CHAPTER ONE

1. Introduction and Literature Review

1.1 The Cupper (Cu^{++})

Cupper is an important trace element micronutrient that is required for plant, animal, and human health. It is also required for the normal functioning of aerobic (oxygen-requiring) microorganisms and incorporated into a variety of proteins and metalloenzymes which perform essential metabolic functions. [1, 2]

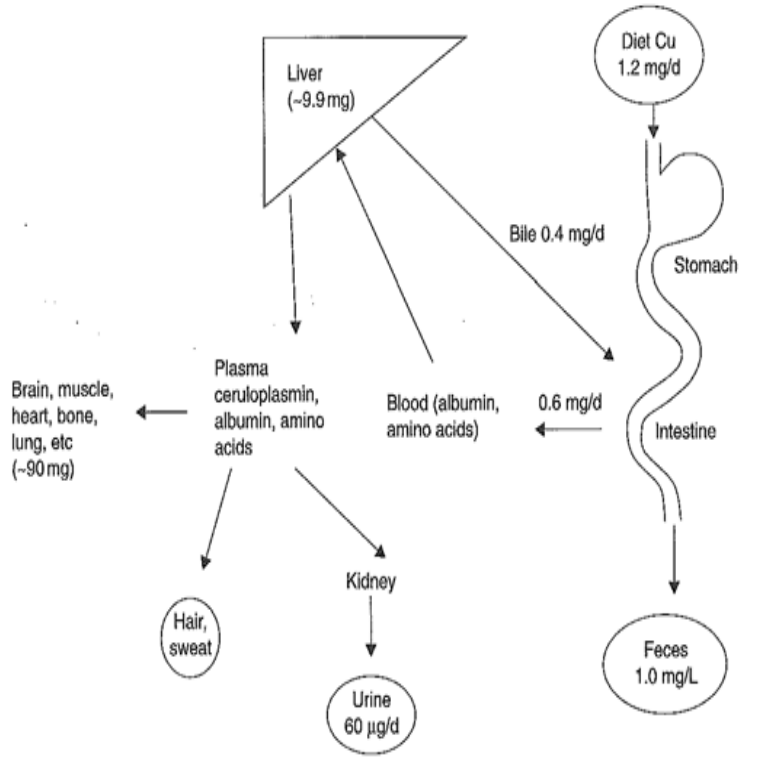
1.1.1 Biochemistry and Homeostasis of Cupper

Cupper, (atomic number 29, relative atomic mass 63.54) act as an electron donor or acceptor as its oxidation state fluxes between Cu^{+1}(cuprous) and Cu^{+2} (cupric). [3] As a component of about a dozen cuproenzymes, cupper is involved in key redox (oxidation-reduction) reactions in essential metabolic processes such as mitochondrial respiration, synthesis of melanin, and cross-linking of collagen.[4] Cupper is an integral part of the antioxidant enzyme, cupper-zinc superoxide dismutase (Cu,Zn-SOD), and has a role in iron homeostasis as a cofactor in ceruloplasmin.[3] The easy exchange between these ions gives the element important redox properties. Because of their high electron affinities, these ions are the most strongly bound to organic molecules of all the essential trace metal. Cupper transport at the cellular level involves the movement of extracellular cupper across the cell membrane and into the cell by specialized transporters.[5]

In the blood stream, cupper is carried throughout the body by albumin, ceruloplasmin, and other proteins. The majority of blood cupper (or serum cupper) is bound to ceruloplasmin. The proportion of ceruloplasmin-bound cupper can range from 70-95% and differs between individuals, depending, for example, on hormonal cycle, season, and cupper status. Intracellular cupper is routed to sites of synthesis of cupper-requiring enzymes and to organelles by specialized proteins called metallochaperones. Another set of these transporters carries cupper into subcellular compartment. [1]

Certain mechanisms exist to release cupper from the cell. Specialized transporters return excess unstored cupper to the liver for additional storage and/or biliary excretion. The extent of small intestinal cupper absorption varies with dietary cupper content and is around 50% at low cupper intake (less than 1mg Cu^{++} per day) but only 20% at higher intakes (>5mg per day), absorption is reduced by other dietary component, such as zinc (via metallo thionein), molybdate and iron, and increased by amino acid. Absorbed cupper is transported to the liver in portal blood bound to albumin where it is

incorporated by the hepatocytes into cuprous enzymes and other proteins and then exported in peripheral blood mainly as ceruloplasmin to tissue and organs. Although two thirds of the 80 to 100 mg total body cupper content is located in the skeleton and muscle, the liver is the key organ in cupper homeostasis. Ceruloplasmin is positive acute phase reactant and increases during infection and after tissue injury. A smaller amount of cupper in plasma (<10%) is bound to albumin .an over review of cupper metabolism is illustrated in Figure (1-1). Between 0.5 and 2.0 mg of cupper per day is excreted via bile into feces. Patients with cholestatic jaundice or other form of liver dysfunction are therefore at risk of cupper accumulation caused by failure of excretion. Urine cupper output is normally less than 60(µg/day). [1]



Metabolism of copper. (Modified from Harris ED. Copper. In: O'Dell BL, Sunde RA, eds. Handbook of nutritionally essential mineral elements. New York: Marcel Dekker, 1997:231-73.)

