1.1. Introduction

Breast cancer is a malignant tumor that starts in the cells of the breast. It is found mostly in women, but men can get breast cancer. Signs of breast cancer may include a lump in the breast, a change in breast shape, dimpling of the skin, fluid coming from the nipple, or a red scaly patch of skin. In those with distant spread of the disease, there may be bone pain, swollen lymph nodes, shortness of breath, or yellow skin [(1)].

Risk factors for developing breast cancer include obesity, lack of physical exercise, drinking alcohol, hormone replacement therapy during menopause, ionizing radiation[(2)].

Copper and zinc are two important minerals that play important roles in a variety of biochemical reactions as cofactors of the superoxide dismutase (SOD) enzyme. This enzyme plays an important role in the protection of the organism against free radicals and, consequently, prevents the initiation and progression of neoplastic events [(3)] [(4)].

Zinc and copper have been recognized to play an important role as cofactors of superoxide dismutase. The enzyme protects cellular components against free radical induced damage, which is considered to be of particular relevance to carcinogenesis [(5)].

Several studies showed that plasma copper concentrations are increased in various carcinomas Low copper intake was reported to result in a higher incidence of carcinogen-induced colon cancer in rats compared with rats fed with a very high copper diet [(6)].

Zinc can be essential for the structure, regulation, and catalytic action of an enzyme. Zinc occurs in enzymes that realize the synthesis and metabolism of DNA and RNA [(7)].

Excess dietary zinc has been shown to be protective against lead toxicity by an inhibition of lead absorption, many trace elements in cancerous breast tissue and suggested that these elements compete for the binding sites in the cell, change its enzymatic activity and exert direct or indirect action on the carcinogenic progress accelerating the growth of tumors and that the inhibition of enzyme(s) caused by variation in trace element concentrations results in immunological breakdown of the body system [(8)].

1.2. Rationale

Breast cancer is the most common invasive cancer in women. (The most common form of cancer is non-invasive non-melanoma skin cancer, non-invasive cancers are generally easily cured, cause very few deaths, and are routinely excluded from cancer statistics).

Breast cancer comprises 22.9% of invasive cancers in women and 16% of all female cancer. In 2008, breast cancer caused 458,503 deaths worldwide (13.7% of cancer deaths in women and 6.0% of all cancer deaths for men and women together).

And there is association between oxidative stress and development of tumors, zinc and copper which both involved in relieve of oxidative stress. That makes this type of research important in order to find a new tool for diagnosis prevention and follow up. Many studies were conducted to evaluate the plasma level of zinc and copper in breast tumor patients, but there were few published studies in Sudan. This study done to evaluate plasma levels of copper and zinc among Sudanese women with breast tumors, in order to develop future prevention and follow up.

1.3. Objectives

1.4. General objective

To evaluate plasma levels of copper and zinc in the Sudanese women with breast tumor patients.

Specific objectives

I. To estimate the plasma levels of copper and zinc in patients with breast tumors

II. To compare plasma levels of copper and zinc in breast tumor patients according to the type of tumor (benign and malignant).

III. To find correlation between the plasma levels of copper and zinc with age.

IV. To find correlation between the plasma levels of copper and zinc with duration of breast tumor.

2. Literature Review

2.1 Breast cancer

Signs of breast cancer may include a lump in the breast, a change in breast shape, dimpling of the skin, fluid coming from the nipple, or a red scaly patch of skin. In those with distant spread of the disease, there may be bone pain, swollen lymph nodes, shortness of breath, or yellow skin [(1)].

Risk factors for developing breast cancer include obesity, lack of physical exercise, drinking alcohol, hormone replacement therapy during menopause, ionizing radiation, early age at first menstruation, and having children late or not at all. About 5-10% of case is due to genes inherited from a person's parents. Breast cancer most commonly develops in cells from the lining of milk ducts and the lobules that supply the ducts with milk. Cancers developing from the ducts are known as ducats carcinomas, while those developing from lobules are known as lobular carcinomas. In addition, there are more than 18 other sub-types of breast cancer. Some cancers develop from pre-invasive lesions such as ductal carcinoma in situ. The diagnosis of breast cancer is confirmed by taking a biopsy of the concerning lump Once the diagnosis is made, further tests are done to determine if the cancer has spread beyond the breast and which treatments it may respond to [(8)].

The balance of benefits versus harms of breast cancer screening is controversial. A 2007 Cochrane review stated that it is unclear if mammographic screening does more good or harm. The medications tamoxifen or raloxifene may be used in an effort to prevent breast cancer in those who are at high risk of developing it. Surgical removal of both breasts is another useful preventative measure in some high risk women. In those who have been diagnosed with cancer, a number of treatments may be used, including surgery, radiation therapy, chemotherapy, and targeted therapy. Types of surgery vary from breast-conserving surgery to mastectomy. Breast reconstruction may

take place at the time or surgery or at a later date. In those in whom the cancer has spread to other parts of the body, treatments are mostly aimed at improving quality of life and comfort. [9]

Outcomes for breast cancer vary depending on the cancer type, extent of disease, and person's age. Survival rates in the developed world are high, between 80% and 90% of those in England and the United States alive for at least 5 years, but in developing countries survival rates are poorer. Worldwide, breast cancer is the leading type of cancer in women, accounting for 25% of all cases. In 2007 it resulted in 1.68 million cases and 522,000 deaths. It is more common in developed countries and is more than 100 times more common in women than in men [9].

2.1.1 Signs and symptoms:

Breast cancer showing an inverted nipple, lump and skin dimpling. The first noticeable symptom of breast cancer is typically a lump that feels different from the rest of the breast tissue. More than 80% of breast cancer cases are discovered when the woman feels a lump. The earliest breast cancers are detected by a mammogram. Lumps found in lymph nodes located in the armpits can also indicate breast cancer [10].

Indications of breast cancer other than a lump may include thickening different from the other breast tissue, one breast becoming larger or lower, a nipple changing position or shape or becoming inverted, skin puckering or dimpling, a rash on or around a nipple, discharge from nipple/s, constant pain in part of the breast or armpit, and swelling beneath the armpit or around the collarbone. Pain ("mastodynia") is an unreliable tool in determining the presence or absence of breast cancer, but may be indicative of other breast health issues [10].

Inflammatory breast cancer is a particular type of breast cancer which can pose a substantial diagnostic challenge. Symptoms may resemble a breast inflammation and may include itching, pain, swelling, nipple inversion, warmth and redness throughout the breast, as well as an orange-peel texture to the skin referred to as peau d'orange; as

inflammatory breast cancer doesn't shows as a lump there's sometimes a delay in diagnosis [11].

Another reported symptom complex of breast cancer is Paget's disease of the breast. This syndrome presents as skin changes resemblingeczema, such as redness, discoloration, or mild flaking of the nipple skin. As Paget's disease of the breast advances, symptoms may include tingling, itching, increased sensitivity, burning, and pain. There may also be discharge from the nipple. Approximately half of women di agnosed with Paget's disease of the breast also have a lump in the breast [10].

In rare cases, what initially appears as a fibroadenoma (hard, movable non-cancerous lump) could in fact be a phyllodes tumor. Phyllodes tumors are formed within the stroma (connective tissue) of the breast and contain glandular as well as stromal tissue. Phyllodes tumors are not staged in the usual sense; they are classified on the basis of their appearance under the microscope as benign, borderline, or malignant [12].

Occasionally, breast cancer presents as metastatic disease that is, cancer that has spread beyond the original organ. The symptoms caused by metastatic breast cancer will depend on the location of metastasis. Common sites of metastasis include bone, liver, lung and brain. Unexplained weight loss can occasionally herald an occult breast cancer, as can symptoms of fevers or chills. Bone or joint pains can sometimes be manifestations of metastatic breast cancer, as can jaundice or neurological symptoms. These symptoms are called non-specific, meaning they could be manifestations of many other illnesses [12].

Most symptoms of breast disorders, including most lumps, do not turn out to represent underlying breast cancer, Fewer than 20% of lumps, for example, are cancerous, and benign breast diseases such as mastitis and fibroadenoma of the breast are more common causes of breast disorder symptoms. Nevertheless, the appearance of a new symptom should be taken seriously by both patients and their doctors, because of the possibility of an underlying breast cancer at almost any age [13].

