Chapter one

Introduction

1.1 Background

The breasts are external symbol of beauty and womanhood in women; however cancer of the breast is responsible for the death of millions of women worldwide every year. Malignancy of the breast is one of the commonest causes of death in women aged between 40-45 years \(^1\).

The etiology of the disease is unknown, although both low radiation and oncogenic viruses may play a role. A variety of interrelated hormonal, genetic, environmental, and physiological factors exert an influence on the development of this disease \(^1\).

Epidemiological and experimental evidence implicates estrogens in the etiology of breast cancer. Most established risk factors for breast cancer in humans are thought to influence risk through hormone-related pathways \(^2\).

Estrogens have an essential role, together with other hormones, in the development of the female sex organs and secondary sex characteristics, the regulation of the menstrual cycle and reproduction. Thus, it has been proposed that the effects of many established reproductive risk factors for breast cancer are mediated by hormonal mechanisms, for the most part involving estrogens \(^2\).

Diet may also be a factor in the variation of the incidence of breast cancer among women from different racial or ethnic communities. There has been much debate regarding the correlation between the intake of total and saturated fat and the risk of breast cancer. Epidemiological studies have provided evidence on the postulated association between fat intake and breast cancer risk \(^3\).
1.2 Rationale:
Breast cancer is one of the most serious problems worldwide, and there is strong association between estrogen and lipid profile and breast cancer which is give a reason to be a target for researchers to find out a new ways for early diagnosis, prevention, treatment and follow up. Many studies had conducted to determine the association between the estrogen and lipid profile in breast cancer in different parts of the world, but there are rare published studies in Sudan. Therefore this study is conducted to determine the association between estrogen and lipid profile in Sudanese women with breast cancer, in order to develop future prevention strategies for breast cancer in this country.

1.3 Objectives:
1.3.1 General objective:-
-To study the association between serum estradiol and lipid profile in breast cancer.

1.3.2 Specific objectives:-
- To estimate serum estradiol level in study and control groups.
- To estimate serum lipid profile level in study and control groups.
- To compare serum estradiol, lipid profile and body mass index levels between study and control groups.
- To relate serum estradiol and lipid profile level between study and control groups divided into different ranges of age.
- To compare the pre- and post-menopausal serum estradiol and lipid profile values to the control group.
- To correlate between age, estradiol and lipid profile levels.
Chapter two

Literature review

2.1 Breast cancer:

2.1.1 Breast cancer definition:
Breast cancer is a malignant tumor that starts in the cells of the breast. A malignant tumor is a group of cancer cells that can grow into (invade) surrounding tissues or spread (metastasize) to distant areas of the body. The disease occurs almost entirely in women, but men can get it, too (4).

2.1.2 Breast cancer epidemiology:
Breast cancer continues to remain the most lethal malignancy in women across the world. In 2008, approximately 1.4 million women were diagnosed with breast cancer worldwide with corresponding 460000 deaths (5). Of these, approximately 450000 women were diagnosed with the disease in Europe with a corresponding 140000 deaths, while 68000 women were reportedly diagnosed with the disease in Africa with a corresponding 37000 deaths (5). A number of studies have suggested that there are epidemiological differences between breast cancers among women in Europe and Africa. Risk factors such as menopause, oral contraceptive use, cigarette smoking, and family history of breast cancer have been shown to have different relations to breast cancer among blacks and whites (6).

2.1.3 Breast cancer Incidences:
Breast cancer is a leading cause of death among women in West Africa with an approximately 30000 new cases in 2008 and more than 16000 deaths (5). The incidence appears to be significantly lower in Eastern Africa with approximately 18000 new cases and a corresponding 10000 deaths during the same year (5). In Western Europe, the incidence is five times higher than
that in West Africa. Furthermore, approximately 40000 deaths from breast cancer were recorded in 2008 \(^{(5)}\). The incidence is similar in Central and Eastern Europe with approximately 115000 new cases and more than 47000 deaths in 2008 \(^{(5)}\).

2.1.4 Causes of Breast cancer:
There are several causes of breast cancer which include:

2.1.4.1 Heredity: Being born with mutate, or damage breast cancer gene cause from 5 to 10 percent of breast cancer cause. The breast cancer genes breast cancer 1 (BRCA1) and breast cancer 2 (BECA2) are supposed to stop tumor growth. When these genes are abnormal, they can't carry out their function \(^{(7)}\).

2.1.4.2 Radiation: Radiation is particles send out from radioactive substances. Exposure to high dose of ionizing, or electrically charging radiation has been proven to increase cancer risk \(^{(7)}\).

2.1.4.3 Environment: Chemicals used in factories and on farms and chemicals in every day household products may cause breast cancer. Pesticides for killing bugs, as well as fuel, plastics, detergents, and other poisonous substance may damage breast cancer gene. These chemicals haven't been proven to cause breast cancer. However, studies are currently being done to determine their effect \(^{(7)}\).