Figure 1.1Cupper metabolism.

1.1.2 Biochemical Functions of Cupper

Cupper is a catalytic component of numerous enzymes and is also a structural component of other important proteins in humans, animals, plant, and microorganism.

1.1.2.1 Energy Production

Cytochrome c oxidase is a multi-subunit complex containing cupper and iron located on the external face of mitochondrial membranes, the enzyme catalyzes a four–electron reduction of molecular oxygen, which is necessary for ATP production. (1)

1.12.2 Connective Tissue Formation

Protein -lysine 6 -oxidase (Lysol oxidase) is cupro enzyme that is essential for stabilization of extracellular matrixes, specifically the enzymatic cross linking of collagen and elastin. The enzyme is highly associated with connective and located in the aorta, dermal connective tissue, fibro blast, and cytoskeleton of many other cells. (1)

1.1.2.3 Iron Metabolism

Cupper containing enzymes, namely ferroxidase 1 (ceruloplasmin), ferroxidase 2 and hephaestin in the entrecyte - oxide ferrous iron to ferric iron, this allows incorporation of fe^{+3} into transferrin and eventually in hemoglobin. (1)

1.1.2.4 Central Nervous System

Dopamine monoxygenase (DOM) is an enzyme that requires cupper as a cofactor and uses ascorbate as an electron donor. This enzyme catalyzes the conversion of neither dopamine to nor epinephrine. The important neurotransmitter monoamine oxidase is a cupper-containing enzyme that catalyzes the degradation of serotonin in the brain. (1)

1.1.2.5 Melanin Synthesis

Tyrosinase is a cupper-containing enzyme that is present in melanocyte and catalyzes the synthesis of melanin. (1)

1.1.2.6 Antioxidant Function`

Both in intracellular and extracellular (SOD) are cupper and zinc containing enzymes, able to convert superoxide radicals to hydrogen peroxide, which is subsequently removed, ceruloplasmin also binds cupper ions and thus prevent oxidative damage from free cupper ions which generate hydroxyl radicals. (1)

1.1.2.7 Regulation of Gene Expression and Intracellular Cupper

Metallo thionein synthesis is controlled by cupper responsive transcription factor s and this protein is important in regulation the intracellular distribution of cupper additional specialized proteins acts as cupper chaperones to deliver cupper to intracellular sites and prevent oxidative damage by free cupper ions. (1)

1.1.2.8 Hemoglobin Synthesis

The finding, in rats, that cupper and only cupper prevented an iron resistant anaemia has been followed by other work that has revealed a remarkable interdependence between cupper and iron. In pigs, for example, cupper deficiency can lead to defective absorption of iron from the gastrointestinal tract, a restricted flow of iron from reticuloendothelial cells to the plasma, excessive retention of iron in the liver, and an impaired production of haem. Ceruloplasmin plays a key role in mobilising iron from the reticuloendothelial cells. (1)

Table 1.1 showed key cupper-containing enzymes and their functions. (6)

Enzymes	**Function**
Amine oxidases	Group of enzymes oxidizing primary amines (e.g., tyramine, histidine and polylamines).
Ceruloplasmin (ferroxidase I)	Multi-cupper oxidase in plasma, essential for iron transport.
Cytochrome c oxidase	Terminal oxidase enzyme in mitochondrial respiratory chain, involved in electron transport.
Dopamine β-hydroxylase	Involved in catecholamine metabolism, catalyzes conversion of dopamine to norepinephrine.
Hephaestin	Multi-cupper ferroxidase, involved in iron transport across intestinal mucosa into portal circulation.
Lysyl oxidase	Cross-linking of collagen and elastin.
Peptidylglycine alpha-amidating mono-oxygenase (PAM)	Multifunction enzyme involved in maturation and modification of key neuropeptides (e.g., neurotransmitters, neuroendocrine peptides).
Superoxide dismutase (Cu, Zn)	Intracellular and extracellular enzyme involved in defense against reactive oxygen species (e.g., destruction of superoxide radicals).
Tyrosinase	Enzyme catalyzing melanin and other pigment production.