2.1.2. Risk factors:

The primary risk factors for breast cancer are female sex and older age. Other potential risk factors include: genetics, lack of childbearing or lack of breastfeeding, higher levels of certain hormones, certain dietary patterns, and obesity. Recent studies have indicated that exposure to light pollution is a risk factor for the development of breast cancer [(14)].

2.1.2.1. Life style:

Smoking tobacco appears to increase the risk of breast cancer, with the greater the amount smoked and the earlier in life that smoking began, the higher the risk. In those who are long-term smokers, the risk is increased 35% to 50%. A lack of physical activity has been linked to 10% of the cases [(14)].

There may be an association between use of oral contraceptives and the development of premenopausal breast cancer, but whether oral contraceptives use may actually cause premenopausal breast cancer is a matter of debate. If there is indeed a link, the absolute effect is small. In those with mutations in the breast cancer susceptibility genes BRCA1 or BRCA2, or who have a family history of breast cancer, use of modern oral contraceptives does not appear to affect the risk of breast cancer.

The association between breast feeding and breast cancer has not been clearly determined; some studies have found support for an association while others have not. In the 1980s, the abortion breast cancer hypothesis posited that induced abortion increased the risk of developing breast cancer. This hypothesis was the subject of extensive scientific inquiry, which concluded that neither miscarriages nor abortions are associated with a heightened risk for breast cancer.

There is a relationship between diet and breast cancer, including an increased risk with a high fat diet, alcohol intake, and obesity, related to higher cholesterol levels. Dietary iodine deficiency may also play a role [(15)].

Other risk factors include radiation, and shift-work. A number of chemicals have also been linked including: polychlorinated biphenyls, hydrocarbons, organic and a number of pesticides. Although the radiation from mammography is a low dose, it is estimated that yearly screening from 40 to 80 years of age will cause approximately 225 cases of fatal breast cancer per million women screened [14].

2.1.2.2. Genetics

Some genetic susceptibility may play a minor role in most cases. Overall, however, genetics is believed to be the primary cause of 5–10% of all cases. In those with zero, one or two affected relatives, the risk of breast cancer before the age of 80 is 7.8%, 13.3%, and 21.1% with a subsequent mortality from the disease of 2.3%, 4.2%, and 7.6% respectively. In those with a first degree relative with the disease the risk of breast cancer between the age of 40 and 50 is double that of the general population [16].

In less than 5% of cases, genetics plays a more significant role by causing a hereditary breast–ovarian cancer syndrome. Thisincludes those who carry the BRCA1 andBRCA2 gene mutation. These mutations account for up to 90% of the total genetic influence with a risk of breast cancer of 60–80% in those affected. Other significant mutations include: p53 (LiFraumeni syndrome), PTEN (Cowden syndrome), and STK11 (Peutz–Jeghers syndrome), CHEK2, ATM, BRIP1, and PALB2. In 2012, researchers said that there are four genetically distinct types of the breast cancer and that in each type, hallmark genetic changes lead to many cancers [12] [17].

2.1.2.3. Medical conditions

Breast changes like atypical ductal hyperplasia and lobular carcinoma in situ, found in benign breast conditions such as fibrocystic breast changes, are correlated with an increased breast cancer risk. Diabetes mellitus might also increase the risk of breast cancer [11].

2.1.3. Pathophysiology:

Breast cancer, like other cancers, occurs because of an interaction between an environmental (external) factor and a genetically susceptible host. Normal cells divide as many times as needed and stop. They attach to other cells and stay in place in tissues. Cells become cancerous when they lose their ability to stop dividing, to attach to other cells, to stay where they belong, and to die at the proper time [18].

Normal cells will commit cell suicide (apoptosis) when they are no longer needed. Until then, they are protected from cell suicide by several protein clusters and pathways. One of the protective pathways is the PI3K/AKT pathway; another isthe RAS/MEK/ERK pathway. Sometimes the genes along these protective pathways are mutated in a way that turns them permanently "on", rendering the cell incapable of committing suicide when it is no longer needed. This is one of the steps that cause cancer in combination with other mutations. Normally, the PTEN protein turns off the PI3K/AKT pathway when the cell is ready for cell suicide. In some breast cancers, the gene for the PTEN protein is mutated, so the PI3K/AKT pathway is stuck in the "on" position, and the cancer cell does not commit suicide [18].

Mutations that can lead to breast cancer have been experimentally linked to estrogen exposure.

Failure of immune surveillance, the removal of malignant cells throughout one's life by the immune system. Abnormal factor signaling in the interaction between stromal cells and epithelial cells can facilitate malignant cell growth. In breast adipose tissue, over expression of leptin leads to increased cell proliferation and cancer. [13]

In the United States, 10 to 20 percent of patients with breast cancer and patients with ovarian cancer have a first- or second-degree relative with one of these diseases. The familial tendency to develop these cancers is called hereditary breast–ovarian cancer syndrome. The best known of these, the BRCA mutations, confer a lifetime risk of breast cancer of between 60 and 85 percent and a lifetime risk of ovarian cancer of between 15

and 40 percent. Some mutations associated with cancer, such as p53, BRCA1 andBRCA2, occur in mechanisms to correct errors in DNA. These mutations are either inherited or acquired after birth. Presumably, they allow further mutations, which allow uncontrolled division, lack of attachment, and metastasis to distant organs. However there is strong evidence of residual risk variation that goes well beyond hereditaryBRCA gene mutations between carrier families. This is caused by unobserved risk factors. This implicates environmental and other causes as triggers for breast cancers. The inherited mutation in BRCA1 or BRCA2 genes can interfere with repair of DNA cross links and DNA double strand breaks (known functions of the encoded protein). These carcinogens cause DNA damage such as DNA cross links and double strand breaks that often require repairs by pathways containing BRCA1 and BRCA2. However, mutations in BRCA genes account for only 2 to 3 percent of all breast cancers. Levin et al. say that cancer may not be inevitable for all carriers of BRCA1 and BRCA2mutations. About half of hereditary breast–ovarian cancer syndromes involve unknown genes [19].

GATA-3 directly controls the expression of estrogen receptor (ER) and other genes associated with epithelial differentiation, and the loss of GATA-3 leads to loss of differentiation and poor prognosis due to cancer cell invasion and metastasis [18].

2.1.4. Diagnosis:

Most types of breast cancer are easy to diagnose by microscopic analysis of a sample or biopsy of the affected area of the breast. There are, however, rarer types of breast cancer that require specialized lab exams. The two most commonly used screening methods, physical examination of the breasts by a healthcare provider and mammography, can offer an approximate likelihood that a lump is cancer, and may also detect some other lesions, such as a simple cyst. When these examinations are inconclusive, a healthcare provider can remove a sample of the fluid in the lump for microscopic analysis (a procedure known as fine needle aspiration, or fine needle

aspiration and cytology FNAC) to help establish the diagnosis. The needle aspiration may be performed in a healthcare provider's office or clinic using local anaesthetic if required. A finding of clear fluid makes the lump highly unlikely to be cancerous, but bloody fluid may be sent off for inspection under a microscope for cancerous cells. Together, physical examination of the breasts, mammography, and FNAC can be used to diagnose breast cancer with a good degree of accuracy. Other options for biopsy include a core biopsy or vacuum-assisted breast biopsy, which are procedures in which a section of the breast lump is removed; or an excisional biopsy, in which the entire lump is removed. Very often the results of physical examination by a healthcare provider, mammography, and additional tests that may be performed in special circumstances (such as imaging by ultrasound or MRI) are sufficient to warrant excisional biopsy as the definitive diagnostic and primary treatment methods [20].

2.1.5. Classification:

Breast cancers are classified by several grading systems. Each of these influences the prognosis and can affect treatment response. Description of a breast cancer optimally includes all of these factors :

Histopathology.

Breast cancer is usually classified primarily by its histological appearance. Most breast cancers are derived from the epithelium lining the ducts or lobules, and these cancers are classified as ductal or lobular carcinoma. Carcinoma in situ is growth of low grade cancerous or precancerous cells within a particular tissue compartment such as the mammary duct without invasion of the surrounding tissue. In contrast, invasive carcinoma does not confine itself to the initial tissue compartment [17].

