess and deliver its message to the cells that contain more estrogen receptor. Estrogen metabolism and elimination of estrogen influences the total amount of estrogen in the body (Taylor and BellTaylor, 2009).

1.2.1.6. Factors That Affect Serum Estrogen Levels

Serum estrogen levels increase when increased lifetime exposure to estrogen and progesterone. The factors that expose for estrogen as discussed by several studies are delayed childbearing, sedentary lifestyle, stress and poor diet (Taylor and Bell-Taylor, 2009) (high fat diet), lack or short duration of breast feeding, family history of breast cancer, environmental factors like exposure to radiation and chemicals (Laisupasin, 2013; Jamall *et al.,* 2010). Reproductive events that are early menarche, late menopause, alcohol intake, in active physical activity /sedentary life style and high circulating estradiol levels in premenopausal and postmenopausal women are associated with an increased incidence of breast cancer (Yue *et al.*, 2010).

1.2.1.6a. Life Style

Changes in lifestyle is associated with urban migration that is, a more economically developed one, and associated with a decline progesterone levels or an increased estrogen concentration, results increase breast cancer risk in premenopausal women (Houghton *et al.*, 2016). But, Rohariya *et al.*, (2017) stated that there is no effect whether the patient

lives in rural or urban area. Breast cancer incidence rates indicate that within different countries are increasing and appear to be higher in urban than rural areas. Immigrant studies have given evidences that westernized lifestyle increases the level of estrogen levels and results in a higher incidence of breast cancer (Kim *et al*., 2009). Although, the change of hormonal levels are increased risk of breast cancer in Mongol women is lower when compared to British, and United States of America, especially in women born in the Mongolian countryside (Houghton *et al.,* 2016).

1.2.1.6b. Stress

Stress increased releasing of cortisol. Cortisol decreases SHBG, this consequently increase the amount of free estrogen level. The elevated level of estrogen can block ovulation. Thus, progesterone production is absent and this can become the cause of breast cancer (Taylor B. and Bell-Taylor A., 2009). Therefore, cortisol has an impact on estrogen activity in the mammary gland, which may initiate protumorigenic changes during periods of stress (Antonova *et al*., 2011).

1.2.1.6c. Obesity

Obese women have a 2.5 times greater chance to develop breast cancer than women with a normal body mass index (BMI). Obesity is a recognized risk factor for postmenopausal breast cancer (Kim *et al.* 2009; Rohariya *et al*., 2017). It is correlating with a 30– 50% higher relative risk of breast cancer in menopausal women (Coscia *et al.,* 2017). The increased risk in postmenopausal women is probably due to increased level of circulating estradiol, and reduced level of SHBG by the conversion of androgens to estrone in adipose tissue, which is increased biologically active unbound form of estradiol (Rohariya *et al.,* 2017; Howe *et al.,* 2013; Kim *et al*., 2009; Owiredu *et al*., 2009; Carpenter *et al*., 2003; and Travis and Key, 2003). Peripheral aromatization of androgen precursors in adipose tissue is responsible for estrogen biosynthesis after menopause because elevation of aromatase expression in subcutaneous of these tissue (Subbaramaiah *et al.,* 2011). Obesity is characterized by high levels of estrogen, which increases the growth of endocrine responsive of breast cancer (Newman and Gonzalez-Perez, 2014).

Obese postmenopausal women have more estrogens, insulin growth factor-I, and insulin than thin women. Crosstalk between the insulin growth factor pathways do and estrogen-mediated signalling may favour an increased risk of breast cancer largely in those women. The increased circulating concentrations of insulin and insulin growth factor-I cause a reduction in blood levels of SHBG with a consequent elevation in the bioavailable fraction of circulating estradiol. Additionally, SHBG may act directly on breast cancer cells to inhibit estradiol-induced proliferation, loss of which in obese women can contribute to tumorigenesis (Macciò and Madeddu, 2011).

In obesity was increasingly recognized as a mild inflammatory condition, deregulated secretion of proinflammatory cytokines and adipokines such as interleukin- 1, interleukin- 6, and tumour necrosis factor alpha (Newman and Gonzalez-Perez, 2014; Arcidiacono *et al.*, 2012). Obese postmenopausal women are at increased risk of developing hormone receptor positive breast cancer (Subbaramaiah *et al.*, 2011), through a hormonal mechanism involving the metabolism of an androgenic precursor to estrogen in adipose tissue (Ha *et al*., 2009). BMI is recognized as determinant of serum estradiol in postmenopausal women (Howe *et al*., 2013). Therefore, leads to an overall increase in the active levels of circulating estrone and estradiol, which may promote the growth, and metastatic potential of breast tumour in obese women (Owiredu *et al*., 2009; Carpenter *et al*., 2003).

1.2.1.6d. Body Weight

Higher body weight means more fat tissues and higher estrogen level. This may increase breast cancer in women who are higher in body weight after menopause (komen, 2017). The higher BMI is increased breast cancer risk in postmenopausal women (Owiredu *et al.*, 2009). Because, when BMI increased the main source of estradiol in postmenopausal women via the extra glandular aromatization of androstenedione and estradiol also increases in postmenopausal women. This clearly indicated that the relationship between BMI and breast cancer risk can mainly connected on the relationships of BMI with serum estradiol concentrations (Owiredu *et al.*, 2009; Key, 1999). However, changes in plasma

estradiol levels in breast cancer patients were not associated with BMI (Coscia *et al*., 2017). In comparison of BMI values, between controls and breast cancer patients based on menstrual status BMI of Premenopausal cases were higher than controls. But, it was found to be non-significant (Rohariya *et al.,* 2017).

1.2.1.6e. Alcohol Consumption

Alcohol appears to increase plasma estrogen levels. Alcohol consumption maybe a small extent increased breast cancer risk. However, studies show that women who consume three or more alcohol drinks per day increase about 50-70% breast cancer risk when compared to non-drinkers (Feigelson and Henderson, 1996). Alcohol intake decreases the liver ability to eliminate estrogen from the blood. Because the liver damage influence estrogen metabolism when it is in excess. In addition, alcoholic diet lack vitamin and fiber that is important for estrogen metabolism in liver and in stomach (Taylor and Bell-Taylor, 2009).

1.2.1.6f. Reproductive Events

Reproductive factors that increase the duration and/or levels of exposure to ovarian hormones, which stimulate cell growth, have been associated with an increase in breast cancer risk (NCI, 2016). A woman has an increased risk of breast cancer during lifelong estrogen exposure.; this is increased in case of early menarche, a late menopause, and/or an absence of childbearing, age at first birth /age of first full pregnancy, low/or absence of breast feeding, number of births, and gap of birth (Dall and Britt, 2017 ; Kapil *et al.*,2014). At first age menstruation/ menarche, women who began menstruating before ages 11 or 12 years and at late menopause, women go through menopause after age 55 year have somewhat higher risk of breast cancer. Because, their breast cells have been expose to estrogen and progesterone for a longer time. Longer exposure to these hormones increases breast cancer risk (ASOC, 2019), due to maximize the number of ovulations (Rohariya *et al.,* 2017; Santen *et al.,* 1999).

Pregnancy may help protect against breast cancer because it pushes breast cells into their final phase of maturation (ASCO, 2019). Study also showed that breast feeding may delay return of regular ovulatory cycles and decrease endogenous sex hormone levels

(Shah *et al.,* 2014). Moreover, pregnancy and breastfeeding, which both reduce a woman‘s lifetime number of menstrual cycles and reduced cumulative exposure to endogenous hormones (NCI, 2016). Thus, decrease breast cancer risk. Timing of pregnancy, Women who had their first pregnancy after age 35 or who have never had a full-term pregnancy have a higher risk of breast cancer (ASCO, 2019; Dall and Britt, 2017). An early first full-term birth is the most effective modifiable breast cancer prevention (Katz, 2016). Lactation, differentiation of the cells of the mammary gland, prior to exposure to a carcinogen, protects them from malignant transformation. During pregnancy, estriol is increased largely than estrone and estradiol which can reduce the risk of breast cancer (Kapil *et al*., 2014).

However, the protective effect of early age at first birth is complex. During the first trimester of pregnancy, the level of free estradiol rises rapidly. However, pregnancy is continuing free estradiol levels lower, while SHBG levels rise, giving a net overall benefit with respect to the endogenous estrogen profile which permanently reduces breast cancer risk. Prolonged lactation is more importantly to reduce the risk (Feigelson and Henderson, 1996). Parity has also associated with breast cancer risk; higher parity was associated with a decrease in the risk of breast cancer (Kapil *et al*., 2014). An increased number of births are also associated with decreased breast cancer risk, additional births providing an extra 10% reduction in risk (Dall and Britt 2017; Katz, 2016).

1.2.1.6g. Age

Age is a crucial factor in malignancy, most of the studies show malignancy presents more in elder population when compared to young ones (Rohariya *et al.*, 2017). Researchers indicated that no influence of age on the plasma levels of steroids hormones. The study predominantly included older women in the group of patients. An expected characteristic due to the fact that, age is a risk factor for breast cancer (Coscia *et al*., 2017). However, studies showed that in Sub-Saharan Africa the youngest women developed breast cancer. Thus, age does not concern as breast cancer risk factor (Hadgu *et al*., 2018).

1.2.1.6h Physical Activity

Physical activity can reduce the number of ovulatory cycles. The risk of breast cancer among women who exercised for more hours per week during their reproductive years was nearly 60% lower than that of inactive women (Feigelson and Henderson, 1996).

1.2.1.7. Serum Estrogen Hormone Levels and Breast Cancer Risk

Endogenous sex hormones and endogenous hormonal milieu predicts the chances of breast cancer in females. Serum estrogen levels can account for differences in breast cancer risk (Cleary and Grossmann, 2009). Breast tumors have aromatase activity and can produce estrogen even after circulating estrogen levels have dropped to very low levels. The amount of estrogen in the blood is linked to an increased risk of breast cancer in women (Quigley, 2017). For instance, high circulating levels of estrogens are associated with increased risk of breast cancer in Caucasian women (Yu *et al.,* 2003), specifically women with higher blood level of the estradiol have an increased risk of breast cancer (komen, 2017; Key, 1999).

Studies indicated that circulating estrogen levels are highest in the pre-ovulatory phase of the menstrual cycle. During pregnancy and post-menopause time mostly which is around age of 50 decrease sharply. Overall, several studies have shown that high levels of estrogen are associated with increased risk of breast cancer in women before and after menopause. Higher levels of total estradiol and bioavailable estradiol were associated with higher breast cancer risk in postmenopausal women (Farhat *et al.,* 2013). Contrary, a meta-analysis of nine prospective studies observed that premenopausal women with relatively high serum estrogen concentrations had an approximately two fold risk of breast cancer compared with postmenopausal women with relatively low serum concentrations of sex hormones. Although, other studies have indicated that high serum levels of estradiol in pre-menopausal women elevate the breast cancer risk in comparison with the much lower levels of these hormones in post-menopausal women (Colditz, 1998). A pooled analysis of data from seven studies found that higher blood estrogen level might be modestly increase breast cancer risk before menopause (Komen, 2017). However, Breast cancer patients also have a lower level of both estrogen as compared to

controls (Hussain *et al*., 2013). No significant changes were observed in serum estradiol levels between the premenopausal breast cancer patients and the controls (Owiredu *et al.*, 2009). Although, In a case control study of Sub- Saharan Africa country, Uganda there was no association between level of estradiol and breast cancerrisk (Awio *et al*., 2012).

However, the studies indicate that measuring of serum estrogen level in premenopausal women is a challenged. Because, estrogens levels are vary throughout the menstrual cycle (Komen, 2017; Becker *et al.,* 2007). Thus, hormonal exposure in premenopausal women is more complex and difficult to estimate in epidemiological studies (Travis and Key, 2003). In postmenopausal women, serum estrogen levels are the risk of breast cancer. When the elevation of the mean values of serum estradiol levels above 20–25% the incidence of breast cancer is 2 to 3 times higher (Coscia *et al*., 2017). Estradiol levels in postmenopausal women are generally less than 10 to 20 pg/mL (Dixon, 2014; Hammond *et al.*, 2010). However, it was not possible to quantify plasma estradiol level when below 5 pg/mL. The ability to determine plasma steroid levels below this limit might have resulted in a greater percentage reduction in these hormones age (Coscia *et al*., 2017). Menopause results in the loss of ovarian estrogen production and a consequent drop in circulating levels of estradiol. During menopausal, serum estradiol level is drop well below the lower end seen in premenopausal women (Travis and Key, 2003). The premenopausal women reference ranges of serum estradiol level are widely varying from source to source (Becker *et al.*, 2007). Therefore, its level is variable at different phase of the cycle (Travis and Key, 2003). Studies show that, the mean integrated levels of estradiol during a full menstrual cycle have been reported variously by different sources. That is, 80, 120, and 150 pg/mL.This study used the reference ranges of serum estradiol level as determined in a Caucasian population of healthy women and premenopausal women are :- during follicular phase 12.4 -233 pg/ml, Ovulation phase41.0-398 pg/ml, Luteal phase 22.3-341 pg/ml, for postmenopausal < 5-138 pg/ml (Cobas, 2016).

1.2.2. Serum Progesterone and Breast Cancer Risk

Progesterone is one of the hormones in our bodies that stimulates and regulates various functions (Goldstein, 2019). Progesterone is a steroid hormone and it has 21 carbon atoms. It is a precursor or building block for estrogen but also transmit its own unique message to cells (Taylor.and BellTaylor, 2009). Progesterone governs the second half of the menstrual cycle, it is becomes known as a major regulator of cell proliferation and stem cell activation in the adult mammary gland (Brisken *et al.,* 2015). Which regulate mammary gland through influencing physiological and endocrine related processes including mammary development (Singh *et al.,* 2017). Progesterone and PRs are essential for the development and cyclical regulation of hormone-responsive tissues including the breast (Mauvais-Jarvis *et al.,* 1986). The actions of progesterone are primarily mediated by its high-affinity receptors, which include the classical PR-A and PR -B isforms, located in diverse tissues where progesterone control the breast tissues (Lange *et al.,* 2008).

Progesterone inhibits estrogen-driven growth (Mauvais-Jarvis *et al.,* 1986). Thus, it decreases breast cell growth, involved the maturation of breast cells, and decreases the rate of multiplication. Progesterone also promotes normal cell death in tissues of the breast, which is important in the prevention of cancer (Creative Diagnostics Blog, 2018; Bettuzzi *et al.*, 1991). Hence, specific actions of progesterone in breast cancer are poorly understandable. However, the studies recognized that the role of progesterone in mammary gland proliferation and differentiation (Singh *et al*., 2017). Progesterone is required for proliferative activity of the breasts tissue may be involved directly or indirectly in either stimulating or inhibiting breast cancer. The dual, and paradoxical, actions of progesterone suggest the possibility that progesterone may be converted to two types of metabolites (5α-pregnane and 4α-pregnene) (Wiebe *et al*., 2000). This biphasic effect of progesterone is due to higher level of 5αpregnane results in increased proliferation and promotes breast cancer progression (Singh *et al*., 2017) ,and exposure to the 4-pregnenes results in opposite (anticancer-like) effects by causing suppression of cell proliferation (Lewis *et al.,* 2004).

1.2.2.1. Mechanism and Action of Progesterone in Breast Cancer

Progesterone directs cells to slow their growth and mature normally. The major function of progesterone is to regulate the activity of estrogen and it is able to decrease the production of ER (Taylor and Bell-Taylor, 2009). The mechanisms by which progesterone exert their anti-estrogenic action in women include a reduction of estrogen secretion in the systemic circulation through increased 17β-hydroxysteroid dehydrogenase which leads to accelerated metabolism of estradiol to estrone in the target organ (Mauvais-Jarvis, 1986). And decreases the target organs response to estrogen by a reduction of estrogen receptor levels in the organ and a direct effect on cell multiplication (Creative Diagnostics Blog, 2018; Bettuzzi *et al.,* 1991). This action decrease the cell's ability to over react to excessive levels of estrogen. Thus, these mechanisms, by which progesterone protects the body from estrogen- induced breast cancer proliferation (Taylor.and Bell-Taylor, 2009).

1.2.2.2. Progesterone Metabolism in Breast Cancer

Progesterone primarily produced in the corpus lutem during luteal phase, lesser extent in adrenal and during pregnancy from placenta. Thus this cyclical hormone exposure beginning at menarche and ending at menopause, occurs monthly and differentiation of specialized tissue within reproductive tract and breast tissue (Goldstein, 2019; Graham and Clarke 1997). Corpus luteum procedures progesterone after ovulation and the levels begin to increase maximum (6–10 ng/m). On the 14th day of the cycle progesterone level is reach to10 ng/mL. This level starts to decrease on the 15th day of the cycle. The key regulated step in luteal progesterone production appears to be regulation of transport of cholesterol to the inner mitochondrial membrane (Kuru, 2018). The corpus lutem production of Progesterone is stimulated, and maintained by various hormones, specific by estradiol (Diaz *et al*, 2002). Progesterone biosynthesis requires only two enzymatic steps; the conversion of cholesterol to pregnenolone, catalyzed by P450 side chain cleavage located on the inner mitochondrial membrane, (Christenson and Devoto, 2003; (Diaz *et al.,* 2002). P450 side chain cleavage catalyzes three oxidation steps: hydroxylations at the 20 and 22 positions and then cleavage between these two carbons (Kuru, 2018). P450 side chain cleavage dramatically increases in curpus luetum after the

Lutenizing hormone surge and luteinization (Diaz *et al.,* 2002). Pregnenolone diffuses out of the mitochondria to the smooth endoplasmic reticulum where it is converted to progesterone by the enzyme 3-β hydroxysteroid dehydrogenase (Christenson and Devoto, 2003). Because, pregnenolone has been two hydrophilic residues, that make it less stable in cellular membrane and more readily mobile through the cell. Progesterone then diffuses out of the luteal cell and into the bloodstream to be transported to target tissues (Kuru, 2018). The levels of 3-β hydroxysteroid dehydrogenase increase dramatically following the luetilizing hormone surge and ovulation to a maximum at days 8 /11 after estrus.

Metabolism of progesterone is also important for determining the circulating concentrations of progesterone (Diaz *et al.,* 2002). Breast tissue can convert progesterone into two classes of metabolites: 4-pregnenes and 5α–pregnanes (Wiebe *et al.,* 2000). The process requires the action of the enzymes, 5α- reductase, 3α-hydroxysteroid oxidoreductase and 20α-hydroxysteroid oxidoreductase. The enzyme activities are differences in normal and tumor mammary gland tissues. 5α-pregnanes are produced at a significantly higher rate in tumorous than in normal human breast tissue, because 5α-reductase activity increased in the carcinoma (Lewis *et al.,* 2004). 5 α- reductase is convert progesterone into 5α– pregnane irreversible, then 5α-pregnane can be further converted reversibly in to 3α- and 20α-hydroxy pregnanes by the action of 3α/ß- and 20α-hydroxysteroid oxidoreductase (figure 4) (Wiebe *et al*., 2000). However, the activities of 3α-hydroxysteroid oxido-reductase and 20α-hydroxysteroid oxido-reductase were higher in non-tumorous breast tissues, Progesterone is converted directly and reversibly to more δ-4-pregnenes (Lewis *et al.,* 2004) (figure 4). The concentration of 5α pregnanes exceeds greatly in respect to 4α-pregnene which is attributed due to increased activity of steroid 5α-reductase. These metabolites act on almost in all breast cancer cell lines irrespective of their tumorigenicity. The relative concentrations 4α pregnenes and 5α- pregnane are used to be responsible for determining shift of normal cells towards neoplasia (Singh *et al.,* 2017).

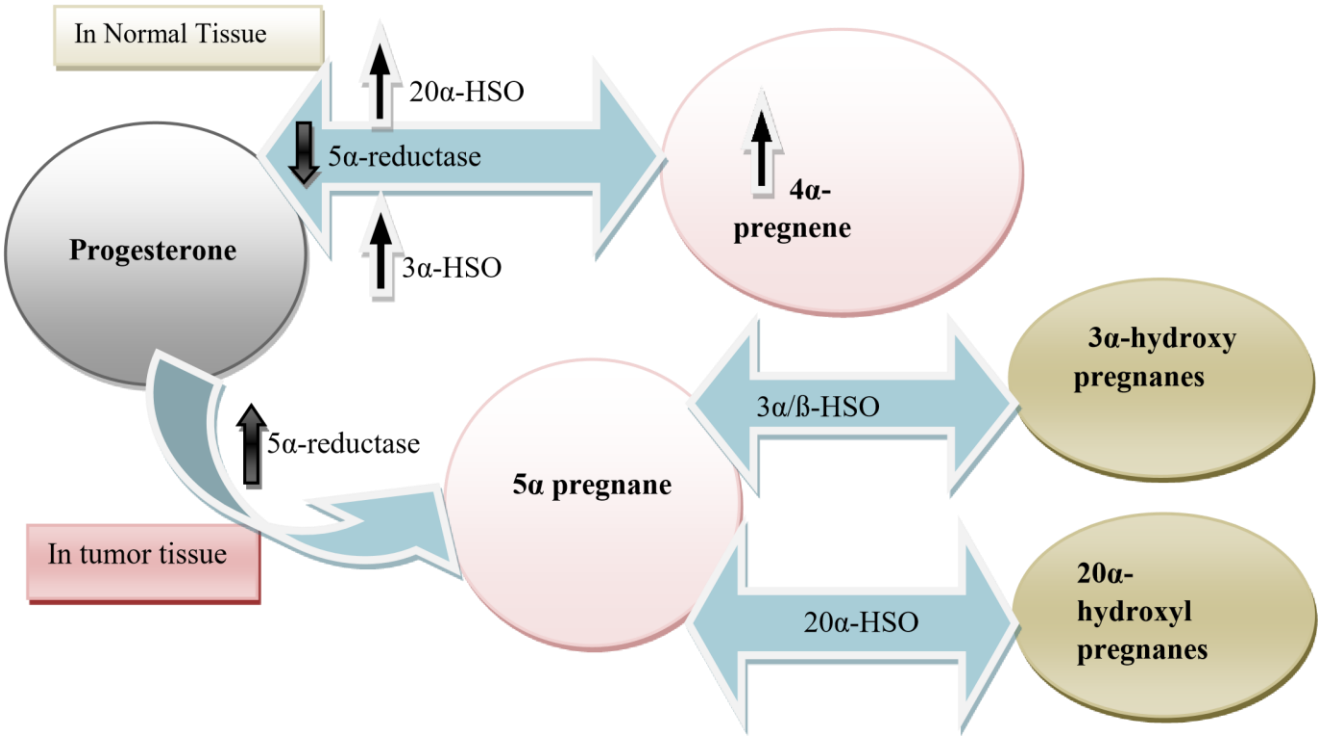

Figure 4: progesterone metabolism in normal and tumour tissue. Adapted from (Singh *et al.*, 2017).5α-reductase, 3α-hydroxysteroidoxid reductase (3α-HSO), 20α-hydroxysteroid oxidoreductase (20αHSO), 3α/ ß -hydroxysteroid oxidoreductase (3α/ß-HSO) enzymes are found in normal breast tissues and breast cancer cells, catalyses the conversion of progesterone to active metabolites, such as 4α pregnenes in normal tissue and 5α-pregnane in tumor tissue. 5α-pregnane further metabolized in to 3α and 20α-hydroxyl pregnanes.

1.2.2.3. Factors Affecting Progesterone Level

One cause of progesterone imbalance is estrogen dominance by outside factors such as environmental hormones (i.e., xenoestrogen) found in the foods you eat. Other causes of diminished progesterone include insulin resistance, chronic stress, poor nutrition, and insufficient exercise (Lee & Hopkins, 2004). When the body is stressed, it works to produce higher levels of the hormone cortisol that manages stress in the body. Because, progesterone is the precursor to cortisol, when cortisol levels increase, progesterone levels decrease. Therefore, too much stress in a woman's life can lead to a progesterone deficiency**,** causing the estrogen dominance symptoms mentioned above (BodyLogicMD, 2020)**.**

1.2.2.4. Levels of Progestrone and Estradiol in Normal Menstrual Phase

Estrogen and progesterone work synergistically when present in a proper ratio. The normal ratio of free estrogen to free progesterone is approximately 20 to 1. After ovulation progesterone is 20 times higher than estrogen. In menstrual cycle the first bleeding day both are low. During follicular or proliferation phase, estrogen is abundant hormone, under the influence of follicular stimulation hormone produce by the ovary and responsible for the development of follicle. During Ovulation occurs estrogen and progesterone working in partnership. The egg is released from the follicular cysts. This cyst can transformed in to corpus luteum at luteal phase and responsible for production of progesterone. Progesterone is the dominant hormone and instructs the uterine lining to stop growing and multiply but start to maturation (Taylor and Bell-Taylor, 2009).

Thus, the levels of serum estrogen and progesterone hormone are varying for premenopausal women as depend to menstrual phase. However, in post-menopausal women the ovary not produces estrogen but from peripheral cell estrogen produces directly and in directly through other steroid hormone metabolism mechanism. Estrogen is dominant hormone at this time (Bernstein and Ross, 1993).

1.2.2.5. The Relationship between Progesterone and Estrogen in Breast Cancer

Estrogen and progesterone play a major role in the etiology of breast cancer (Buijs *et al.*, 2008). High estrogen levels in postmenopausal women are associated with an increase in breast cancer risk. But, such a relation has not yet been established in premenopausal women, despite biologic evidence that breast epithelial cell division rates are high during the luteal phase of the menstrual cycle when estradiol and progesterone levels are high (Bernstein and Ross, 1993). However, the effects of estrogen alone do not fully account for the relationships observed between breast cancer and hormone-related risk factors. Other hormones, such as progesterone may also be important (Howe *et al*., 2013). Estrogen and progesterone promote cellular and epithelial growth in the normal mammary gland and are positively associated with an increased risk for breast cancer in both premenopausal and postmenopausal women (Iversen *et al*., 2016; Furberg *et al*., 2005).

However, progesterone may also have an antagonistic activity against estradiol, may be conditional long periods of luteal phase defect leading to an unopposed estrogen effect on the breast might promote breast carcinogenesis. Thus, estrogen and progesterone have many different roles in the body and are equally important for reaching and maintaining optimal health. A woman‘s life can be explained by the fluctuations of these two hormones. So these hormones are in balance a woman‘s life is normal, but out of balance the problems are follow and when secreted in an adequate balance, permit the complete and proper development of the mammary gland (Mauvais-Jarvis *et al*., 1986). The balance can be easily disturbed by excessive exposure to environmental toxins, diet, medications, poor sleep, stress, and poor elimination of excess hormones (The women‘s wellness collective, 2019). However, Low or absent of progesterone level in the body may play a role in the development of cancer in women. Thus, in the absence of sufficient quantities of progesterone, the body begins to make androgens to regulate the effect of estrogen. Estrogen dominance is one of the most common hormonal imbalances women experiences due to either excess estrogen or a lack of progesterone in the body. This imbalance can negatively affect health in numerous ways (The women‘s wellness collective, 2019). When ovulation does not occur in bodies do not produce enough progesterone to keep estrogen levels in patterned (Taylor.and BellTaylor, 2009), which is sensitive to the risk of breast cancer (NUNM, 2020). Therefore, an excess of estrogen and/or a deficiency of progesterone are responsible for breast cancer (Taylor.andBell-Taylor, 2009). When estrogen is excess in the body, inversly progestrone decreased because estrogen increased the production of cortisol binding globulin in the body. Cortisol binding globulin is important for binding of progestrone from the blood (Holst *et al.*, 2004). Therefore, at last involved in abnormal breast cell development and enhanced free radical or toxic substances in breast cancer cell (Taylor and Bell-Taylor, 2009). Breast cancer cell proliferation is promoted by a variety of mitogenic signals. Estrogen is considered as most predominant mitogenic signal in hormone-dependent breast cancer. Progesterone is mainly considered to have protective effect (Singh *et al.,* 2017).

1.2.2.6. Serum Progesterone Levels and Breast Cancer Risk Factors

Progesterone levels appear to be an unsure risk factor for both pre and postmenopausal breast cancer with significant inverse association among progesterone levels and breast tumor risk (Atoum *et al*., 2014). However, other studies indicate that breast cancer patients also have a lower level of both estrogen and progesterone as compared to controls. In cases, this may be due to the failure of proper matching between the cases and the controls with respect to their different phases of menstrual cycle during the measurements of blood hormones (Hussain *et al*., 2013).

A case – control study within the European Prospective Investigation of Cancer and Nutrition in examine relationships among premenopausal serum concentrations of sex steroids. The results demonstrated an elevated serum progesterone concentration was associated with a statistically significant reduction in breast cancer risk (Kaaks *et al.*, 2005). A low progesterone/estrogen ratio and anovulatory cycles are more frequent in breast cancer patients than in a normal population. Even, it has been suggested that a relatively low progesterone secretion might favor the development of cancer (Saez *et al*., 1978). There is a direct association between the risk of breast cancer and progesterone level (Missmer *et al.*, 2004).

Serum progesterone is low during the follicular phase less than 1.5ng/mL, Levels begin to increase just before the onset of the luteinizing hormone surge and then increase progressively to peak levels 6 to 8 days after ovulation. After menopause, serum progesterone of adrenal origin is less than 0.5ng/mL (Staruss and Barbieri, 2013). However, the reference ranges as determined in a Caucasian population healthy woman of premenopausal used in this study are :- during follicular phase 0.2 -1.5 ng/ml, Ovulation phase 0.8-3.0 ng/ml, Luteal phase 1.7-20 ng/ml , for postmenopausal 0.1- 0.8 ng/ml (cobas,2016).

1.2.3. Lipid Profile Levels in Breast Cancer Patients

Lipids are essential for various biological functions including cell growth and division of normal and malignant tissues and the major cell membrane components (Laisupasin, 2013). There is a high requirement of lipids for new membrane biogenesis during neoplastic processes. Cells realize these necessities from the circulation, by synthesis through the metabolism, or from degradation of major lipoprotein fractions such as High-density lipoproteins (HDL), low-density lipoproteins (LDL), and very low-density lipoproteins (VLDL) (Taha *et al*, 2014). Malignant cells appear to metabolize lipids differently from the normal cells (Shah *et al*., 2008). Its proliferation of breast tissue are associated with changes in plasma lipid and lipoproteins levels (Furberg *et al.*, 2005; Nayak *et al*., 2016). On the other hand, the developing breast cancer also might be considered as one of the factors in alterations of lipid profile levels (Abdelsalam *et al.*, 2012).