1.1.3 Dietary Source of Cupper

Cupper is an essential trace mineral that cannot be formed by the human body. It must be ingested from dietary sources. As an essential trace element, daily dietary requirements for cupper have been recommended by a number of governmental health agencies around the world. [2] Foods contribute virtually all of the cupper consumed by humans. The best dietary sources include seafood (especially shellfish), organ meats (e.g., liver), whole grains, legumes (e.g., beans and lentils) and chocolate. Nuts, including peanuts and pecans, are especially rich in cupper, as are grains such as wheat and rye, and several fruits including lemons and raisins. Other food sources that contain cupper include cereals, potatoes, peas, red meat, mushrooms, some dark green leafy vegetables (such as kale), and fruits (coconuts, papaya and apples). Tea, rice and chicken are relatively low in cupper, but can provide a reasonable amount of cupper when they are consumed in significant amounts. Eating a balanced diet with a range of foods from different food groups is the best way to avoid cupper deficiency. [1]

1.1.4 The Recommended Dietary Allowance (RDA)

The RDA for cupper reflects the results of depletion –repletion studies and is based on the prevention of deficiency. [1]

Table 1.2 The Recommended Dietary allowance of cupper in all stage of life.

Life stage	Age	Male(µg/day)	Female (µg/day)
Infant s	0-6month	200	200
Infants	7-12month	220	220
Children	1-3years	340	340
Children	4-8years	440	440
Children	9-13years	700	700
Adolescents	14-18years	890	890
Adult	19years	900	900
Pregnancy	All Age	---	1000
Breast feeding	All Age	__	1300

Products the average dietary intake of cupper is approximately 1.0 to 1.1mg/day for adult women and 1.2 to 1.6 mg /day for adult men.[1]

1.1.5 Pathology of Cupper

Cupper's essentiality was first discovered in 1928, when it was demonstrated that rats fed a cupper-deficient milk diet were unable to produce sufficient red blood cells. The anemia was corrected by the addition of cupper-containing ash from vegetable or animal sources. [2] Cupper is absorbed, transported, distributed, stored, and excreted in the body according to complex homeostatic processes which ensure a constant and sufficient supply of the micronutrient while simultaneously avoiding excess levels. If an insufficient amount of cupper is ingested for a short period of time, cupper stores in the liver will be depleted. Should this depletion continue,a cupper health deficiency condition may develop? If too much cupper is ingested, an excess condition can result. Both of these conditions, deficiency and excess, can lead to tissue injury and disease. However, due to homeostatic regulation, the human body is capable of balancing a wide range of cupper intakes for the needs of healthy individuals. [5]

1.1.6 Cupper Deficiency

A deficiency of cupper has been noted in a number of conditions:

1.1.6.1 Malnourished Infant

When malnourished infants with a history of chronic diarrhea where rehabilitated using a formula based upon cow’s milk they developed an iron resistant anemia neutropenia and other hematological disorder and bone lesions cupper supplementation of milk feeds reversed these abnormalities. [1]

1.1.6.2 Premature Infant

Most of the accumulation of cupper in the fetal liver occur in the last 3months of pregnancy and premature infant fed formula lacking sufficient cupper are at risk of deficiency disease since they lack adequate liver cupper storess hematological abnormalities and easily fractured brittle bones have been described. [1]

1.1.6.3 Nutritional Support

Adult and children fed intravenously without addition of sufficient cupper to the nutrient regimen develop symptomatic cupper deficiency the hematological changes of hypo chromic anemia and neutropenia are reversed by cupper supplementation. Similar effects have been reported during prolonged enternal feeding via jejunostomy children may also develop the typical bone changes mentioned above. [1]

1.1.6.4 Menkes Syndrome

This syndrome typically occurs in male infant at 2to 3 months who present with loss of previously normal development, hypotenia, seizures and failure to thrive, physical changes in the hair (pili torti /corkscrew hair), in facial appearance and neurological abnormalities suggest the diagnosis local first -line tests would be likely to find plasma cupper of less than 10 (μmol) ceruloplasmin less than 220 mg/L and demonstration of pili torti by microscopic examination of hair. [1]

1.1.6.5 Malabsorption Syndrome

Patients at risk include those with celiac disease, sprue, cystic fibrosis and short bowel syndrome in some cases excessive intake of oral zinc supplements has caused cupper deficiency by zinc induction of metallothionine in the intestinal mucosa which then sequesters dietary cupper blocking its absorption. [1]

