CHAPTER ONE

1. Introduction and Literature Review

1.1 Introduction:

Iron is an essential element for cells but in excess may be harmful. Iron overload is connected with increased risk for some malignant diseases, among them breast cancer (Cujic *et al*., 2011). Iron is necessary for cell proliferation and iron metabolism is influenced by estrogen hormones. Interactions between iron and estrogen may synergistically promote breast cancer (Liehr and Jones, 2001).

Breast cancer is the most common malignancy in females, the average middle-aged woman having approximately a 1 in 10 chance of developing the disease at some point during her lifetime (Neal and Hoskin, 2009).

Each year in the UK there are 45 000 cases of breast cancer, making it the commonest form of cancer, accounting for 16.0%of all cancer cases and leading to a total of 12 000 deaths per annum(Neal and Hoskin, 2009).

In Sudan, breast cancer incidence is increasing. Its frequency was 610 (10.01% of registered cancer cases) in 2003 and 1015 (16.7% of registered cancer cases in 2010. Moreover, the incidence of breast cancer showed high percentage in young and middle age group (27% at 41-50 years old and 26% at group 31-40 years old) (Saeed *et al*.,2014).

1.2 Literature review:

Iron is essential for human life. Most functional iron is in the form of hemoglobin and myoglobin, but a small, significant portion of iron is used to bind with cofactors essential to basic metabolic oxidative and reduction reaction.

The regulation of body iron is complex and exquisitely done so as to preserve iron needed but not to allow highly toxic excess (Rodak, 1995).

1.2.1 Physiological locations of iron:

Iron is present throughout the body. Most physiologically active, but some is stored for future use. Most of the stored iron is found in the intracellular space of the liver and bone marrow. Iron is stored for the most part in the form of ferritin, which is composed of iron and a protein called apoferritin, when apoferritin is unavailable, iron is stored as hemosiderin (Steine *et al.*, 1998).

1.2.2 Absorption of iron:

Iron is absorbed most efficiently in the duodenum of the gastrointestinal tract. It is absorbed in two forms: heme and non heme iron. Heme iron is well absorbed. It is present in forms of hemoglobin, myoglobin, and heme enzymes in meat sources. Approximately 20-30% of heme iron is absorbed. Non heme iron, found in non meat sources such as legumes and leafy vegetable, accounts for approximately 90% of dietary iron, but only about 10% of it is absorbed(Rodak, 1995).

An average American diet may contain 10-20mg of iron per day, but of that, only 1-2mg is absorbed.The amount of iron absorbed is dependent on the composition of the meal. Caffeine, fiber, phylate, tomatoes, and

phosphate hamper iron absorption. Ascorbic acid enhances iron absorption (Rodak, 1995).

Cooking in iron pots and pans increase the amount of iron found in food. Iron absorption is also influenced by gastrointestinal factors such as gastric secretion, intestinal motility, and consequence of surgery or bowel disease. Upon entering the gastrointestinal tract, iron is most effectively absorbed in the duodenum and less so in the lower gastrointestinal tract (Rodak, 1995).

Iron is absorbed in both the heme and non heme forms and must traverse the mucosal epithelium and pass into the sub mucosal capillary network. Heme iron is absorbed intact and processed within the mucosal cell. It is degraded to free iron and a teterapyrrole by heme-splitting enzyme, heme oxygenase (Rodak, 1995).

Non heme iron either may be absorbed bound to transferrin or may pass the mucosa by diffusion. Upon entering the mucosal cell, both heme and non heme iron enters a common pool (Rodak, 1995).

Some of the absorbed iron is held by ferritin, a storage form of iron, until the cell is exfoliated .Some iron is only temporarily stored as ferritin to be released and absorbed over a period of a few hours. Iron enters the circulation bound to transferrin, nonspecifically bound to albumin, or perhaps in the form of low-molecular-weight iron chalets, iron may be also be taken up by macrophages to be carried back into the intestinal lumen (Rodak., 1995).

1.2.3 Regulation of iron:

Humans are unique in their lack of any effective means to secrete excess iron. Therefore they regulate iron by controlling absorption.

The amount of iron absorbed is inversely related to the amount of iron stores and the rate of erythropoiesis (Rodak, 1995).

Both in vivo and in vitro animal studies, as well as studies of human duodenal mucosal obtained by biopsy, show this to be true. Some evidence suggests that this is achieved, at least in part, by altering the number of specific iron receptors on mucosal surface. Normal absorption of iron is 1-2 mg/day. With decrease iron stores, iron absorption may be 3-4mg/day. In iron overload, only 0.5mg/day is absorbed (Rodak, 1995).

Human conserve iron very efficiently. There is a 35-40mg/day turnover of iron, and a normal male may lose 1 mg/day, mostly through normal shedding of the epithelial cells from the intestine. A menstruating female lose, on the average, 2mg/day. Patients with iron overload may lose up to 4mg/day (Rodak, 1995).

1.2.4 Cycle and Transport of iron:

Iron cycles through the body moving from absorption in the gastrointestinal tract via the circulation to the bone marrow, where it is incorporated with protoporphyrinIX in the mitochondria of the erythroid precursors to make heme .Iron circulate in red blood cells in the ferrous form in the hemoglobin molecule. The iron is senescent red blood cells is turned over to macrophages and reused. A glycoprotein, transferrin, has a key role in the iron cycling process (Rodak, 1995).

The transferrin molecule contains two terminal lobes, an N and a C, each of which can bind independently to ferric Fe^{+3} ions. A bicarbonate ion locks the iron in place within the molecule by serving as a bridging ligand between the proteins and iron. The transferrin molecule can exist as apoferritin, as a single chain glycoprotein with no iron attached, or in a monoferric or diferric form (Rodak, 1995).

The transferrin gene is located on the long arm of chromosome 3,3q21-qter.Most transferrin is produced by hepatocytes. The transferrin receptor is a glycoprotein dimer located on virtually all cells and present in large numbers in erythroid precursors, the placenta, and the liver (Rodak, 1995).