Grade:

Grading compares the appearance of the breast cancer cells to the appearance of normal breast tissue. Normal cells in an organ like the breast become differentiated, meaning that they take on specific shapes and forms that reflect their function as part of that organ. Cancerous cells lose that differentiation. In cancer, the cells that would normally line up in an orderly way to make up the milk ducts become disorganized. Cell division becomes uncontrolled. Cell nuclei become less uniform. Pathologists describe cells as well differentiated (low grade), moderately differentiated (intermediate grade), and poorly differentiated (high grade) as the cells progressively lose the features seen in normal breast cells. Poorly differentiated cancers (the ones whose tissue is least like normal breast tissue) have a worse prognosis [(15)].

Stage:

Breast cancer staging using the TNM system is based on the size of the tumor (**T**), whether or not the tumor has spread to the lymph nodes (**N**) in the armpits, and whether the tumor has metastasized (**M**) (i.e. spread to a more distant part of the body). Larger size, nodal spread, and metastasis have a larger stage number and a worse prognosis.

The main stages are:

Stage 0 is a pre-cancerous or marker condition, either ductal carcinoma in situ (DCIS) or lobular carcinoma in situ (LCIS).

Stages 1–3 are within the breast or regional lymph nodes.

Stage 4 is 'metastatic' cancer that has a less favorable prognosis.

Where available, imaging studies may be employed as part of the staging process in select cases to look for signs of metastatic cancer. However, in cases of breast cancer with low risk for metastasis, the risks associated with PET scans, CT scans, or bone scans outweigh the possible benefits, as these procedures expose the patient to a substantial amount of potentially dangerous ionizing radiation [(19)].

Receptor status:

Breast cancer cells have receptors on their surface and in their cytoplasm and nucleus. Chemical messengers such as hormones bind to receptors, and this causes changes in the cell.

Breast cancer cells may or may not have three important receptors: estrogen receptor (ER), progesterone receptor (PR), and HER2.

ER+ cancer cells (that is, cancer cells that have estrogen receptors) depend on estrogen for their growth, so they can be treated with drugs to block estrogen effects (e.g.tamoxifen), and generally have a better prognosis. Untreated, HER2+ breast cancers are generally more aggressive than HER2- breast cancers, but HER2+ cancer cells respond to drugs such as the monoclonal antibody trastuzumab (in combination with conventional chemotherapy), and this has improved the prognosis significantly. Cells that do not have any of these three receptor types (estrogen receptors, progesterone receptors, or HER2) are called triple-negative, although they frequently do express receptors for other hormones, such as androgen receptor and prolactin receptor [(10)].

DNA assays:

DNA testing of various types including DNA microarrays has compared normal cells to breast cancer cells. The specific changes in a particular breast cancer can be used to classify the cancer in several ways, and may assist in choosing the most effective treatment for that DNA type.

2.1.6. Prevention:

Women may reduce their risk of breast cancer by maintaining a healthy weight, drinking less alcohol, being physically active and breastfeeding their children. These modifications might prevent 38% of breast cancers in the US, 42% in the UK, 28% in Brazil and 20% in China. The benefits with moderate exercise such as brisk walking are seen at all age groups including postmenopausal women. Marine omega-3 polyunsaturated fatty acids appear to reduce the risk.

Removal of both breasts before any cancer has been diagnosed or any suspicious lump or other lesion has appeared (a procedure known as prophylactic bilateral mastectomy) may be considered in people with BRCA1 and BRCA2 mutations, which are associated with a substantially heightened risk for an eventual diagnosis of breast cancer. BRCAtesting is recommended in those with a high family risk after genetic counseling. It is not recommended routinely. This is because there are many different forms of changes inBRCA genes, ranging from harmless polymorphisms to obviously dangerous frameshift mutations. The effect of most of identifiable changes in the genes is uncertain. Testing in an average-risk person is particularly likely to return one of these indeterminate, useless results [(20)].

The selective estrogen receptor modulators (such as tamoxifen) reduce the risk of breast cancer but increase the risk of thromboembolism and endometrial cancer. There is no overall change in the risk of death. They are thus not recommended for the prevention of breast cancer in women at average risk but may be offered for those at high risk. The benefit of breast cancer reduction continues for at least five years after stopping a course of treatment with these medications [(21)].

2.1.7. Screening:

Breast cancer screening refers to testing otherwise-healthy women for breast cancer in an attempt to achieve an earlier diagnosis under the assumption that early detection will improve outcomes. A number of screening test have been employed including: clinical and self breast exams, mammography, genetic screening, ultrasound, and magnetic resonance imaging.

A clinical or self breast exam involves feeling the breast for lumps or other abnormalities. Clinical breast exams are performed by health care providers, while self breast exams are performed by the person themselves. Evidence does not support the effectiveness of either type of breast exam, as by the time a lump is large enough to be found it is likely to have been growing for several years and thus soon be large enough to be found without

an exam. Mammographic screening for breast cancer uses X-rays to examine the breast for any uncharacteristic masses or lumps. During a screening, the breast is compressed and a technician takes photos from multiple angles. A general mammogram takes photos of the entire breast, while a diagnostic mammogram focuses on a specific lump or area of concern [21].

A number of national bodies recommend breast cancer screening. For the average woman, the U.S. Preventive Services Task Force recommends mammography every two years in women between the ages of 50 and 74, the Council of Europe recommends mammography between 50 and 69 with most programs using a 2 year frequency, and in Canada screening is recommended between the ages of 50 and 74 at a frequency of 2 to 3 years. These task force reports point out that in addition to unnecessary surgery and anxiety, the risks of more frequent mammograms include a small but significant increase in breast cancer induced by radiation.

The Cochrane collaboration (2013) states that the best quality evidence neither demonstrates a reduction in cancer specific, nor a reduction in all cause mortality from screening mammography. When less rigorous trials are added to the analysis there is a reduction in mortality due to breast cancer of 0.05% (a decrease of 1 in 2000 deaths from breast cancer over 10 years or a relative decrease of 15% from breast cancer). Screening over 10 years results in a 30% increase in rates of over-diagnosis and over-treatment (3 to 14 per 1000) and more than half will have at least one falsely positive test. This has resulted in the view that it is not clear whether mammography screening does more good or harm. Cochrane states that, due to recent improvements in breast cancer treatment, and the risks of false positives from breast cancer screening leading to unnecessary treatment, "it therefore no longer seems reasonable to attend for breast cancer screening" at any age. Whether MRI as a screening method has greater harms or benefits when compared to standard mammography is not known [20].

2.1.8. Management:

The management of breast cancer depends on various factors, including the stage of the cancer. Increasingly aggressive treatments are employed in accordance with the poorer the patient's prognosis and the higher the risk of recurrence of the cancer following treatment.

Breast cancer is usually treated with surgery, which may be followed by chemotherapy or radiation therapy, or both. A multidisciplinary approach is preferable. Hormone receptor-positive cancers are often treated with hormone-blocking therapy over courses of several years. Monoclonal antibodies, or other immune-modulating treatments, may be administered in certain cases of metastatic and other advanced stages of breast cancer [(21)].

2.1.8.1. Surgery

Surgery involves the physical removal of the tumor, typically along with some of the surrounding tissue. One or more lymph nodes may be biopsied during the surgery; increasingly the lymph node sampling is performed by a sentinel lymph node biopsy.

Standard surgeries include:

Mastectomy: Removal of the whole breast.

Quadrantectomy: Removal of one quarter of the breast.

Lumpectomy: Removal of a small part of the breast.

Once the tumor has been removed, if the patient desires, breast reconstruction surgery, a type of plastic surgery, may then be performed to improve the aesthetic appearance of the treated site. Alternatively, women use breast prostheses to simulate a breast under clothing, or choose a flat chest. Nipple/areola prostheses can be used at any time following the mastectomy [(22)].

2.1.8.2. Medication

Drugs used after and in addition to surgery are called adjuvant therapy. Chemotherapy or other types of therapy prior to surgery are called neoadjuvant therapy. Aspirin may reduce mortality from breast cancer.

There are currently three main groups of medications used for adjuvant breast cancer treatment: hormone-blocking agents, chemotherapy, and monoclonal antibodies [23].