2.1.4.4 Estrogen: Estrogen is a female sex hormone produced by ovaries. It triggers breast development and helps in menstrual cycle regulation. This is the time from one menstruation to the next. Estrogen increases the number of cells in the breasts during menstruation. It may stimulate growth of breast cancer cells \(^{(7)}\).

2.1.4.5 Diet: A high-fat diet may contribute to breast cancer by rising estrogen levels in the body. As possible evidence, researchers point to Japan,
where the rate of breast cancer is quite low. Women in Japan typically eat less fat than women in the United States and Canada do (7).

**2.1.5 Risk factors of breast cancer:**

The major risk factors are as fallow:

**Person's sex:** Although men can develop breast cancer, it is predominantly a disease of women.

**Age:** Breast cancer risk increases progressively as women age. Two thirds of breast cancer cases occur in women over the age 50, after menopause. It does not occur often in women under the age of 30, but it increases sharply in the early forties. It levels off after age 45 and increase again after age 55. The yearly incidence in 70-years old women is three times greater than in 50- years-olds.

**Family history:** This is may be the most important factor, especially in women who have a history of immediate relatives who have breast cancer. Women whose mothers or sisters have had breast cancer are two to three times more likely to develop it. If it is occurred in both breasts of these relative and before menopause, the risk is increased. A family history of breast cancer need not indicate a genetic cause.

**Previous cancer:** If a woman has had cancer in one breast, the risk of her developing it in other breast in the twenty years following the initial diagnose is between 10 and 15 percent (8).

Other risk factors are pregnancy and menstrual history. Women who have not born a child or whose pregnancy occurred after age 30 have a highest risk. Early onset of the menstrual period along with late menopause also seems to increase the risk, whereas early menopause lessens it (8).
2.1.6 Signs and symptoms of breast cancer:
The widespread use of screening mammograms has increased the number of breast cancers found before they cause any symptoms, but some are still missed. The most common sign of breast cancer is a new lump or mass. A lump that is painless, hard, and has uneven edges is more likely to be cancer. But some cancers are tender, soft, and rounded or even painful. So it's important to have anything new or unusual checked by a doctor\(^9\).

2.1.7 Types of breast cancer:
There are several types of breast cancer, and cancer can be found in different areas of the breast. Generally, the cancer can be non invasive (in situ) or invasive (infiltrating) and can begin in the cells lining the ducts (ductal carcinoma) or the lobules (lobular carcinoma). Most commonly, breast cancer begins in one of the cells lining the ducts\(^{10}\).

Following are some of the main categories of breast cancer:

2.1.7.1 **Infiltrating ductal carcinoma:** This is most common type of breast cancer and accounts for 65 to 85 percent of cases. This cancer starts in the cells lining the wall of a milk duct and spreads through the wall into the surrounding tissue. The cancer becomes surrounded by scar like material, which form the lump that is detected\(^{10}\).

2.1.7.2 **Infiltrating lobular carcinoma:** This type of cancer accounts for 5 to 10 percent of cases. Here the cancer starts in the cells lining the lobules and spread through the lobule walls to the surrounding tissue. The cancer typically forms fingerlike projections rather than a lump and may be more difficult to distinguish and diagnose than infiltrating ductal carcinoma\(^{10}\).
2.1.7.3 Ductal Carcinoma in Situ (DCIC): Combined with lobular Carcinoma in Situ, ductal carcinoma accounts for 15 to 20 percent of cases. It starts in the cells that line the duct walls but not spread outside the ducts. However it may eventually involve a large area of ducts. If not treated, it may spread outside the ducts. It is earliest stage of breast cancer \(^{(10)}\).

2.1.7.4 Lobular Carcinoma in Situ (LCIS): Combined with ductal Carcinoma lobular carcinoma accounts for 15 to 20 percent of cases. LCIS originates in a lobular cell and grows within the lobule but has not spread to tissue outside the lobules. LCIS is considered a precancerous condition in which a premalignant change has occurred in the lobular cells. The cells divide and multiply but do not always become invasive cancer. Thus, LCIS is considered a marker for having an increased risk of developing invasive breast cancer in either breast \(^{(10)}\).

2.1.7.5 Inflammatory breast cancer: This is the rarest form of breast cancer and accounts for 1 to 4 percent of cases. It invades and blocks lymph vessels in the skin, which result in a red, warm, swollen breast \(^{(10)}\).

2.1.8 Stages of breast cancer:
Like other cancers, breast cancer is classified by stages. The stages indicate the size of tumor and how far the cancer has spread. Breast cancer has five stages. It is treated most successfully in the early stages \(^{(7)}\).

Stage 0: this is non invasive breast cancer. The cancer cells haven't gone beyond the ducts or lobules.

Stage 1: the tumor is still within in the breast. It is two centimeters (about 3/4 inch) or less in diameter.

Stage 2: the tumor is larger than two centimeters but smaller than five centimeters (2 inch), and the lymph node under the arm test positive for cancer.
Stage 3A and 3B: in stage 3A, the tumor is larger than five centimeters and spread to lymph node under the arm. In stage 3B the tumor spread to the skin, chest wall, or lymph node near to the sternum, or breastbone.