The transferrin receptor can bind two molecules of transferrin. Transferrin receptor's affinity for transferrin depends on both the iron content and physiological pH. At a PH of 7.4 and with sufficient amount of iron bearing transferrin, the transferrin receptors have a highest affinity. For diferritransferrin, as opposed to monoferric transferrin or

apotransferrin. The transferrin receptor gene is located near the trasferrin gene chromosome 3,3q21-qter (Rodak, 1995).

Cellular uptake of iron is mediated largely by the interaction between transferrin receptor and transferrin molecule. The ferric transferrin receptor is endocytosed, iron is released into the cell, and the receptor-transferrincomplex is returned to the cell surface, whereupon transferrin is released for reuse (Rodak, 1995).

Iron enter a"chelatable" soluble pool, where it is used either for synthesis of essential cellular constitutes or for deposition as ferritin, an apparently non toxic storage form of iron (Rodak, 1995).

1.2.5 Storage of iron:

Iron may be stored in an accessible reserve form as ferritin or as partially degraded or precipitated ferritin called hemosiderin. Apoferritin, the ferritin molecule without iron, is a spherical protein shell about 12nm in diameter and 1nm in width and is composed of mixtures of light and heavy subunits. The liver and spleen, which function as major iron

storage deposits, have a large amount of light subunits. Tissues such as heart tissue that do not act normally as iron storage sites have a higher proportion of heavy subunits (Rodak, 1995).

Genes for the heavy and light chains belong to muligene families with members of several chromosomes. The gene for two types of light chains is on chromosome 19; the gene for heavy chains is on chromosome 11.Interacellular ferritin is synthesized by smooth endoplasmic reticulum to be used in the cell. Small amounts are secreted into the plasma (Rodak, 1995).

It is believed that plasma ferritin is synthesized by rough endoplasmic reticulum and glycoslated by the Golgi apparatus. Within the apoferritin shell, ferric irons, hydroxyl irons, and oxygen are distributed in a lattice-like relationship (Rodak, 1995).

Hemosidern is thought to be a degradation production of ferritin produced by partial digestion of protein and release of iron micelles, which then form insoluble aggregates. Normally, most of the stored iron is in the ferritin form, but as iron stores increase, so does the proportion of hemosiderin in relation to ferritin (Rodak, 1995).

Ferritin and hemosidern stores are found in liver, bone marrow, and spleen. The majority are found in the liver. When iron is required from iron stores, it is returned to transferrin to be used by the cells that need the iron for metabolism (Rodak, 1995).

1.2.6 Iron metabolism:

Abnormal iron metabolism may be the main culprit and player in tumorigenesis, as it is definitely involved in tumor metabolism and cancer mitochondrial dysfunction (Das, 2005).

Iron also plays a significant role in tumor immunosupression. It plays a role in the mechanisms and function of all three partners in tumorgenesis. So, iron (Fe) is an essential metal vital for living cells (Das, 2005).

It is required by a large number of heme and non heme enzymes and proteins, which have essential functions in oxygen transport and oxidative phosphorylation (Sauer, 2000).

Iron is a cofactor for ribonucleotide reductase, an enzyme that converts ribonucleotides to deoxyribonucleotides and thus is a key enzyme in DNA synthesis, and it requires a continuous supply of iron to maintain its activity (Marques *et al*., 2014).

Transferrin (TF), a bilobed glycoprotein is the chief iron transport protein in mammalian blood. It has a molecular weight of about 78,000, and it transports iron from sites of absorption and storage to sites of iron utilization (Finch and Huebers, 1986).

1.2.7 The normal development of breast:

The normal breast, are typical exocrine gland, which may be considered to be a skin appendage, a modified sweat gland, found only in mammals. Breasts first appear on uterine development. In a small number of causes, the breasts fail to develop and the condition and example of agenesis is termed amastia (Vardaxis, 2012).

In the seventh week there is thickening of the mammary ridge to form the precursor of the nipple. In humans only two nipples are normally formed but in lower mammals many nipples form. Not infrequently (5% of female, 1% of male), smaller accessory nipples may also be seen in humans as a congenital anomaly, a condition called polythelia (Varidax, 2012).

Between the twentieth and thirtieth week solid epidermal cords grow into the subcutaneous tissue and form 15-20 lobar ducts. At birth the nipple is formed by evagination of the epidermal bit and in both sex breast enlargement and transitory of secretion may be seen due to the action of maternal hormones (Varidax, 2012).

Furtheter development of the breasts depends up on female sex hormone stimulation such that the male breast remains infertile and rudimentary throughout the life, unless a hormonal disorder causes the male breasts to enlarge a condition called gynaeocmastia (Varidax, 2012).

Osterogene causes development of the large duct in the breast wills progesterone developed the lobules and ductless that will form the acini. The prolactin stimulates the development of the secretary units and causes milk production. And oxytocin stimulates contraction of the myoepithelial cells and expression of milk. During pregnancy, estrogen and progesterone prevent the milk from being produced (Varidax, 2012).

When pregnancy ends, lactation begins. Stimulation of the nipple causes production for both prolactin (which maintains lactation) and oxytocin (which ejects the milk).At puberty in the female, ovarian hormonal stimulation begins and causes an enlargement of the nipple, areola and the entire breast through a process of physiological hyperplasia. Adipose tissue is deposited in large amount in the fibrocollagenous strom (Varidax, 2012).

The lobar ducts elongate and subdivide, forming interlobular and interlobular ducts, lined by 2to 3 cells. The breast then undergoes cyclical hyperplasia and involution during the menstrual cycle, but develops no further until the women becomes pregnant. During the second half of the menstrual cycle progesterone causes some proliferation of the ducts and

stroma in the lobules. When the cycle ends, these changes regress (Varidax, 2012).