Epidemiological studies have proposed that a relationship between lipids and cancer correlating with lipid abnormalities and oncogenesis. The changed lipid profile status may be due to abnormal lipid metabolism associated with tumor pathogenesis and tumor host interactions (Shah *et al*., 2008). Dyslipidaemia can affect cell integrity in patients of breast cancer (Mishra, 2015). Dysregulated lipid metabolism is recognized as hallmark of cancer. Dependable with the importance of lipids in cancer, regulatory factors, enzymes, and transporters involved in lipid transport, lipid synthesis, and lipid degradation are dysregulated in cancer cells. This lipid diversity is still not fully understood and still a subject of controversy (Pandeya *et al.*, 2018; Zhao *et al*., 2016). Factors such as cancer stages, types of cancer, parity, high fat diet, Obesity and menopausal status may affect to lipid profile levels in breast cancer patients (Laisupasin, 2013; Kim *et al*., 2009).

However, studies have shown a strong relationship of plasma lipids changes with risk of breast cancer and conflict in the association of serum levels of various lipid components and breast cancer (Peela *et al.*, 2012). Plasma lipid profile levels may predict the risk of breast cancer (Shah *et al.*, 2008). Total lipids, phospholipids, Triacylglycerol (TG), Total cholesterol (TC), LDL-C and free fatty acids elevated in premenopausal and post-

menopausal breast cancer patients (Abdelsalam *et al*., 2012). Lipid profile is not only associated with etiology but also with prognosis in cancer. Increased lipid levels have a major role in the pathophysiological appearance and progression of breast cancers associated with women (Ghahremanfard *et al*., 2015). The reference range of all lipid profile levels (TC, TG, HDLC, and LDL-C) according to this study is as follows in (table 1).

Table 1: Cobas lipid panel / cut off points of lipid profile levels.

Parameters	Cut point mg/dl			
	Desirable	Border line	Higher	
TC	<200	200-239	>240	
LDL-c	<100	129-130	>160	
TG	<150	150-200	>240	
HDL-C	>65	40-65	>40	

(Adopted from cobas, 2016).

1.2.3.1. Total Cholesterol and Breast Cancer Risk

Cholesterol is a chief lipid constituents of cell and it is critical for physiological functions like maintenance of the structural and functional integrity of all biological membranes (Mishra, 2015). Proliferating cells (cancer cells) increased requirements for cholesterol, to overcome its need. Thus, tumor cells can increase lipid biosynthesis and can also uptake cholesterol from the bloodstream (Taha *et al.*, 2014). A major link has been established between cell growth and cholesterol biosynthesis. Cholesterol inhibition, either by decreasing cholesterol availability (lowering of plasma cholesterol) or by decreasing intracellular cholesterol synthesis can inhibit tumor cell growth and possibly prevent carcinogenesis (Mishra, 2015*;* Owiredu *et al.,* 2009).

Enhancement of utilization of cholesterol by neoplastic cells for new membrane biogenesis, which is the pathogenesis of the decreased cholesterol decreased synthesis or increased catabolism. Cholesterol synthesis by liver can be inhibited by tumour metabolites. A number of epidemiological studies have shown the increased risk of death from cancer with hypocholesterolemia and also proposed that low levels of cholesterol is a predisposing factor for carcinogenesis (Raste and Naik, 2000). However, different

scholars showed that different findings on the level of TC in breast cancer patients. Lower levels of TC are found in cancer patients, and this is much more severe in advancedstage disease but this was not significant. Low levels of cholesterol in the proliferating tissue and in blood compartment could be due to carcinogenesis may contribute to hypocholesterolaemia (Mishra, 2015). In contradict to this, the study showed that high cholesterol levels associated with breast cancer (Llanos *et al.*, 2012). Cholesterol level and ratio of TC/HDL-cholesterol demonstrated significantly increase in breast cancer compared to control (Taha *et al.*, 2014). The elevated levels of TC have been observed in different stages of breast cancer patients in comparison to the control group (Nayak *et al.,* 2016). The same observation is reported in different study the plasma cholesterol levels in breast cancer patients were found significantly higher as compared to the normal healthy individuals (Ghahremanfard *et al*., 2015; Abas *et al*., 2014; Hussain *et al.*, 2013**;** Laisupasin, 2013; Abdelsalam *et al.*, 2012; Jamall *et al*., 2010). The study also demonstrated significantly high serum TC levels of post-menopausal study group as compared to premenopausal control group (Rohariya *et al*., 2017; Nayak *et al.,* 2016; Peela *et al*., 2012). Whereas, the results of meta-analysis suggested that the levels of TC in premenopausal and postmenopausal breast cancer patients were not statistically significant when compared with controls (Zhao *et al*., 2016).

1.2.3.2. Triglyceride and Breast Cancer Risk

TG serve as an independent source for fatty acid oxidation, an important process promoting cell proliferation and tumor growth. Hence, proposing a carcinogenic potential of TG (Lofterød *et al*., 2018). The increased levels of TG were closely related to decreased concentrations of SHBG, which increased the amount of free estradiol and developed breast cancer risk (Zhao *et al*, 2016; Taha *et al*, 2014). TG levels in breast cancer patients were found to be significantly higher as compared to the normal healthy individuals (Nayak *et al* 2016; Abdelsalam *et al*., 2012; Jamall *et al.*, 2010; Shah *et al*., 2008). Furthermore, there is a significant variation between the TG levels of postmenopausal women and the premenopausal women. Postmenopausal women have shown an increase in serum TG levels when compared to that of premenopausal women (Peela *et al*., 2012). The serum TG level in postmenopausal breast cancer patients was higher than control (Mishra, 2015; Owiredu *et al.*, 2009). Although, the results of this

meta-analysis suggested that TG levels was higher in both premenopausal and postmenopausal barest cancer compared with normal controls (Zhao *et al*, 2016). However, other studies did not demonstrate a statistically significant increase in TG. In the case of premenopausal women, serum TG level had not shown any statistically significant variation.

1.2.3.3. HDL-Cholesterol and Breast Cancer Risk

HDL-C possesses anti-inflammatory properties. It has inversely associated with breast cancer risk (Lofterød *et al*., 2018). Due to, increased serum HDL-C is associated with greater production of anti-inflammatory cytokines such as interleukin 10, which thought to play a protective role against breast cancer (Bernstein and Ross,1993; Boyd and McGuire,1991). HDL-C plays a key protective role against oxidative membrane damage by inhibiting LDL-C oxidative damage (Kim *et al*., 2009). The function of HDL–C is reverse cholesterol transport and the pleiotropic properties of the HDL-C particle including its anti-oxidative function (Abas *et al*., 2014). It is reasonable that as total cholesterol levels increase, potentially stimulating increases in HDL-C levels, breast cancer risk subsequently decreases (and vice versa)(Kshirsagar *et al*., 2016;Llanos *et al*., 2012). Particles of HDL-C are thought to be derived from lipolysis of TG and the LPL activity is decreased in cancer, increased plasma TG levels may be one of the factors for a lower concentration of HDL-C (Shah *et al*., 2008). However, decreased concentrations of HDL-C and vitamin C and E are not likely to be sufficient enough to counter higher reactive oxygen metabolites production in breast cancer patients that may cause oxidative stress leading to cellular and molecular damage thereby resulting in cell proliferation and malignant conversions (Taha *et al*, 2014).

However, the pathogenesis of breast cancer by HDL-C is still not confirmed (Kim *et al*., 2009). The observation that low HDL-C levels may be associated with an increased risk of breast cancer, this indicated that high HDL-C levels may cause a protective effect (Llanos *et al*., 2012). Low HDL-C was significantly associated with stage (stage III and IV). The cancer patients are characterized by lower levels of HDL-C (Nayak *et al*., 2016; Abas *et al*., 2014; Hussain *et al*., 2013).

Studies on breast cancer showed that low HDL-C among premenopausal women might be a marker of increased breast cancer risk (Abas *et al*., 2014). A high HDL-C level is increased risk of breast cancer. The association between serum HDL-C level and risk for breast cancer can be influenced by menopausal status, so premenopausal cases have mean HDL-C levels lower than matched controls. Whereas, in postmenopausal cases had higher levels than controls (Ghahremanfard *et al*., 2015; Peela *et al.*, 2012). However, the plasma HDL-C levels in breast cancer patients were no significantly lower (Taha *et al*, 2014; Jamall *et al.*, 2010) and also no significant difference was observed in HDL-cholesterol levels between the breast cancer patients and controls (Owiredu *et al.*, 2009). No significant difference between serum levels of HDL-C in pre and postmenopausal control groups and study groups, while post-menopausal breast cancer patients had slightly lower values as compared to premenopausal controls (Rohariya *et al.*, 2017).

1.2.3.4. LDL-Cholesterol and Breast Cancer Risk

The elevated plasma LDL-C is more susceptible to oxidation, may result in high lipid peroxidation in breast cancer (Pandeya *et al.*, 2018 and Hussain *et al*., 2013). This may be cause of oxidative stress leading to cellular and molecular damage, resulting in cell proliferation and malignant (Zhao *et al*., 2016; Abas *et al*., 2014 and Owiredu *et al.*, 2009). Higher LDL-C levels result are higher levels of oxidized, which increases reactive oxygen species levels and subsequently damage DNA, delay DNA repair, activate oncogenes, and inactivate tumor suppressor genes (Kim *et al.*, 2009).

However, the inverse association between LDL-C and breast cancer cannot be as easily explained and suggested that might be due to increased activity of the LDL receptor, which promotes the removal of LDL-C from circulation. LDL-C levels are affected by the presence of the disease (Kshirsagar *et al*., 2016). The levels of serum LDL-C were higher in breast cancer patients as compared to control group (Rohariya *et al*, 2017; Hussain, *et al*., 2013 and Abdelsalam *et al.*, 2012). The level of LDL-C was significantly higher at VI stages compared with the normal control group (Zhao *et al.*, 2016). There was a significant increase in LDL-C in all stages of breast cancer and LDL-C was higher in post-menopausal cases (Nayak *et al.*, 2016). The increase in LDL-C levels of premenopausal patients was (22%) and that of postmenopausal patients was (12%)

compared with the controls (Owiredu *et al.*, 2009). However, serum LDL-C had not showed significant variation in breast cancer **(**Peela *et al*., 2012).

1.2.3.5. The Relationship between Estradiol, Progesterone and Lipid Profile Levels in Breast Cancer

Lipids and steroid hormones are closely linked, while cholesterol is the substrate of all classes of steroid hormones synthesis (Pecks *et al.*, 2016; Atanassova and Koeva, 2012), such as: glucocorticoids (e.g. cortisol), mineralocorticoids (e.g. aldosterone), and sex hormones (androgen, estrogen and progesterone) (figure 5) (Kuru, 2018).

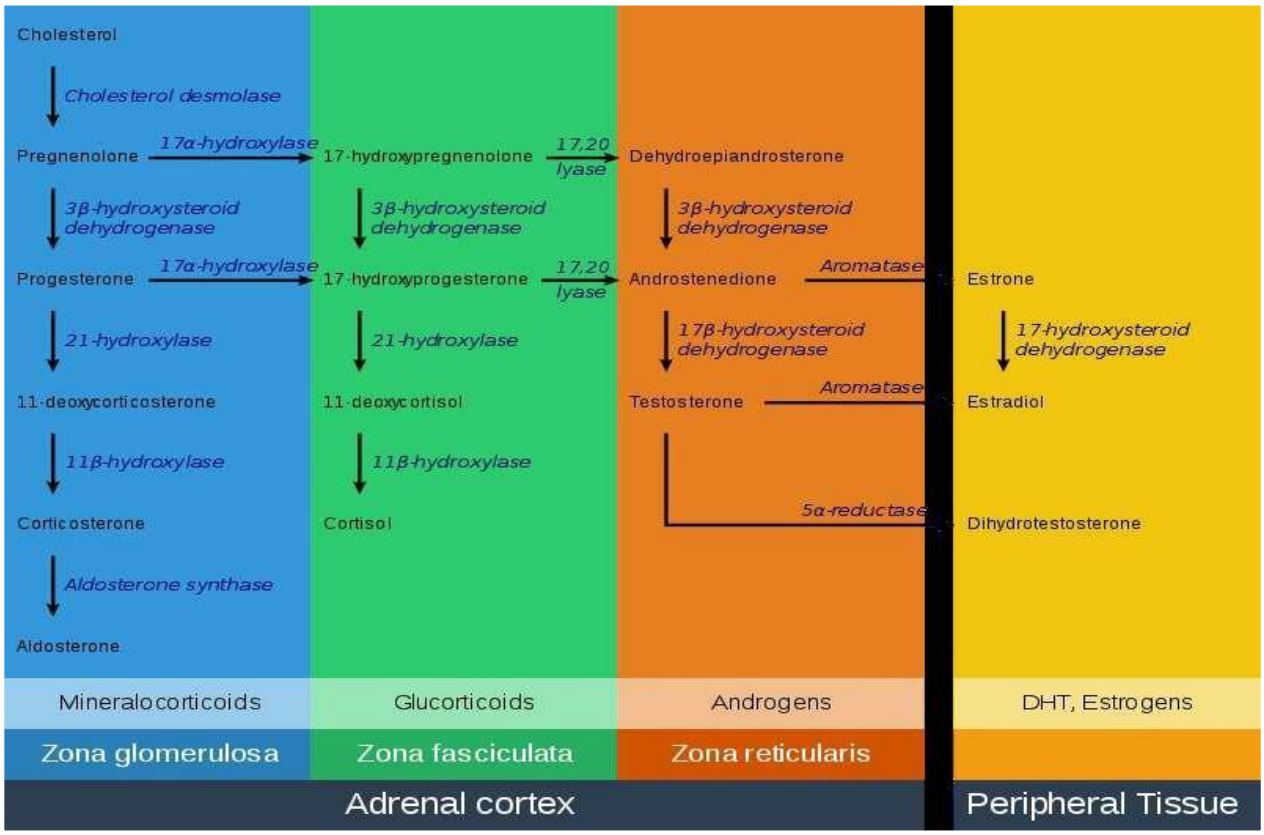

Figure 5: The relationshipbetween steroid hormones and lipid profile in synthesis pathway. Adopted from Shawaka (2018). Cholesterol is the precursor of all classes of steroid hormones: glucocorticoids (cortisol), mineralocorticoids (aldosterone), and sex hormones (androgen, estrogen, and progesterone) with different enzymes. Specialy, hydroxysteroid dehydrogenase, P450ccs (desmolase/hydroxylase), and another aromatase enzymes are the most important in the synthesis estradiol from androgen precursor.

The synthesis take in the adrenal gland, ovary, testis, and placenta. The three principal sources of cholesterol are de novo synthesis of cholesterol from acetate, circulating lipoproteins, cholesterol ester stores and plasma membranes (Kuru, 2018).The major source of cholesterol for luteal cells is circulating lipoproteins, particularly HDL-C and LDL-C. Receptors for HDL and LDL have been identified in the curpus luetum. The HDL receptor increases dramatically after luteinization of granulosa cells *in vitro or in vivo* (Diaz *et al.,* 2012). LDL receptor number and uptake of LDL-C and Storage of cholesterol esters in lipid droplets is controlling by two opposing enzymes, cholesterol esterase (cholesterol ester hydrolase) and cholesterol synthetase (Hu *et al.,* 2010).

The enzymes involved in the synthesis of steroid hormones can be divided into two major classes of proteins: the cytochrome P450 heme-containing proteins (CYP) and the hydroxysteroid dehydrogenases (Miller and Auchus, 2011). The first and rate-limiting step of steroidogenesis is convertion of cholesterol to pregnenolone after cleavage of side chain, catalyzed by cytochrome P450scc enzyme (also called 20, 22-desmolase, or 20, 22-lyase) names hydroxylases (figure 5), located in the inner mitochondrial membrane (Hu *et al.,* 2010). This critical step is control by the adrenocorticotropic hormone in the adrenals and by the luteinizing hormone in the gonads. The reactions consume molecular oxygen and nicotinamide adenine dinucleotide phosphate (NADPH, reduced) as the source of reductive potential, reduced form (NADPH) as a cofactor and are linked to an electron transport system (Steimer, 2020). In the synthesis several enzymes are involved: cytochrome P450 cholesterol side-chain cleavage enzyme (P450scc, 11β-hydroxylase), cytochrome P450 17α-hydroxylase (P450c17, 17α-hydroxylase, 17-20 lyases, P450 aromatase (aromatase, *CYP19A1*), 21α-hydroxylase, 11β-hydroxylase and aldosterone synthase (figure 5) (Atanassova and Koeva, 2012).

Next, Pregnenolone diffuses across the mitochondrial membrane and further metabolized by 3βhydroxysteroid dehydrogenase enzymes (figure 5) associated with the smooth endoplasmic reticulum (Miller and Auchus, 2011). A Hydroxysteroid dehydrogenase enzyme is the second class of steroidogenic enzymes; called alcohol oxydoreductases catalyze the dehydrogenation of hydroxysteroids. Acting as oxydoreductases and require nicotinamide adenine dinucleotide (NAD, oxidized) and/or NADPH as electron

acceptor/donor, include: 3β-hydroxysteroid dehydrogenase, 11β -hydroxysteroid dehydrogenase and 17β-hydroxysteroid dehydrogenase (figure 5) (Atanassova and Koeva, 2012).

Pregnenolone can be converted either to progesterone, which branches to the glucocorticoid and androgen/estrogen pathways, or to 17α-hydroxypregnenolone, which is another route for the formation of androgens and estrogens. Androgen formed in the adrenal in form of dehydroepiandrosterone, androstenedione and testosterone. Estrogen formation requires another P-450 enzyme, the aromatase complex (P-450Arom). The substrate is either androstenedione (for estrone) or testosterone (for estradiol). Estrone and estradiol are interconvertible through a reversible reaction involving another 17ß-hydroxysteroid dehydrogenase, as in the androstenedione-testosterone conversion (figure 5) (Steimer, 2020).

Steroids are highly lipid soluble, once they are synthesized can simply diffuse across the cell membrane and enter the interstitial fluid then enter to the blood (Hall, 2016). Steroid hormones regulate hepatic lipid production. Lipid metabolism and steroid hormone biosynthesis are closely linked, thus human mammary tissue metabolizes lipids from plasma affected by female gonadal hormones (Pecks *et al*., 2016; Ghahremanfard *et al*., 2015). Changes in the lipid profile can be described by an increased estrogen activity, which is involved in the development of breast cancer and in the modulation of lipid metabolism (Jamall *et al.,* 2010). Lipid profile can reflect higher levels of, or greater response to estrogens, which are associated with an increased risk of breast cancer, particularly ER+ disease (Martin *et al*., 2015). Even if, lipid profile and steroid hormone relation not only risk of breast cancer but also influence of hyperlipidemia on the prognosis of breast cancer. This might be indicated by several mechanisms, one of the mechanisms is cholesterol is a precursor of steroid hormones and increased steroid hormone production, including estrogen, which might promote the growth of hormone-dependent tumors (Taha *et al.*, 2014). Other mechanism may be the small HDL-C particles transporting excess cholesterol for excretion and have antiinflammatory properties. Thus, the breast tissue may experience higher levels of circulating cholesterol, increased low-grade inflammation, and higher levels of total endogenous estradiol and estradiol locally produced in the breast (Flote *et al*., 2015).

The estrogen concentration threshold was affecting cholesterol metabolism (Sheeba, 2013). Excess free serum estrogen levels countable for high rate of immature cell replication and increase fat metabolism in the body (Feigelson and Henderson, 1996). Estradiol control not only the formation but also the breakdown of lipid droplets by regulating either the activities of cholesterol esterase, the cholesterol side chain cleavages enzymes or the level of cholesterol transport protein (Miller and McLean, 1987).

For instance, estrogen reduce serum LDL-C levels by increasing LDL particle clearance through LDL receptor up regulation (Filippatos *et al*., 2009). The inhibiting effect of estrogen on hepatic lipase (HLP) activity contributes to a reduction of TG catabolism (Pecks *et al.*, 2016). Consequently, estrogen decreases the TC and LDL levels and increases the HDL levels (Callejon *et al.*, 2009). However, several studies reported that estrogen inhibit LPL activity in plasma. LPL is the major enzyme responsible for hydrolysis of circulating TG. It hydrolyzes the TG moiety of chylomicrons and VLDL, thus resulting in the biogenesis of HDL particles (Sawadas *et al.,* 2005). Although, estradiol regulates the uptake and storages of cholesterol, also rate of metabolism in to progesterone. LDL-C and HDL-C can increase progesterone in vitro (Miller and McLean, 1987). Progestogens inhibit estrogen effects on the lipid profile, which is based on the progestogen androgenicity. Testosterone-derived progestogens highly interfere the estrogen effects on the lipid profile, decreasing the beneficial estrogen effect (Jiang and Tian, 2017 and Callejon *et al.*, 2009).).

Low HDL-C and sex hormones (estrogen and progesterone) levels may, in combination, stimulate growth of epithelial and stromal tissues, influencing both absolute and percent mammographic density. Furthermore, hypercholesterolemia, strongly associated with low HDL-C, may be accelerating breast cell growth and metastasis (Flote *et al*., 2015). Serum HDL-C and salivary estradiol are biologically reasonable as sex steroids are physiologic regulators of serum lipids. The changes by sex steroids may be mediated, in part by the lipolytic enzyme, HLP; the activity of this enzyme is regulated by sex steroids (Furberg *et al.*, 2005). Estrogen levels are positively associated with HDL-C levels and inversely associated with LDL-C levels in some. But not all studies (Martin *et al*., 2015).

1.2.4. Hormonal Therapy and Breast Cancer Patients

Breast cancer is the most common malignancy in women and causes more than half a million deaths annually worldwide (Verma *et al*., 2016). But due to earlier diagnosis and improvements in treatment, mortality rates have declined (Buijs *et al.*, 2008). It is a clinically heterogeneous disease for which best treatment must be personalized to a specific breast cancer type (Yoshimura and Furuya, 2014) and with different responses to therapy (Lin *et al*., 2014). Endocrine treatment significantly reduces morbidity and mortality in patients with breast cancer (Hammond *et al.*, 2010). Tumors that express ER and/or PR are likely to respond to endocrine treatment (Althuis *et al*., 2004 and Angelopoulos *et al*, 2004). The detection of the ER and PR is crucial for prognostic evaluation and treatment choice of breast cancer for clinical practice (Effi *et al.*, 2017). This is mostly based on eliminating estrogen production or blocking the ER (Buijs *et al.*, 2008).

In addition, change of plasma lipid profile levels in breast cancer patients are regressed by effective treatment of the tumor. Thus, successive monitoring of lipid profile levels can be useful as a simple, noninvasive, rapid, and dependable method for the prediction of risk, diagnosis, and for treatment outcome in breast cancer patients receiving anticancer therapy (Shah *et al*., 2008). Stopping the action of estrogen in the breast is a known therapeutic approach for preventing recurrence, and it can be achieved by blocking the hormone‘s action on the ERs or stopping its synthesis (ASCO, 2019). Hormonal therapy is slow or stops the growth of hormonal sensitive tumors by blocking the ability to produce hormones or by interfering with effects of hormones on breast cancer cell (NCI, 2017). The two most commonly used alternative strategies of endocrine treatment are either the interference with estrogen signaling by a selective estrogenreceptor modulator (non-steroidal) such as tamoxifen, or the inhibition of endogenous estrogen production via an aromatase inhibitor (steroidal) (Markopoulos *et al.*, 2010; Buijs *et al.*, 2008).

1.2.4.1. Tamoxifen and Breast Cancer Patients

Tamoxifen is the most common hormone therapy drug (Breast Cancer Org., 2020; NBCF, 2016). It blocks ER in breast cancer cell via stopping estrogen from binding to the cancer

cells and inducing them to grow and divide. While tamoxifen act like anti-estrogen in breast cells and it acts like estrogen in other tissues. Because of this, it is called Selective estrogen receptor modulator (ASCO, 2019). Selective estrogen receptor modulators are anti estrogens blocks the effects of estrogen by binding to the ER and interfering with receptor-mediated transcriptional events (Sawada *et al.,* 2005). Its antitumor effect is based on competition with estrogen in binding to its receptor. Tamoxifen can exert their effect depending on serum estrogen levels. Tamoxifen is still the agent of choice in the first-line adjuvant and metastatic treatment of premenopausal patients (Buijs *et al.*, 2008). Because of, tamoxifen is appropriate in both, prevention and treatment of breast cancer (Khetrapal, 2018).

Blockade of estrogen action with tamoxifen reduces the incidence of breast cancer by 50–75% in high-risk women (Hammond *et al*., 2010). Tamoxifen was associated with a highly significant improvement in relapse free and overall survival. Its efficacy in the treatment and prevention of ER+ breast cancer was unquestionably (Angelopoulos *et al*., 2004). For almost 30 years, tamoxifen has continued the "gold - standard"endocrine treatment of breast cancer (Sawada *et al.*, 2005). However, use of tamoxifen has been questioned in recent years (Ntukidem *et al*., 2008) .These treatments may induce severe side effects such as endometrial cancer, thromboembolism, osteoporosis, and vaginal atrophy (Bendrik and Dabrosin, 2009). The most frequent side effects are hot flashes, fatigue and nausea. Less frequently observed side effects include uterine bleeding, vaginal dryness, dyspareunia, impaired sexual desire and optical (cataract) problems. Rare are deep venous thrombosis and thromboembolism, pulmonary embolism, cardiovascular and ischemic cerebrovascular events (Buijs *et al*., 2008). Furthermore, development of resistance to tamoxifen limits, the duration of effective treatment to 5 years (Ntukidem et al., 2008), thus typically recommended for 5 years (Khetrapal, 2018).
Tamoxifen is known to stimulate the synthesis of many "estrogen-sensitive" liver proteins including SHBG. The association between the concentrations of ER protein in breast cancer tissue and the "estrogenic" liver response to tamoxifen as reflected by the increase in SHBG levels during treatment (Lofgren *et al*., 2006).

1.2.4.2. Mechanism and Action of Tamoxifen in Breast Cancer

Tamoxifen have both agonist and antagonist estrogenic actions in various organs, inhibits the growth of breast tumors by competitively antagonizing estrogen binding to its receptor (Angelopoulos *et al*, 2004). Tamoxifen has a complex mechanism of action owing to its molecular structure. It is chemically very similar to estradiol, but tamoxifen has an extra chain, which is important for its antagonistic action. Studies indicated that tamoxifen itself is a pro-drug with a relatively low affinity for the ER. The cytochrome P450 family CYP2D6 activate as endoxifen (4-OH-Ndesmethyl-tamoxifen). This metabolite bind the ER with an almost 100-fold greater affinity than tamoxifen (Gjerde *et al.*, 2010). Tamoxifen's pro- and anti-estrogenic actions are mediated by its competitive binding to the ERα and/or β) which then undergo a conformational change. The nuclear complexes that form change the expression of estrogen-dependent genes to generate multiple growth-promoting signals both inside and outside the nucleus (Figure 6) (Khetrapal, 2018).

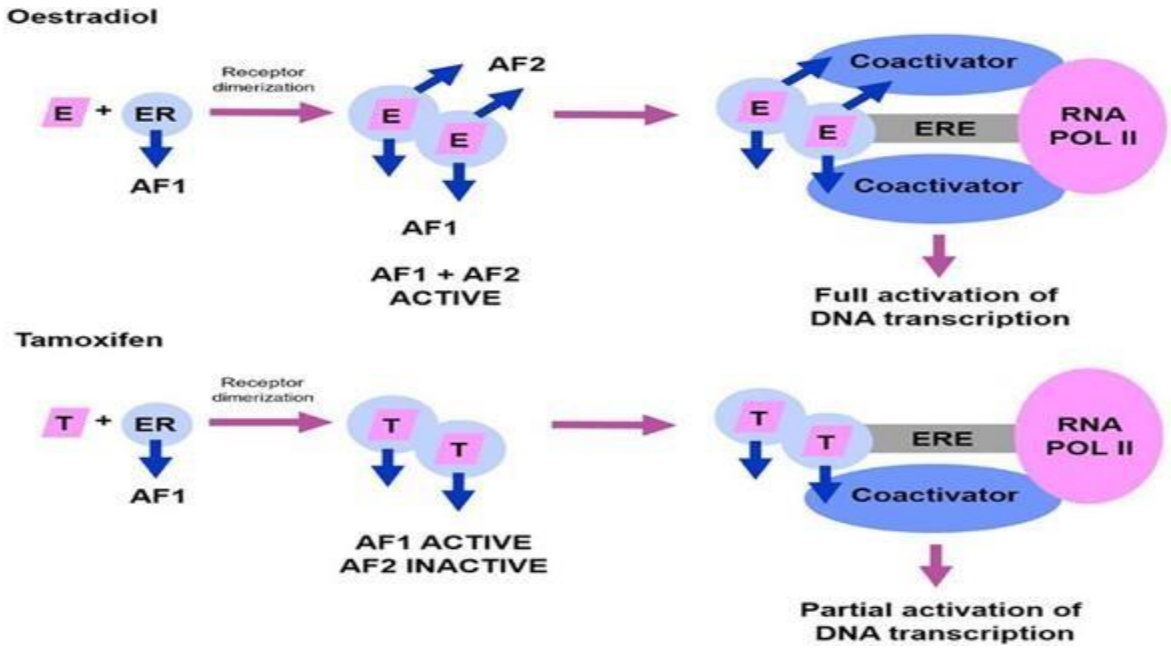

Figure 6: Tamoxifen's mechanism and action in breast tissue. Adopted from (Boér, 2017).
Tamoxifen block in the proliferative actions of endogenous estrogen on mammary epithelium by reducing DNA polymerase activity. Tamoxifen (T), Estrogen Receptor(ER), Estrogen (E), Activation Function 1 (AF1)domain, Activation Function 2 (AF2)domain, Estrogen Response Element (ERE).

Tamoxifen decelerates the proliferation of breast cancer cells by inhibiting their progression and induces apoptosis (Angelopoulos *et al*, 2004). The prolonged binding of tamoxifen to specific genes can block in the proliferative actions of estrogen on mammary epithelium by reducing DNA polymerase activity (Figure 5), both genomic and additional non-genomic effects actually have been demonstrated with estradiol administration (Khetrapal, 2018).

1.2.4.3. Tamoxifen and Lipid Profile in Breast Cancer

Tamoxifen exhibits different effects on the lipid profile (Lin *et al.,* 2014). The effects of tamoxifen vary from patient to patient and this variability is specifically seen in the response of lipid parameters. The causes are not completely understood (Ntukidem *et al*., 2008). Study showed activity of tamoxifen is considered as strong estrogen agonistic effect on lipoprotein metabolism (Czyżykowski *et al*., 2014; Sawada *et al*., 2005) and suggested that, like estrogens increases the plasma level of TG and liver secretion of VLDL by inhibits the key enzymes of TG metabolism (decreases the activity of LPL and HTGL) (Czyżykowski *et al.,* 2014; Markopoulos *et al*., 2010; Sawada *et al.,* 2005). Tamoxifen decrease TC by 13.4% and LDL-C by 23.5%. This may be due to its partial estrogenic activity, since estrogen is known to reduce serum LDL-C levels by increasing LDL particle clearance through LDL receptor up regulation (Sawada *et al.,* 2005). There is also a tendency for HDL-C levels to increase and increased TG levels in postmenopausal women (Markopoulos *et al*., 2010).