1.1.6.6 Cardiovascular Disease

Animal studies show that severe cupper deficiency causes cardiac damage but the abnormality differs from that seen in human cardiovascular disease epidemiological surveys have also shown that increased plasma cupper value are a positive cardio vascular risk factor an increase in plasma ceruloplasmin and hence plasma cupper may be anon specific response to the inflammation of arteries fond in arteriosclerosis. [1]

1.1.6.7 Signs and Symptoms of Cupper Deficiency

The signs and symptoms include anemia: (the most common clinical signs of cupper deficiency that is resulted from defective iron mobilization due to decrease ceruloplasmin activity), abnormally low numbers of white blood cells (neutropenia) a condition that may be accompanied by increased susceptibility to infection, osteoporosis and other abnormalities of bone development, low birth weight infant, loss of pigmentation, neurological symptoms and impaired growth. [1]

1.1.7 Toxicity

1.1.7.1 Wilson Disease

Is genetic disorder of cupper metabolism that causes an increase in cupper toxic concentrations the incidence of Wilson disease is estimated to be 1/30000 live birth. [1] It is inherited as an autosomal recessive trait having a defect in the metabolism of cupper with accumulation of cupper in the liver, brain, kidney, cornea and other tissue. Cupper

transporting P-types ATPase known as ATP7A and ATP7B are essential factor in maintaining cupper balance impaired intestinal transport of cupper caused by a mutation in the ATP7A gene leads to the sever cupper deficiency disease seen in menkes – syndrome a defect in the ATP7B gene affects both incorporation of cupper into ceruloplasmin and cupper excretion via bile and it the basis of Wilson disease. (1)

The presentation is highly variable so adolescents or young adult with other wise unexplained liver disease or neurological symptoms should be screened especially where there is a family history of suspected Wilson disease initial local investigation would include plasma cupper and ceruloplasmin which will usually be low (<50µg/dl,8µmol cu^{++}/L and <2oomg/L ceruloplasmin). (1)

Although the total plasma cupper is decreased the non ceruloplasmin bound fraction is increased, allowing deposition of cupper in the brain, eyes, and kidney. Slit lamp eye examination may detect cupper deposit in the eye (kayser-Fleischer ring) and there may be abnormalities in liver function test with an increased urine cupper output (>500 µg Cu^{++}/L) Liver biopsy for cupper analysis is use full in suspected cases and results above 250 µg/g cu dry weight are usually found (normal 8 to 40 µg Cu^{++}per dry weight) gene tracking and mutation detection are now possible. Diagnosis often is difficult in the Wilson disease cases involving acute failure a greatly increased plasma cupper will be found but without an appropriately increased ceruloplasmin. The chronic form of Wilson disease is treated by oral chelating agent such as penicillamine and trientine which remove excess cupper from tissue and increase urine cupper excretion. Toxicity can also arise directly from cupper contamination of water supplies. (1)

1.1.7.2 Cancer

Is a complicated disease that is not well understood? Some researchers are investigating the possible role of cupper in angiogenesis associated with different types of cancers (hepatocellular carcinoma, pleural mesothelioma, colorectal cancer, head and neck squamos cell carcinoma, breast cancer, and kidney cancer).The trace element cupper had been found promoting tumor growth, several evidence indicates that tumors concentrate high levels of cupper. (9)

1.1.7.3 Symptoms of Cupper Toxicity

The symptoms of cupper toxicity include-vomiting, severe liver damage, abdominal pain, coma, diarrhea, kidney failure, nausea and may lead to death. (1)

1.2 Breast Cancer

Breast cancer is a disease in which malignant (cancer) cells form in the tissues of the breast usually carcinoma, rarely a sarcoma. The most common type of breast cancer is ductal carcinoma75%, which begins in the cells of the ducts. Cancer that begins in the lobes or lobules is called lobular carcinoma and is more often found in both breasts than are other types of breast cancer. Inflammatory breast cancer is an uncommon type of breast cancer in which the breast is warm, red, and swollen. (10)

Breast is the mammary gland of a woman in front part of chest each breast consist of glandular lobules (the milk secreting area) embedded in fatty tissue the milk passes from the lobules into ducts which join up to form 15-20 lactiferous ducts which dilated into ampullae which act as reservoirs for milk near the front of breast, each duct discharges through a separate orifice in the nipple, the dark area through nipple is called the areola.(11)

1.2.1 Epidemiology

Worldwide, Breast cancer comprises 22.9% of invasive cancers in women and 16% of all female cancers.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). 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. (12)