Stage 4: cancer cells break away from the tumor. The lymph system and blood stream carry them to other parts of the body. Cancer cells may be carried to places such as the bone, liver, lungs or brain. The spread of cancer from the original tumor to other body parts called metastasis (7).

2.1.9 Molecular subtypes of breast cancer:
Today, breast cancer is considered to be a heterogeneous disease, consisting of different molecular subtypes which correlate with disease outcomes independent of other factors. Gene expression microarrays have identified distinct breast cancer molecular subtypes, including two types of estrogen receptor (ER) negative tumors:

- Basal-like and human epidermal growth receptor (HER2) enriched, and two types of ER positive tumors: luminal A and luminal B. A simpler method employs immune histochemistry (IHC) to identify these molecular subtypes, using antibodies to ER, progesterone receptor (PR), HER2, and myoepithelial markers such as cytokeratin (CK) 5/6. Although gene microarray analysis is more accurate, it is not routinely used in clinical practice, as it is more expensive (11).

Using these surrogate IHC markers, breast cancer is classified into luminal A (ER+ and/or PR+, HER2−), luminal B (ER+ and/or PR+, HER2+), HER2 type (ER−, PR−, and HER2+), basal-like (ER−, PR−, HER2−, and CK 5/6 positive), and unclassified (negative for all markers) (11).

2.1.10 Breast cancer treatment:
The main types of treatment for breast cancer are:

1. Surgery
2. Radiation
3. Chemotherapy
4. Hormone therapy

Treatments can be put into broad groups based on how they work and when they are used. Local treatment is used to treat a tumor without affecting the rest of the body.

Surgery and radiation are examples of local treatment. Systemic treatment is given into the bloodstream or by mouth and goes throughout the body to reach cancer cells that may have spread beyond the breast. Chemotherapy and hormone therapy are systemic treatments (9).

2.2 Estradiol:
The steroid hormone estradiol is the most potent among the estrogens (24).

2.2.1 Synthesis of estradiol:
Estradiol, like other steroids, is derived from cholesterol. After side chain cleavage and using the delta-5 or the delta-4 pathway, androstenedione is the key intermediary. A fraction of the androstenedione is converted to testosterone, which in turn undergoes conversion to estradiol by an enzyme called aromatase. In an alternative pathway, androstenedione is aromatized to estrone, which is subsequently converted to estradiol (12).

2.2.2 Production of estradiol:
During the reproductive years, most estradiol in women is produced by the granulosa cells of the ovaries by the aromatization of androstenedione (produced in the theca folliculi cells) to estrone, followed by conversion of estrone to estradiol by 17β-hydroxysteroid dehydrogenase. Smaller amounts of estradiol are also produced by the adrenal cortex, and (in men), by the testes. Estradiol is not produced in the gonads only: In both sexes, testosterone is converted by aromatization to estradiol. In particular, fat cells
produce active precursors to estradiol, and will continue to do so even after menopause \(^{(13)}\).

Estradiol is also produced in the brain and in arterial walls, though it cannot be readily transferred from the circulatory system into the brain \(^{(14)}\).

**2.2.3 Metabolism of estradiol:**

The liver is the principal site of metabolic destruction of estrogens. Estradiol is completely cleared from the blood by a single passage through the liver and is inactivated by hydroxylation and conjugation with sulfate and glucuronide. About half of the protein-bound estrogen in blood is conjugated with sulfate or glucuronide. Although the liver may excrete some conjugated estrogens in the bile, they are reabsorbed in the lower gut and returned to the liver in portal blood in a typical enterohepatic circulatory pattern. The kidney is the chief route of excretion of estrogenic metabolites \(^{(15)}\).

**2.2.4 Measurement of estradiol:**

Accurate determination of estradiol concentrations in human serum and plasma is important in many clinical settings. In most clinical laboratories, estradiol is measured by immunoassay methods in conjunction with sensitive detection technologies and automated instrumentation. Such measurements are usually robust, economical, and precise. Estradiol is classified as a “type A” analyte by the WHO because it is a chemically well-defined compound \(^{(24)}\).

**2.2.4.1 Principle of immunoassay method:**

The E2 EIA is based on the principle of competitive binding between E2 in the test specimen and E2-HP conjugate for a constant amount of rabbit anti-Estradiol. In the incubation, goat anti-rabbit IgG-coated wells are incubated with E2 standards, controls, samples, Estradiol-HP Conjugate Reagent and rabbit anti-Estradiol reagent at room temperature for 90
minutes. During the incubation, a fixed amount of HRP-labeled E2 competes with the endogenous E2 in the standard, sample, or quality control serum for a fixed number of binding sites of the specific E2 antibody. E2 peroxidase conjugate immunologically bound to the well progressively decreases as the concentration of E2 in the specimen increases. Unbound E2 peroxidase conjugate is then removed and the wells washed. Next, a solution of TMB Reagent is added and incubated at room temperature for 20 minutes, resulting in the development of blue color. The color development is stopped with the addition of stop solution, and the absorbance is measured spectrophotometrically at 450 nm\(^{(29)}\).