1.2.4.4. Aromatase Inhibitors and Breast Cancer

Aromatase inhibitors belong to a group of drugs widely used for breast cancer hormone therapy, that decrease estrogen levels and deactivate the aromatase complex, in order to stop the conversion of adrenal androstenedione into estrone, and testosterone (Hammond *et al*., 2010) into estradiol in peripheral adipose tissue (Buijs *et al*., 2008) and within breast cancers themselves (Coscia *et al*., 2017). Aromatase is a cytochrome-P450 enzyme-complex, responsible for the conversion of androgen into estrogen, the final step in the estrogen biosynthesis pathway (Buijs *et al*., 2008). Excessive or in appropriate aromatase expression was demonstrated in adipose fibroblasts surrounding breast

carcinoma. Such activity contributes substantially to intra-tumoral estrogen levels (Angelopoulos *et al*., 2004). These drugs reduces plasma and intratumoral estrogen levels and block the rate-limiting step in the synthesis of estrogens by the cytochrome P-450 enzyme aromatase (Figure.7) (Sawada *et al*., 2005). For postmenopausal women with hormone-receptor positive breast cancer, aromatase inhibitors are well tolerated (Buijs *et al*., 2008). Because of, aromatase inhibitors might be more effective in preventing breast cancer because of their dual role to block both initiation by reduce levels of the genotoxic metabolites of estradiol by lowering estradiol concentrations in tissue and promotion of breast cancer by lowering tissue levels of estradiol and thus blocking cell proliferation (Coscia *et al*., 2017). The third-generation aromatase inhibitors armidex have been shown to be effective in postmenopausal women with hormone-sensitive breast cancer (Ahmad, 2013; Ntukidem *et al*., 2008**).** Armidex is non-steroidal aromatase inhibitors (Angelopoulos *et al.*, 2004). It is an oral inhibitor that significantly lowers both circulating plasma estrogens and intratumoral estrogen levels (Sawada *et al.*, 2005). Armidex‘s action is exerted through a competitive link with the aromatase enzyme.

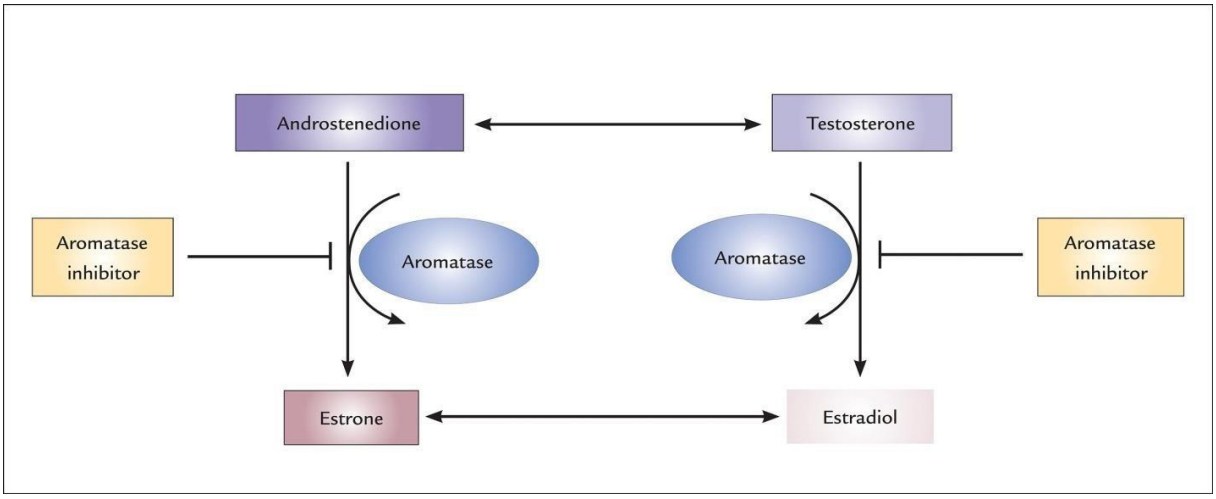

Figure 7: Armidex‘s action in biosynthesis of estradiol. Adopted from (Barros-Oliveira *et al.*, 2017). Armidex inhibited competitively aromatase enzyme, and decreases estrogen (estrone and estradiol) biosynthesis of estradiol from androstenedione and testosterone.

Armidex at 1mg/day inhibits aromatization by 96.7%, and suppresses plasma estrone and estradiol concentrations by 84–94%. The time required for armidex to promote estrogen suppression is two to four days. After approximately 7 days of administration, armidex

reaches 90 to 95% of its plasma concentrations (Coscia *et al*., 2017). Thus might be providing adverse effect and secondary complications (Angelopoulos *et al*, 2004). As with most therapies, the use of aromatase inhibitors poses its own challenges. Aromatase inhibitors affect cardiovascular and bone health (Ahmad, 2013; Ntukidem *et al.*, 2008). Their short-term adverse effects include fatigue, back pain, dyspnoea, headache and hot flashes and associated with an increased risk of osteopenia, osteoporosis, and /or bone fracture. Long-term effects are estrogen deprivation, including cognitive, vascular, skin, and urogenital tract changes (Buijs *et al.*, 2008).

1.2.4.5. Armidex and Lipid Profile in Breast Cancer

Armidex had a beneficial effect on lipid profiles of postmenopausal women with early breast cancer after 12 weeks of treatment significantly reduced levels of TG and levels of HDL-C significantly increased via increased activity of LPL. This enzyme is mainly produced in adipose tissue (Sawadas *et al*, 2005). However, some literatures indicated that for the postmenopausal patients with breast cancer, taking armidex might lead to an abnormal lipid metabolism and significantly increases the levels of LDL-C, TC and HDL-C, and significantly reduces the level of TG (Lu *et al*., 2011).

1.2.4.6. Serum Estradiol and Progesterone Level after Hormonal Therapy in Breast Cancer

Understanding of breast cancer biology have led to the development of a wide selection of systemic therapies for patients with breast cancer relapse. One of the major therapeutic option is hormonal therapy, focused at interfering the estrogen-based signal that stimulates growth in the majority of breast cancers (Peterson *et al.*, 2008). In a cohort study show that there were statistically significant reduction in estradiol levels between baseline and three months after starting the armidex treatment (Coscia *et al.*, 2017). In other findings, 4 of the 66 patients the serum estradiol level was decreased at 3 months but had then increased at 6 months, while in 2 other patients estradiol was decreased at both 3 and 6 months but had increased at 9 months (Nagao, 2009).

However, tamoxifen induced ovarian hyperstimulation in premenopausal patients, with increasing circulating estrogens (Madeddu *et al*., 2014). In Japanese women, tamoxifen could stimulate the ovarian function; high values of serum estradiol during post-operative tamoxifen therapy were employed in the study (Yamazaki *et al*., 2015), long-term tamoxifen therapy can be associated with increased serum levels of estradiol, Elevated serum estrogens may explain tamoxifen's estrogenic effects (Lum, 1997). Ovarian stimulation by tamoxifen may bring about changes on serum progesterone; the levels of estradiol, and progesterone were elevated one fold to threefold after tamoxifen (Sherman *et al*., 1979; Jordan *et al.,* 1991).

1.2.4.7. Estradiol, Progesterone, Lipid Profiles and Hormonal Therapy on Breast Cancer

The studies suggested that estrogen has been shownb a beneficial effect on lipid metabolism, while the reduction of estrogen at the menopause has been associated with an increased rate of hyperlipidemia and a risk of hypoestrogenic-related disease, including myocardial infarction and stroke. Estrogen deficiency following treatment for breast cancer may not be important in the metastatic setting. But, adjuvant setting very important (Sawadas *et al*., 2005). While tamoxifen has been shown to improve lipid profiles, the aromatase inhibitors have a very different mode of action and do not possess the estrogen-agonistic effects of tamoxifen. So few data show that the effects of these agents on lipid profiles and suggest that the different aromatase inhibitors have different effects on lipid profiles. Also indicate that it have little effect and adverse effects /increased hypercholesterolaemia on lipid profiles (Bundred, 2005). Arimidex through the effect of estrogen levels may increase LPL activity and hydrolysis of TG-rich lipoprotein, generating HDL in the process (Sawadas *et al*, 2005). In addition, might be decreased activity of LDL clearances (Lu *et al*., 2011). TC and LDL-C rapidly decreased and TG increased in patients treated with tamoxifen at 3 months in Comparison of armidex and tamoxifen patients (Hozumi, 2011). Estrogen levels increase the activity of the HTGL-C enzymes and, consequently, the levels of TC and LDL will decrease and the synthesis of HDL will be stimulated. Progestogens with high interference on the estrogen effects on the lipid profile (Callejon *et al*., 2009).

1.3. Statement of the Problem

Breast cancer is a major public health problem in women population worldwide and affecting developing and developed countries (Yadav *et al.*, 2012) and it causes more than half a million deaths annually (Verma *et al*., 2016). In Ethiopia, annual incidence and mortality rate of breast cancer accounts for 34% of female cancer cases, followed by cervical cancer at 16% (Hadgu *et al*., 2018).

Breast cancer is a heterogeneous and a hormone-dependent disease (Effi *et al*., 2017). Levels of endogenous hormones have been associated with the risk of breast cancer (Missmer *et al.*, 2004). Several studies reveal that high estrogen levels and low progesterone enhance breast cancer risk. Estrogen can make more estrogen receptors then directly induce cell proliferation and tumour growth. But, progesterone concentration is regulating estrogen activity in the breast tissue. Subsequently breast cancer cells contain large number of hormonal receptor that are sensitive to imbalanced hormone level (NCI, 2017). The relationship between lipids metabolism and breast cancer is not undistinguished until now (Abdelsalam *et al.*, .2012) but some works have shown that the altered lipid profile status in breast cancer patients may be due to abnormal lipid metabolism associated with tumor pathogenesis and tumor-host interactions (Shah *et al*., 2008). The pathogenesis may decrease cholesterol, decreased synthesis or increased catabolism; this also may contribute to hypocholesterolemia (Mishra, 2015). The etiology of lipid changes associated with breast cancer is multi factorial and relationship of lipid changes to breast cancer is still a subject of controversy. However, an unbalanced lipid profile was observed in disease situation with high TC, LDL-C, TG, and low HDL-C (Li, 2018). Gonadal hormones are affected lipid metabolism in mammary tissue (Zhao *et al.*, 2016). Estrogen, has beneficial effects on lipid metabolism that can decreases TC and LDL-C, by LDL particle clearance through LDL receptor up regulation (Filippatos *et al*.,2009) and increased HDL-C through inhibing LPL activity in plasma, which hydrolysis circulating TG (Banerjee *et al*., 2005). Estradiol regulates the progesterone production by controlling the breakdown of esterified cholesterol. LDL-C and HDL-C can increase production of progesterone *in vitro* (Miller and McLean, 1987). Estrogen and progesterone associated with only with low HDL-C levels in women may reflect

complex biologic processes. This association stimulates growth of epithelial and stromal tissues, and also induces breast cellular proliferation (Flote *et al.,* 2015).

Some studies indicated that several therapeutic approaches are available for blocking estrogen action in breast cancer patients (Singh *et al.,* 2017). Hormonal therapies are systemic treatment for hormone-dependent breast cancers and administered in two ways:

(a) Tamoxifen act like antiestrogen; block the effect of serum estrogens in breast cells (ASCO, 2017). It can increase circulating estrogens (Madeddu *et al*., 2014), whereas reduced plasma LPL and HLPL activities (Elisaf *et al*., 2000; Milionis *et al*., 2001) and increased serum TG concentration (Filippatos *et al.*, 2009). Thus, induce hypertriglyceridemia in breast cancer by stimulating hepatic synthesis and secretion of VLDL (Elisaf *et al*., 2000; Milionis *et al*., 2001). This dangerous lipid abnormalities may occur months or years during therapy and may provide severe hypertriglyceridemia which can be associated with life-threatening complications (Elisaf, 2000), like acute pancreatitis (Filippatos *et al*., 2009). Long-term administration of tamoxifen increase ovarian steroidogenesis specifically high estradiol levels due to the presence of hyperactive ovaries, result development of drug resistance (Lum, 1997, Madeddu *et al.,* 2014), endometrial cancer, thromboembolic events and so on (Markopoulos *et al.,* 2010).

(b) The second drug aromatase inhibitors may deactivate the aromatase complex (Coscia *et al.*, 2017), induces lowering the level of estrogen in the body and decreases the risk of breast cancer (komen, 2020). Arimidex through decreasing the level of estradiol may increase LPL activity. As a consequence, increased HDL-C and reduced TG concentration (Sawadas *et al.*, 2005), may decrease activity of LDL clearances and enhanced LDL-C concentration (Lu *et al*., 2011) which may leads to hyperlipidemia. However, studies have shown that armidex with little effect and adverse effect on lipid profiles /increased hypercholesterolaemia (Bundred, 2005). In addition, provided secondary complications, inducing cardiovascular diseases. Arimidex is to inhibit *in vivo* aromatization by 96–97% and to suppress plasma estrogen levels by 84–94% (Geisler *et al*., 2001). This condition may be to make hypoestrogenic effect and leads other secondary complications.

However, Studies indicated that breast cancer occurrence has a relationship with exposure to higher estradiol, deficiency of progesterone, and abnormality in lipid profiles level.The effect of hormonal therapies with changes in levels of estradiol, progesterone, and lipid profiles, and can develop endocrine resistance and secondary complications. Yet the presence of controversial ideas around this critical issue required further research and also the results might be varying with genetic and environmental factors. In addition, many studies have done on hormone receptor status in breast cancer patients, but no study was under taken on the assessment of the relationship between serum estradiol, progesterone and lipid profiles level in breast cancer patients before and after hormonal therapy by comparing with healthy controls at TASH in Ethiopia. Hence, this research is expected to provide very important background information for physican and other reasercher about the issue. In summary, the following basic questions were set at the beginning of the study:

1. What is the effect of serum estradiol, progesterone and lipid profile levels in breast cancer patients?
2. Is there association between serum estradiol, progesterone and lipid profile levels in breast cancer patients before hormonal therapy?
3. What is the effect of hormonal therapy on serum estradiol, progesterone and lipid profile levels in breast cancer patients?
4. Is there a relationship between serum estradiol, progesterone and lipid profile levels in breast cancer patients after hormonal therapy?

1.4. Significance of the Study

Around 80% of breast cancer is hormone receptor positive. Women who were be diagnosed with ER+ breast cancer have conditions associated with excess estrogen (GSN, 2016). An increase in level of serum estrogen is one marker of breast cancer risk. Most of the risk factors for breast cancer that have been identified manifest their effects by influencing the level of exposure to sex or steroid hormones (Feigelson *et al.,* 1998). This type of breast cancer can be managed with endocrine therapy, by lowering of estrogen level and or by blocking the acting of estrogen hormone on breast cancer cells (ASCO, 2017). Some evidences indicated that the endogenous sex steroid hormones maybe increased the risk of breast cancer directly, for the reason that serum cholesterol was the precursor to steroid hormone synthesis. Gonadal hormones was affected lipids metabolism in mammary tissue, the lipids promote tumor growth and metabolic abnormality of it's, which provided evidence for public health implications for breast cancer diagnosis and prevention (Zhao *et al,* 2016). Any change of lipid profile in breast cancer cases can increase its risk status. Its measurement may be helpful in evaluation of prognostic and diagnostic of the disease (Peela *et al.,* 2012).

There are also indications of estrogen concentration threshold affect cholesterol metabolism. Since, a clear variation was found in the level of estrogen before and after treatment. This may serve as a powerful predictive parameter of breast cancer and in disease prognosis (Sheeba, 2013). Estrogen levels may serve as a clinical indicator of breast cancer risk once laboratory methods to measure estrogens are adequately standardized and the best estrogen fraction to predict risk is identified (Colditz, 1998). Therefore, breast cancers have abnormalities in hormone levels. These abnormalities may be considered in the pathogenesis of the disease and should be taken into account in the treatment of patients of breast cancer. It may also be helpful to delay the onset of cancer by normalizing the levels of these hormones and in deciding the treatment modality for the patients once breast cancer has been diagnosed.

However, further studies are required to prove the benefit of measuring serum hormone levels as a screening test (Trehan *et al.*, 2012). Effective treatment of breast cancer has now become a worldwide challenge. Even though, significance advance have been made

in cellular, biochemical and clinical level, still there is a need of understanding of cancer in all aspects. Scientists and clinicians are putting huge efforts to overcome the challenges that come on the way to treatment of breast cancer (Ray and Hussain, 2001).

To overcome the indicated problems, further understanding of the relationship between serum estrogen, progesterone and lipid profile levels in breast cancer and uses of hormonal therapy for hormone dependent breast cancer patients and, measuring of estradiol, progesterone and lipid profile levels are unquestionable during diagnosis for screening of breast cancer before and after hormonal therapies during follow up of the patients. Because, this phenomenon can significantly help: for early screening methods of cases and early treatment, which provide appropriate relief to patients through specific medications. This can prevent relapse in patients and can protect the patients from secondary complications like drug resistance, these methods are cost effective and can be applied in many hospitals for a developing country, Ethiopia. Thus, the data obtained/ presented in this study will provide background information for management of breast cancer patients, helps as guideline to oncologists and preventing adverse effects of drugs used during treatment. Finally, this study is part of the ongoing thematic projects on the breast cancer at TASH, Ethiopia and can help the policy makers and other responsible bodies to formulate further investigations.

2. Objectives of the Study

2.1. General Objective of the Study

- ❖ To assess the relationship between serum estradiol, progesterone and lipid profile levels among breast cancer patients before and after hormonal therapy as compared with healthy control groups.

2.2. Specific Objectives of the Study

- ➢ To measure mean serum estrogen, progesterone and lipid profile levels before and after hormonal therapy.
- ➢ To compare the association between serum estradiol progesterone and lipid profile levels before and after hormonal therapy in breast cancer patients.
- ➢ To determine the effect of hormonal therapy on the serum estradiol, progesterone and lipid profile levels in breast cancer patients.
- ➢ To determine the strength of the association between serum estradiol progesterone and lipid profile levels before and after hormonal therapy in breast cancer patients.

3. Materials and Methods

3.1. Study Area and period

The study was conducted at TASH, oncology unit from July 1/2017 – April 30 /2018. TASH is a large referral teaching hospital, under the administration of Addis Ababa University, located in Addis Ababa, Ethiopia. The hospital has 700 beds of which 19 are allocated for cancer treatment, and give diagnostic and treatment service for about 370,000-400,000 patients per year. The oncology unit of TASH is the only oncology unit for the country and has an outpatient department which gives service to new and follow-up patients and an in-patients department (Abate *et al.,* 2016). From 201 physicians at the hospital, only two are hematologists, two are surgical oncologists, and two are paediatric oncologist. Three palliative care specialists also work at the hospital. From 627 nurses of black lionony 26 are dedicated oncology nurses. Rradiology department have one CT scanner and one MRI scanner. TASH offers comprehensive cancer treatment within the country. Being a public referral hospital, TASH offers the lowest cost for these services compared to the private hospitals (Woldeamanuel *et al.,* 2014).

3.2. Study Design

Descriptive cross-sectional study design was used to assess the relationship between serum estradiol, progesterone and lipid proile levels in breast cancer patients before and after hormonal therapy at TASH.

3.3. Source Population

All female breast cancer patients who were attendingat TASH during the study period was included in the study.

3.3.1. Study Subjects

Female breast cancer patients newly diagnosed who were not receiving any treatments (chemo, surgery, radiation and hormonal therapy), who were treated with tamoxifen, armidex and healthy controlsat TASH.

3.4. Eligibility Criteria

3.4.1. Inclusion Criteria

All females breast cancer patients who were;

- Willing to participate in the study,
- All confirmed newly diagnosed and not receiving any treatment (chemo, surgery, radiation and hormonal therapy),
- On tamoxifen and armidex for three months and above three month and,
- Other healthy females as control were included this study.

3.4.2. Exclusion Criteria

- All female breast cancer patients who were/ have:
- Refused to participate in this study.
- Other diseases/ contradicting with breast cancer.
- Taking medicines antagonistic with tamoxifen and armidex.
- Lipemic serum
- A family history of breast cancer.

3.5. Sample size and sampling method

Convenience sampling method would be applied to employee study subjects. The sample size was calculated based on an estimated 22.8 % prevalence of breast cancer in Ethiopia. The desired sample size was determined by the single population formula as follow:

$$n = (Z\alpha/2)^2 \, p \, (1-p)/d^2$$

Where

P = estimates of prevalence rate for the population = 22.8 %

D = the margin of sample error tolerated = 5 %

$Z\alpha/2$ is the standard normal variable at 1- α % confidence level = 1.96 and α =5%

q = 1-p

n = minimum sample size

$n = (1.96)^2 \, 0.228(1- 0.228)/(0.05)^2$

n = 276

The calculated sample size, using the above formula was 276 breast cancer female patients selected as sample size. However, due to financial and time constraints the sample size was purposely assigned to be 120. Out of this, 40 females were breast cancer patients who were not receiving hormonal therapies and 40 were breast cancer patients who were medicated with tamoxifen and armidex and 40 were normalfemales, who weredid not have any breast cancer problems and included as healthy control groups.

3.6. Study Variables

3.6.1. Dependent Variables

Steroid hormone test results (normal/higher/lower)

- Serum estradiol level
- Serum progesterone level
- And serum lipid profile levels

3.6.2. Independent Variables

- Socio-demographic characteristics: Age, BMI, Drinking alcohol, Smoking status, Physical activity, Educational status, Residence
- Reproductive condition: Current menses condition, Menstruation phases, Menopausal status, parity, and
- Clinical characteristics: Treatment, Stage and Duration of treatment

3.7. Operational and Standard Definitions

- ✓ **Dyslipidaemia**:-synonymous with hyperlipidemia, or hypolipidemia abnormal lipid profiles, which is higher or below normal reference range like Hypocholesterolemia: - low levels of cholesterol in the proliferating tissue and in blood compartment,
- ✓ **Lipid profile levels**: - which is consists of TC, LDL-C, TG and HDL-C levels.
- ✓ **Progestogens**:- consist natural progesterone
- ✓ **Estrogen**: - which is contained the most potency /strongest form of estradiol and calls as estradiol.

- ✓ **Hormonal therapy:** - endocrine therapy that interrupting the estrogen-based signal that stimulates cell growth and cell proliferation, which is consists of aromatase inhibitors/armidex/ and tamoxifen. Aromatase inhibitors:-inhibit the conversion of androstenedione and testosterone in to estradiol, which is consists armidex etc.
- ✓ **Anastrozole: -** in other name of armidex, an example of the third-generation aromatase inhibitors that is lowers both circulating plasma estrogens and intratumoral estrogen levels.
- ✓ **Breast cancer patients with medication**: - females breast cancer patients who received chemo, surgery, radiation and hormonal therapy and hormonal therapy only.
- ✓ **Breast cancer patients without medication**: - the breast cancer patients who did‘t received chemo, surgery, radiation and hormonal therapy and hormonal therapy only.
- ✓ **Healthy control groups**: - females who did‘t has breast cancer and /or a family history.
- ✓ **Stages of breast cancer** :- grade of cancer that are categoriezed in to two: lower stage (stage I and II) and higer stage(stage III and IV)
- ✓ **Menarche** :- the time of women starting menstruation (menstrual cycle)

3.8. Data Source

The study instruments consist of patients‘card and a survey based on questionnaire that was divided into the following sections:

- Socio-demographic characteristics
- Reproductive data
- Clinical characteristics

3.9. Data Collection Procedure and Processing

3.9.1. Data Collection Procedure

In this study, quantitative data was collected cross-sectionally using blood sampling based on questionnaire that filled about patients and control group history by the oncology nurses and investigator. The data collections were mainly focus on the

objective of the study. Relevant information about each patient was collected based on their characteristics like age, and reproductive events, medication histories by using recorded data and primary data.

3.9.2. Blood Sample Collection

Steroid sex hormones dissolve in fat but not in water. Blood tests give important information about the function of the blood cells and levels of chemicals in the blood. The blood measurements reflect the total amount of hormone present (Taylor and Bell-Taylor, 2009). Studies indicated that Lipid measurements are used in the diagnosis of various endocrine disorders. The circulating lipids are carried on lipoproteins. For TC, LDL-C fasting and non-fasting samples can be used (Gundersen health system, 2019).

Owing to this, for determination of serum estradiol, progesterone and lipid profiles level, blood samples was collected by oncology nurses from cases and control group within ten-month period. Blood sample was collected under septic conditions; skin was cleaned by 70% alcohol and blood drawn from patient vein, then whole blood was transferred to serum separator yellow top test tube and centrifuged at 3000 rpm for 10 minutes. The separated serum would be transferred in to sterile nuck tube and stored at – 80°C. Then, serum estradiol, progesterone and lipid profiles level were measured by fully automated immunochemistry analyzer (Elecsys 2010, cobas e 411, Roche Diagnostics International,Switzerland) and serum lipid profiles level was measured by fully automated Roche/ Chemistry Cobas 6000 Analyzer based on the reagent manufacturer‘s instruction in clinical chemistry laboratory of Ethiopian public health institute. Values were considered within the normal range for premenopausal and postmenopausal.

3.9.3. Methods of Immunochemistry Assays

Immunochemistry analysis specifically enzyme link fluorescent assay technique was used for analyzing serum estradiol and progesterone hormone level. TC, TG and HDL-C were determined by standard enzymatic method (Hopkins, 2003)

3.10. Determination of the Analytes

3.10.1. Determination of Estradiol

Immunoassay was used for *in vitro* quantitative determination of serum estradiol level. The electrochemiluminescence immunoassay is intended for use Elecsys® and cobas e411immunoassay analyzers and employs a competitive test principle using two monoclonal antibodies specifically directed against 17β-estradiol. Endogenous estradiol released from the sample by mesterolone complexes by added estradiol derivative labeled with a ruthenium complex for the binding sites of the biotinylated antibody.

Test principle. Competitive immunoassay with analyte liberation total duration of assay: 18 minutes.

- First incubation: By incubating the sample (25 μL) with two estradiol-specific biotinylated antibodies, immuno complexes (mesteorlone complexs) were formed, the amount is depend up on the analyte concentration in the sample. The higher the estradiol concentration in the patients'sample, the lower is the amount of unbound biotinylated antibody.
- Second incubation: after addition of streptavidin-coated microparticles and an estradiol derivative labeled with a ruthenium complex, the still-vacant sites of the biotinylated antibodies become occupied with formation of an antibody-hapten complex. The entire complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- Measurement: the reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured on to the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. The signal yield is roughly reversely proportional to the total estradiol concentration in the sample.
- Results are determined via a calibration curve, which is instrument-specifically generated by 2 point calibration, and a master curve provided via the reagent barcode (figure 8).

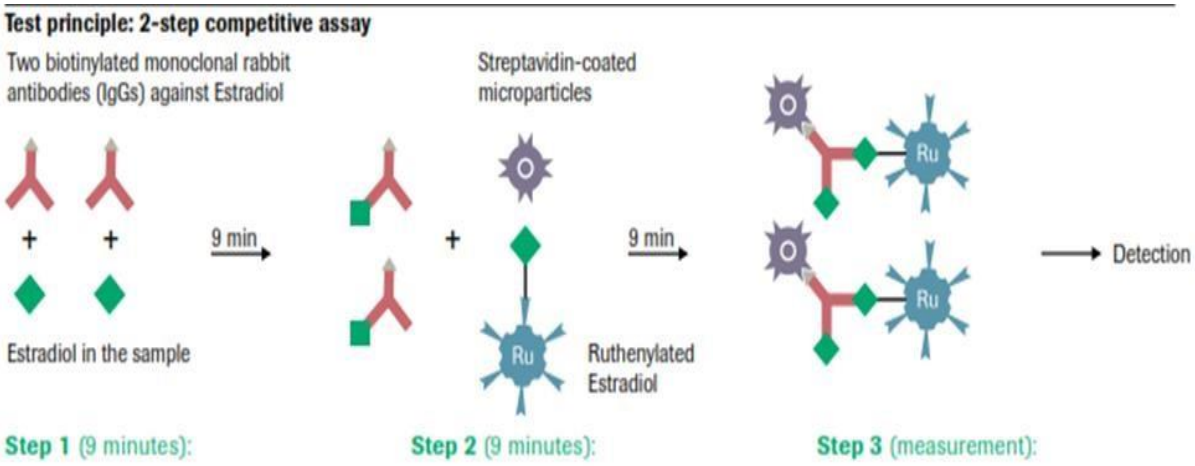

Figure 8: Electrochemiluminescence immunoassay (ECLIA) for the *in vitro* quantitative determination of estradiol in human serum and plasma. Adopted from (Elecsys 2010 analyzer, cobas e 411 analyzer, 2016).

3.10.2. Determination of Progesterone

Electrochemiluminescence immunoassay was used for *in vitro* quantitative determination of serum progesterone level.

Test principle: competitive immunoassay with analyte liberation total duration of assay: 18 minutes.

- First incubation: 30 µL of the sample is incubated with a biotinylated, monoclonal progesterone-specific antibody, ruthenylated progesterone derivative, and danazol (to release progesterone). The antibody binding sites are occupied by either progesterone or the ruthenylated derivative, with the proportion of each depending on the concentration of progesterone in the sample.

- Second incubation: Streptavidin-coated microparticles are added to the reaction mixture and the immune complexes bind to the solid phase via biotin–streptavidin interactions.
- Measurement: The reaction mixture is transferred to a measuring cell and the microparticles are magnetically captured on the surface of an electrode; unbound sample is washed away before a chemiluminescent reaction is induced by applying a voltage to the electrode. Chemiluminescence is measured by a photomultiplier and the concentration of Progesterone within the sample is calculated using a calibration curve (figure 9).