Hormone blocking therapy

Some breast cancers require estrogen to continue growing. They can be identified by the presence of estrogen receptors (ER+) and progesterone receptors (PR+) on their surface (sometimes referred to together as hormone receptors). These ER+ cancers can be treated with drugs that either block the receptors, e.g. tamoxifen, or alternatively block the production of estrogen with an aromatase inhibitor, e.g. anastrozole or letrozole. The use of tamoxifen is recommended for 10 years. Aromatase inhibitors, however, are only suitable for post-menopausal patients. This is because the active aromatase in postmenopausal women is different from the prevalent form in premenopausal women, and therefore these agents are ineffective in inhibiting the predominant aromatase of premenopausal women [23].

Chemotherapy

Chemotherapy is predominantly used for cases of breast cancer in stages 2–4, and is particularly beneficial in estrogen receptor-negative (ER-) disease. The chemotherapy medications are administered in combinations, usually for periods of 3–6 months. The most common regimens, known as "AC", combines cyclophosphamide with doxorubicin. Sometimes a taxane drug, such as docetaxel (Taxotere), is added, and the regime is then known as "CAT". Another common treatment, which produces equivalent results, is cyclophosphamide, methotrexate, and fluorouracil (or "CMF"). Most chemotherapy medications work by destroying fast-growing and/or fast-replicating cancer cells, either by causing DNA damage upon replication or by other mechanisms. However, the medications also damage fast-growing normal cells, which may cause serious side effects. Damage to the heart muscle is the most dangerous complication of doxorubicin, for example [23].

Monoclonal antibodies

Trastuzumab, a monoclonal antibody to HER2 (a cell receptor that is especially active in some breast cancer cells), has improved the 5-year disease free survival of stage 1–3 HER2-positive breast cancers to about 87% (overall survival 95%). When stimulated by certain growth factors, HER2 causes cellular growth and division; in the absence of stimulation by the growth factor, the cell will normally stop growing. Between 25% and 30% of breast cancers overexpress the HER2 gene or its protein product, and over expression of HER2 in breast cancer is associated with increased disease recurrence and worse prognosis. When trastuzumab binds to the HER2 in breast cancer cells that overexpress the receptor, trastuzumab prevents growth factors from being able to bind to and stimulate the receptors, effectively blocking the growth of the cancer cells. Trastuzumab, however, is very expensive, and its use may cause serious side effects (approximately 2% of patients who receive it suffer significant heart damage). Further, trastuzumab is only effective in patients with HER2 amplification/over expression [(22)].

2.1.8.3. Radiation

Radiotherapy is given after surgery to the region of the tumor bed and regional lymph nodes, to destroy microscopic tumor cells that may have escaped surgery. It may also have a beneficial effect on tumor microenvironment. Radiation therapy can be delivered as external beam radiotherapy or as brachytherapy (internal radiotherapy). Conventionally radiotherapy is given after the operation for breast cancer. Radiation can also be given at the time of operation on the breast cancer- intraoperatively. The largest randomised trial to test this approach was the TAR-GIT-A Trial which found that targeted intraoperative radiotherapy was equally effective at 4-years as the usual several weeks' of whole breast external beam radiotherapy. Radiation can reduce the risk of recurrence by 50–66% (1/2 – 2/3 reduction of risk) when delivered in the correct dose and is considered essential when breast cancer is treated by removing only the lump (Lumpectomy or Wide local excision) [(24)].

2.1.9. Prognosis:

Prognosis is the long term outcomes of the condition. This includes the probability of progression-free survival (PFS) or disease-free survival (DFS). These predictions are based on experience with breast cancer patients with similar classification. A prognosis is an estimate, as patients with the same classification will survive a different amount of time, and classifications are not always precise. Survival is usually calculated as an average number of months (or years) that 50% of patients survive, or the percentage of patients that are alive after 1, 5, 15, and 20 years. Prognosis is important for treatment decisions because patients with a good prognosis are usually offered less invasive treatments, such as lumpectomy and radiation or hormone therapy, while patients with poor prognosis are usually offered more aggressive treatment, such as more extensive mastectomy and one or more chemotherapy drugs [(24)].

2.1.9.1. Prognostic factors

Prognostic factors are reflected in the classification scheme for breast cancer including stage, (i.e., tumor size, location, whether disease has spread to lymph nodes and other parts of the body), grade, recurrence of the disease, and the age and health of the patient. The Nottingham Prognostic Index is a commonly used prognostic tool.

The stage of the breast cancer is the most important component of traditional classification methods of breast cancer, because it has a greater effect on the prognosis than the other considerations. Staging takes into consideration size, local involvement, lymph node status and whether metastatic disease is present. The higher the stage at diagnosis, the poorer the prognosis. The stage is raised by the invasiveness of disease to lymph nodes, chest wall, and skin or beyond, and the aggressiveness of the cancer cells. The stage is lowered by the presence of cancer-free zones and close-to-normal cell behaviour (grading). Size is not a factor in staging unless the cancer is invasive. For example, Ductal Carcinoma In Situ (DCIS) involving the entire breast will still be stage

zero and consequently an excellent prognosis with a 10-year disease free survival of about 98% [20].

Stage 1 cancer (and DCIS, LCIS) have an excellent prognosis and are generally treated with lumpectomy and sometimes radiation. HER2+ cancers should be treated with the trastuzumab (Herceptin) regime. Chemotherapy is uncommon for other types of stage 1 cancers [21].

Stage 2 and 3 cancers with a progressively poorer prognosis and greater risk of recurrence are generally treated with surgery (lumpectomy or mastectomy with or without lymph node removal), chemotherapy (plus trastuzumab for HER2+ cancers) and sometimes radiation (particularly following large cancers, multiple positive nodes or lumpectomy).

Stage 4, metastatic cancer, (i.e. spread to distant sites) has poor prognosis and is managed by various combination of all treatments from surgery, radiation, chemotherapy and targeted therapies. 10-year survival rate is 5% without treatment and 10% with optimal treatment.

The breast cancer grade is assessed by comparison of the breast cancer cells to normal breast cells. The closer to normal the cancer cells are, the slower their growth and the better the prognosis. If cells are not well differentiated, they will appear immature, will divide more rapidly, and will tend to spread. Well differentiated is given a grade of 1, moderate is grade 2, while poor or undifferentiated is given a higher grade of 3 or 4 (depending upon the scale used). The most widely used grading system is the Nottingham scheme; details are provided in the discussion of breast cancer grade [23].

The presence of estrogen and progesterone receptors in the cancer cell is important in guiding treatment. Those who do not test positive for these specific receptors will not be able to respond to hormone therapy, and this can affect their chance of survival depending upon what treatment options remain, the exact type of the cancer, and how advanced the disease is.

In addition to hormone receptors, there are other cell surface proteins that may affect prognosis and treatment. HER2 status directs the course of treatment. Patients whose cancer cells are positive for HER2 have more aggressive disease and may be treated with the 'targeted therapy', trastuzumab (Herceptin), a monoclonal antibody that targets this protein and improves the prognosis significantly [22].

Younger women tend to have a poorer prognosis than post-menopausal women due to several factors. Their breasts may change with their menstrual cycles, they may be nursing infants, and they may be unaware of changes in their breasts. Therefore, younger women are usually at a more advanced stage when diagnosed. There may also be biologic factors contributing to a higher risk of disease recurrence for younger women with breast cancer [19].

High mammographic breast density, which is a marker of increased risk of developing breast cancer, may not mean an increased risk of death among breast cancer patients, according to a 2012 report of a study involving 9232 women by the National Cancer Institute (NCI).

Since breast cancer in males is usually detected at later stages, outcome is typically worse. [18]

2.1.9.2. Psychological aspects

The emotional impact of cancer diagnosis, symptoms, treatment, and related issues can be severe. Larger hospitals are associated with cancer support groups which provide a supportive environment to help patients cope and gain perspective from cancer survivors.

Not all breast cancer patients experience their illness in the same manner. Factors such as age can have a significant impact on the way a patient copes with a breast cancer diagnosis. Premenopausal women with estrogen-receptor positive breast cancer must confront the issues of early menopause induced by many of the chemotherapy regimens used to treat their breast cancer, especially those that use hormones to counteract ovarian function [20].