2.2.4.2 Normal range:

**Table 2.1: expected value for the estradiol test.**\(^{(30)}\)

<table>
<thead>
<tr>
<th></th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
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<td></td>
<td></td>
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<tr>
<td>Follicular phase</td>
<td>48</td>
<td>9 – 175</td>
</tr>
<tr>
<td>Luteal phase</td>
<td>103</td>
<td>44 – 196</td>
</tr>
<tr>
<td>Periovulatory</td>
<td>209</td>
<td>107 – 281</td>
</tr>
<tr>
<td>Treated menopausal</td>
<td>122</td>
<td>42 – 289</td>
</tr>
<tr>
<td>Untreated menopausal</td>
<td>7.3</td>
<td>ND – 20</td>
</tr>
<tr>
<td>Oral contraceptives</td>
<td>13</td>
<td>ND – 103</td>
</tr>
<tr>
<td><strong>Males</strong></td>
<td>19</td>
<td>4 – 94</td>
</tr>
</tbody>
</table>

2.2.5 Mechanism of action on cancer cell proliferation:

Estradiol has been tied to the development and progression of cancers such as breast cancer, ovarian cancer and endometrial cancer. Estradiol effects target tissues by interacting with two nuclear hormone receptors called estrogen receptor \(\alpha\) (ER\(\alpha\)) and estrogen receptor \(\beta\) (ER\(\beta\))\(^{(16)}\).
Estrogens have a marked proliferative effect on breast epithelial tissue in model systems. Both endogenous and exogenous estrogens stimulate breast epithelial cell mitosis, increasing the number of cell divisions and thus the opportunity for random genetic errors. Estrogen concentrations may be important at all stages in the development of breast neoplasm because the hormonal stimulus to cell division continues all along the progression pathway. However, even if estrogens can induce genetic damage, the data overall suggest that proliferative effects are likely to be the most important mechanism by which this hormone acts to influence the development of breast cancer (2).

2.3. Lipids profile:
Lipids are fat substances that provide energy to the body; are necessary for the production of steroid hormones and bile acids; and have a role in creating cell membranes. Two dominant lipids are cholesterol and triglyceride. Cholesterol and triglycerides are transported in the bloodstream by lipoproteins, which are complex molecules consisting of plasma proteins and lipids. Lipoproteins are categorized as high-density lipoproteins (HDL), cholesterol-rich plasma proteins; very-low-density lipoproteins (VLDL), triglyceride-rich plasma proteins; and low-density lipoproteins (LDL), the cholesterol-rich product of very-low-density lipoprotein breakdown.
A lipid profile includes measuring plasma levels of cholesterol, triglycerides, HDLs, LDLs, and VLDLs. The purpose of the lipid profile is to detect disorders of lipid metabolism and to assess the risk of atherosclerosis, arteriosclerotic heart disease (ASHD), and peripheral vascular disease (20).
2.3.1 Cholesterol:
The cholesterol molecule is asteroid lipid, found in the cell membrane of all body tissues, and transported in the blood plasma, of all animals. Most cholesterol is produced internally, not dietary in origin (21). Cholesterol, however, is an important component of the body and is necessary for the production of bile acids, steroids, and cellular membranes. In addition, cholesterol plays a role in maintaining the skin's resistance to water-soluble substances and prevents excess evaporation of water from the body. About 75% of cholesterol is transported in the bloodstream via low density lipoproteins, and the remaining 25% is bound to high-density lipoproteins (20).

2.3.1.1 Synthesis of cholesterol:
Cholesterol is synthesized endogenously by the liver and other tissue from simple molecule, particularly acetate. About 300 to 1000 mg of the total cholesterol synthesized daily, is derived from dietary intake (22).

2.3.1.2 Methods of estimation of cholesterol:

2.3.1.2.1 Chemical method:
- Libermann-Burchard method: this method measured the cholesterol extracted into cold chloroform and then treated with acetic anhydride, acetic acid, and concentrated sulphoric acid to form a green color complex.
- Zak’s ferric chloride method
- Henly method (21).

2.3.1.2.2 Principle of Enzymatic method:
Cholesterol esters are hydrolyzed by cholesterol esterase enzyme to free cholesterol and free fatty acids. Free cholesterol is oxidized by cholesterol oxidase enzyme to form cholesta-4-ene-3-one and hydrogen peroxide. The hydrogen peroxide is reduced by hydrogen peroxidase enzyme to water and
oxygen that is received by oxygen receptor (4-amino antipyrine), and in the presence of phenol as indicator- quinonimine red is formed and it is measured at 515nm green filter (21).