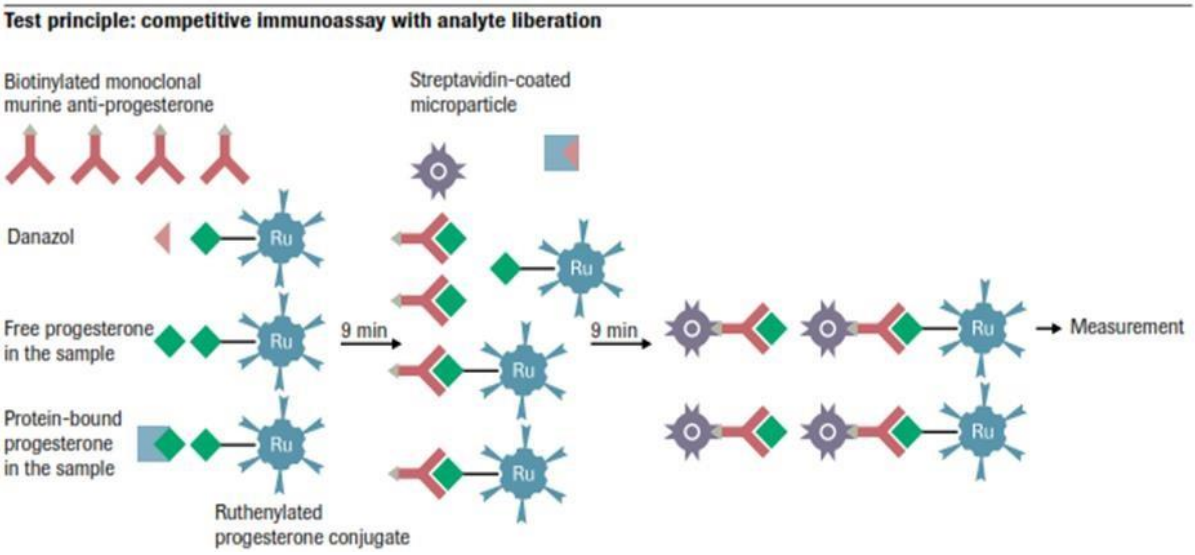

Figure 9: Electrochemiluminescence immunoassay (ECLIA) for the *in vitro* quantitative determination of progesterone in human serum and plasma. Adopted from (Elecsys 2010 analyzer, cobas e 411 analyzer, 2016).

3.10.3. Determination of Lipid Profile Levels

3.10.3.1. Determination of TC by Enzymatic Colometric Method

Test principle: Cholesterol esters cleaved by the action of cholesterol esterase (CE) to yield free cholesterol and fatty acids. Cholesterol oxidase (CHOD) then catalyzes the oxidation of cholesterol to cholest-4-en-3-one and hydrogen peroxide. In the presence of peroxidase (POD), the hydrogen peroxide formed effects the oxidative coupling of phenol and 4-aminophenazone to form a red quinone-imine dye. The color intensity of the dye formed is directly proportional to the cholesterol concentration. It is determined by measuring the increase in absorbance. The intensity of the color formed is measured at 500 nm (500-550nm). The reactions are described as follows:

$$\text{Cholesterol esters} + H_2O \xrightarrow{CE} \text{cholesterol} + \text{RCOOH}$$

$$\text{Cholesterol} + O_2 \xrightarrow{CHOD} \text{cholest-4-en-3-one} + H_2O_2$$

$$2\,H_2O_2 + \text{4- AAP} + \text{phenol} \xrightarrow{POD} \text{quinone-imine dye} + 4\,H_2O$$

3.10.3.2. Determination of TG

Test Principle: LPL release glycerol and free fatty acids from TG in serum. Glycerol is converted to glycerol-3 phosphate (G3P) and adenosine-5- diphosphate (ADP) by glycerol kinase and ATP. Glycerol-3-phosphate (G3P) is then converted to dihydroxyacetone phosphate (DAP) and hydrogen peroxide (H_2O_2) by glycerol phosphate dehydrogenase (GPO). In the last reaction, H_2O_2 reacts with 4-aminophenazone (4-AP) and P-chlorophenol in presence of peroxidase (POD) to give a red colored dye whose intensity is measured at 500nm and directly proportional to the TG concentration in present the sample. The reactions are summarized as follows:

$$\text{Triglycerides} + H_2O \xrightarrow{\text{LPL}} \text{Glycerol} + \text{free fatty acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerol kinase}} \text{G3P} + \text{ADP}$$

$$\text{G3P} + O_2 \xrightarrow{\text{GPO}} \text{DAP} + H_2O_2$$

$$H_2O_2 + 4 - \text{AP} + \text{p- Chlorophenol} \xrightarrow{\text{POD}} \text{Quinone} + 2H_2O$$

3.10.3.3. Determination of HDL-C

Test Principle: In this method a magnesium/dextran sulfate solution is first added to the specimen to form water-soluble complexes with non-HDL cholesterol fractions. These complexes are not reactive with the measuring reagents added in the second step with addition of reagent 2, HDLcholesterol esters are converted to HDL-cholesterol by polyethylene glycol-modified cholesterol esterase (PEG-cholesterol esterase). The HDL-cholesterol is replaced upon by by PEG-modified cholesterol oxidase (PEG-cholesterol oxidase), and the hydrogen peroxide produced from this reaction combines with 4-amino-antipyrine and HSDA under the action of peroxidase to form a purple/blue pigment that is measured photometrically at 600 nm (secondary wavelength = 700 nm). When the cholesterol measuring enzymes are modified with (PEG,) they are preferentially more reactive with HDL-cholesterol than the other cholesterol fractions. This is an end point reaction that is specific for HDL-cholesterol. The sequences of reactions are summarized as follows:

- ApoB containing lipoproteins + α-cyclodextrin + Mg^{+2} + $\underrightarrow{dextranSO_4}$ soluble nonreactive complexes with apoB-containing lipoproteins.
- HDL-cholesteryl esters $\xrightarrow{\text{PEG- cholesteryl esterase}}$ HDL-Unesterified cholesterol + fatty acid
- Unesterified cholesterol+ O_2 $\xrightarrow{\text{PEG-cholesterol oxidase}}$ cholestenone + H_2O_2.
- H_2O_2 + 5-aminophenazone + N-ethyl-N-(3-methylphenyl)-N'-succinyl ethylene diamine+H_2O+ H^+ $\xrightarrow{\text{peroxidase}}$ qunoneimine dye + H_2O.

3.10.3.4. Determination of LDL-Cholesterol

Test principle: Homogeneous enzymatic colorimetric assay used for determination of LDL- cholesterol without any preparatory steps or calculation. This automated method for the direct determination of LDL-C takes advantage of the selective micellary solubilization of LDL-C by a nonionic detergent and the interaction of a sugar compound and lipoproteins (VLDL and chylomicrons). When a detergent is included in the enzymatic method for cholesterol determination (cholesterol esterase, cholesterol oxidase coupling reaction), the relative reactivities of cholesterol in the lipoprotein fractions increase in this order: HDL < chylomicrons < VLDL< LDL. In the presence of Mg++, a sugar compound markedly reduces the enzymatic reaction of the cholesterol measurement in VLDL and chylomicrons. The combination of a sugar compound with detergent enables the selective determination of LDL-C in serum.

a) HSDA = Sodium N-(2-hydroxy-3-sulfopropyl)-3,5-dimethoxyaniline In the presence of peroxidase, the hydrogen peroxide generated reacts with 4-aminoantipyrine and HSDA to form a purple- blue dye. The color intensity of this dye is directly proportional to the cholesterol concentration and is measured photometrically.

$$\text{LDL-cholesterol esters} + H_2O \xrightarrow[\text{Cholesterol esterase}]{\text{Detergent}} \underset{\text{(selective micellary solubilization)}}{\text{Cholesterol + free fatty acids}}$$

Cholesterol esters are broken down quantitatively into free cholesterol and fatty acids by cholesterol esterase.

Cholesterol oxidase

LDL-Cholesterol + O_2 ⟶ Δ^4-cholestenone + H_2O_2

In the presence of oxygen, cholesterol is oxidized by cholesterol oxidase to Δ^4-cholestenone and hydrogen peroxide.

$2\ H_2O_2$ + 4- aminoantipyrine + HSDA[a] + H_2O + H^+ ⟶ (Peroxidase) Purple- blue pigment + $5\ H_2O$

(Abs. max = 585 nm)

3.11. Data quality Assurance and Management

In order to assure its quality, the questionnaire was translate to Amharic language, and then back to English. At the end, another person checked the consisteny. The data collection questionnaire was well prepared and all variables were filled on the data extraction format daily.The data would be filled by investigators immediately at blood sample collection time and. The information gathered from patients would be well secure. Blood sample was collected by oncology nurses who have basic knowledge on cancer therapy care services. The blood samples for immunochemistry assay were collected by observation with standard operation procedures and measurements of analyses were carried out after running quality control samples. All the laboratory procedures were handled by professional laboratory technologist. All the tests were standardized and automated. The sample test result cross checked with questionnaire. At the end data analysis and interpretation would be rechecked repeatedly.

3.12. Data Processing and Analysis

Followed checking for completeness and cleaning, the data obtained from laboratory analyses of the blood samples, questionnaires coded and entered into SPSS version 20 were performed, and Statistical data was analyzed using appropriate tests. Simple descriptive statistics test was used to present the breast cancer risk factors and general clinical characteristics of the study participants. Categorical variables were summarized by using number, percentage and frequency. Continuous variables were summarized by using the mean ±SD (standard deviation). Comparison of mean value of serum steroid

hormones and lipid profile levels among the three groups and among breast cancer risk factors and general clinical characteristics of the study participants were performed. By using different inferential statistics at 95%confidence interval such as independent sample *t*-test, One-way ANOVA with Gabriel post hoc test used for unequal sample size and with Tukey post hoc test used for equal sample size. Pearson's correlation coefficient, and multiple and binary logistic regression was employed to examine the association between independent variables with serum estradiol, progesterone, TC, LDL-C and HDL-C level in three examined groups. For all study participants the significance of mean differences between all groupswas tested. In Statistical analysis all p-values less than 0.05 was considered as significant and $P \leq 0.01$ as highly significant.

3.13. Ethical Consideration

Before starting data collection and preliminary study, ethical clearance letter with reference number SOM/BCHM/149/2009 meeting No. of DRERC 02/17 and protocol No. of M.Sc. 6/17 issued on March 24/2017 was obtained from the Department Ethics and Research Committee, Department of Biochemistry, College of Health Sciences, Addis Ababa University. Then a letter informing the department of Oncology about the study was written from ethical Committee of department of Medical biochemistry and permission was obtained from department of hormonal therapy unit to access data from study population. The objective of the study was briefly clarified for each participant before enrolling any of the fit study participants. Samples and data were collected after informed consent had been obtained from the study participants. Confidentiality, anonymity, neutrality, accountability and academic honesty was maintained throughout the study. The findings of the study were disseminated for health care professionals and other concerned bodies for better care of breast cancer patients.

4. Results

Out of the total 120 study participants, healthy controls 40 and cases of breast cancer patients 80 (before hormonal therapy or without medication 40 and after hormonal therapy or with medication 40) were enrolled in the study.

4.1. Breast Cancer Risk Factors and General Clinical Characteristics of Participants.

Breast cancer risk factors (age, BMI, reproductive characteristics, smoking, alcohol drinking) and general clinical characteristics of participants are indicated in table1.The mean age of healthy controls, breast cancer patients without medication and with medication were 39.6 ± 14.6, 41 ±11.3 and 45.3±12.9 respectively. There were no significant differences between age and three examined groups (p=0.135). However, most of the participants were found in the age of between 20-45 years. The majority of the study participants were lived in urban area. The average BMI of healthy controls, breast cancer patients without and with medication were 20.6± 6.3, 22.8±4.8 and 23.4± 4.8 respectively, and there were no significant differences between three examined groups (P=0.052). Under reproductive characteristics in the age of first menarche, most of the study participants of the examined groups were above 12 years. The average ages of premenopausal status of healthy controls were (28.4±4.9), breast cancer patient without medication (33.7±5.9) and with medication (34.2±5.6). Whereas, the average age of postmenopausal status of healthy controls was (54.8±7.7), breast cancer patients without medication were (52.6±6) and patients with medication (53.4±10.4). In birth condition or parity, most the study participants had a child at an early age (13-25) years. All of the study participants were non-smokers, had no alcohol drinking habit and most of those participants were not involved physical activity.

According to general clinical characteristics, almost all of the study participants had no chance to check their hormones level before and after follow up. Only two (5%) of breast cancer patients with medication get a chance to know their hormonal level. (60%) and (32.5%) of breast cancer patients without medication and with medication were found within three month follow up. Breast cancer patients with medication (82.5%) were take surgery, radiation, chemotherapy in addition to hormonal therapy. However, breast

cancer patients with medication only 5% was identified their types of breast cancer i.e. ER+ and PR+. (42.5%) and (57.5%) of breast cancer patients with medication have taken tamoxifen and armidex. However, 37.5 % of breast cancer patients without medication could not identify with the stage of the disease (table 2)

Table 2: Socio-demographic and clinical characteristics of study participants

Variables	Classification	Hc N=40	Wom N=40	Wm N=40	Wom+Wm = 80	P-value
Age, Year, n (%)	20-35	21(52.5)	13(32.5)	12(30)	25(31.3)	
	36-45	3(7.5)	15(37.5)	10(25)	25(31.3)	
	Above 45	16(40)	12(30)	18(45)	30(37.5)	
Mean ± SD		39.6±14.6	41.1±11.3	45.3±12.9	43.2 ±12.1	0.135
Min-max		20-70	22-61	26-75	24-68	
Residence, n (%)	Urban	34(85)	26(65)	31(77.5)	57(71.3)	
	Rural	6(15)	14(35)	9(22.5)	23(28.75)	
BMIKg/m^2	<18	7(17.5)	3(7.5)	3(7.5)	6(7.5)	
	18-24.5	29(72.5)	26(65)	23(57.5)	24.5(61.25)	
	24.6-30	4(10)	8(20)	8(20)	16(20)	
	>30	0(0)	3(7.5)	6(15)	9(11.25)	
mean±SD		20.6±6.3	22.8±4.8	23.4±4.82	23.1±4.8	0.052
Age 1st menarche, year, n (%)	After 12	36(90)	38(95)	36(90)	37(92.5)	
	Befor 12	4(10)	2(5)	4(10)	6(7.5)	
Current monthly menstrual status, n (%)	Yes	23(57.5)	24(60)	15(37.5)	39(48.8)	
	No	17(42.5)	16(40)	23(57.5)	39(48.75)	
	No/chemo			2(5)	2(5)	
Phase of menstruation, n (%)	1st and 2nd week (foll)	19(82.6)	13(54.2)	5(33.3)	18(43.8)	
	14th day and last week(lut)	4(17.4)	11(45.8)	10(66.7)	21(56.25)	
		23(100)	24(100)	15(100)	39(100)	
Birth condition Have child n (%)	Yes	36(90)	31(77.5)	35(87.5)	66(82.5)	
	No	4(10)	8(20)	5(12.5)	13(16.25)	
	menopausal		1(2.5)		1(2.5)	
Age at first live birth, year, n (%)	13-25	36(100)	31(100)	32(91.4)	31.5(95.7)	
	26-35	0(0)	0(0)	2(5.7)	2(5.7)	
	After 35	0(0)	0(0)	1(2.9)	1(2.9)	
Menopausal status, n (%)	Pre	23(57.5)	24(60)	17(42.5)	41(51.25)	0.237
	Post	17(42.5)	16(40)	23(57.5)	39(48.8)	

Variables	Classification	Hc N=40	Wom N=40	Wm N=40	Wom+Wm = 80	
Age at menopausal status mean ± SD	Pre	28.4±4.9	33.7±5.9	34.2±5.6	33.95±5.8	
	Post	54.8±7.7	52.6±6	53.4±10.4	53.5±8.2	
Min-max	Pre	20-39	22-40	26-48	24-44	
	Post	45-70	42-61	39-75	40.5-68	
Drinking, n (%)	Yes	0(0)	0(0)	0(0)		
	No	40(100)	40(100)	40(100)	80(100)	
Smoking, n (%)	Yes	0(0)	0(0)	0(0)	0(0)	
	No	40(100)	40(100)	40(100)	40(100)	
Exercise, n (%)	Yes	6(15)	1(2.5)	7(17.5)	8(20)	
	No	34(85)	39(97.5)	33(82.5)	72(90)	
Time of identification BRCA, n (%)	3month		24(60)	8(20)	32(40)	
	3month-5year		11(27.5)	27(67.5)	38(47.5)	
	Above 5year		5(12.5)	5(12.5)	5(12.5)	
Stage of disease, n (%)	I and II		14(35)	19(47.5)	33(41.25)	
	II and V		11(27.5)	19(47.5)	30(37.5)	
	Not classified		15(37.5)	2(5)	17(21.25)	
Identification of hormone level, n (%)	Yes			2(5)	2(5)	
	No		40(100)	38(95)	78(97.5)	
Types of BRCA, n (%)	ER+		0(0)	0(0)	0(0)	
	PR+		0(0)	0(0)	0(0)	
	ER+ and PR+		0(0)	2(5)	2(5)	
	Not classified		40(100)	38(95)	78(97.5)	
Medication of BRCA, n (%)	Hormone			7(17.5)		
	Sur, Chemo, Rad and Hormona			33(82.5)		
Duration of hormonal therapy, n (%)	3month			13(32.5)		
	3month-5year			27(67.5)		
	Above 5year			0(0)		
Types of hormonal therapy, n (%)	Tamoxifen			17(42.5)		
	Armidex			23(57.5)		

Age and BMI =body mass index Kg/m^2 continuous variable were expressed as mean ±SD (standard deviation) used one way ANOVA; for categories variables were expressed n (numbers) and % (percent) out of the total (40,40 and 40) for three groups respectively. For all P value< 0.05 was statistically significant indicated by *. BRCA=breast cancer. Hc=Healthy controls. Wm=with medication. Wom=without medication.

4.2. Serum Estradiol, Progesterone and Lipid Profiles Levels among Healthy Controls, Breast Cancer Patients Without and With Medication, based on Breast Cancer Risk Factors.

A one-way analysis of variance was indicated that the average serum progesterone level at age categories above 45 years showed a statistically significant difference between breast cancer patients without medication and with medication groups at (p= 0.032). The actual differences mean serum scores of progesterone level in breast cancer patients with medication were observed higher by 0.26 when compared to patients without medication in Post hoc multiple comparisons using the Gabriel test. In breast cancer patients without medication were showed insignificantly higher mean serum estradiol level than healthy control groups at age categories 36-45 (table 3).

For serum TC, LDL-C and HDL-C levels there were a statistically significant differences observed (p= 0.026; p=0.001and p=0.002) in the age categories 20-30 years of the three groups, in post hoc multiple comparison these stastically significant difference found that between breast cancer patients without medication and with medication (p= 0.044; p=0.011and p=0.005) correspondingly. Breast cancer patients with medication contained greater mean serum of these levels were 31.4, 28.3 and 11.4 than without medication respectively. Even though, significant differences observed among breast cancer patients with medication and healthy control groups (p= 0.001) for LDL-C level and among breast cancer patients without medication and healthy control groups (p= 0.003) for HDL-C level. The differences mean score serum LDL-C of breast cancer patients with medication was greater by 32.3 than healthy control groups and HDL-C level of healthy controls was greater by 10.4 than breast cancer patients without medication. In addition, at the age categories of above 45 years TC and HDL-C levels showed a statistically significant differences among the three examined groups at (p= 0.026) and (p=0.024) respectively. For TC level these significant difference indicated between breast cancer patients with medication and without medication at (p=0.021) and for HDL-C level indicated in breast cancer patients without medication and healthy control groups at (p=0.021).The actual difference in mean serum scores between groups were 32 and 9 respectively (table 3).

Table 3: Comparison of serum estradiol, progesterone and lipid profile levels with age of healthy controls and breast cancer patients

variables	20-35 years				36-45 years				Above 45			
	Hc N= 21	Wom N=13	Wm N=12	Pvalue	Hc N=3	Wom N=15	Wm N=10	P-value	N=16	N=12	N=18	P-Value
Etradiol pg/ml	108.6±75.9	150.2±100.7	161.9±166.2	0.360	93.5±66.3	89.7±53.4	64.1±81.8	0.606	15.8±16	20±18.8	21.3±30	0.775
PgR ng/ml	2.9±4.3	0.73±0.76	1.72±0.92	0.124	0.6±0.7	0.3±0.2.6	1.2±2.2	0.288	0.3±0.2	0.2±1[c]	0.5±0.4[c]	**0.030***
TC mg/dl	162.7± 19.4	136.2±42.5c	167.6±33.1[c]	**0.026***	170.7±22.2	157.2±28.6	166.6±38.9	0.701	159.8±28.7	141.8±30.8[c]	174±32[c]	**0.026***
LDL-C mg/dl	99.6±25.7[b]	103.6±11.8c	131.9±26.5bc	**<0.001**	105.3±1.2	92.7.9±21.4	107.7±32.9	0.340	101.7±27	104.5±16.7	109±22	0.706
TG mg/dl	108.7±30.5	113.7±24.1	131.2±66.6	0.327	102.2±23.	108.7±26.7	129.6±67	0.486	121.7±31.4	101.8±20	131±48	0.122
HDL-C mg/dl	44.5±7.9[a]	34.1±8.2[ac]	45.5±9.7[c]	**0.002***	57.3±2.3	38.2±10.2	35.9±8.1	0.820	41±9.6[a]	31.5±6.9[a]	38.2±9	**0.024***

*Key: All values were given by Mean± SD (standard deviation), age in year. P value < 0.05 is statistically significant indicated by *. Superscript letters (a, b and c) indicate significant differences compered to healthy controls versus breast cancer patients without medication, healthy controls versus breast cancer patients with medication ,breast cancer patients without medication and with medication respectively*

One-way ANOVA with Gabriel post hoc test which showed that there was significantly higher mean serum TC and HDL-C level in healthy control groups than that of breast cancer patients without medication at 18-24.5 BMI categories (p= 0.040) and (p= 0.024). The mean differences between the two groups were 20.4 and 7.1 respectively. Although, there was significantly higher mean serum LDL-C level showed in breast cancer patients with medication than breast cancer patients without medication at (p= 0.004) the mean difference was 22.4. In addition to this, statistically significant difference was found to be lower mean serum progesterone and HDL-C level in breast cancer patients without medication than healthy control groups at 24.6-30 BMI categories (p=0.011and 0.027). The actual mean difference in score was 1.5 and 15.2 correspondingly (table 4).

Table 4: Comparison of serum estradiol, progesterone and lipid profile levels with BMI in healthy controls, breast cancer patients without and with medication.

	Underweight (< 18)				**Normal (18-24.5)**			
Variables	HcN =7	Wom N=3	Wm N=3	P-value	Hc N=29	Wom N=26	Wm N=23	P-value
Estradiol pg/ml	71±99	10.7±6.6	8.5±37	0.380	71±66.8	93.8±85.2	102.7±140.7	0.489
PgR ng/ml	0.4±0.6	0.2±0.1	1.1±1.7	0.450	2±3.8	0.4±0.5	1.3±1.6	0.083
TC mg/dl	156.3±24.8	157.6±18.9	166.7±15.1	0.790	165.5±23.6[a]	145.1±32.4[a]	165.4±34	**0.022***
LDL-C mg/dl	100.1±36.3	102.8±9.6	89±10.6	0.816	103.7±22.6	95.7±15.9[c]	118.2±30.5[c]	**0.005***
TG mg/dl	112.4±30	105.2±2.3	141.2±63.4	0.452	113.7±32.7	106.4±27.5	119.9±47.8	0.428
HDL-C mg/dl	39.8 ±5.4	35.1±11.3	38.8±5	0.627	42.6±18.6[a]	35.6±9.6[a]	41.5±10.9	**0.020***
	0ver weight (24.6 -30)				Obese (>30)			
	N=4	N=8	N=8		N=0	N=3	N=6	
Estradiol pg/ml	64.6±89.8	87.7±65.5	54.5±64.2	0.635	0	121.8±137	24±41.9	0.134
PgR ng/ml	1.8±1.7[a]	0.29±0.2[a]	0.63±0.28	**0.014***	0	0.83±1	0.38±0.26	0.296
TC mg/dl	148.3±12.1	144.1±45.6	167.1±21.7	0.366	0	144.5±49.7	193.8±45	0.181
LDL-C mg/dl	81.73±11.5	102.7±19.5	124.8±40	0.069	0	124.8±19.8	106.6±22.1	0.267
TG mg/dl	109.8±15.9	111.7±16.4	159.1±65.3	0.083	0	118.7±20.7	126.8±78.4	0.869
HDL-C mg/dl	47±12.3[a]	31.8±4.9[a]	40.8±9.5	**0.024***	0	36.8±11.8	32.8±4.4	0.471

Key: PG=progesterone, TC = Total Cholesterol, HDL-C = High-Density Lipoprotein-Cholesterol, LDL-C = Low Density LipoproteinCholesterol, TG = triglyceride. The values were given by Mean ± SD (standard deviation), BMI = body mass index by Kg/m2. Hc=Healthy controls. Wm=with medication. Wom=without medication. P value < 0.05is statistically significant indicate by*. Superscript letters (a, b and c) indicate significant differences compared to healthy controls versus breast cancer patients withou medication, healthy controls versus breast cancer patients with medication, breast cancer patients without medication and with medication respectively

The independent samples t-test was performed in the comparison of the two groups of breast cancer patients with healthy controls and themselves based on menopausal status (table 5). In premenopausal status, statistically significant lower mean serum progesterone level found in breast cancer patients without medication compared to healthy control groupsat (P=0.015) and also there was significant differences observed between the two breast cancer patient groups (p=0.001). But breast cancer patients without medication have lower mean serum progesterone level than that of breast cancer patients with medication. Although, in breast cancer patients with medication was observed statistically insignificant higher mean serum estridiol level when compared to breast cancer patients without medication and healthy control groups. In case of lipid profile levels, significantly lower mean serum HDL-C level was observed in breast cancer patients without medication when related to healthy controls (P=0.014). Inaddition, significantly higher mean serum LDL-C level exhibited in breast cancer patients with medication compared with healthy control groups (P<0.005). There was significant difference and higher mean serum LDL-C and mean serum HDL-C levels was shown in breast cancer patients with medication when compared to breast cancer patients without medication (p=0.003) and (p=0.038) respectively.

Regarding Postmenopausal status, there was no significant difference observed in higher serum estradiol level of breast cancer patients with medication when compared to without medication and healthy control groups. However, there was significant difference observed in mean serum progesterone level among breast cancer patients without medication and with medication (P=0.007). Higher mean serum TC, TG and HDL-C level were observed and a significant difference was noticed for above parameters among breast cancer patients with medication than without medication at (P=0.010; 0.019 and P=0.044) correspondingly. Although, there was significant difference observed in lower mean serum HDL-C level of breast cancer patients without medication when compared to healthy control groups at (P=0.002). Therefore, mean serum steroid hormones and lipid profile levels in the three examined groups of premenopausal status were greater than that of postmenopausal status, exceptional to serum TG level in healthy controls and breast cancer patients with medication, and mean serum LDL-C level of breast cancer patients without medication (table 5).

Table 5: Comparison of serum estradiol, progesterone and lipid profile levels with menopausal status in healthy controls, breast cancer patients without and with medication.

Premenopausal						
Variables	Hc N= 23	Wom N=24	P-value	Wm N=17	P-value Hc vsWm	P-value Wo vsWm
Estradiol pg/ml	110±72.8	134.1±78.6	0.293	149.9±146.8	0.271	0.657
PgR ng/ml	2.7±4.1[a]	0.57±0.61[ac]	**0.015***	1.9±1.7[c]	0.420	**0.001***
TC mg/dl	163.9±19.8	146.3±38.6	0.056	167.3±35.7	0.707	0.085
LDL-C mg/dl	100.1±24.6	97.9±18.5[c]	0.727	121.1±29.2[c]	0.018	**0.003***
TG mg/dl	109.3±29.2	113±24.1	0.632	121.9±61.6	0.392	0.532
HDL-C mg/dl	43.9±7.7[a]	37.2±9.8[ac]	**0.014***	43.7±9.1[c]	0.944	**0.038***
Post-menopausal						
	N= 17	N=16		N= 23		
Estradiol pg/ml	15.9±15.6	20.1±17.1	0.473	18.2±20.2	0.699	0.769
PgR ng/ml	0.27±0.21	0.2±0.09[c]	0.218	0.4±0.3[c]	0.099	**0.007***
TC mg/dl	159.8±27.8	145±29.1[c]	0.145	172.1±32[c]	0.212	**0.010***
LDL-C mg/dl	101.9±26.1	102.6±16.8	0.925	115.5±32.5	0.321	0.322
TG mg/dl	118.3±32.2	101.9±26.1[c]	0.086	136.7±54.6[c]	0.226	**0.019**
HDL-C mg/dl	40.8±9.3[a]	31.4±6.1[ac]	**0.002***	37±9.5[c]	0.210	**0.044***

MS= menopausal status. HC vsWm= healthy controls versus without medication Wo vsWm = without medication versus with medication. P value < 0.05 is statistically significant indicated by *. Superscript letters (a, b and c) indicate significant differences compared to healthy controls versus breast cancer patients without medication, healthy controls versus breast cancer patients with medication, breast cancer patients without medication and with medication respectively.

Independent samples t-test showed stastically significant difference in the higer average serum progesterone and HDL-C levels of healthy controls correlated with breast cancer patients without medication at follicular phase (P=0.022) and (P=0.001). And lower level of progesterone was observed high significant among breast cancer patients without medication than patients with medication (P<0.001). Slightly significant differences were observed in lower mean serum TC level of breast cancer patients with medication with when compared to healthy control groups at (P=0.043) in follicular phase. Like as

follicular phase, significantly higher mean serum progesterone level was observed in healthy controls than breast cancer patients without medication at (P=0.009). Although, there were significant difference observed lower mean serum progesterone and LDL-C levels in breast cancer patients without medication when compared to patients with medication (P=0.009) and (P=0.025) at luteal phase. However, no stastically significant differences were detected in mean serum estradiol levels among the three different examined groups at the luteal phases

Table 6: Comparison of serum estradiol, progesterone and lipid profile levels with menstrual phases of healthy controls, breast cancer patients without and with medication.

Follicular phase						
Variables	**HC,N=19**	**Wom, N=13**	**P-value**	**Wm, N= 5**	**P-value HCvsWm**	**P-value WovsWm**
Estradiol pg/ml	111.7±73.3	114.2±49.6	0.918	124.6±108.6	0.754	0.778
PgR ng/ml	1.68± 2[a]	0.3±0.17[ac]	**0.022***	0.96±0.25[c]	0.443	**<0.001**
TC mg/dl	164.1± 20.9[b]	143±41.2	0.065	141.4±21.4[b]	**0.043***	0.938
LDL-C mg/dl	100.6± 26.7	102.1±11.5	0.856	120.6±30.9	0.163	0.073
TG mg/dl	109.1±29.1	114±25.5	0.634	138±99	0.256	0.406
HDL-C mg/dl	43.6± 7.6[a]	33.6±7.1[a]	**0.001***	40.2±7.4	0.392	0.096
Luteal phase						
	N= 4	N=11		N= 10		
Estradiol pg/ml	105±80.8	157.6±100.7	0.367	190.9±162.4	0.341	0.575
PgR ng/ml	7..7±7.8[a]	0.87±00.8[ac]	**0.009***	2.6±1.8[c]	0.065	**0.009***
TC mg/dl	163.±16.1	150.2±36.8	0.521	175.8±38.5	0.539	0.136
LDL-C mg/dl	97.8± 12.3	93±24.1[c]	0.713	122.5±31.1[c]	0.160	**0.025***
TG mg/dl	109.7±34.3	111.9±23.5	0.888	117.1±44.4	0.772	0.739
HDL-C mg/dl	45.1± 10.3	41.4± 11.2	0.578	45.9±10.3	0.888	0.349

Superscript letters (a, b and c) indicate significant differences compered to healthy controls versus breast cancer patients without medication, health control versus breast cancer patients with medication ,breast cancer patients without medication and with medication respectively.