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. (13) The American Cancer Society's estimates for breast cancer in the United States are for 2013; About 232,340 new cases of invasive breast cancer will be

diagnosed in women. About 64,640 new cases of carcinoma in situ (CIS) will be diagnosed (CIS is non-invasive and is the earliest form of breast cancer). About 39,620 women will die from breast cancer. Breast cancer is the second leading cause of cancer death in women, exceeded only by lung cancer. The chance that breast cancer will be responsible for a woman's death is about 1 in 36 (about 3%). Death rates from breast cancer have been declining since about 1989, with larger decreases in women younger than 50. These decreases are believed to be the result of earlier detection through screening and increased awareness, as well as improved treatment. (14)

1.2.2 Pathophysiology

Ductal carcinoma originate in the lactiferous ducts where the cancer cells form a dense fibrotic core with radiating tentacles that invade surrounding breast tissue .the tumor mass is generally solid, nonmobile, irregular shaped, poorly defined and unilateral, Lobular carcinoma originates in breast lobules and are bilateral, Nipple carcinoma originate in the nipple and often occur in conjunction with invasive ductal carcinoma. The lungs, bones and liver are common sites of breast cancer metastasis. (15)

Although the cause of breast cancer is unknown the following risk factors are considered significant in its etiology; Menstruating at an early age, older age at first birth or never having given birth, A family history (first-degree relative, such as mother, daughter, or sister) of breast cancer, Treatment with radiation therapy to the breast/chest, Breast tissue that is dense on a mammogram, Taking hormones such as estrogen and progesterone for symptoms of menopause, obesity, not getting enough exercise, drinking alcoholic beverages andbeing white.(10)

1.2.3 Clinical Staging of Breast Cancer

Stage I tumor 2 cm or less in diameter, no nodal spread. Stage II tumor > 2 cm in diameter, regional lymph nodes involved. Stage IIIA tumor ≥ 5cm in diameter, regional lymph nodes involved. Stage IIIB tumor ≥ 5 cm in diameter, supraclavicular and infraclavicular lymph node involved, Stage IV tumor of any size, with or without regional spread but with distant metastasis. (28)

1.2.4 Diagnostic Tests

A mass detected by breast self-examination, physical examination, mammogram or needle biopsy of the mass. (15)

Current treatment of localized tumors usually by surgery with or without radiotherapy, cytotoxic drugs and hormone therapy are used as adjuvant therapy and for metastatic disease. Tamoxifen is the hormonal of choice in postmenopausal women with metastatic disease; it is also used as first line treatment for premenopausal women. [11]

1.2.5 Cupper and cancers

Cupper is an essential trace metal. The amount of cupper in an organism is tightly regulated. [16, 17] Angiogenesis, the growth of a tumor blood supply, is essential for tumor growth, invasion, and metastasis. [18] It has been shown that tumors, without a blood supply, do not grow larger than 1 to 2 mm^3.[19] Molecular processes of angiogenesis that require cupper as an essential cofactor include stimulation of endothelial growth by tumor cytokine production (i.e., vasoendothelial growth factor), degradation of extracellular matrix proteins by metalloproteinases, and migration of endothelial cells mediated by integrins. Consistently high levels of cupper have been found in many types of human cancers, including breast, prostate, colon, lung, and brain. [17]

Breast lesions were studied for the first time to access the association between breast cancers and to determine the potential of changes in trace elements concentration as a diagnostic marker and/or its etiological involvement in the disease. Cu level was significantly higher in tumor of cases when compared with those of their respective normal tumor free breast tissue. Further, modulation of trace elements level in both benign and malignant breast diseases patients may be of potential to be used as diagnostic marker of the disease process and its possible relationship etiologically. [20] Three anti-cupper drugs have been tested in clinical trials particularly tetrathiomolybdate (TM), a cupper chelator, which was originally used for patients with Wilson's disease. TM has been found to be effective in impairing the growth of mammary tumors in HER2/neu transgenic mice and lung metastatic carcinoma in C557BL6/J mice. In a phase I clinical trial with patients suffering from metastatic cancers, TM therapy achieved stable disease in five of six patients who were cupper-deficient .However, the disease advanced in some other patients before cupper levels were sufficiently lowered. These reports support the idea of cupper control as an anticancer strategy. [21, 22, 23, 24]

Another study concluded that Patients with advanced breast cancer had higher serum copper levels than did patients with early breast cancer (177.9 μg/dl vs 130.4 μg/dl). The

precise mechanisms responsible for the alterations in trace element levels in breast cancer patients are still unclear and require further evaluation. However, the serum copper levels may be used as biochemical markers in these patients. [29]

1.3.1 Rationale

Breast cancer mortality rate is about 458,503 deaths worldwide and it is the second leading cause of cancer death in women. [12] Angiogenesis, the growth of a tumor blood supply, is essential for tumor growth, it has been shown that tumors, without a blood supply, do not grow larger than 1 to 2 mm^3. [19] Molecular processes of angiogenesis that require cupper as an essential cofactor include stimulation of endothelial growth. Previous studies worldwide link between copper and breast cancer risk factors. In the Sudan little is known about the copper and breast cancer, accordingly present study conducted to evaluate copper level in Sudanese breast cancer women.