On the other hand, a small 2007 study conducted by researchers at the College of Public Health of the University of Georgia suggested a need for greater attention to promoting functioning and psychological well-being among older cancer survivors, even when they may not have obvious cancer-related medical complications. The study found that older breast cancer survivors showed multiple indications of decrements in their health-related quality of life, and lower psychosocial well-being than a comparison group. Survivors reported no more depressive symptoms or anxious mood than the comparison group, however, they did score lower in measures of positive psychosocial well-being, and reported more depressed mood and days affected by fatigue. As the incidence of breast cancer in women over 50 rises and survival rates increase, breast cancer is increasingly becoming a geriatric issue that warrants both further research and the expansion of specialized cancer support services tailored for specific age groups.

2.1.9.3. Epidemiology:

Worldwide, breast cancer is the most common invasive cancer in women. (The most common form of cancer is non-invasive non-melanoma skin cancer; non-invasive cancers are generally easily cured, cause very few deaths, and are routinely excluded from cancer statistics.) Breast cancer comprises 22.9% of invasive cancers in women and 16% of all female cancer.

In 2008, breast cancer caused 458,503 deaths worldwide (13.7% of cancer deaths in women and 6.0% of all cancer deaths for men and women together). [(5)]

Lung cancer, the second most common cause of cancer-related death in women, caused 12.8% of cancer deaths in women (18.2% of all cancer deaths for men and women together).

The incidence of breast cancer varies greatly around the world: it is lowest in less-developed countries and greatest in the more-developed countries. In the twelve world regions, the annual age-standardized incidence rates per 100,000 women are as follows: in Eastern Asia, 18; South Central Asia, 22; sub-Saharan Africa, 22; South-Eastern Asia,

26; North Africa and Western Asia, 28; South and Central America, 42; Eastern Europe, 49; Southern Europe, 56; Northern Europe, 73; Oceania, 74; Western Europe, 78; and in North America, 90 [5].

The number of cases worldwide has significantly increased since the 1970s, a phenomenon partly attributed to the modern lifestyles. Breast cancer is strongly related to age with only 5% of all breast cancers occurring in women under 40 years old. There were more than 41,000 newly diagnosed cases of breast cancer registered in England in 2011, around 80% of these cases were in women age 50 or older [5].

2.2 Copper

Copper (Cu) is a relatively soft yet tough metal with excellent electrical and heat conducting properties. Copper is widely distributed in nature both in its elemental form and in compounds. Copper forms alloys with zinc (brass), tin (bronze), and nickel (cupronickel, widely used in coins) [25].

2.2.1 Health Effects

The copper content in the normal human adult is 50–120mg. Copper is distributed through the body with the highest concentrations found in liver, brain, heart, and kidneys. Hepatic copper accounts for about 10% of the total copper in the body. Copper is also found in cornea, spleen, intestine, and lung [3].

Copper is a component of several metalloenzymes, including ceruloplasmin, cytochrome C oxidase, superoxide dismutase, tyrosinase, metallothionein, dopamine hydroxylase, lysyl oxidase, clotting factor V, and an unknown enzyme that cross-links keratin in hair. Ceruloplasmin is the best known yet the least understood copper protein. It is a 2-globulin, and each 132,000-molecular-weight molecule contains six atoms of copper.46 Ceruloplasmin levels are influenced by hormones [26].

2.2.2 Absorption, Transport, and Excretion

An average day's diet may contain 10 mg or more of copper. The amount of copper absorbed from the intestine is 50%–80% of ingested copper. About half of dietary copper is excreted in feces. The exact mechanisms by which copper is absorbed and transported by the intestine are unknown. Copper absorption is impaired in severe diffuse diseases of small bowel, lymph sarcoma, and scleroderma [(27)].

Copper losses in the urine and sweat are approximately 3% of dietary intake. Menstrual losses of copper are minor [(28)].

2.2.3 Deficiency

Copper deficiency is observed in premature infants. Copper deficiency is related to malnutrition, malabsorption, chronic diarrhea, hyperalimentation, and prolonged feeding with low-copper, total-milk diets. Signs of copper deficiency include:

(1) Neutropenia and hypochromic anemia in the early stages [(4)].

(2) Osteoporosis and various bone and joint abnormalities that reflect deficient copper-dependent cross-linking of bone collagen and connective tissue [(4)].

(3) decreased pigmentation of the skin and general pallor [(4)].

(4), in the later stages, possible neurologic abnormalities (hypotonia, apnea, psychomotor retardation) [(4)].

Subclinical copper depletion contributes to an increased risk of coronary heart disease. An extreme form of copper deficiency is seen in Menkes disease. This invariably fatal, progressive brain disease is characterized by peculiar hair, called kinky or steely, and retardation of growth. Clinical forms include progressive mental deterioration, coarse feces, and disturbance of muscle tone, seizures, and episodes of severe hypothermia. Symptoms of Menkes disease usually appear at the age of 3 months and death usually occurs in 5-year-olds [(4)].

2.2.4 Toxicity

Wilson's disease is a genetically determined copper accumulation disease that usually presents between the ages of 6 and 40 years. Its manifestations include neurologic disorders, liver dysfunction, and Kayser-Fleischer rings (green-brown discoloration) in the cornea caused by copper deposition. Early diagnosis of Wilson's disease is important because complications can be effectively prevented and in some cases the disease can be halted with use of zinc acetate or chelation therapy [(29)].

2.2.5 Laboratory Evaluation of Copper Status

Copper is measured by flame AAS, ICP-MS, ICP-AES, and ASV. Serum copper and urine copper are used to monitor the nutritional adequacy and to screen for Wilson's disease, copper toxicity in premature children, and in children with Indian childhood cirrhosis (ICC), which is not limited to Indian children [(30)].

2.2.6. Relationship between breast cancer and copper

Several studies showed that plasma copper concentrations are increased in various carcinomas Low copper intake was reported to result in a higher incidence of carcinogen-induced colon cancer in rats compared with rats fed with a very high copper diet [(6)].

2.3 Zinc

Zinc (Zn) is a bluish white, lustrous metal. Zinc is stable in dry air and becomes covered with a white coating when exposed to moisture. Zinc is the fourth most used metal (after iron, aluminum, and copper). Zinc and its compounds are used in a production of alloys, especially brass (with copper), in galvanizing steel, in die casting, in paints, in skin lotions, in treatment of Wilson's disease, and in many over-the-counter (OTC) medications [(31)].

2.3.1 Health Effects

Zinc is second only to iron in importance as an essential trace element. The main biochemical role of zinc is its influence on the activity of more than 300 enzymes (from

the classes of oxireductases, transferases, hydrolases, lyases, isomerases, and ligases). Zinc can be essential for the structure, regulation, and catalytic action of an enzyme. Zinc occurs in enzymes that realize the synthesis and metabolism of DNA and RNA. Zinc influences the synthesis and metabolism of proteins, participates in glycolysis and cholesterol metabolism, maintains membrane structures, effects functions of insulin, and affects growth factor. [4] Chronic oral zinc supplementation interferes with copper absorption and may cause copper deficiency. This ability to interfere with copper absorption is also the basis for using zinc to treat Wilson's disease. Copper status should be monitored in patients on long-term zinc therapy [32] [33].

2.3.2 Absorption, Transport, and Excretion

The body content in a normal individual is about 2.5 g zinc, which is mainly in muscles (60%) and skeleton (30%). The remaining 10% is distributed in all tissues with highest concentrations in eyes, prostate, and hair. All tissue levels depend on age [34]. Zinc absorption mainly occurs in the small intestine and especially in the jejunum in blood, the absorbed zinc is distributed between RBCs (80%), plasma (17%), and white blood cells (3%). [31] Different factors modify the absorption of zinc. The factors increasing zinc absorption include: presence of animal proteins [35] and amino acids in a meal, [36] intake of calcium, [37] and unsaturated fatty acids[38].

The factors decreasing zinc absorption include intake of iron, [39] [40] taking zinc on empty stomach,[16] presence of copper at high levels,[41] and age.74 In normal dietary circumstances, about 90% of zinc is excreted in feces[41].

2.3.3 Deficiency

Nutritional zinc deficiency is widespread all over the world. Zinc deficiency causes growth retardation, slows skeletal maturation, causes testicular atrophy, and reduces taste perception. Old age, pregnancy, lactation, and alcoholism are also associated with poor zinc nutrition [21].

Infants with acrodermatatis enteropathica (zinc malabsorption) usually first develop characteristic facial and diaper rash. Untreated, symptoms progress and include growth retardation, diarrhea, impaired T-cell immunity, insufficient wound healing, infections, delayed testicular development in adolescence, and early death. Zn deficiency in adolescents is manifested by slow growth or weight loss, altered taste, delayed puberty, dwarfism, impaired dark adaptation, alopecia, emotional instability, and tremors. In severe cases, lymphopenia may occur; death follows an overwhelming infection [(42)].