Some breast parenchyma extends toward the axilla as the tail of Spence. Generally the upper outer quadrant of the breast contains most tissue which probably explains why most breast disease is most common here. In rare cases, several well-developed breasts will be seen along the milk line, a condition termed polymastia (Varidax, 2012).

1.2.8 Normal structure of the breast:

The breast is a modified skin appendage which is functional in the females during lactation but is rudimentary in the males. Microanatomy of the breast reveals 2 types of tissue components; epithelial and stromal. In a fully developed non-lactating female breast, the epithelial component comprises less than 10% of the total volume but is more significant pathologically since majority of lesions pertain to this portion of the breast (Mohan, 2010).

Among disease-prone organs, the breast is unique because it is affected by one of life-threatening disease: cancer, which, in the UK, kills 28 per100, 000 women annually (Levison *et al*., 2008).

1.2.8.1 Epithelial component of the breast:

The epithelial component of the breast consists of 2major parts; terminal duct- lobular unit (TDLU) which performs the main secretary function during lactation, and large duct system which perform the function of collection and drainage of secretion; both are interconnected to each other (Mohan, 2010).

The breast is divided into about 20 lobes. each lobe consist of breast lobules which drain their secretion through its collecting duct system and

open into the nipple through its own main excretory duct, lactiferous duct, the segment of lactiferous duct subjacent to the nipple shows a small dilation called lactiferous sinus. Each lactiferous duct has its own collecting duct system which has branches of the smaller diameter, ultimately terminating peripherally as terminal ducts (or TDLU) in the breast lobules (Mohan, 2010).

The entire ductal-lobular epithelial system has bilayered lining: the inner epithelial with secretary and absorptive function, and an outer supporting myoepithelial lining, both having characteristic ultra-structure and immunoreativitytroma (Mohan, 2010).

The inner epithelium stains positive for epithelial membrane antigen (EMA) and lactalbumin while the myoepithelium is positive for smooth muscle actin (SMA) and S-100 (Mohan, 2010).

1.2.8.2 Stromal components of the breast:

The supportive stroma of the breast consists of variable amount of loose connective tissue and adipose tissue during different stages of reproductive life. The stromal tissue of the breast is present at 2 location; interlobular and interlobular stroma. Interlobular stroma encloses each lobule, and its acini and ducts, and is chiefly made of loose connective tissue, myxomatous stroma and a few scattered lymphocytes (Mohan, 2010).

Interlobular stroma separates one lobule from the other and is composed mainly of adipose tissue and some loose connective tissue. The most important disease of the breast is cancer. However, there are a few inflammatory lesions, benign tumors and tumors-like lesions which may be confused clinically with breast cancer (Mohan, 2010).

1.2.9 Cancer:

Is a broad group of disease involving cells divide and grow uncontrolled forming malignant tumors and invading nearby parts of the body. The cancer may also spread to more distant parts of the body through the lymphatic system or blood stream (National Cancer Institute, 2013).

Not all tumors are cancerous; benign tumors don't invade neighboring tissue and don't spread throughout the body. There are over 200 different known cancers that affect humans (National Cancer Institute, 2013).

1.2.10 Genetics of breast cancer:

The development of malignancy is related to abnormalities and structure and or function of tumor suppressor gene and oncogenes. A great many genes have been found to show aberrations in invasive breast carcinoma including members of the tyrosine kinase family of growth factor and HER2, c-myc, the tumor suppressor gene p53 and members of ras family. Many of these abnormalities are seen in sporadic cases of breast cancer. Although inherited cases account only 5-10% of breast cancer, it clear that some families have a significantly greater risk than the general population for development of breast cancer (Levison *et al*., 2008).

In particular it is likely that true hereditary factor is implicated if the disease is diagnosed at young age, is bilateral or if many family members are affected. In some families with breast cancer, ovarian carcinoma may also be seen and in others both males and females may be affected (Levison *et al*., 2008).

It is clear that there is not a single gene which is mutated in all cases of familial breast cancer. One of the first abnormalities to be identified was a mutation in the tumor suppressor genep53(also seen in sporadic breast

cancer) on the short arm of chromosome 17.Li- fraumeni syndrome is a cancer family syndrome with a germ-line mutation in p53 causing breast cancer but also sarcomas, various childhood malignancies and gliomas. This autosomal dominant inherited condition is, however, associated with less than 5% of familial breast cancers. However, breast cancer associated gene 1(BRCA1) and subsequently gene2 (BRCA2) have been cloned (Levison *et al.*, 2008).

These are also of autosomal dominant inheritance. It remains uncertain precisely what functions these gene products play in normal tissue .It is nevertheless clear that mutations in these genes are associated with differing risk of breast and ovarian cancers. BRCA1 is associated with an overall lifetime risk of 85% developing breast cancer but the site of the mutation appear to be important with relation to the differing risk of breast and ovarian cancer (Levison *et al.*, 2008).

1.2.11 Risk factors of breast carcinoma:

Causes of breast cancer have been identified by epidemiologic studies and while several etiological and predisposing factors investigated have been implicated, various other have been exonerated (Vardaxis, 2012).

1.2.11.1Geographic/ ethnic influences:

Breast cancer is 5 times more common in Australia, the USA and other western countries than in Asia. It is possible that exogenous factors, such as diet and carcinogens in the environment, play a role in different regions and also social factors, such as parity (Varidax, 2012).

1.2.11.2 Genetic predisposition:

The degree of risk is proportional to the number of close relatives with breast cancer. The younger the age incidence of breast cancer and the

higher the incidence of bilateral breast cancer in close relatives, the greater the genetic predisposition. Occasionally there are seen high- risk families with apparent autosomal- dominant transmission and familial association of breast and ovarian carcinoma. Early- onset familial breast cancer is usually due to germ-line mutations of the genes BRCA1 or BRCA2 (Varidax, 2012).

1.2.11.3 Increasing age:

Breast cancer is uncommon before the age of 20, and then there is a steady increase until menopause, and a slower rise through late life (Varidax, 2012).