1.3.2 Objectives

1.3.2.1 General Objective

To evaluate plasma cupper levels in breast cancer women patients in Khartoum State.

1.3.2.2 Specific Objectives

1- To estimate plasma cupper levels using atomic absorption in study groups.

2- To compare plasma cupper levels between patients and controls.

3- To correlate between plasma cupper levels and risk factors of breast cancer (BMI, age, parity and duration of disease) among patients groups.

CHAPTER TWO

2. Materials and Method

2.1 Materials

2.1.1 Study Design

This is a descriptive cross sectional study conducted in Borg Alamal during the period from May 2013 to April 2014.

2.1.2 Study Group

A study group of randomly selected 60 subjects at Khartoum state who were diagnosed as breast cancer patients and 30 healthy individual matched with patient's variables as control group.

2.1.3 Sample Collection

Blood samples were collected from subjects (patients and control) after fulfillment of questionnaire under septic condition. Blood sample was drawn in heparinized container then centrifuged at 3000 RPM for 3 minute to obtain plasma. Hemolyzed samples were excluded from study, and then 3.0 ml plasma samples were preserved at -20 prior to processing. Obtained plasma was tested for cupper by using atomic absorption spectroscopy.

2.1.4 Ethical Consideration

The present study was approved by the Ethics Committee of Sudan university of Science and Technology, all patients enrolled in this study were fully informed by the aim of the study and verbally informed consent has been obtained from all participants in the study.

2.2 Methods

2.2.1 Estimation of Plasma Cupper

2.2.3 Principle of Flame Atomic Absorption Spectroscopy

The electrons of the atoms in the atomizer can be promoted to higher orbital's (excited state) for a short period of time (nanoseconds) by absorbing a defined quantity of energy (radiation of a given wavelength). This amount of energy, i.e. Wavelength, is specific to a particular electron transition in a particular element. In general, each wavelength corresponds to only one element, and the width of an absorption line is only of the order of a few picometers (pm), which gives the technique its elemental selectivity. The radiation flux without a sample and with a sample in the atomizer is measured using a detector, and the ratio between the two values (the absorbance) is converted to analyte concentration or mass using Beer's- Lambert law. [(26)]

2.2.4 Procedure

Plasma samples diluted 1:1 with deionized water. Established instrumental and gas-flow settings and aspiration rate precisely, then signal optimized and minimized background noise. After that the instrumental set and then the table below applied to the instrument according to protocol in this study. Once the aspiration rate is optimized with 10-ml aliquots of water, locked the nebulizer flow adjusted in place. Aspirated glycerol/water solution (5/95 by vol) into the luminescent flame and set the baseline to read 0.000±0.001 absorbance *(A)*. Baseline reading obtained before and after each sample and reset the baseline as required. [27]

Table 1.3 showed Standard atomic absorption conditions for cu:

Wave length (nm)	Slit (nm)	Relative noise	Characteristic concentration (mg/L)	Characteristic concentration check(mg/L)	Linear rage (mg/L)
324.8	0.7	1.0	0.077	4.0	5.0
327.4	0.7	1.1	0.17	8.0	5.0
216.5	0.2	7.2	0.117	20.0	20.0
222.6	0.2	5.9	1.1	50.0	50.0
249.2	0.7	1.7	5.8	300.0	100.0
24.42	0.2	6.0	14.0	650.0	-----
244.2	0.7	2.2	24.0	1000.0	-------

Table 1.4 showed Standard flame emission conditions for cu:

Wave length (nm)	Slit (nm)	Flame
327.4	0.2	Nitrous oxide –acetylene

2.2.5 Reference Intervals

For adults plasma copper is usually in the interval of 70 to 140 µg/dl (10 to 22µmol/L 0.7-1.4 mg\l).Values in women of child bearing age and especially in pregnancy are higher. Urine copper output is normally less than 60 µg/24hrs (<1.0 µmol/ 24hrs) and values above 200 µg/24hrs (3 µmol/ L) are found in Wilson disease. [1]

2.2.6 Atomic Absorption Spectroscopy

Atomic absorption spectroscopy device principally based on the technique makes use of absorption spectrometry to assess the concentration of an analyte in a sample. It requires standards with known analyte content to establish the relation between the measured absorbance and the analyte concentration and relies therefore on Beer's-Lambert law. [25]

Figure 2.1 Atomic absorption spectroscopy devices

2.2.7 Statistical Tests

As appropriate descriptive and analytical procedure was followed using SPSS package (version 14).independent t –test was applied to show the mean of plasma copper between study group who are breast cancer patients and control .the level of significance was expressed as P-value less than 0.05 for highly significant.