2.3.1.2.3 Normal range:
Total cholesterol: Less than 200 mg/dl
Border line: 200-240 mg/dl
High risky: > 240 mg/dl (21).

2.3.2 Triglyceride:
Triglycerides, the main form of stored fat in humans, are an important source of energy. Triglycerides exist in the bloodstream and are transported throughout the body by VLDLs and LDLs. Excess plasma triglycerides are stored in the body's adipose tissue. The triglyceride test is used to evaluate the individual's risk of coronary and vascular disease, and to identify atherosclerosis. The test can also provide information about the body's ability to metabolize fat (20).

2.3.2.1 Methods of estimation of triglyceride:
Measurement of serum triglycerides in conjunction with cholesterol is useful in detecting certain genetic and other types of metabolic disorders, as well as in characterizing risk of CVDs. The triglyceride value is also commonly used in the estimation of LDL cholesterol by the Friedewald equation (23).

2.3.2.1.1 Principle of enzymatic method:
Triglycerides are broken down by lipase enzyme to glycerol and free fatty acids. And in the presence of ATP, the glycerol is phosphorilated by glycerol kinase enzyme to glycerol-3-phosphate.

Then, the reaction can be completed by one of the following methods:
glycerol-3-phosphate reduces NAD+ catalyzed by glucose-6-phosphate dehydrogenase enzyme to give dihydroxyacetone phosphate (DHAP), hydrogen ions and NADH that read at 340nm.

glycerol-3-phosphate by an enzyme that called L-glycerophosphate oxidase enzyme gives dihydroxyacetone phosphate (DHAP) and hydrogen peroxide. The hydrogen peroxide is reduced by hydrogen peroxidase enzyme to water and oxygen that is received by oxygen receptor (4-amino antipyrine), and in the presence of phenol as indicator quinonimine red is formed and it is measured at 515nm green filter (21).

2.3.2.1.2 Normal range:
- Normal: <150 mg/dl
- Border line: 150-199mg/dl
- High: 200-499 mg/dl
- Very high: >500 mg/dl (21).

2.3.3 Low Density Lipoprotein (LDL):
The breakdown of VLDLs is a major source of low-density lipoproteins (LDLs), which are cholesterol-rich plasma proteins. Increased levels of very-low density lipoprotein are accompanied by increased levels of low-density lipoproteins (20).

Low-density lipoprotein, a primary transporter of cholesterol, delivers and deposits the cholesterol into the peripheral tissues. Because of this function, LDLs are sometimes referred to as "bad" cholesterol and are associated with atherosclerosis, ASHD, and peripheral vascular disease (20).

2.3.3.1 Measurement of LDL-cholesterol:
Several methods have been used to measure LDL-C. The first reference laboratory procedure, involve ultracentrifugation to separate LDL from other lipoproteins, followed by analysis as cholesterol (21).
Also recently developed homogeneous methods for measuring LDL-C are now used. A much more common second method uses the friedewald formula to calculate LDL-C \(^{(21)}\).

\textbf{Friedewald’s equation:} \(LDL= T.\text{cholesterol} - (HDL+TG/5)\) \(^{(21)}\).

2.3.3.1 Normal level:

60-180 mg/dl \(^{(20)}\).

2.3.4 High Density Lipoprotein (HDL):

High-density lipoproteins (HDL) are plasma proteins that function as carriers of plasma cholesterol. Measuring the cholesterol contained in the HDL molecule is predictive of the individual's risk for coronary artery disease. It is believed that the HDL molecule carries cholesterol from the peripheral tissues of the body to the liver, where the cholesterol is converted into bile acids and eventually excreted. Cholesterol that is part of the high-density lipoprotein molecule will not be deposited in blood vessel walls. Because of this, HDL is sometimes referred to as the "good" cholesterol and is believed to have a protective effect on the circulatory system \(^{(20)}\).

2.3.4.1 Measurement of HDL Cholesterol:

2.3.4.1.1 Principle:

HDL-C is measured as cholesterol in the supernatant of samples following the precipitation of apoB-containing lipoproteins by several methods \(^{(21)}\).

- Polyanion precipitation methods:

Policyanion precipitation was most commonly used to remove apoB-containing lipoproteins prior to analysis HDL-C. It required a sample pretreatment and was not fully automate. Most clinical laboratories have replaced precipitation techniques with automated homogeneous assay for
HDL-C and LDL-C. HDL-C has been measured in the supernatant of samples following the precipitation of apoB-containing lipoproteins by polyanion (as heparin sulphate, dextrane sulphate, phosphotungstate)-divalent cations(Ca2+,Mg2+ and Mn2+) (21).

2.3.4.1.2 Normal level:
Male: 29-62 mg/dl
Female: 34-82 mg/dl (21).

2.3.5 Lipid profile and breast cancer:
Lifestyle and diet are frequently indicated as reasons for the global distribution of breast cancer incidence. Nevertheless, while dyslipidemia [high LDL-C (low density lipoprotein cholesterol) and low HDL-C (high density lipoprotein cholesterol) levels] was already shown to play a major role in the etiopathogenesis of cardiovascular diseases, mainly attributed to diet, the specific influence of dyslipidemia in breast cancer initiation and progression is not completely understood (25).