1.2.11.4 Exogenous estrogen:

This is controversial, but some evidence shows moderately increased risk with high dose estrogen therapy of menopausal symptoms. More recent treatments with balanced preparations do not predispose. The oral contraceptive pill is not a risk factor and it has been shown to protect against many of benign breast lesions (Varidax, 2012).

1.2.11.5 Endogenous estrogens (hyperestronisim):

In the conditions where there are high estrogen levels in the body (e.g. ovarian or adrenal tumors) there is an increased risk (Varidax, 2012).

1.2.11.6 Length of reproductive life:

The risk increases with early menarche and late menopause. As women today commence menace earlier than their predecessors in the 1800s and enter menopause later, they are more predisposed to breast carcinoma (Varidax, 2012).

1.2.11.7 Parity:

It is more common in nultiparous than parous or multiparous women (Varidax, 2012).

1.2.11.8 Age at which women has her first child:

There is increased risk when a mother is over 30 years of age at time of having her first child (Vardaxis, 2012).

1.2.11.9 Obesity:

There is an increased risk associated with the increased estrogen synthesis in fat depots (Varidax, 2012).

1.2.11.10 Dietary factors:

Some studies showed that women with a high saturated fat diet and much red meat in the diet have a higher risk (Varidax, 2012).

1.2.12 Histopathology of breast cancer:

Breast cancer is usually classified primarily by its histological appearance. Most breast cancers are derived from the epithelium lining the ducts or lobules, cancer from other tissue are considered rare cancer, carcinoma in situ is proliferation of cancer cells within the epithelial tissue without the invasion of the surrounding tissue. Invasive carcinoma invades surrounding tissue cells that dividing more quickly have wars prognosis. One way to measure tumor cell growth is with presence of protein k67. Which indicate that the cell is in S phase, and also indicates susceptibility to certain (Giordano *et al.*, 2004).

1.2.13 Classification of breast carcinoma:

Almost all breast malignances are adenocarcinomas, all other types (i.e. squamaous cell carcinoma, phyllodes tumors, sarcomas, and lymphomas) making up fewer than 5% of the total (Grag and Gupta, 2011).

Carcinomas are divided into *insitu* carcinomas and invasive carcinomas.

1.2.13.1 Non invasive carcinomas:

Non invasive carcinomas (carcinoma *insitu*) may be located within the ducts (intraductal carcinoma al papillary carcinoma. comedocarcinom grows as a solid intraductal sheet of cells with a central area of necrosis that commonly undergoes classification (Garg and Gupta, 2011).

It is frequently associated with the erb B2\neu oncogene and a poor prognosis. Cribriform carcinoma is characterized by round, duct like structure within the solid intraductal sheet of epithelial a) or within the lobules (lobular carcinoma *insitu*). There are several variants of intraductal carcinoma, including comedocarcinoma, cribriform carcinoma, and intraduct cell, while intraductal papillary carcinoma has a predominant papillary pattern (Garg and Gupta, 2011).

Infiltration of the nipple by large cells with clear cytoplasm is diagnostic of Paget's disease. These cells are usually found both singly and in small cluster in the epidermis. Paget's disease is always associated with (in fact, it begins with) and underling intraductal carcinoma that extent to infiltrate the skin of nipple and areola. Paget cells may resemble the cells of superficial spreading melanoma, but they are PAS positive diacetase – resistant (mucopolyschariде-or mucin positive) unlike melanoma cell (Garg and Gupta, 2011).

1.2.13.2 Invasive (infiltrating) carcinoma:

Invasive breast carcinoma is divided into two main types

No- special type carcinoma (intraductal) and Special carcinoma which includes:

Lobular, Cribriform, Colloid, Modularly, papillary and Metaplastic carcinoma (Garg and Gupta, 2011).

1.2.14 Staging of breast cancer:

The TNM staging is the most frequently used and can be used as a guide to management and Prognosis. It is as follows:

T0 – No evidence of primary tumor

TX – Primary tumor cannot be assessed

Tis – Carcinoma *in situ*

T1 – 2 cm or less in greatest dimension

1a – 0.5 cm or less in greatest dimension

1b – >0.5 cm but not >1 cm in greatest Dimension

1c – >1 cm but not >2 cm in greatest Dimension

T2 – >2 cm but not >5 cm in greatest Dimension

T3 – >5 cm in greatest dimension

T4 – Tumor of any size with extension to Chest wall and/or skin

4a- Invasion of chest wall (ribs, serratus anterior, intercostals muscle)

4b- Oedema/‘peau d’orange’, ulceration, satellite nodules confined to same breast

4c – Both 4a and 4b

4d– Inflammatory carcinoma N0 – No lymphadenopathy

N1 – Ipsilateral mobile axillary nodes

N2 – Ipsilateral axillary nodes fixed to one another or to adjacent structures

N3 – Ipsilateral internal mammary node Metastases

M1– Involvement of supraclavicular nodes or distant metastases (Neal and Hoskin, 2009).

1.2.15 Signs and symptoms of breast cancer:

The lump is usually non-tender, well defined and most likely to be located in the upper outer quadrant, which contains the majority of the breast tissue. Breast discomfort is occasionally a presenting symptom. In advanced cases, the overlying skin can be dimpled or frankly invaded by tumor leading to reddening indurations and nodular irregularity (Neal and Hoskin, 2009).

Fixation to the skin or chest wall will limit mobility of the lump, and this should be sought by the clinician during physical examination. A very large lump will lead to obvious asymmetry of the breasts (Neal and Hoskin, 2009).

There may be enlargement of the ipsilateral should be assessed as part of the clinical staging and less frequently enlargement of the supraclavicular lymph nodes. Hepatomegally could suggest metastatic infiltration while intrathoracic signs of collapse, consolidation or pleural signs (Neal and Hoskin, 2009).

The majority present with a painless breast lump or distortion of the breast, which might be associated with a blood-stained nipple discharge. Patients detected by screening are likely to be asymptomatic. Less frequently the presentation is with diffuse enlargement or reddening of the breast, and occasionally lymphadenopathy or symptoms from distant metastases (Neal and Hoskin, 2009).