Also to compare mean of plasma copper concentration in patients with age <45 years and >45, patients who have <3 children with > 3 children, patients with BMI <25 and >25 and who have cancer for more than 2 years with less than 2 years.

CHAPTER THREE

3. Results

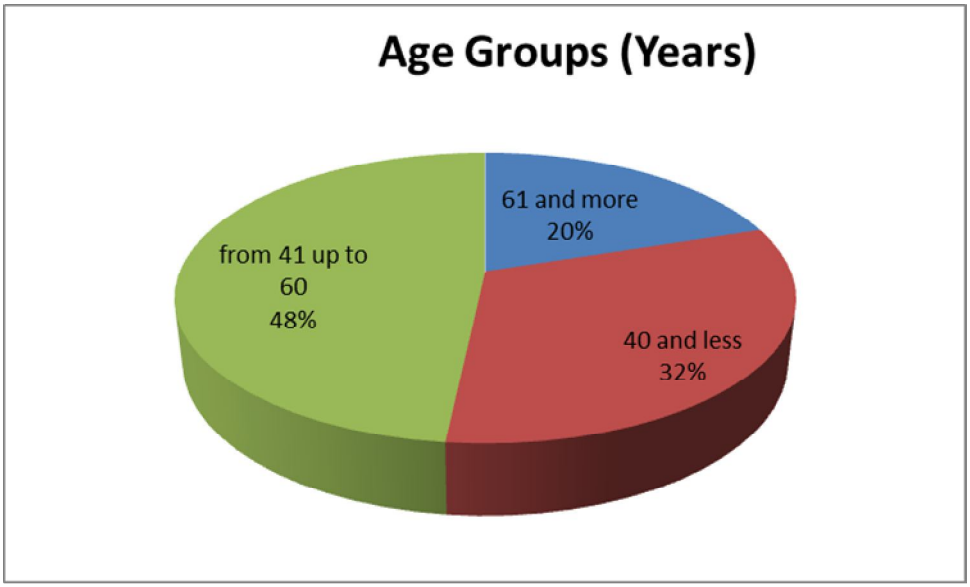


Figure 3.1 showed percentage of breast cancer among age groups (years)

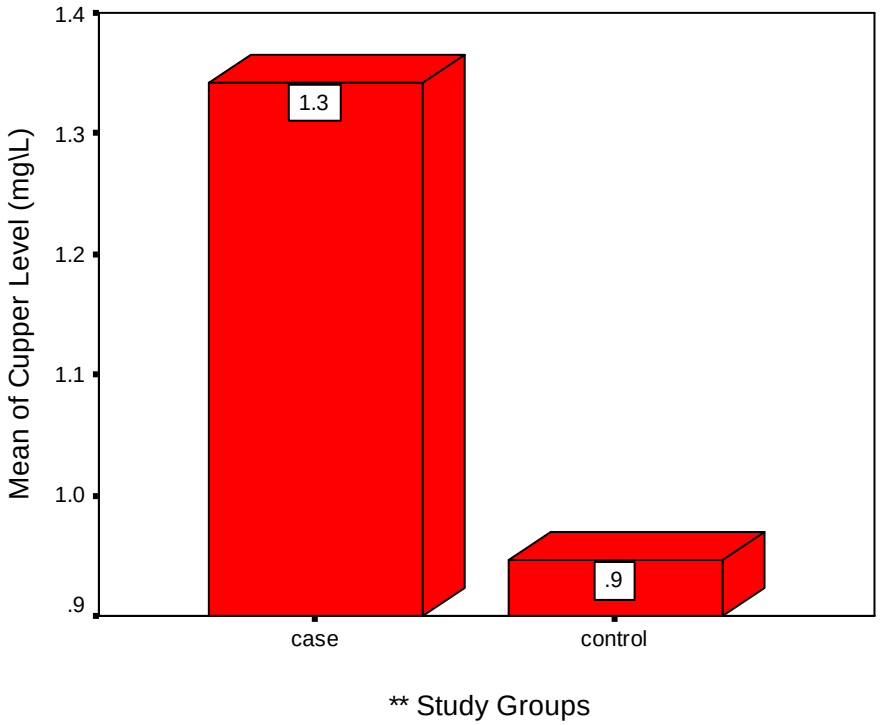


Figure 3.2 showed mean concentration of cupper levels (mg\L), in patients and control groups, result express as (M ± SD), (*P*-value < 0.05) = (0.000).