2.3.4. Toxicity

Zinc is relatively nontoxic. Nevertheless, high doses (1 g) or repetitive doses of 100 mg/day for several months may lead to disorders, especially gastrointestinal tract symptoms, decrease in heme synthesis due to an induced copper deficiency, and hyperglycemia. Exposure to ZnO fumes and dust may cause “zinc fume fever.” The symptoms include chemically induced pneumonia, severe pulmonary inflammation, fever hyperpnea, coughing, pains in legs and chest, and vomiting [(31)].

2.3.5 Laboratory Evaluation of Zinc Status

Zinc is measured by flame AAS, ICP-AES, and ICP-MS. Low urine zinc levels in presence of low serum zinc levels, usually confirms zinc deficiency.[(35)] Low serum zinc in an apparently healthy (nonstressed nonseptic) patient who has normal serum albumin levels can be used as evidence of zinc deficiency, especially if urine zinc levels are also low. Normal serum zinc cannot be interpreted as evidence of normal zinc stores.

Zinc concentration in red blood cells is approximately 10 times that in serum [(42)].

2.3.6 Relationship between Zinc and breast tumor

Many reports have suggested an involvement of zinc in cancer development, and shown that the levels of zinc in the serum and malignant tissues decreases in patients with various malignancies, such as carcinoma of the breast, liver, gallbladder, digestive tracts or prostate. In contrast with these observations, in breast cancer patients the zinc levels are lower in the serum and elevated in malignant tissue. In fact, studies of the role of zinc

in malignant diseases have a long history of reporting contradictory and ill-defined biological [43].

Chapter Three

3. Materials and Methods

3.1. Study approach and design

This is analytical case-control study.

3.2. Study area and period

The study carried out in Radio Isotope centre of Khartoum (RICK) at Khartoum state, during the period from March to June 2014.

3.3. Target population and sample size

The study covered 85 individuals randomly selected from whole population with different age. 50 were patient with breast tumor and the rest were healthy women (control group).

3.4. Selection criteria

3.4.1. Inclusion criteria

Test group: patient with breast tumors (benign and malignant).

Control group: healthy women subjects.

3.5. Ethical consideration

- The aims and benefits of this study were explained to the participants.
- An informed consent was obtained from each participant.
- Health education was provided to each participant.

3.6. Data collection and analysis

3.6.1. Interview with a questionnaire

An interview with a questionnaire to obtain the clinical data was used for each participant in this study.

3.6.2. Blood samples and collection

Blood samples (5ml) were collected from each patients as well as control subject using disposable syringe. Blood samples were collected in heparin containers. Lipemic sample were cleared by centrifugation. Hemolyzed samples were disqualified. Specimen of about 1ml heparinized plasma were preserved at -20◦C prior to processing.

3.7. Biomedical measurements and instruments used

Cotton, 70% Alcohol, disposable syringes, tourniquet, heparin containers, atomic absorption spectrophotometer device, test tubes, automatic pipette (10-100µl), automatic pipette (1000 µl) and distilled water.

3.7.1 Estimation of copper:

I. Sample preparation:

For the determination of plasma copper, samples diluted with an equal volume of deionized water (1:1) by using atomic absorption Perkin-Elmer ,according to manufacture‘s instruction.

II. Procedure:

Determination of copper using the conditions, copper standards are prepared by diluting the copper stock standard solution with 10% (v/v) glycerol. A 10% (v/v) glycerol solution should also be used as a blank solution when determining copper.

III. Reference range:

Normal plasma copper: 0.7-1.4 mg/L.

3.7.1 Estimation zinc:

I. Sample preparation:

For the determination of plasma zinc, samples diluted 1: 5 with deionized water (1:4).

II. Procedure:

Determination of zinc using the conditions, copper standards are prepared by diluting the copper stock standard solution with 10% (v/v) glycerol. A 10% (v/v) glycerol solution should also be used as a blank solution when determining zinc.

III. Reference range:

Normal plasma zinc: 0.5-1.2 mg/L.

3.8. Quality Control

The precision and accuracy of all methods used in this study were checked each time a batch was analyzed by including commercially prepared control sera.

2.9. Statistical analysis

Statistical package for social science (SPSS version 11.5) computer software was used for data analysis. The means and standard deviation of the plasma copper and zinc were calculated. T-test was used for comparison (significant level was set at $p \leq 0.05$).

Person correlation analysis was used to asses relationship between the age, duration of breast cancer and the plasma levels of copper and zinc, the results presented in form of tables and figures.

Chapter four

4. Results

This study was conducted on 50 patients with breast tumor as test group and 35 healthy women as control group. Age of the test group was matched with the control group.

In this study the test group was composed of 15 benign breast tumor patients and 35 malignant breast cancer patients.

Table (4-1) shows no significant difference between the mean of age of the test group and the control group (P= 0.369).

As demonstrated in table (4-2) there were a significant reduction in the means of plasma levels of copper and zinc of test when compared to control group (P= 0.000), (0.007), respectively.

As showed in table (4-3) there were a significant decrease in means of plasma levels of copper and zinc in the malignant breast tumor patients than in benign breast patient (P = 0.000), (p= 0.000), respectively.

As showed in figure (4-1) there was positive non significant correlation between plasma levels of copper (mg/L) and age (r =0.069, P value =0.717).

As demonstrated in figure (4-2) there was negative non significant correlation between plasma levels of copper (mg/L) and duration of tumor (r = -0.04, P value =0.764).

As also showed in figure (4-3) there was positive non significant correlation between plasma levels of zinc (mg/L) and age (r =0.028, P value =0.847).

As demonstrated in figure (4-4) there was negative non significant correlation between plasma levels of zinc (mg/L) and duration of breast tumor (r = -0.063, P value =0.664)

Table (4-1) Comparison of means of age in the test group and control group (per years).

Variable	Test group n =50	Control group n =35	P value
Age	**38 ± 11**	**36 ± 8**	**0.369**

The tables shows the mean ± SD, and the probability (P).

The value ≤ was considered significant.

Table (4-2) Comparison of means of plasma levels of copper and zinc (mg/L) of the breast cancer patients (test group) and control group.

Variable	Test group n =50	Control group n =35	P value
Copper mg/L	0.593 ± 0.141	0.809 ± 0.135	0.000
Zinc mg/L	0.439 ± 0.143	0.687±0.104	0.00

The tables shows the mean ± SD, and the probability (P).

The value ≤ was considered significant.

Table (4-3) Comparison of means of plasma levels of copper and zinc (mg/L) of the breast cancer patients according to types of breast cancer.

Variable	Benign tumor n =15	Malignant tumor n =35	P value
Copper mg/L	0.740 ± 0.127	0.579 ± 0.134	0.007
zinc mg/L	0.752 ± 0.139	0.406 ± 0.234	0.000

The tables shows the mean ± SD, and the probability (P).

The value ≤ was considered significant.