1.2.16 Diagnosis of breast cancer:

Triple assessment comprises physical examination, mammography and ultrasonography.

1.2.16.1Mammography:

This comprises radiographic examination of the breasts using low energy X-rays to allow definition of the soft tissue detail and breast architecture (Neal and Hoskin, 2009).

1.2.16.2 Breast ultrasound:

This enables the radiologist to determine whether a lump is solid or cystic. It provides images that are complementary to those obtained by mammography (Neal and Hoskin, 2009).

1.2.16.3 Magnetic resonance imaging:

This is reserved for the investigation of more difficult cases. It is also useful in excluding multifocal disease in mammographically dense breasts and in excluding occult contralateral breast cancer in those with invasive lobular carcinoma (Neal and Hoskin, 2009).

1.2.16.4 Fine needle aspiration (FNA) cytology:

This is a rapid, safe, relatively non-traumatic procedure which can be performed at an outpatient consultation and can provide a tissue diagnosis

within hours. It should be performed whenever a palpable lump or suspicious area of indurations is found, and is applicable to the primary tumor, regional lymph nodes or suspicious skin lesion (Neal and Hoskin, 2009).

1.2.16.5 Needle biopsy:

This is performed under local anesthetic and is a little more traumatic than FNA but gives a core(s) of tissue for histological analysis (Neal and Hoskin, 2009).

1.2.16.6 Nipple discharge cytology:

This is useful in women presenting with a bloody nipple discharge in the absence of a palpable lump (Neal and Hoskin, 2009).

1.2.17 Previous studies:

The results of several studies pointed to the association between increased serum iron and breast cancer. A case-control study was done by Pavithra *et al* in which serum level of metal ions in female patients with breast cancer was measured. The study was conducted on 54 female patients with breast cancer and 54 female controls. They found there was statistically significant increase in serum levels of iron in patients with breast cancer when compared to controls (Pavithra *et al*., 2015).

Study conducted in India by Dhankhar *et al*. The study was conducted on ninety females. Out of these, 60 were patients of newly diagnosed/ untreated histopathological proven breast cancer and 30 were healthy, non-anemic females of age more than 15 years as control group.

In study, iron, ferritin and TIBC were analyzed in 30 patients of early stage, 30 of advanced stage breast carcinoma before and after treatment

and the results were compared with 30 healthy controls. The levels of all the three parameters were found to be significantly higher in breast cancer patients as compared to healthy controls and patients with advanced disease showed greater values as compared to early stage disease. These levels decreased after treatment and the difference was more significant in patients with complete response. They concluded that serum analyses of iron, ferritin and TIBC may help in assessing the severity and prognosis of the disease (Dhankhar *et al*., 2014).

Furthermore, a cohort study done by Pang *et al* in 309,443 adults in Taiwan who had no history of cancer had serum iron levels tested at the time of recruitment. Initially measured iron levels were associated with subsequent cancer risk by linking individuals with the National Cancer Registry and National Death File. The relationship between serum iron and cancer risk was a J-shaped one, with higher cancer risk at both ends, either at lower than 60 mg/dL or higher than 120 mg/dL. This study reveals that high serum iron is both a common disorder and a marker of increased risk for several cancers (Pang *et al*., 2014).

A cross-sectional study done by Mazdak H1 *et al* the study was conducted on 51 patients with bladder cancer and 58 healthy volunteers after age, sex, and smoking habits were matched. They found that the serum iron level was significantly lower in the patients than the control group ($P < 0.001$) (Mazdak H1 *et al*., 2010).

1.3 Rationale

Breast cancer is one of the most common types of cancer worldwide and frequently occurs during the prime of life. Cancer patient suffers from many abnormalities.

Female with breast cancer suffer from anemia which is related directly to cancer, such those patients when given therapeutic iron to treat anemia may impact with prognosis from breast cancer. A few published studies showed a relationship between serum iron, TIBC and transferrin saturation percent and breast cancer and pointed to the need for more studies. To the best of our knowledge there are no published studies in Sudan.

This study was expected to raise the attention to the consideration of serum iron status as the diagnosis of breast cancer. Whether high or low levels were encountered, a balance should be taken for the best outcome of the patient.

1.4 Objectives:

1.4.1 General objective:

To study changes in serum iron, TIBC and transferring saturation percent in Sudanese females newly diagnosed with breast cancer at the Radio Isotope Center –Khartoum, Sudan.

1.4.2 Specific objective:

1. To measure serum iron in case and control samples.

2. To measure total iron binding capacity in case and control samples.

3. To calculate transferrin saturation percent in case and control samples.

4. To compare the changes in serum iron, TIBC and transferring saturation percent between case and control groups.

5. To find out the effect of the type of breast cancer, histopathological grade and menopausal status on level of serum iron, TIBC and transferring saturation percent.

CHAPTER TWO

2. Materials and methods

2.1 Study design area and duration:

This is a case control study to measure serum iron, TIBC and transferring saturation percent in newly diagnosed breast cancer in the Radio Isotope Center Khartoum (RICK) and apparently healthy Sudanese females in Khartoum State, in the period from February to August 2015.

2.2 Study population:

Fifty Females newly diagnosed with breast cancer at RICK while the seventy control group were recruited from Primary Health Centers in Khartoum State.

2.3 Inclusion criteria:

Female newly diagnosed with breast cancer within one month of the date of histopathology report regardless of the menopausal status, with no other chronic condition or not under treatment with iron supplements which affect the result were included in this study. The control group includes apparently healthy females matched to patients in age group and parity.

2.4 Exclusion criteria:

Patients with breast cancer diagnosed histological more than one month, patients with breast cancer under- radiation and hormonal or chemotherapy and patients receiving therapeutic iron were excluded from this study.