Cholesterol is a steroid hormone precursor and the vast majority of breast cancer is known to be hormone responsive (26). The peak incidence of breast cancer occurs in the premenopausal age (27). When women dyslipidemia prevalence also rises (28).
Chapter three

Materials and methods

3.1 Study Design:
Quantitative, observational, analytical, case control and hospital based study design to determine the association between serum estradiol and lipid profile in breast cancer.

3.2 Study area:
This study was done in Khartoum state during the period from February 2014 to July 2014, in Radio Isotope Centre Khartoum.

3.3 Study Population:
The targeting group in this study was Sudanese women who have breast cancer.

3.4 Sample size:
The size included 90 samples, 50 samples from women patients with breast cancer (case group) and 40 samples from healthy non cancerous women (control group).

3.5 Inclusion and exclusion criteria:
Samples were collected from breast cancer women as case, and from non cancerous women as control. Women have hypertension, diabetes or thyroid abnormalities were excluded.

3.6 Collection of samples:
Early morning blood samples were collected by using sterile dry, plastic syringes, tourniquet used to make the veins more prominent, Puncture sites were cleaned with 70% ethanol and blood sample (5ml) were collected in plane containers from each volunteer. All blood samples were allowed to
clot at room temperature. Then they were centrifuged at 4000 rpm to obtain the serum, and stored in freezer until the analysis.

3.7 Ethical Considerations:
This study was ethically approved by ethical committee of the Sudan University of Science and Technology. Then the verbal informed consent was agreed by the general managers of the Radio Isotope Centre Khartoum and the participant.

3.8 Methods:-
3.8.1 Estradiol estimation:
Serum estradiol (E2) was determined by competitive enzyme linked immunoassay (ELISA) according to the reagent manufacturer’s instruction.

3.8.2 Estimation of lipid profile:
Lipid profile was estimated photometrically by enzymatic method according to the reagent manufacturer’s instruction.

3.9 Statistical analysis:
The data analyzed by using SPSS computer program.

3.10 Quality control:
Normal and elevated control sera were estimated before determination of lipid profile for the accuracy of lipid profile measurement. Also six ELISA controls were used for the accuracy of estradiol measurement.
Chapter four

Result

This study included 90 women comprising 50 breast cancer patients and 40 controls aged between 24 – 75 years.

In table 4.1 the levels of total cholesterol, triglycerides, low density lipoprotein and BMI in breast cancer patients were increased as compared to the control group.

In table 4.2 there was trend increase in TC, TG and LDL-cholesterol in the breast cancer patients up to age of 50 years and in the control group up to 60 years of age. And the breast cancer patients have higher values than the control group at corresponding age group in all parameters except HDL-cholesterol.

In Fig. 4.1 estradiol level decreased as the age progresses in both the breast cancer patients and the control group and breast cancer patients have higher level than the control at the corresponding age.

In Fig. 4.2 BMI showed little variation with age in both the breast cancer patients and the control group and the breast cancer patients have slightly higher BMI than their corresponding control at the various age groups.

In table 4.3 there was a significant positive correlation between age and TC, age and LDL-cholesterol and significant but negative correlation between age and estradiol and between LDL-cholesterol and estradiol.

In table 4.4 the breast cancer patients have significantly higher BMI, TG and LDL-cholesterol than the control group during premenopausal stage. However TC is significantly raised during both pre and postmenopausal stages.
### Table 4.1: characteristics of study population

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Total</th>
<th>Control</th>
<th>Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years mean)</td>
<td>41.6±12.4</td>
<td>37.6±12.6</td>
<td>44.9±11.4</td>
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<tr>
<td>BMI (Kg/m²)</td>
<td>25.2±5.3</td>
<td>23.8±4.7</td>
<td>26.3±5.4*</td>
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<tr>
<td>TC (mg/dl)</td>
<td>153.5±43.4</td>
<td>127.4±39.0</td>
<td>174.4±34.9**</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>112.8±65.3</td>
<td>88.9±52.7</td>
<td>132.0±68.5*</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>66.6±22.6</td>
<td>68.6±22.3</td>
<td>65.1±23.0</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>63.8±40.1</td>
<td>41.1±29.4</td>
<td>82.1±38.3**</td>
</tr>
<tr>
<td>E₂(IU/ml)</td>
<td>113.1±131.6</td>
<td>92.1±108.9</td>
<td>129.9±146.2</td>
</tr>
</tbody>
</table>

The data are presented as Mean ± SD, BMI: Body Mass Index, TC: Total serum cholesterol, TG: Serum triglycerides, HDL: High density Lipoprotein, LDL: Low Density Lipoprotein, E₂: Estradiol, *P<0.05 and **P<0.001 when the patients group was compared to control group.
**Table 4.2:** Comparisons of biochemical parameters between breast cancer patients and control group divided into different ranges of age (years)