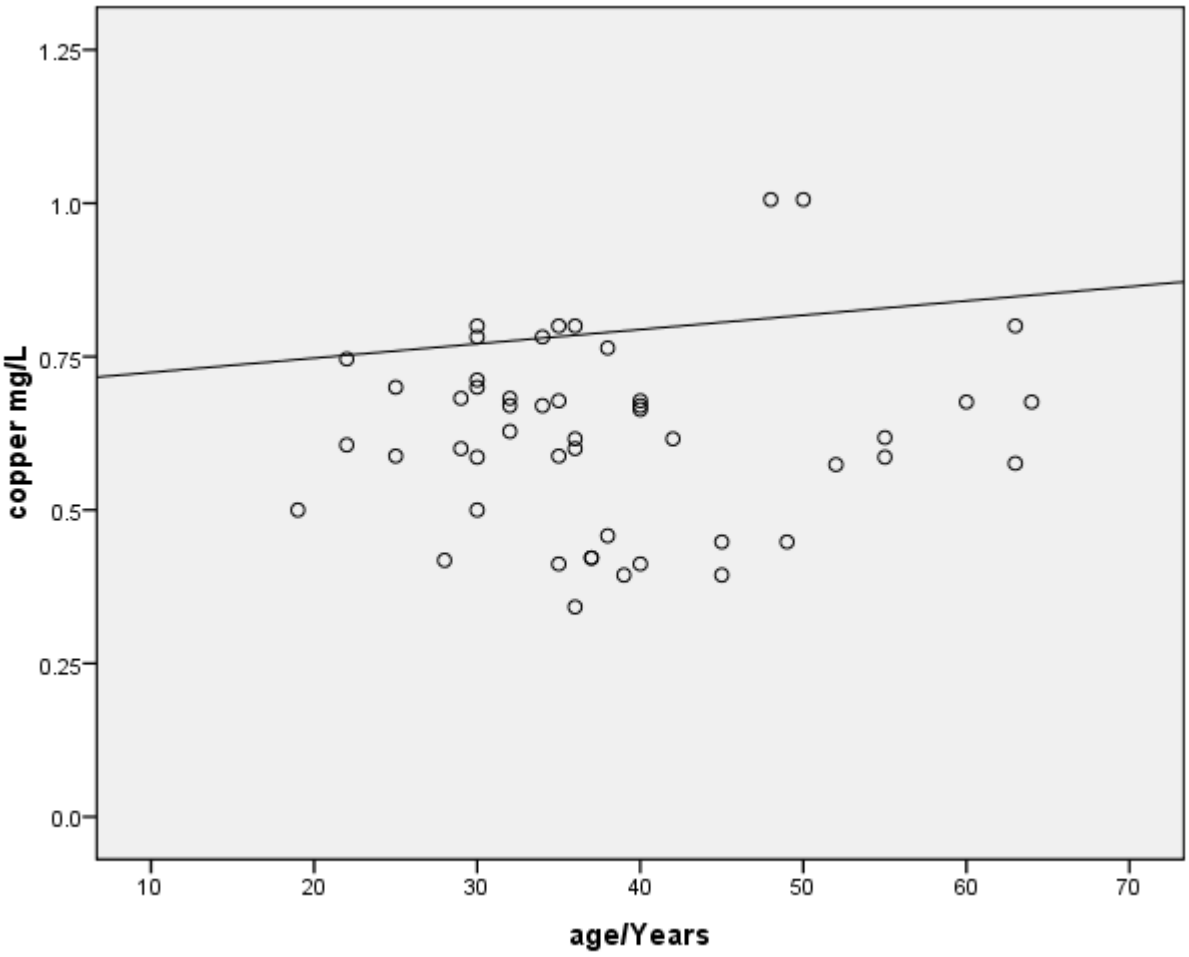

Figure (4-1) A scatter plot shows the relationship between plasma level of copper (mg/L) and different age group (r =0.069, P value =0.717).

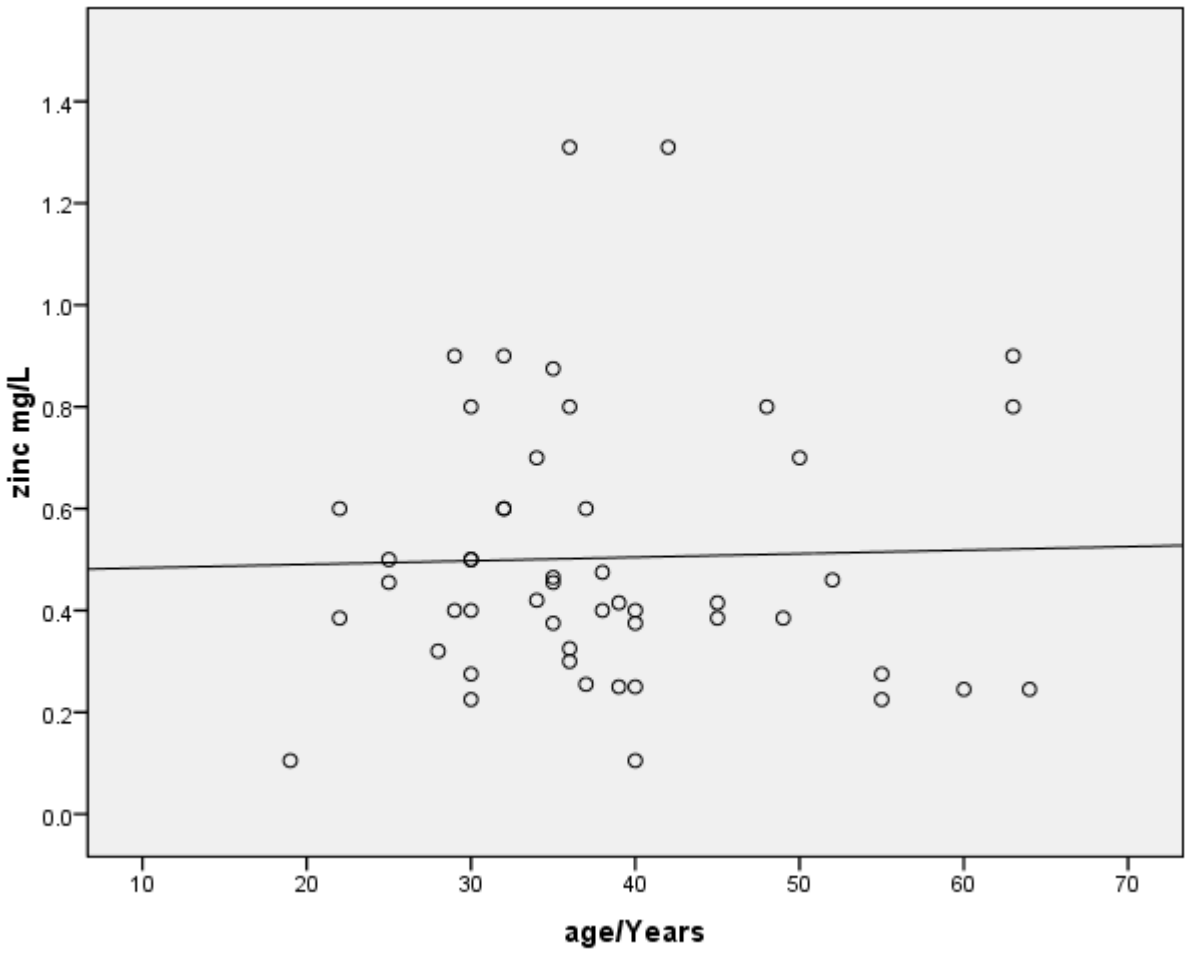

Figure (4-2) A scatter plot shows the relationship between plasma level of zinc (mg/L) and different age group (r =0.028, P value =0.847).