2.5 Sampling technique:

Simple random sampling method was used for selection of cases. The control group was selected using stratified sampling technique from the 41 Primary Health Centers (PHCs) distributed among the seven localities of Khartoum State. Data concerning population size and PHCs were collected from the website of Ministry of Health –Khartoum State.

2.5.1 Sample size:

The samples size was set by using the formula:

$$n=\frac{N}{N+(d^2)}$$

n= sample size

N= total number of female newly diagnosed with breast cancer per year

d=degree of precision (0.05) (Taro,1967).

According to formula, sample size is 100 samples and was set to be 120 (50 as cases and70 as controls).

2.6 Sample collection:

Five ml of the Blood were collected from the superficial vein in the anticupital fossa from the study population under sterile condition and collected using the following procedure.

2.7 Data collection:

Data was collected by using a questionnaire which contains the following variables, age, marital status,parity, type of breast cancer, histopathological grade and menopausal status.

2.8 Method of sample collection:

2.8.1 Requirement:

Plain container.

Syringe.

Cotton.

70% Alcohol.

A tourniquet.

2.8.2 Procedure:

1. Female was set up at right position for the collection.

2. The skin was cleaned by 70% alcohol and allowed to dry, to avoid stinging when the skin is penetrated.

3. A tourniquet was applied to the arm, tight sufficiently to distend the vein, but not so tightly to cause discomfort.

4. The needle was inserted and 5ml of the blood sample were collected in plain container.

5. Blood samples were centrifuged to get serum and kept at -20 till the samples were analyzed.

2.9 Methods:

2.9.1 Serum iron:

Estimation of serum iron was performed by using a manual method (IRON-FERROZINE).

2.9.1.1 Principle:

Transferrin-bound ferric ions in the sample are released by guanidinium and reduced to ferrous by means of ascorbic acid. Ferrous ions react with ferrozine forming colored complex that can be measured by a spectrophotometer (CECIL instrument, Cambridge England 1000 series).

2.9.1.2Reagents:

Reagent A: guanidinium chloride and acetate buffer.

Reagent B: ferrozine and ascorbic acid.

Iron standard.

2.9.1.3 Working reagent:

Mix between reagent A and reagent B.

2.9.1.4 Procedure:

- Three test tubes were labeled (sample-sample blank-standard).
- 1.0 ml from working reagent was placed in a tube labeled with the sample and the standard.
- 1.0 ml from reagent A was placed in the tube labeled with sample blank.

- 200µL from sample was added to the tube labeled with sample and sample blank.
- 200µL from standard was added to the tube labeled with standard.
- All tubes were mixed thoroughly and let stand for 5 minutes at room temperature.
- Absorbance (A) for all tubes was read at 560 nm against distilled water.

Serum iron concentration was calculated using the following general formula:

Concentration of serum iron in sample=

$$\frac{\text{Absorbance of sample–Absorbance of sample blank}}{\text{A of standard}} \times \text{Concentration of standard}$$

2.9.1.5 Reference values:

Women 50-170 µg/dL.

2.9.2 Total iron binding capacity:

Estimation of total iron binding capacity (TIBC) was performed by using a manual method (IRON-FERROZINE).

2.9.2.1 Principle:

Excess of Fe^{+3} is added to the sample to saturate serum transferring. Un complexed Fe^{+3} is precipitated with magnesium hydroxide carbonate and the iron bonded to protein in the supernatant is then spectrophotometrically measured.

2.9.2.2 Reagents:

Reagent A: iron chloride.

Reagent B: magnesium hydroxide carbonates (powder).

2.9.2.3 Procedure:

- 1.0 ml from reagent A was placed in a clean dry test tube.
- 0.5 ml from sample was added.
- The test tube was mixed thoroughly and let stand for 5-30 minutes at room temperature.
- One spoonful of reagent B was added.
- The test tube was mixed thoroughly and let stand for 30-60 minutes at room temperature.
- The test tube was centrifuged at a minimum 3000 round per minute for 10 minutes.
- Supernatant was carefully collected.
- The serum iron concentration in the supernatant was measured using the kit of iron from Biosystems Company Barcelona (Spain).

Total iron binging capacity (TIBC) =

Serum iron concentration in supernatant×3

3= dilution factor

2.9.2.4 Reference values

Adult 250-425 µg/dL.

2.9.3 Tansferrin saturation percent

$$\text{Transferrin saturation percent} = \frac{\text{serum iron concentration}}{\text{TIBC}} \times 100$$

2.9.3.1Reference values

Women 15-50%

2.10 Ethical consideration:

Informed consent was written and signed by each participants following explanation of the study and sample collection procedure. The participants' information was kept confidential. Ethical approval was obtained from the Ministry of Health, Khartoum State. A permission of study conduction was taken from the Head Administrative of RICK.

2.11 Data analysis:

Statistical Package for Social Science (IBM SPSS version 22.0) computer software was used to analyze the data collected. Pearson Chi squire, Student t test and one way ANOVA were used. P.value at 0.05 was considered statistically significant.

CHAPTER THREE

3. Results

The study was conducted on 120 samples, 50 breast cancer cases and 70 non-cancer controls.

The mean age of the studied case group was 47years and 42 in the control group with the most common age group 45-54 years and 35-44 years, respectively, while the mean of parity number was 4 in both groups (Table 3.1).

There were 49.0(98.0%) married females and 1.0 (2.0%) was single in the case group, 43.0(61.4%) married, 11.0 (15.7%) single, 8.0(11.4%) divorced and 8.0(11.4%) widow in the control group (Table 3.1).

The mean of serum iron in the case group was 244.30(µg/dL) and 57.59(µg/dL) in the control group (P.value 0.00), mean of TIBC in case group 412.98(µg/dL) and 403.71(µg/dL) in the control group (P .value 0.838) and mean of transferring saturation percent in the case was 61.08 % and 223.23% in the control group (P.value 0.00) as shown in Table 3.2.