As independent sample t-test results showed, there were significant difference showed that among breast cancer patients without medication and with medication in serum TC, LDL-C and HDL-C level at stages I and II (p=0.027; 0.037; 0.030) respectively. Though, all biochemical parameters were higher in breast cancer patients without and with medication at stage I and II when compared to stage III and IV. However, the mean serum LDL-C and TG levels of breast cancer patients without medication were lower at stage I and II when compared to breast cancer patients of stage III-IV (table 7).

Table 7: Comparison of serum estradiol, progesterone and lipid profile levels with stages of breast cancer in patients without and with medication.

	Stage I and II			Stage III and V		
variables	Wom N= 14	Wm N=19	P-Value	Wom N= 11	Wm N= 19	P-Value
Estradiol pg/ml	87.7±91.5	95.2± 134.9	0.859	89±87.3	46.1±84.1	0.195
PgR ng/ml	0.4± 0.6	1.2±1.7	0.095	0.5±0.5	0.8±0.9	0.316
TC mg/dl	141.7±34.4[a]	170.1±34.4[a]	**0.027***	158±37.3	169.3±34.4	0.407
LDL-C mg/dl	99.6±21.2[a]	122.6±34.8[a]	**0.037***	103.9±15.6	107.3±26.4	0.702
TG mg/dl	111.3± 21.3	141.5±59.2	0.079	102.4±16.3	119.5±56.3	0.337
HDL-C mg/dl	32.7± 7.4[a]	39.6±9.3[a]	**0.030***	34.5±11.4	38.5±9.5	0.311

Key: PgR=progesterone, TC = Total Cholesterol, HDL-C = High-Density Lipoprotein-Cholesterol, LDL-C = Low Density Lipoprotein-Cholesterol, TG = triglyceride. The values were given by Mean± (SD) standard deviation. Wm=with medication. Wom=without medication. P value < 0.05 is statistically significant indicated by *.

Independent sample t-test was carried out for mean serum estradiol, progesterone and lipid profile levels of breast cancer patients with medication based on types of hormonal therapy and categories of medication i.e.breast cancer patients under surgery, radiation, chemotherapy and with additional hormonal therapy and only hormonal therapy.

There were statistically significant differences in mean serum progesterone and HDL-C levels among breast cancer patients when treated with surgery, radiation, chemotherapy and addition with hormonal therapy and treated with only hormonal therapy at (p=0.020) and (p=0.027). Breast cancer patients were treated with surgery, radiation, chemotherapy and addition with hormonal therapy were found lower mean serum estradiol, progesterone, and HDL-C levels than that of breast cancer patients treated with only hormonal therapy. In the case of types of hormonal therapy there was highly significant differences exhibited among breast cancer patients treated with armidex and tamoxifen in mean serum estradiol and progesterone level (p= < 0.001). Statistically significant differences observed in mean serum HDL-C level among those groups (p=0.032). The

mean serum of these three significant parameters levels of breast cancer patients treated with tamoxifen were higher than that of armidex (table 8).

Table 8: Comparison of serum estradiol, progesterone and lipid profile levels with medication of breast cancer and type of hormonal therapy in breast cancer patients with medication.

Variables	Wm N=40		p-value	Variables	Wm N=40		p-value
Medication of BRCA	Wm^1 N=33	Wm^2 N=7		Types of HT	Tamoxifen N=17	Armidex N=23	
Estadiol pg/ml	60.4±1o7.7	139.2±139.3	0.103	Estradiol pg/ml	149.9±146.8	18±20.2	**<0.001**
PgRng/ml	0.8± 0.8	2.1±2.5	**0.020***	PG ng/ml	1.9±1.7	0.4±0.3	**<0.001**
TC mg/dl	167.9±29.8	180.7±48	0.389	TC mg/dl	167.3±35.7	172.9±31.9	0.656
LDL-C mg/dl	114.2±28.7	1222.2±43	0.547	LDL-C mg/dl	121.1±29.1	111.5±32.5	0.342
TG mg/dl	132.4±58.8	121.2±53.4	0.645	TG mg/dl	121.9±61.6	136.7±54.6	0.428
HDL-C mg/dl	38.3±9.7	47.2±7	**0.027***	HDL-C mg/dl	43.7±9.1	37±9.5	**0.032***

BRCA= breast cancer, Su, R, Ch& HT= surgery, Radiation, chemotherapy and hormonal therapy. HT= hormonal therapy only. Subscript numbers (1and 2) indicate breast cancer patients with medication treated with surgery, radiation, chemotherapy and with additional hormonal therapy and breast cancer patients with medication treated with only hormonal therapy respectively.Wm=with medication. P value < 0.05 is statistically significant indicated by *.

4.3. Serum Estradiol, Progesterone and Lipid Profile Levels among Healthy Controls, Breast Cancer Patients Without and With Medication

One-way ANOVA with Tukey post hoc test results indicated that there were statically significant difference in breast cancer patients without medication and breast cancer patients with medication as compared with healthy controls in serum levels of progesterone and all lipid profile levels ($p<0.05$). Although, multiple logestic regression analysis was done to shows the associtionn of these biochemical parameters levels among those groups. However, there were no significant differences between three groups in mean serum estradiol levels (p= 0.654). There were significant difference in higher mean serum (24.3) TC, (15.8) LDL-C, (22.1) TG and (5.0) HDL-C levels of breast cancer patients with medication than that of without medication at (p= 0.002; p= 0.016; p= 0.039; p= 0.042). There were significant differences among breast cancer patients without

medication and have lower mean serum (1.26) progesterone, (16.4) TC and (7.71) HDL-C level when compared with healthy controls (p= 0.022) (0.049) and (p=0.001) respectively. Breast cancer patients with medication were found to have higher mean serum (14.7) LDL-C level than healthy controls (p= 0.027) (table 9). Therefore, except for mean serum estradiol level OR= 1.015 (95%CI: 1.006-1.024, P= 0.001) the remaining biochemical parameters mean serum level were found to be lower in breast cancer patients without medication when compared to the other two examined groups. Such as progesterone OR=0.109 (95% CI: 0.025 - 0.467, P=0.003), TC OR=0.979 (95% CI: 0.962-0.997, P=0.019), LDL-C OR= 0.978 (95% CI: 0.957- 0.999, P=0.043) OR = 0 .096 (95%CI: 0.022 - 0.412). Even, insignificantly lower levels of progesterone OR=0.879 (95% CI: 0.690 -1.121) and HDL-C OR= 0.950 (95% CI: 0.901-1.002) (table10) were observed in breast cancer patients with medication when compared to healthy control groups. However, higher mean serum estradiol, TC, TG and LDL-C levels were indicated in breast cancer patients with medication when compared to healthy control groups in (tables 9).

Table 9: Mean comparison of serum estradiol, progesterone and lipid profile levels among healthy controls, breast cancer patients without and with medication

Variable	**Hc N=40 Mean± SD**	**Wom N=40 Mean± SD**	**Wm N=40 Mean± SD**	**p-value**
Estradiol Pg/ml	70.4±72.6	88.5±83.4	74.2±115.8	0.654
PgRng/ml	1.68±3.3[a]	0.4±0.5[a]	1.03±1.3	**0.029***
TC mg/dl	162.2±23.3[a]	145.8±34.7[ca]	170±33.2[c]	**0.002***
LDL-C mg/dl	100.9±25[b]	99.8±17.8[c]	115.6±31.1[cb]	**0.009***
TG mg/dl	113.1±30.5	108.3± 23.9[c]	130.4±57.4[c]	**0.037***
HDL-C mg/dl	42.6±8.5[a]	34.3±8.9[ac]	39.8±9.8[c]	**< 0.001***

Key: SD (standard deviation).HC=Healthy controls. Wm=with medication. Wom=without medication. P value < 0.05 is statistically significant indicated by *. Superscript letters (a, b and c) indicate significant differences compared to healthy controls versus breast cancer patient without medication, healthy controls versus breast cancer patients with medication ,breast cancer patients without medication and with medication respectively.

Table 10: The correlation strength of estradiol, progestrol, and lipid profile levels of healthy controls; breast cancer patients without medication and with medication by adjustment with some breast cancer risk factors

variable		OR(95%CI)	pvalue	variables	OR(95%CI)	p-value
HC		**(1.00)***		Wm	(1.00)*	
Wom	Estradiol Pg/ml	1.015 (1.006 -1.024)	0.001	HC	0.996 (.990 - 1.003)	0.240
	PgR ng/ml	0.096 (.022- .412)	0.002		1.137 (.892-1.449)	0.299
	TC mg/ml	0.987 (.970 -1.005)	0.163		0.992 (.975 -1.008)	0.312
	LDL-C mg/ml	1.001 (.978-1.024)	0.930		0.977 (.958 - .996)	0.019
	TG mg/ml	1.001 (.985-1.016)	0.937		0.987 (.974 - .999)	0.040
	HDL-C mg/ml	0.918 (.862-.978)	0.008		1.053 (.998 - 1.110)	0.061
Wm	Estradiol Pg/ml	1.004 (.997-1.010)	0.240	Wom	1.011 (1.003 - 1.019)	0.009
	PgRng/ml	0.879 (.690 -1.121)	0.299		0.109 (0.025 - 0.467)	0.003
	TC mg/ml	1.009 (.992 -1.025)	0.312		0.979 (0.962-0.997)	0.019
	LDL-C mg/ml	1.024 (1.004 -1.044)	0.019		0.978 (0.957- 0.999)	0.043
	TG mg/ml	1.014(1.001-1.027)	0.040		0.987 (0.973 - 1.002)	0.092
	HDL-C mg/ml	0.950(.901 -1.002)	0.061		0.966 (0.908-1.028)	0.280

Key: PgR=progesterone, TC = Total Cholesterol, HDL-C = High-Density Lipoprotein-Cholesterol, LDL-C = Low Density Lipoprotein-Cholesterol, TG = triglyceride.OR= odd ratio, 95% CI=95% confidence interval. HC=Healthy controls. Wm=with medication. Wom=without medication. P value < 0.05 is statistically significant. (1.00)* indicated that reference category

4.4. Abnormality in Concentration of Serum Estradiol, Progesterone, TC, LDL-C and HDL-C Levels in Healthy Controls, Breast Cancer Patients Without and With Medication when compared to Cut off Points.

In premenopausal status mean serum progesterone level was observed higher abnormality (62.5%) below reference range in breast cancer patients without medication than breast cancer patients with medication (32.5%) and healthy controls (12.5%). Most of these abnormality was found in premenopausal status when compared with the cut-off points. Higher abnormality or below(< 40mg/dl) of mean serum HDL-C level was observed in (72.5%) breast cancer patients without medication when compared to (55%) breast cancer patients with medication and (25%) healthy controls (table 11).

Table 11: Cut off points and abnormal levels of serum estradiol, progesterone and lipid profile in healthy controls, breast cancer patients without and with medication.

Variables	Cut off points	Hc N=40	Wom N=40	Wm N=40
Estradiol pg/ml Based on menopausal status				
1.premenopause				
1.1.Follicular Phase n,%	≥232.5	1(2.5)	4(10)	1(2.5)
	≤12.35		1(2.5)	1(2.5)
1.2.luteal phase n,%	≥397		3(7.5)	3(7.5)
	≤ 22.28			
2.postmenopuse n,%	≥136			
	≤5			
Total		1(2.5)	7(17.5)	5(12.5)
PgR ng/ml Based menopausal status				
1.premenopause				
1.1Follicular Phase n,%	≥1.4	2(5)		
	≤ 0.2		4(10)	2(5)
1.2.luteal phase n,%	≥2.7	3(7.5)		1(2.5)
	≤0.8		14(35)	7(17.5)
2.postmenopause n,%	≥0.8			3(7.5)
	≤0.1		7(17.5)	
Total		5(12.5)	**25(62.5)**	**13(32.5)**
TC mg/dl, n,%	≥200	2(5)	2(5)	7(17.5)
LDL-C mg/dl, n,%	≥129	2(5)	7(17.5)	**14(35)**
TG mg/dl n,%	≥200	1(2.5)	3(7.5)	5(12.5)
HDL-C mg/dl	<40	10(25)	**29(72.5)**	**22(55)**

Key: PgR=progesterone, TC = Total Cholesterol, HDL-C = High-Density Lipoprotein-Cholesterol, LDL-C = Low Density Lipoprotein-Cholesterol, TG = triglyceride. N=numbers of participants. n, number of abnormal patients.(%) in percentage. HC=Healthy controls. Wm=with medication. Wom=without medication. Higher abnormality of each biochemical parameters indicated by bold.

4.5. The Correlation between Serum Estradiol, Progesterone and Lipid Profile Levels of Healthy Controls, Breast Cancer Patients Without and With Medication based on Breast Cancer Risk Factors.

Pearson correlation analysis were conducted for the correlation between serum estradiol, progesterone and lipid profile levels of the three groups by comparing each other, based on breast cancer risk factors and general clinical characteristics illustrated in tables (12, 13, and 15). Current monthly menstrual status has slightly strong positive correlation with serum estradiol for healthy controls $p<0.001$ $r=0.649$, for breast cancer patients without medication $p<0.001$, $r=0.678$, and for breast cancer patients with medication $p=0.006$ $r=0.427$, It has weak positive correlation with mean serum progesterone level for three groups. Menopausal status has positive correlation with serum estradiol for healthy controls $p<0.001$ $r=0.649$, for breast cancer patients without medication $p<0.001$, $r=0.678$, and for breast cancer patients with medication $p<0.001$ $r=0.569$. It has weak positive correlation with mean serum progesterone level for healthy controls $p=0.020$ $r=0.369$, for breast cancer patients without medication $p=0.023$ $r=0.360$, for breast cancer patients without medication and for breast cancer patients with medication slightly strong $p<0.001$ $r=0.554$. However, significantly negative correlation was observed between age and serum estradiol and progesterone levels in three three groups.

A statistically significant negative correlation was observed between menstrual phase and serum estradiol in healthy controls, breast cancer patients without medication and with medication ($p< 0.001$, $r= -0.650$), ($p=0.001$, $r=-0.511$) and ($p=0.001$, $r=-0.571$) respectively (table 13). But, types of hormonal therapy has slightly strong negative correlation with serum estradiol ($p<0.001$, $r=-0.569$) and progesterone $P <0.001$, $r=-0.554$), HDL-C level, weakly negative associated ($p=0.032$ $r=-0.340$) in breast cancer patients with medication. Medication of breast cancer also showed negatively correlated with those patients mean serum progesterone ($P=0.020$, r -0.368), mean serum HDL-C levels ($P=0.027$, r-0.350) (table 14).

Table 12: Correlation between serum estradiol, progesterone and lipid profile levels of healthy controls and breast cancer risk factors

Variables	Estradiol		PgR		TC		LDL-C		TG		HDL-C	
	r	P	r	P	r	P	r	P	r	P	r	P
Age	-0.610[b]	**<0.001**	-0.383[a]	**0.015**	-0.056	0.733	0.043	0.792	0.190	0.240	-0.205	0.205
Current menstrual status	0.649[b]	**<0.001**	0.369[a]	**0.020**	0.089	0.586	-0.036	0.826	-0.150	0.357	0.177	0.275
Munstral phase	-0.650[b]	**<0.001**	-0.178	0.272	-0.107	0.510	-0.011	0.947	0.160	0.325	-0.135	0.407
Monopausal status	0.649[b]	**<0.001**	0.369[a]	**0.020**	0.089	0.586	-0.036	0.826	-0.150	0.357	0.177	0.275

Key: r= Pearson correlation coefficient, p= p value for Pearson correlation, PG=progesterone TC =Total Cholesterol, TgR= Triglyceride, LDL-C= Low Density Lipoprotein-Cholesterol, HDL-C= High Density Lipoprotein-Cholesterol. BMI=body mass index. Superscript letters a, indicated weak and b, indicate strong and moderate statistically significant.

Table 13: Correlation between serum estradiol, progesterone and lipid profile levels of breast cancer patients without medication and breast cancer risk factors.

variable	Estradiol		PgR		TC		LDL-C		TG		HDL-C	
	r	P	r	p	r	p	r	p	r	P	r	P
Age	-0.624[b]	**<0.001**	-0.431[b]	**0.005**	0.071	0.663	0.014	0.933	-0.199	0.217	-0.114	0.486
Current menstrual status	0.678[b]	**<0.001**	0.360[a]	**0.023**	0.018	0.911	-0.132	0.416	0.246	0.125	0.323[a]	**0.042**
Menstrual phase	-0.511[b]	**0.001**	-0.127	0.435	0.020	0.900	-0.027	0.868	-0.235	0.145	0.131	0.421
Monopausal status	0.678[b]	**<0.001**	0.360[a]	**0.023**	0.018	0.911	-0.132	0.416	0.246	0.125	0.323[a]	**0.042**
Stage	0.006	0.971	-0.011	0.944	-0.019	0.909	-0.067	0.680	-0.027	0.870	0.212	0.190

Key: r= Pearson correlation coefficient, p= p value for Pearson correlation, PgR=progesterone, TC =Total Cholesterol, TG= Triglyceride, LDL-C= Low Density Lipoprotein-Cholesterol, HDL-C= High Density Lipoprotein-Cholesterol.BMI=body mass index.Superscript letters a, indicated weak and b, indicate strong and moderate statistically significant.

Table 14: Correlation between serum estradiol, progesterone and lipid profile levels of breast cancer patients with medication and breast cancer risk factors.

Variables	**Estradiol**		**PgR**		**TC**		**LDL-C**		**TG**		**HDL-C**	
	r	P	r	p	r	p	r	P	r	P	R	P
Age	-0.514[b]	**0.001**	-0.424[b]	**0.006**	0.088	0.589	-0.298	0.062	-0.003	0.983	-0.294	0.123
Current menstrual status	0.427[b]	**0.006**	0.415[b]	**0.008**	-0.011	0.944	0.129	0.427	-0.145	0.372	0.293	0.067
Menstrual phase	-0.571[b]	**0.001**	-0.419[b]	**0.007**	0.246	0.126	-0.136	0.402	0.035	0.832	-0.239	0.137
Monopausal status	0.569[b]	**<0.001**	0.554[b]	**<0.001**	-0.073	0.656	0.154	0.342	-0.129	0.428	0.340[a]	**0.032**
Medication of BRCA	-0.262	0.103	-0.368[a]	**0.020**	-0.140	0.389	-0.098	0.547	0.075	0.645	-0.350[a]	**0.027**
Stage	-0.164	0.313	-0.202	0.211	0.122	0.454	-0.034	0.833	-0.100	0.539	0.120	0.461
Types of HT	-0.569[b]	**<0.001**	-0.554[b]	**<0.001**	-0.073	0.656	-0.154	0.342	0.129	0.428	-0.340[a]	**0.032**
Duration of TH	-0.214	0.185	0.012	0.942	0.162	0.319	-0.182	0.262	-0.006	0.972	-0.180	0.267

Key: r= Pearson correlation coefficient, p= p value for Pearson correlation, PgR=progesterone, TC =Total Cholesterol, TG= Triglyceride, LDL-C= Low Density Lipoprotein-Cholesterol, HDL-C= High Density Lipoprotein-Cholesterol.BMI=body mass index. BRCA= breast cancer,HT= hormonal therapy. Superscript letters a, indicated week and b, indicate strong and moderate statistically significant

5. Discussion

Steroid hormones, estrogen and progesterone are playing a major role in the etiology of breast cancer (Buijs *et al*., 2008). Excess free serum estrogen levels are responsible for high rate of immature cell replication and increase of fat metabolism in the body (Feigelson and Henderson, 1996). Although, plasma lipid profile levels may predict the risk of breast cancer, altered of its plasma levels in breast cancer patients are retreated by effective treatment of the tumor (Shah *et al*., 2008). Hormonal therapy is slow or stops the growth of hormonal sensitive tumors by blocking the ability to produce hormones or by interfering with effects of hormones in breast cancer cells (NCI, 2017). Tamoxifen reduces the incidence of breast cancer by 50–75% in high-risk women (Hammond *et al*., 2010). Armidex inhibits aromatization by 96.7%, and suppresses plasma estradiol concentrations by 84–94% (Coscia *et al*., 2017) and had a beneficial effect on lipid profiles of postmenopausal women (Sawada *et al.,* 2005). So, the assessments of serum estradiol, progesterone and lipid profile levels are predictors of effective treatment up to the level of these biochemical parameters become near to normal range in breast cancer patients. Although, it is used to control the disease, prevent adverse effect of the treatment and provided useful prevention when applied to healthy women.

The present study assesses the serum level of estradiol, progesterone and lipid profile (TC, LDL-C, TG and HDL-C) of the three different groups: healthy controls, breast cancer patients with medication and patients without medication. The data collected from120 study participants which included; 40 healthy controls, 40 breast cancer patients with medication and 40 without medication. According to, this study progesterone and lipid profile levels were demonstrated that lower mean serum concentration in breast cancer patients without medication than patients with medication and healthy control groups. However, mean serum estradiol level was higher in breast cancer patients without medication than that of the two groups. Higher mean serum estradiol, TC, TG and LDL-C levels were observed in breast cancer patients with medication when compared to healty control groups. However, lower levels of progesterone and HDL-C were observed in breast cancer patients with medication when compared to healthy control groups.

The present study shows that statistically insignificantly higher mean serum estradiol levels in breast cancer patients without medication than that of patients with medication and healthy controls. Although, postmenopausal breast cancer patients without medication have shown statistically insignificantly enhancement of the level of estradiol than other two groups and premenopausal breast cancer patients without medication hold insignificant higer mean serum estradiol level than that of healthy controls. The reason for the elevation may be due to increased endogenous production of aromatase /over expression of aromatase activity (Santen *et al*., 1999) in extra ovarian body sites and within the tumor tissue, this may be directly related to increased synthesis of estrogen (Bulun *et al*., 2012 ; Cleary and Grossmann, 2009). This process can also increase the life time exposure on the tissues (Taylor and Bell-Taylor, 2009). Because of direct androgenic effects on breast tissue, which elevated serum androgen level may lead indirectly to increase estrogenic exposure of breast tissue. That is, all steroidogenic enzymes necessary for the formation of estrogens from androgenic precursor molecules are present in normal mammary tissues and breast tumor specimens (Kaaks, *et al*., 2005). The other case of elevation may be the low concentration of SHBG occurs in breast cancer patients and also deficiency of vitamins like B, E; minerals like magnesium and substance like indole 3-carbinol may affect estrogen metabolism and increase free estrogen levels in the bloodstream (Taylor and Bell-Taylor 2009).

Several studies supported the elevation of esteridiols in breast cancer tissues. Like komen (2017), Farhat *et al.,* (2013), Trehan *et al.,* (2012), Blair (2010), and Key (1999). Komen (2017) stated that, pooled analysis of data from studies found higher blood estrogen levels increased breast cancer risk in premenopausal and postmenopausal women. Key (1999) explored evidence in analysis of different researches as breast cancer risk is increased by relatively high estradiol concentrations in premenopausal and postmenopausal women.Trehan *et al.,* (2012) expressed that estrogen level was very significantly higher in patients before treatment as compared to age matched healthy controls. Farhat *et al.,* (2013) demonstrated that higher levels of total estradiol, bioavailable estradiol were observed significantly associated with increased breast cancer risk. Missmer *et al.,* (2004) observed that a statistically significant direct association

between breast cancer risk and the level of estrogens. Blair (2010) also suggested that postmenopausal women with elevated plasma or serum estrogens are at increased risk for breast cancer.

Contrary to the current results, Hussain *et al*., (2013) stated that breast cancer patients also have a lower level of estrogen when compared to controls.This could probably be due to the failure of proper matching between the cases and the controls with respect to their different phases of menstrual cycle during the measurements of blood hormone and the sample size was relatively small. On the other hand, Awio *et al*., (2012) suggested that in Sub-Saharan Africa among Uganda women there were no significant association between level of estradiol and breast cancer. Although, Abdelhadi (2006) stated that in Sudanese females no significance difference was detected in the serum levels of estrogen between the control subjects and the breast cancer patients. YU *et al*., (2003) assessed that in Chinese women with breast cancer no significant association was found for estradiol in either premenopausal or postmenopausal women. This may be due to circulating levels of estrogen in premenopausal women not only differ significantly from person to person but also vary substantially through the menstrual cycle. Ssmall sample size and less-stringent matching conditions on menstruation days may hamper the ability to detect any significant differences in estrogen levels between the study groups. Kaaks *et al.,* (2013) investigated that did not show a significant association of estradiol and progesterone levels with breast cancer overall risk.

However, the present study results indicated that after receiving hormonal therapy insignificantly reduced mean serum estradiol level in breast cancer patients with medication than breast cancer patient without medication, as well as, in postmenopausal status of those patients also had lower mean serum estradiol level than breast cancer patients without medication. This may be estrogenic effect of armidex on the serum level of estradiol, which is blocked the rate-limiting step in the synthesis of estrogens (Sawada *et al*., 2005). This result is in line with Coscia *et al*., (2017), komen, (2017), Banerjee *et al.*, (2005), Sawada *et al*, (2005) Geisler *et al*., (2001).

Komen, (2017) said that aromatase inhibitors lower the level of estrogen in the body and decrease the risk of breast cancer. Banerjee *et al.*, (2005) stated that treatment with anastrozole significantly decreased estradiol serum levels by 87.5%. Sawada *et al*, (2005) indicated that estradiol levels decreased significantly after anastrozole treatmen in Japanese breast cancer postmenopausal women. Geisler *et al.*, (2001) showed that anastrozole given as neoadjuvant therapy causes a profound suppression of tumor levels of estradiol. Coscia *et al*., (2017) stated that in glandular breast tissue, aromatase inhibitors have dual role to block both initiation and promotion of breast cancer by lowering estradiol concentrations.

However, in this study the premenopausal status of breast cancer patients with medication mean serum estradiol levels were insignificantly higher than breast cancer patients without medication. This may be estrogenic effect of tamoxifen, whereas tamoxifen induced ovarian hyperstimulation in premenopausal patients and increase circulating estrogens (Madeddu *et al.*, 2014). The observation was supported by Yamazaki *et al*., (2015), Jordan *et al*., (1991), Sherman *et al*., (1979), and Lum (1997). Yamazaki *et al*., (2015) suggested that in Japanese women, tamoxifen could stimulate the ovarian function and employ high values of serum estradiol level during post-operative tamoxifen therapy. Lum (1997) stated that, long-term tamoxifen can be associated with increased serum levels of estradiol, which may be explain tamoxifen's estrogenic effects. Jordan *et al*., (1991) showed that breast cancer patients stage I or II during with tamoxifen for 4–72 months, the levels of estradiol was elevated one fold to three fold. Sherman *et al*., (1979) also indicated that serum estradiol concentrations were 3 to 10-fold greater during tamoxifen administration. Contrary to this, Lønning (1995) suggested that tamoxifen reduced plasma estradiol; which probably secondary to a fall in plasma testosterone due to a reduced ovarian excretion of this androgen this related with plasma luteinizing and follicle stimulating hormones are decreased. Although, this idea supported by Lofgren *et al*., (2006) after 3 months of tamoxifen treatment (56%) increase in circulating SHBG levels, thus free estradiol levels decreased. Alternatively, Banerjee *et al.*,(2005) reported that no correlation between serum estradiol in treatment with tamoxifen was observed.

The present study demonstrated that significantly lower serum progesterone levels in breast cancer patients without medication when compared to healthy control group. This

condition was observed in premenopausal status and also in luteal phase. This may be due to the ovarian hyperandrogenism hypothesis/ luteal inadequacy hypothesis, which is ovarian androgen excess, chronic anovulation, and an associated reduction of luteal phase progesterone production (Kaaks *et al.*, 2005). Other reason may be the presented of low concentration of TG, LDL-C and HDL-C in the breast cancer patients without medication. Because, HDL and LDL-C may be serve as the source of cholesterol in steroidogenesis (Miller and McLean1987). Especially, for its common precursor steroid pregnenolone, which is formed by the enzymatic cleavage of a 6-carbon side-chain of the 27- carbon cholesterol molecule, a reaction catalyzed by the cytochrome P450 sidechain cleavage enzyme (Ruiz-Cortés, 2012). Although, the elevation level of estradiol can block ovulation, this may Stress affects an ability to ovulate. If not ovulate, not produce progesterone. Its cause, stress increases the release of cortisol. Cortisol decreases SHBG, and this leads to increase of free estrogen level and decreased production of progesterone. Cortisol increased the production of cortisol binding globulin, that deactived free progesterone in plasma by its binding activity (Taylor and Bell-Taylor, 2009).

In agreement with this result, previous studies conducted by different investigators like Houghton *et al.,* (2016), Hussain *et al*., (2013), Kaaks *et al.,* (2005), Saez *et al*., (1978). Hussain *et al*., (2013) have shown that breast cancer patients with a lower level of progesterone as compared to controls. Kaaks *et al.,* (2005) and his colleagues conducted a case – control study within the European Prospective Investigation in cancer and nutrition to examine a relationships among premenopausal serum concentrations of sex steroids and subsequent breast cancer risk, demonstrated that elevation of serum progesterone concentrations was associated with a statistically significant reduction in breast cancer risk**.** Saez *et al*., (1978) proposed that a low progesterone /estrogen ratio and anovulatory cycles are more frequent in breast cancer patients than in a normal population and suggested that a relatively low progesterone secretion might favor the development of cancer. Houghton *et al.,* (2016) also expressed that a decline of progesterone can increase breast cancer risk. However, observation made by Brisken *et al*., (2015), Joshi *et al.*, (2015) and Ho *et al*., (2009) supported that, the idea of progesterone induced breast cancer risk. Brisken *et al*., (2015) suggested that, breast cancer risk increases through

progesterone-induced events during luteal phase. Joshi *et al.*, (2015) showed that, breast cancer risk is constantly elevated with estrogen plus progestrone therapy, while risk is persistently decreased with estrogen alone therapy. Thus, progesterone insertion during a median hormone therapy intervention period of 5-6 years not only increases the breast cancer risk during intervention, but also continued elevated risk for several years even after stopping this regimen. In addition, Ho *et al.*, (2009) also reported that in postmenopausal women serum progesterone levels were significantly associated positively with the odds of having breast cancer. On the other hand, Missmer *et al*., (2004) did not find any significant association between the risk and the progesterone levels. Kaaks *et al.*, (2013) did not show a significant association of progesterone with breast cancer risk overall. Farhat *et al.*, (2013) showed that in pretreatment, a progesterone concentration was not statistically significant associated with breast cancer risk. YU *et al*., (2003) stated that in Chinese premenopausal women by comparing their progesterone levels, no significant differences were found between cancer patients and healthy controls. Ho *et al.*, (2009) also found that there is no correlative significance among the premenopausal cancer cases and serum progesterone levels.