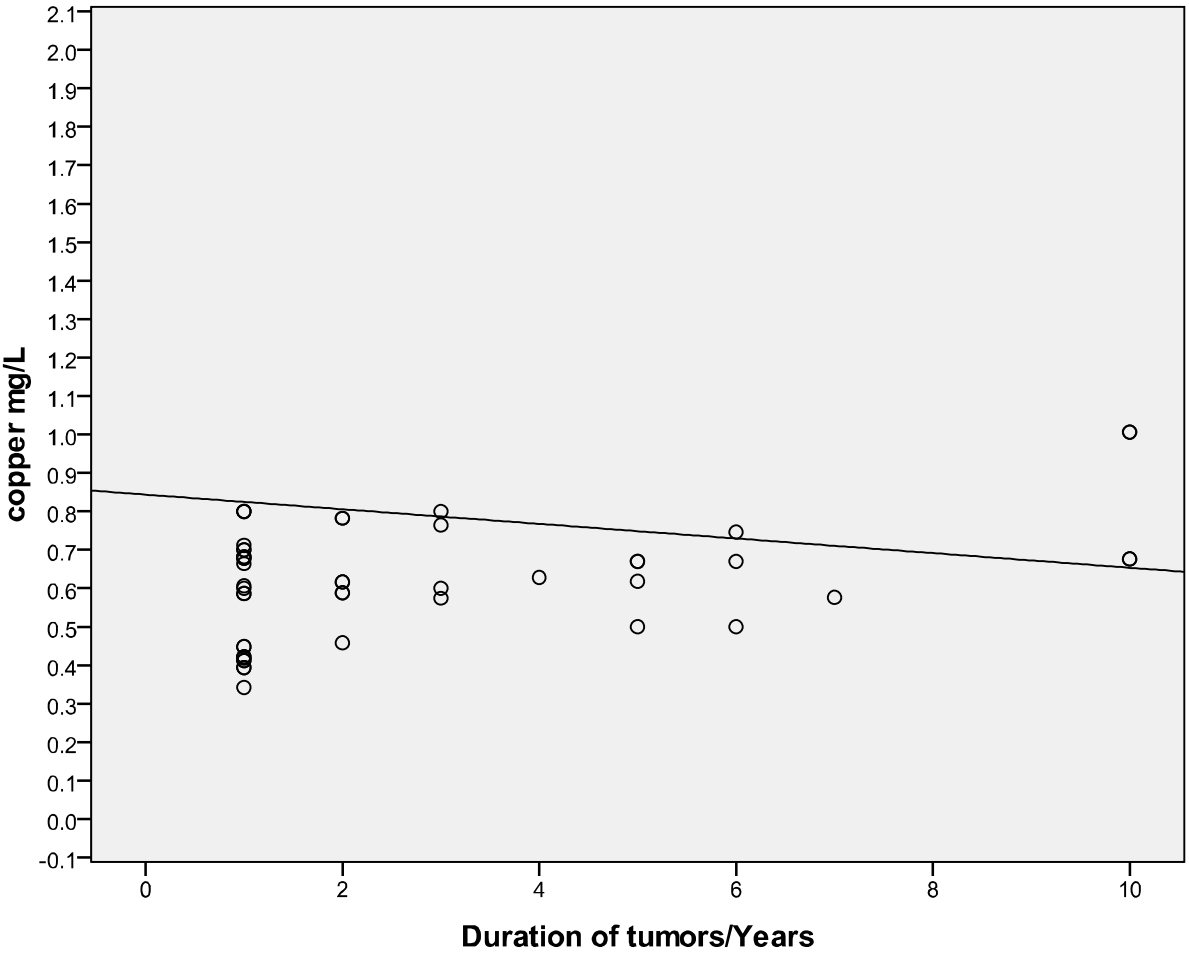

Figure (4-3) A scatter plot shows the relationship between plasma level of copper (mg/L) and duration of tumor (r = -0.04, P value =0.764).

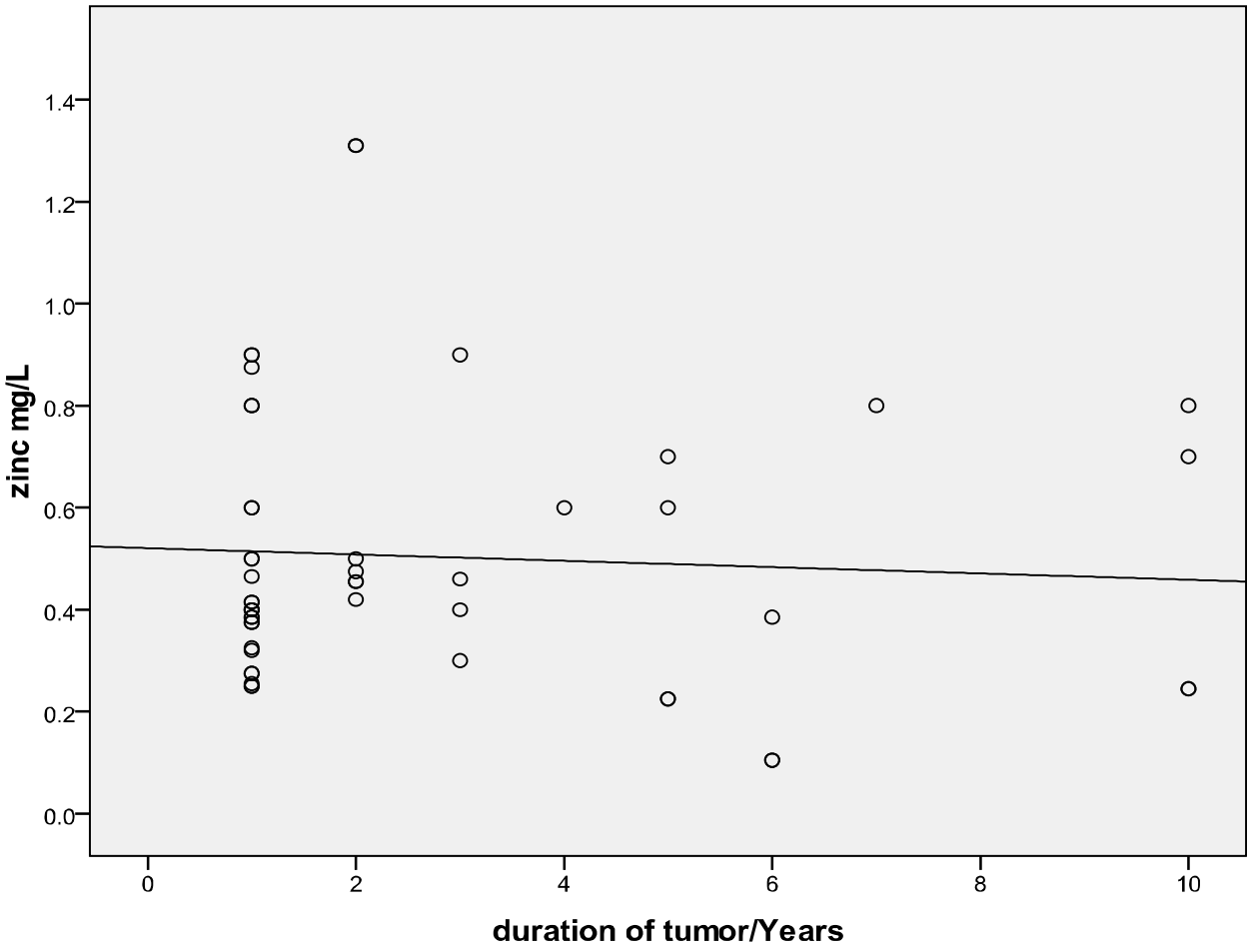

Figure (4-4) A scatter plot shows the relationship between plasma level of zinc (mg/L) and duration of breast tumor (r = -0.063, P value =0.664).

CHAPTER FIVE

5. Discussion, Conclusion and Recommendations

5.1 Discussion

In this study patients with breast tumor has a significant reduction in the means levels of plasma copper and zinc when compared to control group (P =0.000), (P=0.000) respectively, this was agreed with result that reported by Yucel, *et al*, who found that mean of levels of copper was significantly decreased in breast cancer patients than in control group (P =0.001) and this disagree result that reported by Milsolawa, *et al*, who found that mean of levels of zinc was significantly higher in breast cancer patients than in control group (P =0.02) [(43) (44)], this may related to life style, genetic and medical treatment different between group studies of the two researches.

Plasma levels of copper and zinc shows significant reduction in malignant breast cancer patients compared to benign breast cancer patients (P =0.000), (P =0.000), this result is similar to that reported by Siddiqui, *et al*, who found that mean of levels of copper and zinc were significantly lowered in malignant breast cancer patients than benign breast cancer patients (P =0.001)(P=0.000) [(8)].

Plasma levels of copper and zinc in breast tumor patients shows positively no significant correlation between plasma levels of copper (mg/L) with different age (r =0.069, P =0.717) (r =0.028, P =0.847) respectively, this mean levels of plasma of copper and zinc in patients with breast tumor not affected by age.

Also shows negatively no significant correlation between plasma levels of copper and zinc (mg/L) and duration of tumor (r = -0.04, P =0.764) (r = -0.063, P value =0.664) respectively.

5.2 Conclusion

From the results of this study it is concluded that, in breast cancer patients:

1- The means of the plasma levels of copper and zinc are significantly decreased when compared to healthy women.

2- The means of plasma levels of copper and zinc are significantly decreased in patients with malignant breast cancer than patients with benign breast tumor.

3- Age have a positively but insignificant correlation with plasma levels of copper and zinc.

4- Duration of breast tumor has negatively but insignificant correlation with plasma levels of copper and zinc.

5.3 Recommendations

From the results of this study, it is recommended that:

1- Copper and zinc measurement must introduce to medical laboratories.

2- The later studies should include the other trace element, like Lead, Selenium, and Magnesium.

3- Also the later studies should include tumor size and grade of cancer.

4- Patients with breast cancer must supplement with food containing copper and zinc.

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