Table (3.3) described that the mean of serum iron in an invasive ductal carcinoma 247.76 (µg/dL), 188.00(µg/dL) in invasive stromal sarcoma and 254.00(µg/dL) in non invasive ductal carcinoma.

Table (3.4) showed the mean of serum iron in grade I 248.67(µg/dL), 252.06(µg/dL) in grade II and 228.44(µg/dL) in grade III (P.value 0.883).

The mean of TIBC in grade I 343.00(µg/dL), 467.10(µg/dL) in grade II and 321.25(µg/dL) in grade III (P.value 0.019). The mean of transferrin saturation percent in grade I is 71.00%, 55.84% in grade II and 61.08% in grade III (P.value 0.531).

Table (3.5) stated that mean of serum iron in menopausal females 423.0(µg/dL) and 403.0(µg/dL) in non-menopausal (P.value 0.691), mean of TIBC in menopausal females 272.0(µg/dL) and 220.0(µg/dL) in non-menopausal (P.value 0.224) and mean of transferring saturation percent in menopausal females 62.0% and 60.0% in non-menopausal (P.value 0.871).

Table (3.1): Distribution of study population according to marital status, age group and parity.

Variables		Case		Control	
Age group		Frequency	Percent	Frequency	Percent
	15 - 24	0.0	0.0	2.0	2.9
	25 - 34	8.0	16.0	19.0	27.1
	35 - 44	12.0	24.0	21.0	30.0
	45 - 54	13.0	26.0	13.0	18.6
	55 - 64	10.o	20.0	10.0	14.3
	65+	7.0	14.0	5.0	7.1
	Total	50.0	100.0	70.0	100.0
Marital status	Single	1.0	2.0	11.0	15.7
	Married	49.0	98.0	43.0	61.4
	Divorced	0.0	0.0	8.0	11.4
	Widow	0.0	0.0	8.0	11.4
	Total	50.0	100.0	70.0	100.0
	Total	50.0	100.0	70.0	100.0
Parity	< 5	31.0	62.0	49.0	70.0
	5 - 10	17.0	34.0	15.0	21.4
	10 +	2.0	4.0	6.0	8.6
	Total	50.0	100.0	70.0	100.0

Table (3.2): Serum iron concentration, TIBC, and transferrin saturation percent among the study population.

Parameters	status	N	Mean	SD. Deviation	P.value
Serum iron(µg/dL)	Case	50.0	244.30	151.598	0.000
	Control	70.0	57.59	43.191	
TIBC* (µg/dL)	Case	50.0	412.98	177.460	0.838
	Control	70.0	403.71	168.765	
Transferrin Saturation percent (%)	Case	50.0	61.08	41.523	0.000
	Control	70.0	223.23	149.195	

N=Number

*TIBC=Total Iron Binding Capacity

Table (3.3): The effect of types of breast cancer on level of serum iron, TIBC and transferrin saturation percent.

Parameters	Breast cancer	N	Mean	SD deviation	P.value
Serum iron (µg/dL)	Invasive ductal	46	247.76	157.462	0.808
	Invasive stromal sarcoma	3	188.00	10.440	
	Non invasive ductal	1	254.0	-	
TIBC (µg/dL)	Invasive ductal	46	418.61	182.899	0.716
	Invasive stromal sarcoma	3	331.00	89.404	
	Non invasive ductal	1	400.00	-	
Transferrin Saturation percent (%)	Invasive ductal	46	61.35	43.154	0.979
	Invasive stromal sarcoma	3	59.67	17.474	
	Non invasive ductal	1	53.00	-	

N=Number

TIBC=Total Iron Binding Capacity

Table (3.4): The effect of histopathological grade on serum iron concentration, TIBC and transferrin saturation percent.

parameter	Histopathological grade	N	mean	SD deviation	P. value
Serum iron (µg/dL)	I	3.0	248.67	147.873	0.883
	II	31.0	252.06	168.055	
	III	16.0	228.44	123.601	
TIBC (µg/dL)	I	3.0	343.00	104.446	0.019
	II	31.0	467.10	185.205	
	III	16.0	321.25	129.657	
Transferrin Saturation percent (%)	I	3.0	71.00	28.844	0.531
	II	31.0	55.85	37.209	
	III	16.0	69.38	51.015	

N=Number

TIBC=Total Iron Binding Capacity

Table (3.5): Effect of menopausal status on concentration of serum iron, TIBC and transferrin saturation percent in case group.

parameters	Menopausal status	N	Mean	SD deviation	P. value
Serum iron (µg/dL)	Yes	23.0	423.0	150.40	0.691
	NO	27.0	403.0	200.03	
TIBC (µg/dL)	Yes	23.0	272.0	178.48	0.224
	NO	27.0	220.0	122.70	
Transferrin Saturation percent (%)	Yes	23.0	62.0	34.88	0.871
	NO	27.0	60.0	47.09	

N=Number

TIBC=Total Iron Binding Capacity

CHAPTER FOURE

Discussion, Conclusion, Recommendation and References

4.1 Discussion:

This study involved 120 females, 50 as cases with breast cancer and 70 as non-breast cancer controls; for all those women serum iron, TIBC and transferring saturation percent were measured.

This study revealed statistically significant increase in the mean of serum iron and decrease in transferrin saturation percent in women with breast cancer while no significant variation in the mean of TIBC between case and control groups. Finding of Pavithra *et al* in their study is similar to this study in serum iron level; they found a statistically significant increase in levels of serum iron in patients with breast cancer when compared to controls.

In the study conducted by Dhankha *et al* it was found that the levels of serum iron, TIBC and transferrin saturation percent were significantly higher in breast cancer patients as compared to healthy controls. Though agree with results concerning serum iron but disagree in level of TIBC and transferrin saturation percent in the current study. This could be attributed to the methods used.

There is no significant variation in the mean of serum iron and transferrin saturation percent according to type of breast cancer and histopathological grade in this study, but there significant increase in mean of TIBC in females with grade II. That is disagreeing with Dhankhar *et al* study in which they reported that patients with advanced disease showed greater values as compared to early stage, as patients in the current study were in early stage of the disease, because patients taken

in this study within one month of histopathology report. Nevertheless, there is agreement regarding TIBC.