In the present study, the mean progesterone levels of breast cancer patients with mediction were found to be enhanced significantly than that of patients without medication.This also occured in both premenopausal and postmenopausal status. This can be explained by ovarian stimulation during tamoxifen treatment. Jordan *et al*., (1991) and Sheraman (1979) support this view. Sherman (1979) showed that breast cancer patients who were under tamoxifen treatment had elevated serum estrediol and progesterone concentrations at luteal-phase. Similarly, Jordan *et al.*, (1991) could also demonstrate that in cancer patients who were treated with Tamoxifen had 1-3 fold increased levels of estradiol and progesterone.

In the present study, mean serum TC level of breast cancer patients without medication was significantly lower, when compared to patients with medication and healty control groups. This, phenomenon also was observed in both premenopausal and postmenopausal status. This may be explained by the fact that there was a higher amount of estradiol and lower amount of progesterone hormones in the serum of these patients. The increased

estradiol concentrations promoted the activity of HTGL enzymes and consequently decrease in the synthesis of TC. The lowered concentrations of progesterone have an influence on the action of estradiol on lipid profiles of these patients (Callejon *et al.*, 2009). Cholesterol synthes in liver can be affected by tumor metabolites. Perhaps, enhancement of utilization of cholesterol by neoplastic cells for new membrane biogenesis during cell proliferation were decreasing cholesterol synthesis, and increasing catabolism of cholesterol (Mishra, 2015). This supported by Kshirsagar *et al*., (2016) and Llaverias *et al.,* (2011), explain that low cholesterol increase the cancer risk association suggesting that lower cholesterol was not the cause but the result of cancer.

The idea that decreasing levels of TC in breast cancer patients without medication when compared to healty control groups is in line with Li *et al* (2018), Kshirsagar *et al*., (2016), Mishra (2015) Asegaonkar *et al*., (2012), Kim *et al*., (2009), Shah *et al.*,(2008). Kim *et al*., (2009), suggested that in Korea insignificantly lower levels of TC was observed in larger and histologicaly advanced tumors. (Kshirsagar *et al*., 2016 ; Mishra 2015; Asegaonkar *et al*., 2012; Shah *et al.,* 2008) also stated that, significantly lower levels of TC was observed in breast cancer patients when compared to control groups in Indian women. And Li *et al.,* (2018) suggested that the serum levels of TC significantly lower in breast cancer patients than normal control groups in southwest of China.

In contrast to this, (Guan *et al*., 2019; Pandeya *et al.,* 2018*;* Raza *et al*., 2018; Rohariya *et al.,* 2017; Nayak *et al.,* 2016; Ghahremanfard *et al.*, 2015; Taha *et al*., 2014; Hussain *et al*., 2013 and Abdelsalam *et al*., 2012) indicated that an increase of TC levels was observed in breast cancer patients when compared to healthy controls. However, interestingly, there are also reports that TC levels were not significantly different from that of healty control groups (Zhao *et. al.,* 2016; Martin, 2015; Abas *et al*., 2014; Laisupasin *et al.,* 2013; Llanos *et al*., 2012).

The current study results have shown that, mean serum TC levels were significantly higher in breast cancer patients with medication than that of patients without medication. Even if, in premenopausal and postmenopausal women of breast cancer patients with medication also found that higher TC level than patients without medication. This may be explained by the effects of armidex decreased estrogen concentration resulting from

blocking the enzyme aromatase, which activate HTGL and down regulated LDL-C receptor particles clearance i,e increased the substrates (HDL-C & LDL-C) in the synthesis of TC, (Lu *et al*., 2011 & Sawadas *et al.*, 2005). Similar explanation were given by Lu *et al*., (2011), who observed that after treatment with anastrazole for one year significantly increased TC levels in Chinese postmenopausal women with breast cancer. Banerjee *et al., (*2005) also showed that anastrozole was associated with a non-significant increase in TC. Sawada *et al*., (2005) suggested a different view that anastrozole did not induce any changes at all in TC levels btween groups.

However, in the present study the enhancement of TC levels were differently observed in premenopausal women breast cancer patients treated with tamoxifen. This could probably because, effects of tamoxifen vary from patients to patients, and this variability is specifically seen in the reaction of lipid levels in the serum. The reasons of variability are not completely understood, but may be, in part, due to inherited genetic differences in the cellular receptors (Ntukidem *et al.,* 2008). However, Most of the previous studies have shown that the reduction of serum TC levels after tamoxifen treatment in postmenopausal women. For instance, Markopoulos *et al*., (2010) suggested that, tamoxifen decrease TC levels in postmenopausal women. Others study also were shown the reduction of TC level after tamoxifen, like Sawada *et al*., (2005) and Joensuu *et al*., (2000). But, in the present study premenopausal women was treated with tamoxifen, which may be act differently from others studies were observed. In Ntukidem *et al.,* (2008) explained that the differences in the experiences of premenopausal and postmenopausal women with regard to baseline cholesterol. In other view, Hayes *et al*., (2010) reported that in the second reanalysis of the previous research in the associations between ESR1 XbaI genotypes and tamoxifen-induced changes in serum TC levels were no longer observed.

In the present study, significantly higher mean serum TG level was observed in breast cancer patients with medication than that of paients without medication. This may be due to estrogenic effect of tamoxifen on lipid metabolism. Because tamoxifen like estrogen, inhibit the key enzymes of TG metabolism and reduced the activity of LPL and HTGL (Czyżykowski *et al.,* 2014). Although, it is stimulates the synthesis and secretion of TG from VLDL in the liver, VLDL is the main circulating carrier of TG (Milionis *et*

al.,2001; Elisaf *et al*., 2000 ; Hozumi *et al*., 1998). This result are in line with the findings of Czyżykowski *et al*., (2014) Fillipatos *et al.*, (2009), Sawada *et al*., (2005), Sotririou *et al.*, (2003). Filippatos *et al.*, (2009) suggested that tamoxifen induces an increase in TG concentration. Czyżykowski *et al*., (2014.) investigated that in a case of a 55-year-old woman, Tamoxifen, increases the plasma level of TG. Sawada *et al.*, (2005) showed that In Japanese postmenopausal women tamoxifen increased TG levels and also decreased HTGL activity. Sotririou *et al.*, (2003) expressed that the serum TG level was significantly increased after 15 months of treatment and occasionally inducing severe hypertriglyceridemia. By opposing the present result, Ali *et al.*, (2019) reported that postmenopausal patients who received tamoxifen treatment showed significantly decrease in TG from baseline. On the other hand, Lin *et al*., (2014) reported that in Taiwanese breast cancer patients after tamoxifen treatment there was no statistically significant change observed in the mean serum TG level.

The present study data support an inverse association between mean serum HDL-C level and breast cancer risk. That is significantly lower serum HDL-C level exhibited in breast cancer patients without medication than breast cancer patients with medication and healthy control groups.The indication may be due to lowering of serum TC level in these patients, because HDL–C may reverse transport of cholesterol (Abas *et al*., 2014). As the TC levels increase by potentially stimulating HDL levels subsequently decreases breast cancer risk (Kshirsagar *et al*., 2016; Llanos *et al*., 2012). Another reason, perhaps due to lowering of progesterone in relation to excess androgenicity effect on breast cancer patients without medication; HDL-C levels were also significantly associated with levels of free biologically active estradiol. The consequences of excess estradiol level without controlling of progesterone make more inactive LPL activity and reduced the production of HDLC. In addition, the elevation of serum TG level was observed in premenopausal women of breast cancer patients without medication may be another reason fort he lowering of HDL-C level. This may be due to reduction of LPL activity; it regulates the clearance of TG from blood. But, particles of HDL-C are considered to be derived from lipolysis of TG (Shah *et al..*, 2008). Furthermore, the decreased level of HDL-C and the

increased level of TG might result in the neoplastic transformation of the cells within breast cancer patients (Pandeya *et al*., 2018).

The present results are supported by previously reported research by other workers; Li *et al.*,(2018), Pandeya *et al.*,(2018), Raza*et al.*, (2018) Kshirsagar *et al*., (2016), Zhao *et al*., (2016), Abas *et al*., (2014), Llanos *et al.*,**(**2013), Hussain *et al*., (2013), Shah *et al.*, (2008). Zhao *et al*., (2016) suggested that an inverse association between levels of HDL-C and breast cancer was noticed among postmenopausal women. Llanos *et al.,* **(**2013) reported that among African-American women high levels of HDL-C was inversely associated with breast cancer in these groups. Kshirsagar *et al*., (2016) revealed that low HDL-C levels may be associated with an increased risk of breast cancer. Hussain *et al*., (2013) said that low level of serum and HDL-C are associated with breast cancer. Pandeya *et al.,* (2018) suggested that the decreased level of HDL -C were observed in breast cancer patients. Abas *et al*., (2014) said that the level of HDL-C was significantly decreased in cancer patients compared to the control group. Raza *et al.,* (2018) reported HDL-C was found significantly low as compared to control subjects ($p<0.05$). Li *et al.,* (2018) demonstrated that total serum levels of HDL-C was significantly lower in breast cancer patients than normal controls in southwest of China. Shah *et al.,* (2008) also revealed that plasma HDL was significantly lower in breast cancer patients as compared with controls.

Contrary to the above observations, Martin *et al*., (2015) discussed that in a large cohort of women with extensive mammographic density, observed higher levels of serum HDL-C was associated with a higher risk of developing ER+ breast cancer when comparing lipid values across the interquartile range. Peela *et al*., (2012) carried on the study on breast cancer patients in Libyan women, indicated that significantly elevated serum HDL-C in those patient when compared to controls. On the other hand, the results of some researchs, like as Guan *et al.*,(2019) , Rohariya *et al*, (2017), Laisupasin *et al.*,(2013) Abdelsalam, *et al*., (2012) and Owiredu *et al.*,(2009) indicated that there are no significant differences between HDL-C levels between breast cancer patients and control groups.

However, in the present work mean serum HDL-C level of breast cancer patients with medication were significantly found to be higher than that of without medication. This may be due to activity of LPL increased after anastrozole treatment. Because, as results of the present study indicated that after receiving hormonal therapy insignificantly mean serum estradiol level was reduced in breast cancer patients with medication. The effect of anastrozole on estrogen levels may increase LPL activity; this implies that increase in larger HDL particles was observed in breast cancer patients treated with anastrozole (Sawada *et al*., 2005). This idea was also stated by Banerjee *et al*., (2005) and Lu *et al*., (2011) who observed a significant increase in HDL-C after treatment with anastrozole.

In the present study, the data indicated that the enhancement of mean serum HDL-C level after administration of tamoxifen in the premenopausal breast cancer patients. The reasons are not clear for this phenomenon, while according to the current observation it may be associated with the enhancement of TC, LDL-C and progesterone levels after tamoxifen treatment. Because, tamoxifen can be raised serum progesterone level through ovarian stimulation. This may have impact in lipid metabolisms. The result obtained by Banerjee *et al*., (2005) was in agreement with the present studies. Banerjee *et al.,* (2005) demonstrated that after 12 weeks, tamoxifen was associated with a significant increase in HDL-C (26.5%). on the other hand, Lin *et al*., (2014) suggested that there were not statistically significant changes observed in the mean serum HDL-C levels and in Taiwanese breast cancer patients after tamoxifen treatment.

However, in the current study mean serum LDL-C levels were significantly higher in breast cancer patients with medication than breast cancer patients without medication. This condition also observed insignificantly in postmenopausal status. This may be due the effects of armidex with on the synthesis of estradiol. As different researchers reported that estrogen has reduced serum LDL-C levels by increasing LDL particle clearance through LDL receptor up regulation (Lu *et al.,* 2011 and Sawada *et al.,* 2005). This ideas Supported by (Lu *et al*., 2011) suggested that, armidex significantly increases the levels of LDL-C in postmenopausal breast cancer patients.

In addition, in the current study mean serum LDL-C levels were significantly higher in premenopausal breast cancer patients with medication than breast cancer patients without

medication. Yet the reason was unclear as to increment of mean serum TC and HDL-C levels were observed after tamoxifen treatment. In contrast to this findings, Ali *et al.,* (2019) and Sawada *et al.,* (2005) reported that tamoxifen treatment showed a significant decreased in LDL-C later from baseline in postmenopausal patients. This may be due to its partial estrogenic activity, since estrogen is known to reduce serum LDL-C levels by increasing LDL particle clearance through LDL receptor upregulation. Lin *et al.,* (2014) in Taiwanese breast cancer patients showed that lowered levels of the serum concentration of LDL-C after 4 month tamoxifen treatment in both subgroups that is in subgroup 1 and subgroup 2.

In the current work, assessment of abnormality of mean serum estradiol, progesterone and lipid profile levels of breast cancer patients without medication and with medication by comparing healthy controls and reference range /cut off points at different menopausal status was done. Because of the two steroids hormones and also lipid profiles have different levels during premenopausal and postmenopausal status. In both women endogenou hormones production and exposure to estrogen are varies greatly during a woman‘s lifespan (Travis and Key, 2003). Although, the studies indicated that measuring of serum estrogen levels are challenging in premenopausal women. Because, the levels are vary throughout the menstrual cycle (Komen, 2017; Becker *et al.,* 2007). In the assessment, the present study data have shown abnormalities of all biochemical parameter percentages were different. But, in breast cancer patients without medication group level of each biochemical parameter abnormalities were greater than that of breast cancer patients with medication and healthy control groups when compared to reference range /cut off points exceptional to mean serum TC and LDL-C levels. Especially, lower level of progesterone 62.5% and HDL-C (72.5%) exhibited high abnormalities from the remains biochemical parameters. Yet, all of 62.5% progesterone abnormality was observed in premenopausal status below normal reference range. This may be because of estrogen dominance, excess or normal amount estradiol and insufficient amount of progesterone are responsible for breast cancer (Taylor and Bell-Taylor, 2009). And also the ovarian hyperandrogenism hypothesis, which is ovarian androgen excess, chronic anovulation, and associated reduction of luteal phase progesterone production the reason of reduction (Kaaks *et al.,* 2005). Because of direct androgenic effects on breast tissue,

which is elevated serum androgen and indirectly increased estrogenic exposures of breast tissue because all steroidogenic enzymes necessary for the formation of estrogens from androgenic precursor molecules are present in normal mammary tissues and breast tumor specimens (Kaaks, *et al*., 2005). Although, may be due to the present of higher amount of estradiol observed in the breast cancer patients without medication, which leads to lower serum TC, LDL-C and HDL-C levels that required for production of progesterone through inactivated LPL and upregulate LDL-C receptor clearance (Miller and McLean,1987). In the last, the elevation level of estrogen can block ovulation. This may be stress affects an ability to ovulate. Increases cortisol. Cortisol decreases SHBG, and increased CBG, that deactivate PgR (Taylor and Bell-Taylor, 2009).

On the other hand, this finding suggests that higher abnormality was observed in the level of HDL-C in breast cancer patients without medication than the other two groups. This may be in case of reduction of TC level due to increment of neoplastic transformation of the cells within breast cancer and low initiating of TC transportation in serum (Pandeya *et al*., 2018 and Abas *et al*., 2014). Although, may be due to the lipolytic enzyme, LPL and HLP; inactived in case of sex hormone (estradiol) in these patients (Furberg *et al*., 2005). This supported by Flote *et al.*, (2015) showed that estrogen and progesterone associated with only in women with low HDL-C levels may reflect complex biologic processes. Moreover, this abnormality also may be closely linked, in absence of sufficient quantities of progesterone and the body begins to make androgens to regulate the effect of estrogen (Callejon *et al*., 2009). Lowering of progesterone in relation to excess androgenicity effect on breast cancer patients (make free active estradiol & inactive LPL activity (Saha *et al*.., 2008).

The present study assesses that mean serum levels of estradiol, progesterone and lipid profile of the three different groups in relation with some breast cancer factors. Most of participants were found in younger age and the mean age of healthy controls and breast cancer patients was 39.6 ± 14.6 and 43.1±17.8 respectively. Age is not a risk factor in breast cancer. This study is in line with Hadgu *et al.*, (2018), who studied Ethiopian women breast cancer patients, and found that the mean age at diagnosis was 43years

which is relatively considered as a young age. On other hand, Coscia *et al.* (2017) concluded from his study with older women (mean age 64.94 years); that age could be considered as a risk factor for the induction of breast cancer.

However, significantly lower mean serum TC, LDL-C and HDL-C levels in age matching categories of 20-35years and Progesterone, TC and HDL-C levels in age matching categories above 45 years were observed in breast cancer patients without medication when compared to breast cancer patients with medication. Although, in the age categories above 45 years, mean serum HDLC level of breast cancer patients without medication was found to be significantly lower related to healthy controls. Negative correlation was observed between age and estridol, and also progesterone levels in three examined groups. Similary, Owiredu *et al*., (2009) have shown significantly negative correlation between age and mean serum estradiol level, whereas positive correlation among age and TG, LDL-C levels in breast cancer patients.

According to the current study, breast cancer patients of the study participants were lived urban areas. However, it should be noted that most of the Ethiopian population live in rutal areas, and only 19.5% live in urban areas (Weiner *et al*, 2018). This indicates that the rural and the far regions patients can‘t get the chances for diagnosis. Thus, it can‘t be suggested that this cancer appear more in urban area. It was found that in another study (Weiner *et al*., 2018) 60.7% of the breast cancer patients were from Addis Ababa whereas, 39.3% lived in rural areas. And weak negative correlation was found among serum estradiol level of breast cancer patients without medication and positive correlation in serum TC of healthy control groups and residence.

The majiority of the participants of the study were found in normal body weight (that is 18-24.5 BMI categories).This agreed with, Weiner *et al*., (2018) who found that most of breast cancer patients were normal or overweight BMI categories. Even though, no significant differences and there is no any correlation was observed between BMI and the two steroid hormones as well as lipid profiles levels. By supporting to this result, Rohariya *et al*., (2017) also showed that BMI has no relation with breast carcinoma. But, Owiredu *et al*., (2009) showed that BMI has significant positively correlation with TC and LDL-C. However, in the present study significantly mean serum TC and HDL-C

under 18-24.5 BMI categories, and mean serum progesterone and HDL-C in overweight BMI categories of breast cancer patients without medication were shown lower levels than that of healthy controls. In 18-24.5 BMI categories, mean serum LDL-C level significantly lower in breast cancer patients without medication than with medication.

In comparsion of the two menopausal status of mean serum of the two steroid hormones and lipid profile levels of the three examined groups of premenopausal status have greater than that of postmenopausal status, except, that mean serum TG level of healthy controls and breast cancer patients with medication and mean serum LDL-C level of breast cancer patients without medication were lower. Menopausal status has positive correlation with serum estradiol level and progesterone in three examined groups and for serum HDL-C levels in breast cancer patients without medication and patients with medication. Rohariya *et al*., (2017) disagreed with this study results, and demonstrated that significantly high serum TC levels were observed in postmenopausal study group as compared with premenopausal study group.

According to this finding, significantly lower mean serum progesterone levels were observed in breast cancer patients without medication than that of healthy control groups in the two phases of mensturation. Lower mean serum HDL-C statically significant showed in these patients than healthy control at follicular phase. Although, breast cancer patients with medication statistically significant lower mean serumTC level were found when compared to healthy control at follicular phase and also higher mean serum of LDL-C observed when compared to breast cancer patients without medication at luteal phase. The variations of the mean serum levels of these sex steroid hormones and lipid profile in healthy controls and the two breast cancer patients supported by Mumford *et al.*, (2011). During monthly menstrual cycle women's TC levels was rise as estrogen levels increase, drop shortly before ovulation, then decrease more rapidly after ovulation occurs, specifically as the level of estrogen rises, HDL-C also rises, peaking at the time of ovulation. In contrast, TC and LDL-C levels as well as another form of blood fat known as TG declined as estrogen levels rose. The decline was not immediate, beginning a couple of days after the estrogen peak at ovulation TC, LDL-C and TG levels reached their lowest just before menstruation began (NIH, 2005).

In the present study there was significantly lower mean serum TC, LDL-C and HDL-C levels shown in breast cancer patients without medication than that of with medication at stages I and II. This may be enhancement of utilization of cholesterol by neoplastic cells for new membrane biogenesis, which is the pathogenesis decreased synthesis or increased catabolism of TC (Raste & Naik, 2000). Yin, (2016) and Abdelsalam *et al*., (2012) disagreed with this results. Abdelsalam et al., (2012) stated that the values of TC and LDL-C were significantly increased in all the four stages of breast cancer comparing to the control groups. But the value of HDL-C was not affected.

From the assessment of this study significantly lower mean serum progesterone and HDL-C levels were observed in breast cancer patients medicated by surgery, radiation, chemo and with additional hormonal therapy related to medicate with hormonal therapy only. Although, progesterone and HDL-C levels were shows significantly weak negative correlation with medication of breast cancer. However, higher mean serum TG levels were noticed in these patients than that of breast cancer patients medicated only with hormonal therapy. This may be indicate that different therapies have different effect on the levels of serum sex hormone For example, when chemotherapy applied, after three weeks completion to premenopausal women with hormone-responsive breast cancer induced ovarian suppression and stable decline of serum estradiol levels was observed in all patients (Yoshimura and Furuya, 2014). The removal of adipose tissue also have effect on the synthesis of estradiol during surgery. other may be, due to tamoxifen`s ability to reduce LDL-C through LDL-C receptor clearance and LpL activity (Cheung *et al.*, 2019).

In the present study, mean serum estradiol, progesterone and HDL-C levels were found higher in breast cancer patients treated with tamoxifen than that of armidex. Although, a type of hormonal therapy has negative correlation with serum estradiol, progesterone and HDL-C levels in breast cancer patients with medication. This may be ovarian stimulation and increased estradiollevel in the patients during tamoxifen treatment (Jordan *et al*., 1991 and Sheraman 1979), excess estradiol with effect in synthesis of progesterone, (Miller and McLean, 1987). Banerjee *et al*., (2005) also agreed with tamoxifen caused a significant increase in serum HDL-C level.

6. Conclusions

In the face of, the generalization of results using a small sample size from one hospital, the findings of the current study assessed that significantly lower mean serum progesterone, TC and HDL-C level were found in breast cancer patients without medication than healthy control groups. Whereas, insignificantly lower mean serum LDL-C, TG and higher estradiol level also found in breast cancer patients without medication than healthy control groups. Significantly, higher mean serum levels of TC, LDL-C, TG and HDL-C were found in breast cancer patients with medication compared to patients without medication and insignificantly increment of mean serum progesterone and reduction of estradiol levels were observed in breast cancer patients with medication when compared to without medication. Although, significantly lower level of progesterone and HDL-C and insignificantly higher estradiol of breast cancer patients without medication were found than breast cancer patients with medication and healthy controls in different breast cancer risk factors such as age, menstruation phase (at follicular and luteal phase), menopausal status (pre and postmenopausal status).

The present study finging suggest that armidex and tamoxifen inhibit the levels of estradiol, progesterone and lipid profile through its unclear action in steroidogenesis and lipid metabolism. The study did not identify any antioxidant role/ or dyslipidemic role in the case of both therapies by enhancing progesterone levels, which affect estradiol action in breast cancer patients. It could be inferred that estradiol action might be due to reduced oxidative stress by enhancing HDL-C levels in breast cancer patients. However, the study also proposes that further enhancements of TG and estradiol level due to tamoxifen`s estrogenic effect and rising of LDL-C in the case of armidex.

7. Strength and Limitations

7.1. Strength of the Study

- Assessing of the two steroid hormones and lipid profile levels before and after hormonal therapy by using several related breast cancer risk factors and clinical characteristics.
- Healthy control groups were used as additional reference range in comparison to assess the level of these parameters before and after treatment.
- The study tries to explore the relationships of the two steroid hormones and lipid profile levels before and after hormonal therapies of breast cancer patients in the context of Ethiopia.

7.2. Limitations of the Study

- This study was limited by cross-sectional design. However, cohort design especially prospective study would have provided more precise information about the problem.
- Collections of non-fasting samples were not available according to the time of food consumption.
- The sample size of three examined groups in assessment of biochemical parameters was not dependent on large sample size, matched number of participants in menopausal status and menstrual phase.
- Many other steroids hormones, lipid profiles, enzymes and other therapies were not assessed.
- Collection of samples included all types of breast cancer patients and with mixed hormonal therapies /Armidex and Tamoxifen /.
- The present study could not assess the consequences of the change in levels of the study parameters after hormonal therapy like hyper/hypo lipidimea, estrogenic effect and other secondary complications.
- Lack of sufficient documents hampered our study, relating to steroid hormone levels in breast cancer patients of Ethiopia.

8. Recommendations

The following recommendations are forwarded for further investigation and assessment of steroids hormones and lipid profile levels in breast cancer patients, and who are taking hormonal therapies/Tamoxifen or Armidex.

- Further studies should be conducted with larger sample size and equal/ matched number of participants, based on: age, menopausal status, menstrual cycles by using cohort prospective study design to investigate the effect of hormonal therapies with steroid hormones and lipid profile levels in breast cancer patients.
- Further investigations are required in LPL and HLPL, SHBG, cortisol binding globulin, androgen,cortisol, luteinizing and follicle stimulating hormones to show the relationship between serum steroid hormones and lipid profile concentrations with in breast cancer patients and correlated with hormonal therapy
- Further investigations are required based on types, Stages and hormonal therapies separately/ Armidex or Tamoxifen/ in relation to steroid hormone synthesis, lipid profiles and hormonal therapies as independent factors.
- Steroid hormones and lipid profile levels must be measured during diagnosis, before and after hormonal therapies, which might be helpful for progression, preventing relapse, and other secondary complications of breast cancer.
- Further investigations are required for identification of the consequences which cause changes after hormonal therapy in the levels of steroid hormones and lipid profile levels.
- In depth studies should be required in further specific factors like stress, environmental factors, life style and so on associated with the levels of these biochemical parameters.
- For further investigation and medication it is necessary to have country wide reference ranges /cut off point rather than the machine based.
- During measurement of the levels of these biochemicals should be based on types, stages of cancer and menstrual cycle provided the correct information about breast cancer and related diseases are available.

9. References

Abas, A. M., Zaki, H. Y., Faggad, A., Alhaj, A. M., & Abdalla, B. E. (2014). Lipid profiles of Sudanese cancer patients. *Sudan Med J, 11*(2254), 1-14.

Abate, S., Yilma, Z., Assefa, M., & Tigeneh, W. (2016). Trends of breast cancer in Ethiopia. Int *J Cancer Res Mol Mech, 2*(1), 1.

Abdelhadi, H. A., Sirelkhatim, D. A., Eltayeb, E. A., Ahmed, W. A., & Elhussein, B. (2006). Levels of estrogen, carcinoembryonic antigen and cancer antigen of breast in Sudanese female with breast cancer. *Dissertation summited at university of Khartoum, Sudan. 38*(1), 23.

Abdelsalam, K. E. A., Hassan, I. K., & Sadig, I. A. (2012). The role of developing breast cancer in alteration of serum lipid profile. *J Res Med Sci, 17*(6), 562.

Abramovitz, M., Williams, C., Loibl, S., & Leyland-Jones, B. (2016). Dual blockade of HER-2 provides a greater magnitude of benefit in patients with hormone-negative versus hormonepositive breast cancer. *Clin Breast Cancer, 16*(6), 444-455.

Acconcia, F., & Marino, M. (2011). The effects of 17-β estradiol in cancer are mediated by estrogen receptor signaling at the plasma membrane. *Front Physio, 2*, 30.

Ahmad, A. (2013). Pathways to breast cancer recurrence. *ISRN Oncol, 2013*, Article ID 290568, 16.

Ali, L. O. (2014). Study effect of breast cancer on some hematological and biochemical parameters in Babylon Province, Iraq. *IOSR J Pharma Biol Sci (IOSR-JPBS), 9*(3), 20-24.

Ali, Z. A. M., Abbas, H. M., & Alnaqash, M. A. (2019). Comparison between Letrozole and Tamoxifen Effects on the Lipid Profile in Women treated For Breast Cancer. *Iraqi New Med J, 5*(10), 84-88.

Althuis, M. D., Fergenbaum, J. H., Garcia-Closas, M., Brinton, L. A., Madigan, M. P., & Sherman, M. E. (2004). Etiology of hormone receptor–defined breast cancer: a

systematic review of the literature. *Cancer Epidemiol Prev Biomarkers, 13*(10), 1558-1568.

American Society of Clinical Oncology (ASCO), (2019). Breast Cancer: Risk Factors and Prevention. *Cancer. Net.* Available at https://www.cancer.net/cancer-types/breastcancer/risk-factors-and-prevention

American Society of Clinical Oncology (ASCO), (2019). Hormone Therapy for Breast Cancer. Available at https://www.cancer.org/cancer/breast-cancer/treatment/hormone-therapy-forbreast-cancer.html

American Society of Clinical Oncology (ASCO),(2017).Estrogen and progesterone receptor testing for breast cancer. Available at https://ascopubs.org/doi/pdf/10.1200/JCO.19.02309

Angelopoulos, N., Barbounis, V., Livadas, S., Kaltsas, D., & Tolis, G. (2004). Effects of estrogen deprivation due to breast cancer treatment. *Endocr.-Relat. cancer, 11*(3), 523-535.

Antonova, L., Aronson, K., & Mueller, C. R. (2011). Stress and breast cancer: from epidemiology to molecular biology. *Breast Cancer Res, 13*(2), 208.

Aridiacono, B., Iiritano, S., Nocera, A., Possidente, K., Nevolo, M., Ventura, V., ... & Brunetti, A. (2012). Insulin resistance and cancer risk: an overview of the pathogenic mechanisms. *Exp Diabetes Res, 2012*, 1-12.

Asegaonkar, S., Chaudhari, S. C., & Bardapurkar, J. S. (2012). Lipid profile in breast cancer patients from rural India. *J Indian Med Assoc, 110*(11), 831-2.

Atanassova, N., & Koeva, Y. (2012). Hydrohysteroid Dehydrogenases–Biological Role and *Clin. Importance–Rev. Dehydrogenases, 115.*

Atoum, M., Al-Hourani, H., Nimer, N., Almuhrib, T., & Raheem, S. (2014). Endogenous estradiol, estrogen and progesterone receptors increase benign and breast cancer risk among nonfamilial postmenopausal females. *Health Sci. J, 6*:693-702.