<table>
<thead>
<tr>
<th>Age group (years)</th>
<th>&lt;30</th>
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<th>41-50</th>
<th>51-60</th>
<th>&gt;60</th>
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<td>TC (mg/dl)</td>
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<td>186</td>
<td>160</td>
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<tr>
<td>TG (mg/dl)</td>
<td>125</td>
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<td>107</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>59</td>
<td>71</td>
<td>71</td>
<td>50</td>
<td>52</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>73</td>
<td>78</td>
<td>87</td>
<td>84</td>
<td>93</td>
</tr>
<tr>
<td><strong>Controls</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>120</td>
<td>122</td>
<td>138</td>
<td>142</td>
<td>83</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>75</td>
<td>88</td>
<td>106</td>
<td>76</td>
<td>238</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>69</td>
<td>65</td>
<td>78</td>
<td>67</td>
<td>26</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>36</td>
<td>39</td>
<td>39</td>
<td>61</td>
<td>9</td>
</tr>
</tbody>
</table>

The data are presented as Means, TC: Total serum cholesterol, TG: Serum triglycerides, HDL: High Density Lipoprotein, LDL: Low Density Lipoprotein
Figure 4.1: Comparisons of E₂ (IU/ml) between breast cancer patients and control group divided into different ranges of age (years).
Figure 4.2: Comparisons of BMI (Kg/m²) between breast cancer patients and control group divided into different ranges of age (years).
Table 4.3: correlation between age, estradiol and lipid profile

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Age</th>
<th>EST</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>R= 0.213</td>
<td>R= -0.215</td>
</tr>
<tr>
<td></td>
<td>P= 0.044</td>
<td>P= 0.133</td>
</tr>
<tr>
<td>TG</td>
<td>R= 0.158</td>
<td>R= -0.067</td>
</tr>
<tr>
<td></td>
<td>P= 0.137</td>
<td>P= 0.644</td>
</tr>
<tr>
<td>HDL</td>
<td>R= -0.177</td>
<td>R= 0.234</td>
</tr>
<tr>
<td></td>
<td>P= -0.096</td>
<td>P= 0.101</td>
</tr>
<tr>
<td>LDL</td>
<td>R= 0.273</td>
<td>R= -0.295</td>
</tr>
<tr>
<td></td>
<td>P= 0.009</td>
<td>P= 0.037</td>
</tr>
<tr>
<td>E₂</td>
<td>R= -0.348</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>P= 0.001</td>
<td></td>
</tr>
</tbody>
</table>

**Table 4.4:** Comparison of pre and post menopausal biochemical parameters values between the patients and controls.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Pre Mc</th>
<th>Pos MC</th>
<th>Pre Mp</th>
<th>Pos Mp</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (Kg/m²)</td>
<td>24.1±5.1</td>
<td>22.3±2.2</td>
<td>27.2±5.5*</td>
<td>24.7±5.1</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>125.6±36.2</td>
<td>134.6±50.7</td>
<td>174.4±34.8**</td>
<td>174.3±36.0*</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>87.0±51.8</td>
<td>96.3±59.2</td>
<td>133.0±68.9*</td>
<td>130.2±69.7</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>70.4±21.9</td>
<td>61.5±24.0</td>
<td>68.4±21.5</td>
<td>59.6±24.9</td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>37.8±21.9</td>
<td>54.2±49.6</td>
<td>79.4±37.9**</td>
<td>86.4±39.6</td>
</tr>
<tr>
<td>E₂(IU/ml)</td>
<td>108.7±115.7</td>
<td>26.0±22.7</td>
<td>176.6±160.9</td>
<td>53.6±16.4</td>
</tr>
</tbody>
</table>

The data are presented as Mean ± SD, BMI: Body Mass Index, TC: Total serum cholesterol, TG: Serum triglycerides, HDL: High Density Lipoprotein, LDL: Low Density Lipoprotein, E₂: Estradiol, Pre MC: pre-menopausal control, Pre MP: Pre-menopausal patients, Pos MC: Post-menopausal control, Pos MP: post-menopausal patients, *p<0.05 and **p<0.001 when pre-menopausal compared to control, *p<0.05 and **p<0.001 when postmenopausal compared to control.
Chapter five
Discussion- Conclusion- Recommendations

5.1 Discussion:
In this study 90 the mean age at diagnosis of breast cancer patients selected at random was 45.0 years.
Majority of the women with breast cancer were found to be within the age group 30-50 (70%), with 65% of this number not aware that they had breast cancer. It has also been hypothesized that the adult weight gain or increased BMI is a strong predictor of postmenopausal breast cancer risk. Several case-control and prospective studies have also reported that elevated total serum cholesterol is associated with increased breast cancer risk \(^1\).
In this study there was significant increase in body mass index (BMI) and total cholesterol level in breast cancer women when compared to control group which is in agreement with other studies reported by Bhat1 S A (2013) and William K B (2009)\(^{1, 3}\).
This study has also demonstrated a significant increase in total serum cholesterol levels of the premenopausal patients compared to the control group which is in agreement with other studies reported by Bhat1 S A and Abu-Bedair FA (2003) \(^{1, 17}\). And also demonstrated a significant difference between total serum cholesterol levels of postmenopausal cases and the controls which is in agreement with other studies reported by Bhat1 S A(2013), William K B (2009) and Gaard M (1994) \(^{1, 3, 18}\).
It has been reported in this study that the serum triglyceride level was significantly higher in breast cancer women than the controls which is in agreement with other study reported by William K B (2009) \(^3\). And also reported that the serum triglyceride level was significantly higher in
premenopausal patients compared to controls this finding is in agreement with other study reported by Goodwin PJ, et al (1997) \(^{(19)}\).