Furthermore, there is no significant variation in mean of transferrin saturation percent, TIBC and serum iron in menopausal and non-menopausal female in this study. These findings disagree with the findings of Asperen *et al* who concluded that body iron stores are a risk factor for mortality due to cancer in postmenopausal women and justified by accumulation of stored iron among women after menopause. This further supports the findings of Pang *et al* who postulated that high serum iron is both a common disorder and a marker of increased risk for several cancers.

4.2 Conclusion:

It is concluded that:

- There is a statistically significant difference in serum iron and transferrin saturation percent between Sudanese females newly diagnosed with breast cancer and apparently healthy females.
- There is no significant difference in TIBC between Sudanese females newly diagnosed with breast cancer and apparently healthy females.
- Serum iron, TIBC and transferring saturation percent did not vary in regard to type of breast cancer and menopausal status.
- TIBC but not serum iron and transferring saturation percent vary significantly according to histopathological grade.

4.3 Recommendations:

- More studies should be under taken with adequate sample size.
- Estimation of ferritin is recommended.
- Use of more advanced method for measurement of serum iron and TIBC must be used.
- Iron profile must be used as routine test for breast cancer patients.
- Postmenopausal females must be checking the level of iron monthly.

References

Anthony. J. Neal and Peter .J. Hoskin., (2009). Clinical Oncology Basic and Practice. 4th edition. Great Britain by Hodder Arnold and Hachette Company. 90-100.

B.P.Das., (2005). Cancer pattern in Haryana. Radiation Oncology .volume 5 p 22-32.

C.A. finch., and H.A.Huebers., (1986*).* Iron metabolism. volume 4 p 5-10.

Chi Pang Wen, June Han Lee, Ya Ping Tai, Christopher Wen, Shiuan Be Wu, Min Kuang Tsai, Dennis P.H. Hsieh, Hung Che Chiang, Chao Agnes Hsiung, Chung Y. Hsu, and Xifeng Wu.,(2014) High Serum Iron is Associated with Increase Cancer Risk. Cancer Research, 74(22); 6589-97.

Cujic D., Stefanoska I., and Golubovic S., (2011). Serum ferritin in healthy and breast cancer patients. J. Med Biochem,; 30: 33-37.

David A Levison ., Robin Reid., Alastair D Burt., David J Harrison., Stawart Fleming .,(2008). Muri's text book of pathology. 14th edition .great Britain by holder Arnold and Edward Arnold.p 420-421.

E Anne steine-martin., Cheryl A Lotspeich- steininger., and John A Koepke., (1998*).* Clinical Hematology Principles. Procedures and correlation. 2ed edition. Philadelphia New York Lippincott.p 76-77.

Giordano S.H., Cohen Ds., Buzdar UA., perkis G., and Hortobagyi GN., (2004). *Breast carcinoma*: A population –based study.Cancer 101(1):1-7.

Gobind Rai Garg., and Sparsh Gupta., (2011). Review of pathology and genetics.3rd.edition. JAPEE.p 488- 489.

Hamid Mazadak, Faranak Yazdekhasti, Ahmad Movahedian, Nooshin Mirkheshti and Mohammad Shafieian. (2010). The comparative study of serum iron, copper and zinc level between bladder cancer patients and a control group. International Urology and Nephrology march; 42(1):89-93.

Harsh Mohan., (2010). Text Book of Pathology. 6^{th}edition. New Delhi p754-755.

Liehr JG, Jones JS., (2001). Role of Iron in Estrogen Induced Cancer. Curr Med Chem; 8: 839-849.

National Cancer Institute. (2013). what is cancer? .National Cancer Institute www.cancer.gov[Accessed November 15, 2015].

Nicholas .J.Varidax., (2012*).* A text Book of Pathology. 2^{ed}edition. Elsevier Australia. P 557- 558.

O. Marques, B.M, Da Silva, G .Porto, and C. lopes., (2014). Iron metabolism in breast cancer. Cancer let. Volume 34 pp1-14.

Rakesh Dhankhar, Adarsh C, Kiran Dahiya , Veena Singh Ghalaut, Nityasha, Anil Kumar Dhull and Anil Khurana,(2014). Role of Iron Metabolism in Breast Cancer Patients Cancers Review, 1(3): 45-51.

Rodak. B.F., (1995). Diagnostic Hematology. 18^{th} edition .Philadelphia W.B Saunder.p 97- 99.

R. Sauer., (2000) .Adjuvant Radiotherapy after Breast Conserving Surgery for Breast Cancer. Volume 36 p 1073-1078.

Saeed. I. E, Weng. H.Y, Mohamed. K. H, and Mohammed. S. I., (2014). Cancer incidence in Khartoum, Sudan: first results from the Cancer Registry, 2009–2010. Cancer Medicine. *3*(4); 1075–1084.

V. Pavithra,T.G. Sathisha, K. Kasturi, D. Siva Mallika, S. Jeevan Amos, and S. Ragunatha., (2015)Serum Levels of Metal Ions in Female Patients with Breast Cancer Journal of Clinical and Diagnostic Research Jan; 9(1):25–27.

Yamane. Taro., (1967). Statistics: An Introductory Analysis. 2^{ed}. New York: Harper and Row.

Appendixes

Appendix (1): Questionnaire

Sudan University of Science and Technology

Collage of graduate studies

Department of hematology and immunohematology

Measurement of Iron Status in Sudanese Females Newly Diagnosed With Breast Cancer

Questionnaire No ☐ date……

Name …….

Age……..

Contact number……

Marital status ……

Parity number…….

Type of breast cancer……

Histopathological grade.....

Menopausal status: yes ☐ no ☐

Iron profile:

Serum iron……µg /dL

Total iron binding capacity……µg /dL

Transferrin Saturation percent……%