Awio, J. P., Galukande, M., Kituuka, O., & Fualal, J. O. (2012). High serum estradiol confers no risk for breast cancer: another disparity for sub Saharan Africa women. *Pan African Med J, 12*(1).

Banerjee, S., Smith, I. E., Folkerd, L., Iqbal, J., Barker, P., & Dowsett, M. (2005). Comparative effects of anastrozole, tamoxifen alone and in combination on plasma lipids and bonederived resorption during neoadjuvant therapy in the impact trial. *Ann Oncol*, *16*(10), 16321638.

Barros-Oliveira, M. D. C., Costa-Silva, D. R., Andrade, D. B. D., Borges, U. S., Tavares, C. B., Borges, R. S., ... & Silva, B. B. D. (2017). Use of anastrozole in the chemoprevention and treatment of breast cancer: *A Literature Rev Assoc Med Bras, 63*(4), 371-378.

Becker, J. B., Berkley, K. J., Geary, N., Hampson, E., Herman, J. P., & Young, E. (Eds.). (2007). *Sex differences in the brain: from genes to behavior*. Oxford university press. pp.64.

Bendrik, C., & Dabrosin, C. (2009). Estradiol increases IL-8 secretion of normal human breast tissue and breast cancer in vivo. *J Immunology, 182*(1), 371-378.

Bernstein, L., & Ross, R. K. (1993). Endogenous hormones and breast cancer risk. *Epidemiologic Rev, 15*(1), 48-65.

Bettuzzi, S., Robinson, A., Fuchs-Young, R., & Greene, G. L. (1991). *Estrogen and progesterone receptor structure and action in breast cancer cells.* In Genes, Oncogenes, and Hormones (pp. 301-315). Springer, Boston, MA.

Bjornstrom, L., & Sjoberg, M. (2005). Mechanisms of estrogen receptor signaling: convergence of genomic and nongenomic actions on target genes. *Mol Endocrinol*, *19*(4), 833-842.

Blair, I. A. (2010). Analysis of estrogens in serum and plasma from postmenopausal women: past present, and future. *Steroids, 75*(4-5), 297-306.

BodyLogicMD (2020). Bioidentical Hormones for Women: Learn How to Increase Progesterone in Women. Available at https://www.bodylogicmd.com/hormones-for-women/progesterone/

Boér, K. (2017). Fulvestrant in advanced breast cancer: evidence to date and place in therapy. *Ther Adv Med Oncol, 9*(7), 465-479.

Boyd, N. F., & McGuire, V. (1991). The possible role of lipid peroxidation in breast cancer risk. *Free Radical Biol Med, 10*(3-4), 185-190.

Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., & Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: Cancer J Clin, 68*(6), 394-424.

Breast cancer org., (2020) Molecular Subtypes of Breast Cancer. Available at https://www.breastcancer.org/symptoms/types/molecular-subtypes

Brinton, L. A., Figueroa, J. D., Awuah, B., Yarney, J., Wiafe, S., Wood, S. N. ... & Clegg-Lamptey, J. N. (2014). Breast cancer in Sub-Saharan Africa: opportunities for prevention. *Breast Cancer Res Treat, 144*(3), 467-478.

Brisken, C., Hess, K., & Jeitziner, R. (2015). Progesterone and overlooked endocrine pathways in breast cancer pathogenesis. *Endocrinology*, *156*(10), 3442-3450.

Buijs, C. Elisabeth G.E de Vries, Marian J.E. Mourits, Pax H.B. Willemse. (2008). *Long-term side effects of adjuvant breast cancer treatments. The influence of endocrine treatments for breast cancer on health related quality of life.* A review. pp. 90-117.

Bulun, S. E., Chen, D., Moy, I., Brooks, D. C., & Zhao, H. (2012). Aromatase, breast cancer and obesity: a complex interaction. *Trends Endocrinol Metab,* 23(2), 83-89.

Bundred, N. J. (2005). The effects of aromatase inhibitors on lipids and thrombosis. *British J Cancer, 93*(1), S23-S27.

Callejon, D. R., Rios, D. R., Franceschini, S. A., & Toloi, M. R. (2009). Transdermal estradiol and lipid profile: effects on a specific group of Brazilian postmenopausal women. *Arq Bras Cardiol, 93*(6), 571-575.

Carpenter, C. L., Ross, R. K., Paganini-Hill, A., & Bernstein, L. (2003). Effect of family history, obesity and exercise on breast cancer risk among postmenopausal women. *Int J Cancer, 106*(1), 96-102.

Chauhan, P., Yadav, R., Kaushal, V., & Beniwal, P. (2016). Evaluation of serum biochemical profile of breast cancer patients. *Int J Med Res Health Sci, 5*(7), 1-7.

Cheung, Y. M., Ramchand, S. K., Yeo, B., & Grossmann, M. (2019). Cardiometabolic Effects of Endocrine Treatment of Estrogen Receptor–Positive Early Breast Cancer. *J Endocr. Society, 3*(7), 1283-1301.

Cholesterol – Cobas c501/c502, Lab-4285, (cobas, 2016)

Christenson, L. K., & Devoto, L. (2003). Cholesterol transport and steroidogenesis by the corpus luteum. *Reprod Biol Endocrinol, 1*(1), 90.

Cleary, M. P., & Grossmann, M. E. (2009). Obesity and breast cancer: the estrogen connection. *Endocrinology, 150*(6), 2537-2542.

Colditz, G. A. (1998). Relationship between estrogen levels, use of hormone replacement therapy, and breast cancer. *JNCI: J Natl Cancer Inst, 90*(11), 814-823.

Coscia, E. B., Sabha, M., Gerenutti, M., Groppo, F. C., & de Cássia Bergamaschi, C. (2017). Estrone and estradiol levels in breast cancer patients using anastrozole are not related to body mass index. *Rev Bras Ginecol Obstet, 39*(01), 14-20.

Creative Diagnostics Blog (2018). Estrogen and Progesterone. Available at https://www.creativediagnostics.com/blog/index.php/estrogen-and-progesterone/

Czyżykowski, R., Połowinczak-Przybyłek, J., Janiak, A., Herman, J., & Potemski, P. (2014). Tamoxifen-induced acute pancreatitis–a case report. *Przeglad menopauzalny= Menopause Rev, 18(*1), 70-72

Dall, G. V., & Britt, K. L. (2017). Estrogen effects on the mammary gland in early and late life and breast cancer risk. *Front Oncol*, *7*, 110.

Diaz, F. J., Anderson, L. E., Wua Y. L., Rabot, A., Tsai S. J., & Wiltbank, M. C. (2002). Regulation of progesterone and prostaglandin F2a production. *Mol Cellular Endocrinol*, *191*, 65-80.

Dixon, J. M. (2014). Endocrine resistance in breast cancer. *New J Sci*, 2014, |Article ID 390618 | https://doi.org/10.1155/2014/390618

Effi, A. B., Aman, N. A., Koui, B. S., Koffi, K. D., Traoré, Z. C., & Kouyate, M. (2017). Immunohistochemical determination of estrogen and progesterone receptors in breast cancer: relationship with clinicopathologic factors in 302 patients in Ivory Coast. *BMC Cancer*, *17*(1), 115. doi: 10.1186/s12885-017-3105-z.

Egue, M., Gnangnon, F. H., Akele-Akpo, M. T., & Parkin, D. M. (2019). Cancer incidence in Cotonou (Benin), 2014–2016: First results from the cancer Registry of Cotonou. *Cancer Epidemiology*, *59*, 46-50.

Elisaf, M. S., Nakou, K., Liamis, G., & Pavlidis, N. A. (2000). Tamoxifen-induced severe hypertriglyceridemia and pancreatitis. *Ann. Ooncol*, *11*(8), 1067-1069.

Farhat, G. N., Parimi, N., Chlebowski, R. T., Manson, J. E., Anderson, G., Huang, A. J., ... & Jackson, R. (2013). Sex hormone levels and risk of breast cancer with estrogen plus progestin. *J Natl Cancer Inst*, *105*(19), 1496-1503.

Feigelson H.S., Shames L.S., PikeM.C. Coetzee G.A., Stanczyk F.Z. and Henderson B.E. (1998). Cytochrome P450cl7α Gene (CYP1T) Polymorphism Is Associated with Serum Estrogen and Progesterone Concentrations. *Cancer Res, 58*:585-587

Feigelson, H. S., & Henderson, B. E. (1996). Estrogens and breast cancer. *Carcinogenesis*, *17*(11), 2279-2284.

Filippatos, T. D., Liberopoulos, E. N., Pavlidis, N., Elisaf, M. S., & Mikhailidis, D. P. (2009). Effects of hormonal treatment on lipids in patients with cancer. *Cancer Treat. Rev*, *35*(2), 175-184.

Flote, V. G., Frydenberg, H., Ursin, G., Iversen, A., Fagerland, M. W., Ellison, P. T., ... & Furberg, A. S. (2015). High-density lipoprotein-cholesterol, daily estradiol and progesterone, and mammographic density phenotypes in premenopausal women. *Cancer Prev Res*, *8*(6), 535544.

Folk, H., (2019). Estrogen Dominance: 8 Natural Ways to Balance Estrogen Levels. Available at https://www.amymyersmd.com. Retrieved on April 20/2019.

Fuentes, N., & Silveyra, P. (2019). Estrogen receptor signaling mechanisms. *Adv Protein Chem Struct Biol*, *116*, 135-170.

Furberg, A. S., Jasienska, G., Bjurstam, N., Torjesen, P. A., Emaus, A., Lipson, S. F., ... & Thune, I. (2005). Metabolic and hormonal profiles: HDL cholesterol as a plausible biomarker of breast cancer risk. The Norwegian EBBA Study. *Cancer Epidemiol Biomarkers Prev*, *14*(1), 33-40.

Geisler, J., Detre, S., Berntsen, H., Ottestad, L., Lindtjørn, B., Dowsett, M., & Lønning, P. E. (2001). Influence of neoadjuvant anastrozole (Arimidex) on intratumoral estrogen levels and proliferation markers in patients with locally advanced breast cancer. *Clin Cancer Res*, *7*(5), 1230-1236.

Genomic solutions now (GSN). (2016).Women, Estrogen and Breast Cancer: Estrogen, genomic and impact on breast cancer in women. Available at http://genomicsolutionsnow.com. . Retrieved on April 20/2019.

Ghahremanfard, F., Mirmohammadkhani, M., Shahnazari, B., Gholami, G., & Mehdizadeh, J. (2015). The valuable role of measuring serum lipid profile in cancer progression. *Oman Med J*, *30*(5), 353.

Gjerde, J., Geisler, J., Lundgren, S., Ekse, D., Varhaug, J. E., Mellgren, G., ... & Lien, E. A. (2010). Associations between tamoxifen, estrogens, and FSH serum levels during steady state tamoxifen treatment of postmenopausal women with breast cancer. *BMC Cancer*, *10*(1), 313.

Goldstein, S., (2019).progesterone: healthy women. Available at https://www.healthy women.org/condition/progesterone.

Graham, J. D., & Clarke, C. L. (1997). Physiological action of progesterone in target tissues. *Endocr Rev*, *18*(4), 502-519.

Guan, X., Liu, Z., Zhao, Z., Zhang, X., Tao, S., Yuan, B., ... & Ding, Y. (2019). Emerging roles of low-density lipoprotein in the development and treatment of breast cancer. *Lipids Health Dis*, *18*(1), 137.

Gundersen health system, (2019). Standard Operating Procedure, Cobas c501/c502.1of 4. Available at https://www.gundersenhealth.org/app/files/public/12201/Lab-Policies-Lactate---cobasc501--c502.pdf

Ha, M., Sung, J., & Song, Y. M. (2009). Serum total cholesterol and the risk of breast cancer in postmenopausal Korean women. *Cancer Causes Control*, *20*(7), 1055-1060.

Hadgu, E., Seifu, D., Tigneh, W., Bokretsion, Y., Bekele, A., Abebe, M., ... & Karlsson, M. G. (2018). Breast cancer in Ethiopia: evidence for geographic difference in the distribution of molecular subtypes in Africa. *BMC Women's Health*, *18*(1), 40.

Hall, J. E. (2016). *Guyton and Hall Textbook of Medical Physiology, Jordanian Edition E-Book*. Elsevier.

Hammond, M. E. H., Hayes, D. F., Dowsett, M., Allred, D. C., Hagerty, K. L., Badve, S., ... & Hicks, D. G. (2010). American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer (unabridged version). *Arch Pathol Lab Med*, *134*(7), e48-e72.

Hayes, D. F., Skaar, T. C., Rae, J. M., Henry, N. L., Nguyen, A. T., Stearns, V., ... & Flockhart, D. A. (2010). Estrogen receptor genotypes, menopausal status, and the effects of tamoxifen on lipid levels: revised and updated results. *Clin Pharmacol Ther*, *88*(5), 626-629.

HDL-C– Cobas c501/c502, Lab-4287, (cobas, 2016)

Ho, C. C. K., Rohaizak, M., Zulkifli, S. Z., Siti-Aishah, M. A., Nor-Aini, U., & Sharifah-NoorAkmal, S. H. (2009). Serum sex hormone levels in pre-and postmenopausal breast cancer patients. *Singapore Med J*, *50*(5), 513.

Holst, J. P., Soldin, O. P., Guo, T., & Soldin, S. J. (2004). Steroid hormones: relevance and measurement in the clinical laboratory. *Clin Lab Med.*, *24*(1), 105.

Hopkins, J. (2003). Laboratory Procedure Manual. *Analyte: Total Cholesterol, HDL-Cholesterol, Triglycerides, and LDL-Cholesterol NHANES, 2004.*

Houghton, L. C., Ganmaa, D., Rosenberg, P. S., Davaalkham, D., Stanczyk, F. Z., Hoover, R. N., & Troisi, R. (2016). Associations of breast cancer risk factors with premenopausal sex hormones in women with very low breast cancer risk. *J Res Public Health*, *13*(11), 1066.

Howe, L. R., Subbaramaiah, K., Hudis, C. A., & Dannenberg, A. J. (2013). Molecular pathways: adipose inflammation as a mediator of obesity-associated cancer. *Clin Cancer Res*, 19(22), 6074-6083.

Hozumi, Y., Kawano, M., Saito, T., & Miyata, M. (1998). Effect of tamoxifen on serum lipid metabolism. *J Clin Endocrinol Metab*, *83*(5), 1633-1635.

Hozumi, Y., Suemasu, K., Takei, H., Aihara, T., Takehara, M., Saito, T., ... & Ohashi, Y. (2011). The effect of exemestane, anastrozole, and tamoxifen on lipid profiles in Japanese postmenopausal early breast cancer patients: final results of National Surgical Adjuvant Study BC 04, the TEAM Japan sub-study. *Ann. Oncol*, *22*(8), 1777-1782.

Hu, J., Zhang, Z., Shen, W. J., & Azhar, S. (2010). Cellular cholesterol delivery, intracellular processing and utilization for biosynthesis of steroid hormones. *Nutr Metab*, *7*(1), 47.

Hussain, D. A. S., Ahmed, S., Hoque, M., Rabbi, S. M. R., Masood, S., Shafi, T., ... & Chaudhury, H. S. (2013). Biochemical factors associated with breast cancer in Bangladeshi women. *Bangladesh J Med Biochem*, *6*(2), 58-62.

Iversen, A., Frydenberg, H., Furberg, A. S., Flote, V. G., Finstad, S. E., McTiernan, A., ... & Thune, I. (2016). Cyclic endogenous estrogen and progesterone vary by mammographic density phenotypes in premenopausal women. *Eur J Cancer Prev*, *25*(1), 9.

Jaglanian, A., & Tsiani, E. (2020). Rosemary Extract Inhibits Proliferation, Survival, Akt, and mTOR Signaling in Triple-Negative Breast Cancer Cells. *Int J Mol Sci*, *21*(3),

Jamall, S., Ishaq, M., Alam, J. M., & Khadim, M. (2010). CA 15-3 and lipid profile in preoperative breast cancer patients. *Pak J Biochem Mol Biol*, *43*(3), 114-8.

Jiang, Y., & Tian, W. (2017). The effects of progesterones on blood lipids in hormone replacement therapy. *Lipids Health Dis*, *16*(1), 219.

Joensuu, H., Holli, K., Oksanen, H., & Valavaara, R. (2000). Serum lipid levels during and after adjuvant toremifene or tamoxifen therapy for breast cancer. *Breast Cancer Res Treat*, *63*(3), 225-234.

Jordan, V. C., Fritz, N. F., Langan-Fahey, S., Thompson, M., & Tormey, D. C. (1991). Alteration of endocrine parameters in premenopausal women with breast cancer during long-term adjuvant therapy with tamoxifen as the single agent. *JNCI: J Natl Cancer Inst*, *83*(20), 14881491.

Joshi, P. A., Goodwin, P. J., & Khokha, R. (2015). Progesterone exposure and breast cancer risk: understanding the biological roots. *JAMA Oncol*, *1*(3), 283-285.

Kaaks, R., Berrino, F., Key, T., Rinaldi, S., Dossus, L., Biessy, C., ... & de Mesquita, H. B. B. (2005). Serum sex steroids in premenopausal women and breast cancer risk within the European Prospective Investigation into Cancer and Nutrition (EPIC). *J Natl Cancer Inst*, *97*(10), 755-765.

Kaaks, R., Tikk, K., Sookthai, D., Schock, H., Johnson, T., Tjønneland, A., ... & Baglietto, L. (2013). Premenopausal serum sex hormone levels in relation to breast cancer risk, overall and by hormone receptor status—results from the EPIC cohort. *Int J Cancer*, *134*(8), 19471957.

Kapil, U., Bhadoria, A. S., Sareen, N., Singh, P., & Dwivedi, S. N. (2014). Reproductive factors and risk of breast cancer: a review. *Indian J Cancer*, *51*(4), 571.

Katz, T. A. (2016). Potential mechanisms underlying the protective effect of pregnancy against breast cancer: a focus on the IGF pathway. *Front Oncol*, *6*, 228.

Key, T. J. (1999). Serum oestradiol and breast cancer risk. *Endocr-Relat cancer*, *6*(2), 175-180.

Khetrapal A. (2018).Tamoxifen mechanism. Available at https://www.newsmedical.net/health/Tamoxifen-Mechanism.aspx

Kim, Y., Park, S. K., Han, W., Kim, D. H., Hong, Y. C., Ha, E. H., ... & Yoo, K. Y. (2009). Serum high-density lipoprotein cholesterol and breast cancer risk by menopausal status, body mass index, and hormonal receptor in Korea. *Cancer Epidemiol Prev Biomarkers*, *18*(2), 508-515.

Kolan, A., (2014). Whole Health: Change the Conversation: Estrogen Dominance Clinical tool: advancing skills in the delivery of personalized, proactive, patient-driven care Available at. https://docplayer.net/40177758-whole-health-change-the-conversation-estrogen-dominanceclinical-tool.html

Komen, S. G. (2017). Estrogen and Breast Cancer: Blood estrogen level and breast cancer risk. Available at https://ww5.komen.org/BreastCancer/Bloodestrogenlevelsandtheriskofbreastcancerinpostmenopausalwomen.html

Komen, S. G., (2019). Estrogen and Breast Cancer: Blood estrogen level and breast cancer risk. Available at https://ww5.komen.org/BreastCancer/Bloodestrogenlevelsandtheriskofbreastcancerinpostmenopausalwomen.html

Komen, S.G., (2019).The Who, What, Where, When and Sometimes, Why: Tumor Characteristics. Available at https://ww5.komen.org/BreastCancer/Tumor Characteristics. html

Komen, S. G., (2020) The Who, What, Where, When and Sometimes, Why: Blood estrogen levels and breast cancer risk. Available at https://ww5.komen.org/BreastCancer/Table36Estrogenreceptorstatusandoverallsurvival.html

Komen,S.G (2017).The Who, What, Where, When and Sometimes, Why: Body weight and breast cancer risk. Available at https://ww5.komen.org/BreastCancer/Table1Bodyweightandbreastcancerrisk.html

Kshirsagar, V. V., Vaze, , D. D., Dhamane, B. K., Seema G,, Prasanna A Yawalkar, P. A., Pratinidhi, S. A., (2016). Comparative study of lipid profile in patients with carcinoma breast attending a tertiary care hospital of western Maharashtra. *Int J Contemporary Med Res, 3*(4):1093-1095.

Kuru, M., Kükürt, A., Oral, H., & Öğün, M. (2018). *Clinical use of progesterone and its relation to oxidative stress in ruminants. Sex Horm Neurodegenerative Processes Dis,* pp. 303-327.

Laisupasin, P. (2013). Comparison of Serum Lipid Profiles between Normal Controls and Breast Cancer Patients. *J. Lab. Physicians 5*(1): 38–41.

Lange, C. A., & Yee, D. (2008). Progesterone and breast cancer. *Women's Health, 4*(2), 151-162.

LDL-C- Cobas c501/c502, Lab-4287, (cobas, 2016)

Lee, J. R., & Hopkins, V. (2004). *What Your Doctor May Not Tell You About (TM): Menopause: The Breakthrough Book on Natural Progesterone*. Hachette UK.

Lewis, M. J., Wiebe, J. P., & Heathcote, J. G. (2004). Expression of progesterone metabolizing enzyme genes (AKR1C1, AKR1C2, AKR1C3, SRD5A1, and SRD5A2) is altered in human breast carcinoma. *Bmc Cancer*, *4*(1), 27.

Li, X., Liu, Z. L., Wu, Y. T., Wu, H., Dai, W., Arshad, B., ... & Kong, L. Q. (2018). Status of lipid and lipoprotein in female breast cancer patients at initial diagnosis and during chemotherapy. *Lipids Health Dis*, *17*(1), 91.

Lin, C., Chen, L. S., Kuo, S. J., & Chen, D. R. (2014). Adjuvant tamoxifen influences the lipid profile in breast cancer patients. *Breast Care*, *9*(1), 35-39.

Llanos, A. A., Makambi, K. H., Tucker, C. A., Wallington, S. F., Shields, P. G., & AdamsCampbell, L. L. (2012). Cholesterol, lipoproteins, and breast cancer risk in AfricanAmerican women. *Ethn Dis*, *22*(3), 281–287.

Llaverias, G., Danilo, C., Mercier, I., Daumer, K., Capozza, F., Williams, T. M., ... & Frank, P. G. (2011). Role of cholesterol in the development and progression of breast cancer. *Am J Pathol*, *178*(1), 402-412.

Löfgren, L., von Schoultz, E., Fernstad, R., von Schoultz, B., & Carlström, K. (2006). Are estrogen receptor content in breast cancer and effects of tamoxifen on sex hormone-binding globulin markers for individual estrogen sensitivity. *J Steroid Biochem Mol Biol*, *99*(1), 76-79.

Lofterød, T., Mortensen, E. S., Nalwoga, H., Wilsgaard, T., Frydenberg, H., Risberg, T., ... & Stensvold, A. (2018). Impact of pre-diagnostic triglycerides and HDL-cholesterol on breast cancer recurrence and survival by breast cancer subtypes. *BMC cancer*, *18*(1), 654.

Lønning, P. E., Johannessen, D. C., Lien, E. A., Ekse, D., Fotsis, T., & Adlercreutz, H. (1995). Influence of tamoxifen on sex hormones, gonadotrophins and sex hormone binding globulin in postmenopausal breast cancer patients. *J. Steroid Bioehem Molec Biol, 52(5), 491-496.*

Lu, H. H., Li, Q., Xu, B. H., Zhang, P., Yuan, P., Wang, J. Y., & Cai, R. G. (2011). Effects of anastrozole on lipid metabolism in Chinese postmenopausal women with breast cancer. *Zhonghua zhong liu za zhi [Chinese J Ooncol]*, *33*(7), 520-525.

Lum, S. S., Woltering, E. A., Fletcher, W. S., & Pommier, R. F. (1997). Changes in serum estrogen levels in women during tamoxifen therapy. *Am J Surg*, *173*(5), 399-402.

Macciò, A., & Madeddu, C. (2011). Obesity, inflammation, and postmenopausal breast cancer: therapeutic implications. *Scientific World J*, *11*, 2020-2036.

Madeddu, C., Gramignano, G., Kotsonis, P., Paribello, F., & Maccio, A. (2014). Ovarian hyperstimulation in premenopausal women during adjuvant tamoxifen treatment for endocrinedependent breast cancer: A report of two cases. *Oncol letters*, *8*(3), 1279-1282.

Manni, A. (Ed.). (1999). *Endocrinology of breast cancer* (Vol. 11). Springer Science & Business Media. 1sted$^{n.}$ Chapter 4, Humana press. Pp158.

Markopoulos, C. J., Tsaroucha, A. K., & Gogas, H. J. (2010). Effect of aromatase inhibitors on the lipid profile of postmenopausal breast cancer patients. *Clin Lipidology*, *5*(2), 245-254.

Márquez, D. C., Chen, H. W., Curran, E. M., Welshons, W. V., & Pietras, R. J. (2006). Estrogen receptors in membrane lipid rafts and signal transduction in breast cancer. *MCE: Mol Cellular Endocrinol, 246*(1-2), 91-100.

Martin, L. J., Melnichouk, O., Huszti, E., Connelly, P. W., Greenberg, C. V., Minkin, S., & Boyd, N. F. (2015). Serum lipids, lipoproteins, and risk of breast cancer: a nested case-control study using multiple time points. *JNCI: J Natl Cancer Inst*, *107*(5).

Mauvais-Jarvis, P., Kuttenn, F., & Gompel, A. (1986). Antiestrogen action of progesterone in breast tissue. *Breast Cancer Res Treat*, *8*(3), 179-188.

Milionis, H. J., Liberopoulos, E. N., & Elisaf, M. S. (2001). Tamoxifen-induced hypertriglyceridemia in association with diabetes mellitus. *Diabetes Metab*, *27*(2 Pt 1), 160163.

Miller, J. B., & McLean, M. P. (1987). Evidence for the preferential utilization of esterified cholesterol in progesterone production by rabbit corpora lutea. *Biol Reprod*, *36*(3), 729-739.

Miller, W. L., & Auchus, R. J. (2011). The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. *Endocr Rev*, *32*(1), 81–151. https://doi.org/10.1210/er.2010-0013

Mina, A., Yoder, R., & Sharma, P. (2017). Targeting the androgen receptor in triple-negative breast cancer: current perspectives. *Oncol Targets Ther*, *10*, 4675.

Mishra, S. (2015). Lipid profile in breast cancer patients. *Int J Pharma Med. Res*, *3*(1), 29-35.

Missmer, S. A., Eliassen, A. H., Barbieri, R. L., & Hankinson, S. E. (2004). Endogenous estrogen, androgen, and progesterone concentrations and breast cancer risk among postmenopausal women. *J Natl Cancer Inst*, *96*(24), 1856-1865.

Mumford, S. L., Dasharathy, S., & Pollack, A. Z. (2011). Variations in lipid levels according to menstrual cycle phase: clinical implications. *Clin lipidology*, *6*(2), 225-234.

Nagao, T., Kira, M., Takahashi, M., Honda, J., Hirose, T., Tangoku, A., ... & Sasa, M. (2009). Serum estradiol should be monitored not only during the peri-menopausal period but also the post-menopausal period at the time of aromatase inhibitor administration. *World J Surg Oncol*, *7*(1), 88.

National Breast Cancer Foundation (NCBF).(2016).Hormone therapy. Available at www.nationalbreastcancer.org/breast-cancer-hormone-therapy. Retrieved on April 20/2019.

National Cancer Institute at the National Institutes of Health (NCI). (2016). Reproductive History and Cancer Risk. Available at https://www.cancer.gov/about-cancer/causesprevention/risk/hormones/reproductive-history-fact-sheet

National Cancer Institute at the National Institutes of Health (NCI). (2017). Hormone therapy for breast cancer fact sheet. Available at https://www.cancer.gov/breast-hormone-therapy-factsheet. Retrieved on April 20/2019

Nayak, S. R., Rao, G. B., Soren, D. K., & Kiran, J. (2016). Lipid Profile in Newly Detected Carcinoma Breast Patients: A Tertiary Institutional Study. *IJSS J Sur*, *2*(2), 43-46.

Newman, G., & Gonzalez-Perez, R. R. (2014). Leptin–cytokine crosstalk in breast cancer. *Mol Cellular Endocrinol*, *382*(1), 570-582.

Ntukidem, N. I., Nguyen, A. T., Stearns, V., Rehman, M., Schott, A., Skaar, T., ... & Hayden, J. (2008). Estrogen receptor genotypes, menopausal status, and the lipid effects of tamoxifen. *Clin Pharmacol Ther*, *83*(5), 702-710.

Owiredu, W. K. B. A., Donkor, S., Addai, B. W., & Amidu, N. (2009). Serum lipid profile of breast cancer patients. *Pak J Biol Sci*, *12*(4), 332.

Palmisano, B. T., Zhu, L., & Stafford, J. M. (2018). Estrogens in the regulation of liver lipid metabolism. *Adv Exp Med Biol*, 2017 (1043): 227-256.

Pandeya R., Dipendra Raj A., Manoj N., Ezeldine K A., Mahesh B. et al. (2018) .Comparative Study of Serum Lipid Profiles in Nepalese Cancer Patients Attending a Tertiary Care Hospital. *Asian Pac J Cancer Prev*. 19 (2): 491-495.

Pecks, U., Rath, W., Kleine-Eggebrecht, N., Maass, N., Voigt, F., Goecke, T. W., ... & Escher, G. (2016). Maternal serum lipid, estradiol, and progesterone levels in pregnancy, and the impact of placental and hepatic pathologies. *Geburtshilfe und Frauenheilkunde*, *76*(07), 799-808.

Peela, J. R., Jarari, A. M., El Saiety, S. O., El Busaifi, S., & Srikumar, S. (2012). The relationship between serum lipids and breast cancer in Libya. *Biochem Anal Biochem*, *1*(6), 1-3.

Peterson, L. M., Mankoff, D. A., Lawton, T., Yagle, K., Schubert, E. K., Stekhova, S., ... & Krohn, K. A. (2008). Quantitative imaging of estrogen receptor expression in breast cancer with PET and 18F-fluoroestradiol. *J Nuc Med*, *49*(3), 367-374.

Platet, N., Cathiard, A. M., Gleizes, M., & Garcia, M. (2004). Estrogens and their receptors in breast cancer progression: a dual role in cancer proliferation and invasion. *Crit Rev Oncol / Hematol*, *51*(1), 55-67.

Progesterone-Fact-Sheet cobas (2016) Elecsys® 2010 analyzer, cobas e 411 analyzer-PP-US06966.pdfRoche Elecsys estradiol fact sheet cobas (2016) Elecsys® 2010 analyzer, cobas e 411 analyzer PP-US-06968-0416

Quigley, D. A., Tahiri, A., Lüders, T., Riis, M. H., Balmain, A., Børresen-Dale, A. L., ... & Kristensen, V. (2017). Age, estrogen, and immune response in breast adenocarcinoma and adjacent normal tissue. *Oncoimmunology*, *6*(11), e1356142.