On the other hand, there was no significant change in serum triglyceride levels between the postmenopausal patients and controls. Though elevated serum triglyceride levels in postmenopausal breast cancer patients have been reported by Bhat S A (2013) \(^{(1)}\). No significant difference was observed in HDL-cholesterol levels between the breast cancer patients and controls in this study; however LDL-cholesterol levels significantly increased between the patients and the controls this finding is in agreement with other studies reported by Bhat S A(2013) and William K B (2009). \(^{(1, 3)}\). Also LDL-cholesterol levels significantly increased in premenopausal patients compared to controls. The elevated serum LDL-cholesterol, which is more susceptible to oxidation, may result in high lipid peroxidation in breast cancer patients. This may be cause of oxidative stress leading to cellular and molecular damage thereby resulting in cell proliferation and malignant conversions \(^{(1)}\). Although, the relationship between diet and serum lipid levels is complex, diets containing a large amount of saturated fats may lead to higher lipid levels, particularly cholesterol \(^{(17)}\). Elevated lipid levels precede the development of obesity and breast cancer and thus, may have an etiological or predictive significance \(^{(1)}\).

In this study there was increase in estradiol level in breast cancer women in compare to controls group, but it is not statistically significant. Also no significant change was observed in estradiol levels between the premenopausal and postmenopausal cases and the controls though a significant increase in the level of estradiol compared to the controls was observed during postmenopausal phase in studies reported by Bhat S A (2013) and William K B (2009) \(^{(1, 3)}\). It has been hypothesized that the risk
of breast cancer is essentially determined by the intensity and duration of exposure of breast epithelium to menopausal estrogen (1).

Estrogen, like all other steroid hormones is able to cross cell membranes and bind in a specific manner to their receptors to form a specific hormone-receptor complexes. These complexes bind to specific DNA sites in estrogen dependent tissues called Hormone Responsive Elements and cause increased transcription of various genes. The end result is increased cell growth, proliferation and protein synthesis and enzyme synthesis, with concurrent carcinogenesis (1).

In this study there was significant negative correlation between estradiol and LDL cholesterol. Obesity is associated with decreased production of sex hormone binding globulin which results in decrease of follicle stimulating hormone which lower total estradiol production by the ovaries, thus keeping free estradiol relatively constant. Additionally the molecular clearance rate of estradiol is positively associated with weight, also potentially reducing total estradiol level (31). Also it has been hypothesized in other study reported by Bhat S A (2013) that obesity associated with increased production of estrone, which is produced by aromatization of androstenedione in peripheral adipose tissue. It 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 tumor in larger women (1).

The findings of this study suggested the detrimental effect of increased BMI or obesity on breast cancer risk which may promote the growth and metastatic potential of breast tumors in obese women. The results also indicate an increased risk of breast cancer with increasing serum estradiol levels.
5.2 Conclusion:

From this study we conclude the following:

▪ There was significant increase in BMI in breast cancer women when compared to control group.

▪ Lipid profile generally in breast cancer women have significant increased value than control group except HDL-cholesterol have no change between patient and control group.

▪ Total cholesterol and LDL cholesterol increased with age in both patient and control group.

▪ Total cholesterol increased significantly in pre and postmenopausal breast cancer women in compared to control group. But triglyceride and LDL cholesterol increased only in premenopausal phase.

▪ Estradiol hormone have much higher value in breast cancer women than control. And also have negative correlation with age and LDL cholesterol.
5.3 Recommendations:
▪ Regular checkup for lipid profile and estradiol level for women over 45 years.
▪ Diet control and avoidance of highly lipid content for women specially obese one and change of their life style.
▪ Further studies are needed to determine the association between other hormones such as progesterone, prolactin and testosterone and breast cancer risk.
▪ More studies are needed to clarify the effects of hormones on risk for breast cancer and the mechanisms involved, as well as to unravel the complex environmental and genetic determinants of endogenous hormone concentrations.
References:


Appendixes:

Sudan University of Science and Technology
College of Graduate Studies
Clinical Chemistry Department
Association between estradiol and Lipid profile in Breast cancer
Patients among Sudanese women

Questionnaire NO: ............... Date: .............
Name: ................................................................
Age: ................................................................
Body weight (kg): ...........................................
Body height (cm): ............................................
Body mass index: ............................................

Investigations:
Estradiol hormone: ........................................... IU/ml
Cholesterol: ..................................................... mg/dl
TG: ............................................................. mg/dl
HDL-C: ......................................................... mg/dl
LDL-C: ..........................................................mg/dl