Raste, A. S., & Naik, P. P. (2000). Clinical significance of lipid profile in cancer patients. *Indian J med. Sci*, *54*(10), 435-441.

Ray, G., & Husain, S. A. (2001). Role of lipids, lipoproteins and vitamins in women with breast cancer. *Clin biochem*, *34*(1), 71-76.

Raza, U., Asif, M. R., Rehman, A. B., & Sheikh, A. (2018). Hyperlipidemia and hyper glycaemia in Breast Cancer Patients is related to disease stage. *Pak J Med Sci*, *34*(1), 209.

Rohariya, H., Gharde, P., & Gharde, P. M. (2017). Lipid profile and its relevance in carcinoma breast. *Int Sur J*, *4*(7), 2227-2232.

Rosen, E. M., Fan, S., Ma, Y., & Levin, E. R. (2006). Role of plasma membrane ER protein in breast cancer. *Breast Cancer Online*, *9*(3), 1-5.

Ruiz-Cortés, Z. T. (2012). Gonadal sex steroids: production, action and interactions in mammals. *Steroids- Physiol Clin Med*, 3-44.

Saez, S., Martin, P. M., & Chouvet, C. D. (1978). Estradiol and progesterone receptor levels in human breast adenocarcinoma in relation to plasma estrogen and progesterone levels. *Cancer Res*, *38*(10), 3468-3473.

Samavat, H., & Kurzer, M. S. (2015). Estrogen metabolism and breast cancer. *Cancer letters*, *356*(2), 231-243.

Santen, R. J., Yue, W., & Wang, J. P. (2015). Estrogen metabolites and breast cancer. *Steroids*, *99*, 61-66.

Santen, R. J., Yue, W., Naftolin, F., Mor, G., & Berstein, L. (1999). The potential of aromatase inhibitors in breast cancer prevention. *Endocr-Relat Cancer*, *6*(2), 235-243.

Sawada, S., Sato, K., Kusuhara, M., Ayaori, M., Yonemura, A., Tamaki, K., ... & Ohsuzu, F. (2005). Effect of anastrozole and tamoxifen on lipid metabolism in Japanese postmenopausal women with early breast cancer. *Acta Oncologica*, *44*(2), 134-141.

Shah, F. D., Shukla, S. N., Shah, P. M., Patel, H. R., & Patel, P. S. (2008). Significance of alterations in plasma lipid profile levels in breast cancer. *Integrative CancerTher*, *7*(1), 3341.

Shah, R., Rosso, K., & Nathanson, S. D. (2014). Pathogenesis, prevention, diagnosis and treatment of breast cancer. *World J Clin Oncol*, *5*(3), 283-298.

Shawka, Abbas A. A., (2018). Chemistry, mechanism of action & metabolic role of hormones for 2nd year medical students. Available at https://www.slideshare.net/AbbasALRobaiyee1/biochemistry-of-hormones-103837433.

Sheeba, C. A. (2013). Serum hormonal profile and its clinical utility in breast cancer patients among tamil women. *J Adv Sci Res*, 4(3)*:24-26.*

Sherman, B. M., Chapler, F. K., Crickard, K., & Wycoff, D. (1979). Endocrine consequences of continuous antiestrogen therapy with tamoxifen in premenopausal women. *J Clin Invest*, *64*(2), 398-404.

Singh, J., Singh, R., Gupta, P., Rai, S., Ganesher, A., Badrinarayan, P., ... & Panda, G. (2017). Targeting progesterone metabolism in breast cancer with l-proline derived new 14azasteroids. *Bioorganic Med Chem*, *25*(16), 4452-4463.

Solomon, S., & Mulugeta, W. (2019). Diagnosis and Risk Factors of Advantage Cancers in Ethiopia. *J Cancer Prev*, *24*(3), 163-172.

Song, R. X. D., & Santen, R. J. (2006). Membrane initiated estrogen signaling in breast cancer. *Biol Reprod*, *75*(1), 9-16.

Song, R. X., Zhang, Z., & Santen, R. J. (2005). Estrogen rapid action via protein complex formation involving ERα and Src. *Trends Endocrinol Metab*, *16*(8), 347-353.

Sotiriou, C., Neo, S. Y., McShane, L. M., Korn, E. L., Long, P. M., Jazaeri, A., ... & Liu, E. T. (2003). Breast cancer classification and prognosis based on gene expression profiles from a population-based study. *Proceedings Natl Academy Sci*, *100*(18), 10393-10398.

Starek, A. N. D. R. Z. E. J. (2003). Estrogens and organochlorine xenoestrogens and breast cancer risk. *Int J Occup Med Environmental Health*, *16*(2), 113-124.

Steimer, T., (2019). Reproductive health: STEROID HORMONE METABOLISM. Aldo Campana (eds), Available at https://www.gfmer.ch/Books/Reproductive_health/Steroid_hormone_metabolism.html.

Strauss, J. F., & Barbieri, R. L. (2013). *Yen & Jaffe's Reproductive Endocrinology E-Book: Physiology, Pathophysiology, and Clinical Management*. Elsevier Health Sciences. ed. 6th. Chapter 32 Pages 801-823.

Subbaramaiah, K., Howe, L. R., Bhardwaj, P., Du, B., Gravaghi, C., Yantiss, R. K., ... & Kopelovich, L. (2011). Obesity is associated with inflammation and elevated aromatase expression in the mouse mammary gland. *Cancer Prev Res*, *4*(3), 329-346.

Taha A., Mona M. Saad M., Mahmoud NourEldin El-R., and EmanRefaat El-M . (2014). Estrogen receptor gene α polymorphism in relation to lipid profile and Cu, Zn levels in breast cancer patients, *Int Public Health Forum*,1(4),7-12

Taylor E. B. and Bell -Taylor A. (2009). Are Your Hormones Making You Sick? ed 4th. pp. 14-132. Available at https://www.amazon.com/Are-Your-Hormones-Making-Sick/dp/097058590X

Tecalco-Cruz, A. C., & Ramírez-Jarquín, J. O. (2017). Mechanisms that increase stability of estrogen receptor alpha in breast cancer. *Clin Breast Cancer*, *17*(1), 1-10.

TG- Cobas c501/c502, Lab-4455, (cobas 2016)

The women's wellness collective, (2019). A Comprehensive Protocol for Overcoming Estrogen Dominance. Available at https://thewomenswellnesscollective.com/journal/2019/3/9.

Travis, R. C., & Key, T. J. (2003). Oestrogen exposure and breast cancer risk. *Breast Cancer Res: BCR*, *5*(5), 239–247.

Trehan, A. S., Arora, M. K., Seth, S., & Chauhan, A. (2012). Endogenous hormones in postmenopausal females with breast cancer--before and after treatment. *Clin Lab*, *58*(7-8), 771-777.

Velloso, F. J., Bianco, A. F., Farias, J. O., Torres, N. E., Ferruzo, P. Y., Anschau, V., ... & Correa, R. G. (2017). The crossroads of breast cancer progression: insights into the modulation of major signaling pathways. *OncoTargets Ther*, *10*, 5491.

Verma, R., Foster, R. E., Horgan, K., Mounsey, K., Nixon, H., Smalle, N., ... & Carter, C. R. (2016). Lymphocyte depletion and repopulation after chemotherapy for primary breast cancer. *Breast Cancer Res*, *18*(1), 10.

Weiner, C. M., Mathewos, A., Addissie, A., Ayele, W., Aynalem, A., Wondemagegnehu, T., ... & Seidler, A. (2018). Characteristics and follow-up of metastatic breast cancer in Ethiopia: a cohort study of 573 women. *The Breast*, *42*, 23-30.

Wiebe, J. P., Muzia, D., Hu, J., Szwajcer, D., Hill, S. A., & Seachrist, J. L. (2000). The 4-pregnene and 5α-pregnane progesterone metabolites formed in nontumorous and tumorous breast tissue have opposite effects on breast cell proliferation and adhesion. *Cancer Res*, *60*(4), 936-943.

Woldeamanuel, Y. W., Girma, B., & Teklu, A. M. (2013). Cancer in Ethiopia. *The Lancet. Oncol*, *14*(4), 289-290.

Women in Balance Institute/Study Women's Health at National University of Natural Medicine (NUNM) (2020). Hormone Balance and Breast Health Available at https://womeninbalance.org/seventh-woman/hormone-balance-and-breast-health/

Yadav, N. K., Poudel, B., Thanpari, C., & Koner, B. C. (2012). Assessment of biochemical profiles in premenopausal and postmenopausal women with breast cancer. *Asian Pac J Cancer Prev*, *13*(7), 3385-3388.

Yaghjyan, L., & Colditz, G. A. (2011). Estrogens in the breast tissue: a systematic review. *Cancer Causes Control*, *22*(4), 529-540.

Yamazaki, R., Inokuchi, M., Ishikawa, S., Myojo, S., Iwadare, J., Bono, Y., ... & Ohta, T. (2015). Tamoxifen-induced ovarian hyperstimulation during premenopausal hormonal therapy for breast cancer in Japanese women. *SpringerPlus*, *4*(1), 1-5.

Yin, L., Li, Q., Huang, J. M., Lewis, M., Garraway, I. P., Li, Q., ... & Zhau, H. E. (2016). Keratin 13 drives the dissemination of bone and brain metastases of human prostate and breast cancer cells. *Cancer Res;* 76 (14): 4957.

Yoshimura, K., & Furuya, Y. (2014). Changes in ovarian function in premenopausal women with breast cancer undergoing adjuvant TC (docetaxel and cyclophosphamide) chemotherapy during a brief period of amenorrhea around the last chemotherapy cycle. *SpringerPlus*, *3*(1), 1-5.

Yu, H., Shu, X. O., Shi, R., Dai, Q., Jin, F., Gao, Y. T., ... & Zheng, W. (2003). Plasma sex steroid hormones and breast cancer risk in Chinese women. *Int J Cancer*, *105*(1), 92-97.

Yue, W., Wang, J. P., Li, Y., Fan, P., Liu, G., Zhang, N., ... & Santen, R. (2010). Effects of estrogen on breast cancer development: Role of estrogen receptor independent mechanisms. *Int J Cancer*, *127*(8), 1748-1757.

Zhao, Y., Wang, H., Pan, Y., Li, N., & Bian, C. (2016). Association of lipid profile levels in premenopausal and postmenopausal women with breast cancer: a meta-analysis. *Int J Clin Exp Med*, *9*(2), 552-63.

Zhou, Z., Qiao, J. X., Shetty, A., Wu, G., Huang, Y., Davidson, N. E., & Wan, Y. (2014). Regulation of estrogen receptor signaling in breast carcinogenesis and breast cancer therapy. *Cellular Mol Life Sci: CMLS*, *71*(8), 1549.

ZRT Laboratory, (2014). Estrogen metabolites/Urinary Estrogen Metabolites & Their Relevance to Breast Cancer Risk. Available at https://www.zrtlab.com/images/documents/estrogen_metabolites_info_guide.pdf

10. Annexes

Annex 1: Information Sheet for Participants (English Version)

Sponsoring organization: Department of medical Biochemistry, School of graduate studies,

College of Health Sciences, Addis Ababa University

Principal Investigator: Etagegn Tadesse (BSc in chemistry)

Advisors: Dr Menakath Menon (PhD), Dr Daniel Seyifu (PhD), Dr Wondemagegnhu Tigneh (MD) and Mr Mohammed Mehdi (MSc)

Introduction: Dear the participant: if you are voluntarily will be choosen as a study participant. Then you are wright before getting your consent, you need to know all necessary information related to the study which will be detailed and respond freely what the investigator interviews you

Objective of the research project: This study is assess the relationship between serum estradiol, progesterone and lipid profiles level in breast cancer patients before and after hormonal therapy by comparing health control group at TikurAnbessa Specialized Hospital, Addis Ababa, Ethiopia.

Procedure

If you agree to take part in the study, the investigator or a health worker will give you verbal and/or written information about the study and you will be given the consent form to sign, the physician or health professional will ask you some questions about your general health and perform a complete medical examination and assess whether you qualify to participate in the study. If you are fit for the study about 5 ml of blood samples will also be collected for only the laboratory examination of estradiol, progesterone, TC, , LDL-C, TG, HDL-C and face to face interview for additional questions.

Discomforts, risks and benefits from participation

The degree of discomfort you may encounter in giving the sample is no more than when one does in her routine examination. But, there could be cases in which minor pain and change in color of your skin following the blood drawing occur transiently. The blood will be withdrawn by licensed health care professionals (nurses) in the hospital and appropriate care will also be taken.

You will not be provided with any direct incentives for your participation in the research. But the cost for general medical examination will be covered by the project. In addition, based on the results obtained from the research you will be cared accordingly or the results may serve you as a baseline data. In addition, the result of the study will be beneficial for the better prevention and care of breast cancer patients than before. Hence, you are indirectly benefiting other patients and the society in this aspect.

Confidentiality

All pieces of information about the patients will be kept confidential. Log books used in the laboratory will have no names but codes. The information sheet that links the coded number to patient name will be locked inside a box and it will not be revealed to anyone

except your physician and the principal investigator. You have full right to withdraw from participating in this study at any time before and after consent even without explaining the reason. Your decision will not affect your right to get health service you are supposed to get otherwise.

Contact information: If you have any questions contact: ETAGEGN TADESSE: 0911 812365

Annex 2: Informed Consent of Participants (English version)

Department of Biochemistry, School of graduate studies, College of Health Sciences, Addis Ababa

University, Consent form for the participation of the study participants in the research project

Name of the study participant ……

Code number…..……

I have clearly been informed about the research project that it aims to Assessment of the relationship between serum estradiol, progesterone and lipid profiles level in breast cancer patients before and after hormonal therapy by comparing health control group.

The objectives of the research project have clearly been explained to me and I have been told that the results obtained from me will help me as well as the community for better management of the disease. I had been also informed about the confidentiality of this research project. Moreover, I have also been well informed of my right to keep hold of information, decline to cooperate and make myself withdraw from the study. Therefore, with full understanding of the importance of the study, I agreed voluntarily to provide the requested samples and my benefit will be only from the free laboratory investigation result/s.

I_____ hereby give my consent for providing the requested information and blood sample as the doctors find best for me.

Signature: _____ Date_____

Annex 3: Information sheet for participants (Amharic version)

በአዲስ አበባ ዩኒቨርሲቲ የጤና ሳይንስ ኮሌጅ የሕክምና ባዮኬሚስትሪ ትምሕርት ክፍል፡የተሳታፊዎች የፈቃደኝነትና መተማመኛ መረጃ መስጫ ቅፅ

ጥናቱን ስፖንሰር ያደረገዉ ተቋም አዲስአበባ ዩኒቨርሲቲ ጤና ሣይንስ ኮሌጅ ነው፡፡

መረጃመስጫ ቅፅ

በአዲስ አበባ ዩኒቨርሲቲ የጤና ሳይንስ ኮሌጅ የሕክምና ባዮኬሚስትሪ ትምሕርት ክፍል ሁለተኛ ዲግሪ ተማሪ የመመረቂያ ጥናት ጽሁፍ ላይእዲሳተፉ ተጋብዘዋል፡፡

እባክዎ በዚህ ጥናት ለመሳተፍ ከመስማማትዎ በፊት ከዚህ ቀጥሎ የሚገኘዉን ምንባብበ ጥሞና ያንብቡና ግልጽ ያልሆነልዎትን ማንኛዉንም ሃሳብይ ጠይቁ፡፡

Assessment of the relationship between serum estradiol, progesterone and lipid profiles level in breast cancer patients before and after hormonal therapy by comparing health control group at at Tikur Anbesa Specialized Hospital.

የጥናቱ ርእስ ሲሆን አላማውም የጡት ካንሰር ያለባቸው ታካሚዎች በደማቸው ውስጥ ያለውን እስትሮጂን ፕሮጀስትሮን እና የቅባት መጠን እንዲሁም ሌሎች የካንሰሩ ህመማን ከሚወሰዱት ሆረሞናል መደሀኒት ጋር ያላቸውን ግንኙነት እና ተጽዕኖ ለማነጻጸር ሲባል መጠኑንና ሁኔታ መለካት ነው፡፡የጥናቱ ውጤት ለታካሚው ብሎም ለሌላው ማህበረሰብ የሚጠቅምና የተሻለ የጤና እንክብካቤ እንዲኖር የሚያደርግ ነው፡፡ እናም እርስዎ በዚህ ጥናት ለመሳተፍ ጠቃሚና ምቹ ሆነው ተመርጠዋል፡፡የእርስዎ በዚህ ጥናት ላይ የሚያደርጉት ተሳትፎ ሙሉ በሙሉ በበጎፈቃደኝነት ላይ የተመሰረተ ነዉ፡፡በጥናቱ ከተሳተፉ ለናሙና ይሆን ዘንድ 5ሚሊሊትር ያህል ደም በሆስፒታሉ ጤና ባለሙያዎች የሚሰጡ ሲሆን የደም ናሙናውን በሚሰጡበትም ሰዓት ሁልጊዜ ለምርመራ ከሚሰጡበት የተለየ ህመምና አለመመቸት የለውም ለምናልባት ቢኖር ተገቢውን የጤና እንክብካቤ የሚያገኙ ይሆናል፡በዚህ ጥናት ዉስጥ ላለመሳተፍ ወይም ለመሳተፍ ከወሰኑ በኃላ ለማቋረጥ የሚወስኑ ቢሆንም እንኳን በዚህ ሆስፒታል የሚሰጠዎ ማንኛዉም አገልግሎት ላይ ተጽዕኖ የለውም፡፡ በጥናቱ ለመሳተፍ የሚስማሙ ከሆነ የስምምነት ቅጹ ላይ በጽሁፍ ወይም በጣት ፊርማ ማስቀመጥ ይጠበቅበዎታል፡

ግልጽ ያልሆነልዎ ጥያቄ ካለ ሞባል፡ 0911812365

እታገኝ ታደሰ

Annex 4: Informed consent of participants (Amharic version)

የተሳታፊዎች ስምምነት ማረጋገጫ ቅጽ

የሚስጥር ቁጥር----------

የተሳታፊዉ ስም----------

እኔ ስሜ ከላይ የተገለጸዉ ግለሰብየ ተፈለኩት በዚህ ጥናት እንድሳተፍ ሲሆን የጡት ካንሰር ያለባቸው ታካሚዎች በደማቸው ውስጥ ያለውን እስትሮጂን ፕሮጀስትሮን እና የቅባት መጠን እንዲሁም ሌሎች የካንሰሩ ህመማን ከሚወሰዱት ሆረሞናል መደሀኒት ጋር ያላቸውን ግንኙነት እና ተጽዕኖ ለማነጻጸር ሲባል መጠኑንና ሁኔታ መለካት የሚለው ጥናት አላማና ጥቅም ተገልፆልኛል፡፡

ስለዚህ ለዚህ ጥናት መረጃና የስምምነት ቃሌን የምሰጠዉ በአጠቃላይ የጥናቱን አላማና ጥቅም በመረዳትና በፍጹም ፈቃደኝነት ነው፡፡ በመጠይቁ ላይ የምሰጠዉ የእኔ መረጃ እንደ ማይባክና በምስጥር እንደሚያዝም ተነግሮኛል፡፡በተጨማሪም ጥናቱ ዉስጥ ላለመሳተፍ ከፈለኩኝ መብቴ የተጠበቀ እንደሆነና በማንኛዉም ጊዜ ከጥናቱ በራሴ ዉሳኔ መዉጣት ጭምር መብቴ መሆኑንና ከጥናቱ በመዉጣቴ ምንም አይነት ችግር እንደማይደርስብኝ በሚገባ ተገልጾልኛል፡፡ስለሆነም ሁኔታዉን በሚገባ በማጤን በፈቃደኝነት በምርምሩ ላይ ለመሳተፍ ፈቃደኝነቴን ሰጥቻለሁ፡፡

በተጨማሪም የምሰጠዉ የደም ናሙና ለ estradiol, progesterone, TC, TG, HDL-C እና LDL-C ምርመራዎች ብቻ እንደሚዉል ተነግሮኝ ተስማምቻለሁ፡ማንኛዉንም ያልገባኝን ነገር የመጠየቅ እድል ተሰጥቶኝ በሚገባኝ ቋንቋ መልስ አግኝቻለሁ፡በተጨማሪም የሁሉም የላብራቶሪ ምርመራ ዉጤቶች በጊዜዉ ለሀኪሜ እንደሚሰጥልኝ እና ዉጤቱን ማወቅ ከፈለኩ ማግኘት እንደምችል ተነግሮኛል፡፡በአጠቃላይ እኔ ከላይ በመተማመኛ ቅፅ የተጠቀሱትን ሁሉ በሚገባና በተረጋጋ መንፈስ ተገነዝቤአለሁ፡፡ስለዚህ በዚህ ጥናት ለመሳተፍ ፈቃደኛ መሆኔን በፊርማዬ አረጋግጣለሁ፡፡

እኔ_____የተባልኩ ት ግለሰብ ይህን ሁሉ በማገናዘብ በምርምሩ ላይ ስለኔ መረጃ እና የደም ናሙና ለመስጠት ተስማምቻለሁ፡፡

ፊርማ_____ቀን _____

ተሳታፊ _____ _____

Annex 5: - Questionnaire

Date……

Card No ……

Part I: Questionnaire for Female Breast Cancer Patients

1. Age._____
2. Residence A. Urban B. Rural
3. Region: _____
4. Anthropometric measurement:

A. Weight: (in kg) _____

B. Height: (in m _____

C. BMI (kg/m2)_____

5. Educational status A. Primary level B. Secondary level C. Diplomaand above D. Illiterate
6. Menopausal status: A. premenopausal B. postmenopausal
7. Do you drink alcohol? A. Yes B. No

If yes, how much in volume do you drink per day? _____

8. Cigarette smoking: A. Yes B. No C. quitted smoking,

   If yes, how much in per day? specify_____, if quitted when? _____
9. How old you were when you first menstruating? _____
10. Are you menstruating? A. Yes B. No

If yes, currently are you on? A. the first 7days B. The second 7day C. At ovulation/exact 14 day D. post ovulation/14-21days

11. Do you have children? A. YesB. No

    If yes how old you were when you bear your first child? _____

12. When were you examinedbreast cancerfor first time?A. <3 month B. 3month - 5yrsC.>5yrs

13. Which type of breast cancer do you have? A. ER+ B.PR+C. Others D. not classified

14. Specify Clinical stage: _____

15. Did you take any Radiation therapy, Surgery orChemotherapy with related to ovarybreast cancer, colorectal cancer and other organ disease? A. Yes B. No
    If yes, specify_____

16. On which type of hormonal therapy you are? A. Tamoxifen B. Aromatase inhibitor C.Other

17. When did you start treating with tamoxifen or aromatase inhibitor? A. < 3 month B. 3 month-5yrs. C. > 5yrs. Specify the exact day _____/_____/_____

18. Do you have any illness in past? A. yes B. No

    If yes, specify types of illness and medication _____
    If the BIM >25, do you take any medication to reducing obesity problem?
    A. Yes B. No

19. Do you do physical exercise? A. yes B. No

If yes, specify how many time per day_____

20. .Did you get the chance to determine your serum estrogen and progesterone level with related to disease diagnosis? A. Yes B. No
    If yes, specify the disease and hormone_____

21. Do you take any hormonal replacement therapy/contraceptive at this time? A. Yes B. No

If yes, specify type of contraceptive_____

Part II: Questionnaire for Control Group

Date……

Card NO ……

1. Age. _____
2. Residence A. Urban B. Rural
3. Region: _____
4. Anthropometric measurement:

A. Weight: (in kg) _____

B. Height: (in m) _____

C. BMI(kg/m2)_____

5. Educational status A. Primary level B. Secondary level D. Diploma and above E. Illiterate
6. Menopausal status: A. Premenopausal B. Postmenopausal
7. Current alcohol use: A. Yes B. No

If yes, how much volume in glass per day? Specify_____

8. Cigarette smoking: A. Yes B. No

If yes, how much in per day specify_____

9. Are you menstruating? A. Yes B. No

If yes, currently are you on? A. The first 7days B. The second 7day C. At Ovulation /exact 14 day D. Post ovulation/14-21days

10. Do you have the chance to examine breast cancer? A. yes B .No

If yes, specify the time _____

11. Did you take any Radiation therapy, Surgery or Chemotherapy with related to ovary, breast cancer, colorectal cancer and other organ disease? A. Yes B. No

If yes, specify_____

12. Do you have any illness in past? A. yes B. No

If yes, specify types of illness and medication _____

13. If the BIM > 25, do you take any medication to reducing obesity problem?

A. Yes B. No

14. Do you do physical activity? A. yes B. No

If yes, specify how many time per day?_____

15. Did you get the chance to determine your serum estrogen and progesterone level with related to disease diagnosis? A. Yes B. No

If yes, specify the disease and hormone, _____

16. Do you take any hormonal replacement therapy/contraceptive at this time? A. Yes B. No If yes, specify type of contraceptive_____

Annex6: Questionnaire in Amharic

ቀን_____

ካርድቁጥር_____

ክፍል 1፡ ለሴቶች ጡት በሽተኞች የተዘጋጀ መጠይቅ

1. **እድሜ**_____
2. **የወሊድ ሁኔታ ሀ. መውለድ የምትችል ለ. መውለድ ያቆመች**
3. **እስከ አሁን ድረስ የወር አበባ ያያሉ ሀ. አዎ ለ. አላይም**

አዎ ካሉ በየትኛው የወር አበባ ዑደት ውስጥ ነዎት

ሀ. በመጀመሪዉ ለ. የወር አበባ ከመጣ በኋላ እስከ ሰባተኛው ቀን ውስጥ

ሐ. ከመጣ በኋላ 14ኛው ቀን ላይ መ. ከመጣ በኋላ ከ14-21ኛው ቀን ውስጥ

4. **ጡቶትን ከተመረመሩ ምን ያህል ጊዜ ሆኖታል?**

ሀ. ከ3 ወር በፊት ለ. ከ3 ወር እስከ 5 ዓመት ጊዜ ውስጥ ሐ. ከ5 ዓመት በላይ

5. **ምን ዓይነት የጡት ካንሰር በሽታ ነው ያለብዎት?**

ሀ. እስትሮጅን ሪሴፕተር ፖዘቲቭ ለ. ፕሮጀስትሮን ሪሴፕተር ነገቲቭ ሐ. ሌላ ዓይነት

መ. አልተለየም

6. **ከዚህ በፊት ለበሽታዎ ጨረር ህክምና ቀዶጥገና ህክምና እና ኬሞትራፒ ወስድዋል?**

ሀ. አዎ ለ. አልወሰድኩም

7. **የትኛው ኦርሞናል ትራፒ መድኃኒት ነው የሚወስዱት?**

ሀ. ታይሞስፊን ለ. አሮማቴዝእንሂቢተር

8. **የኦርሞናል ትራፒ ሕክምና ክትትል ከጀመሩት ምን ያህል ጊዜ ይሆንዎታል?**

ሀ. ከ3 ወር በፊት ለ. ከ3 ወር እስከ 5 ዓመት ጊዜ ውስጥ ሐ. ከ5 ዓመት በላይ

ትክክለኛውን ጊዜና ቀን በኢትዮጲያ አቆጣጠር ይፃፉ

9. **ከዚህ በፊትም ሆነ አሁን ምንም ዓይነት በሽታ ይዞት አያውቅም?**

ሀ. አዎ ለ. አያውቅም

አዎ ካሉ ምን ዓይነት በሽታ፣መቼ እና ምን ዓይነት መድኃኒት ተጠቅመዋል ቢገልፁልን_____

10. **ከዚህ በፊት ለማንኛውም በሽታ ተብሎ እስትሮጅን እና ፕሮጀስትሮን መጠንን ተመርምረ ውያውቃሉ?**

ሀ. አዎ ለ. አላውቅም

አዎ ካሉ ትክክለኛውን ጊዜ ቢገልፁልን_____

11. **በአሁኑ ጊዜ ማንኛውንም የወሊድ መቆጣጠሪያ መድኃኒት ወስደው ያውቃሉ?**

ሀ. አዎ ለ. አላውቅም

ክፍል ሁለት፡ ለኮንትሮሎች የተዘጋጀ መጠይቅ

ቀን_____

ካርድቁጥር_____

1. እድሜ ሀ. 15-45 ዓመት ለ. 46-55 ዓመት ሐ. ከ55 ዓመት በላይ

2. የወሊድ ሁኔታ ሀ. መውለድ የምትችል ለ. መውለድ ያቆመች

3. እስከ አሁን ድረስ የወር አበባ ያያሉ ሀ. አዎ ለ. አላይም

አዎ ካሉ በየትኛው የወር አበባ ዑደት ውስጥ ነዎት

ሀ. የወር አበባበ መፍሰስ ላይ ለ. የወር አበባ ከመጣ በኋላ እስከ ሰባተኛው ቀን ውስጥ

ሐ. ከመጣ በኋላ 14ኛው ቀን ላይ መ. ከመጣ በኋላ ከ14-21ኛው ቀን ውስጥ

4. ከዚህ በፊት የጡት ህመም በሽታ አጋጥሞት ያውቃል?

ሀ. አዎ ለ. አያውቅም

5. ጡቶትን የመመርመር እድል ገጥሞዎት ያውቃል?

ሀ. አዎ ለ. አላውቅም

6. ለማንኛውም በሽታ ህክምና ተብሎ ጨረር ህክምና ቀዶጥገና ህክምና እና ኬሞትራፒ ወስድዋል?

ሀ. አዎ ለ. አልወሰድኩም

አዎ ካሉ ለምን ዓይነት በሽታ መቼ

7. ከዚህ በፊት ውፍረት ለመቀነስ ብለው ማንኛውንም መድኃኒት ወስደው ያውቃሉ?

ሀ. አዎ ለ. አላውቅም

8. በአሁኑ ወቅት የወሊድ መቆጣጠሪያ ይጠቀማሉ?

ሀ. አዎ ለ. አልጠቀምም

9. በአሁኑ ወቅት ወይም ከዚህ በፊት በሌላ በሽታ ተይዘው ያውቃሉ?

ሀ. አዎ ለ. አላውቅም

አዎ ካሉ የበሽታው ዓይነት መቼ እና ምን ዓይነት መድኃኒት እንደተጠቀሙ ቢገልፁልን?_____

10.ከዚህ በፊት ለማንኛውም በሽታ ብለው እስትሮጂን እና ፕሮጀስትሮን መጠንን ምርመራ አድርገው ያውቃሉ?

ሀ. አዎ ለ. አላውቅም

አዎ ካሉ ትክክለኛውን ጊዜ ይጥቀሱ_____