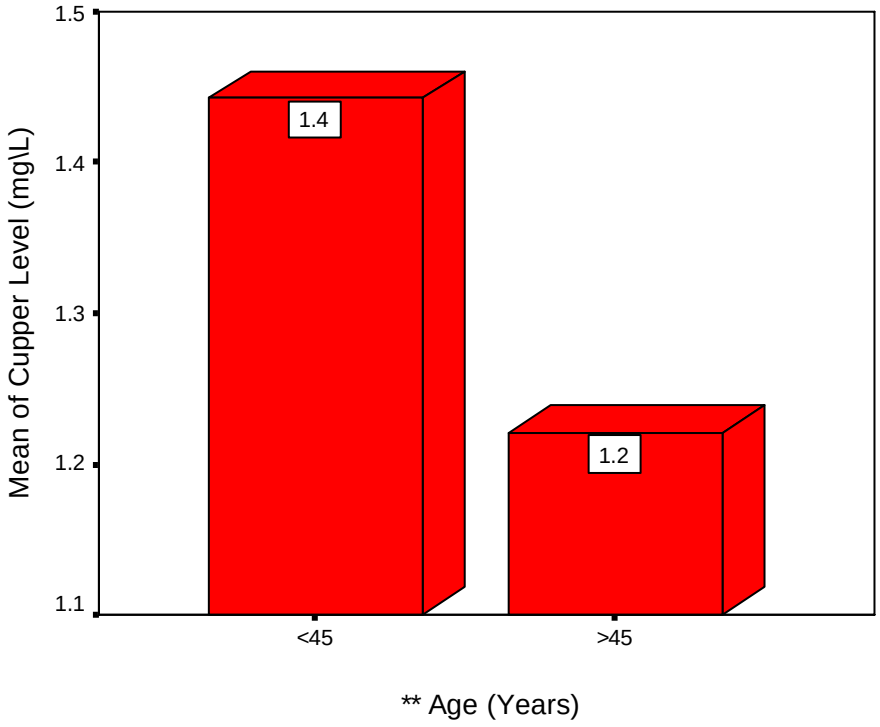


Figure 3.3 showed mean of cupper levels of patients classified according to age < 45 and > 45 years, result express as (M ± SD), (*P*-value < 0.05) = (0.009).

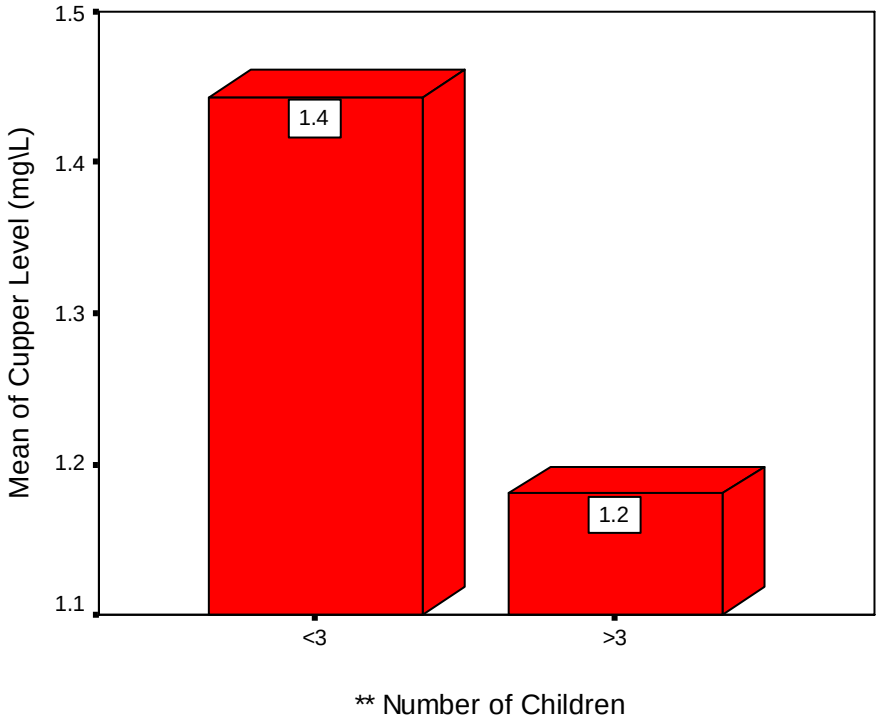


Figure 3.4 showed mean of cupper levels in patients classified according to parity less than 3 and more than 3 children, with mean of patients who have more than 3 children, result express as(M ± SD), (*P*-value < 0.05) = (0.003).

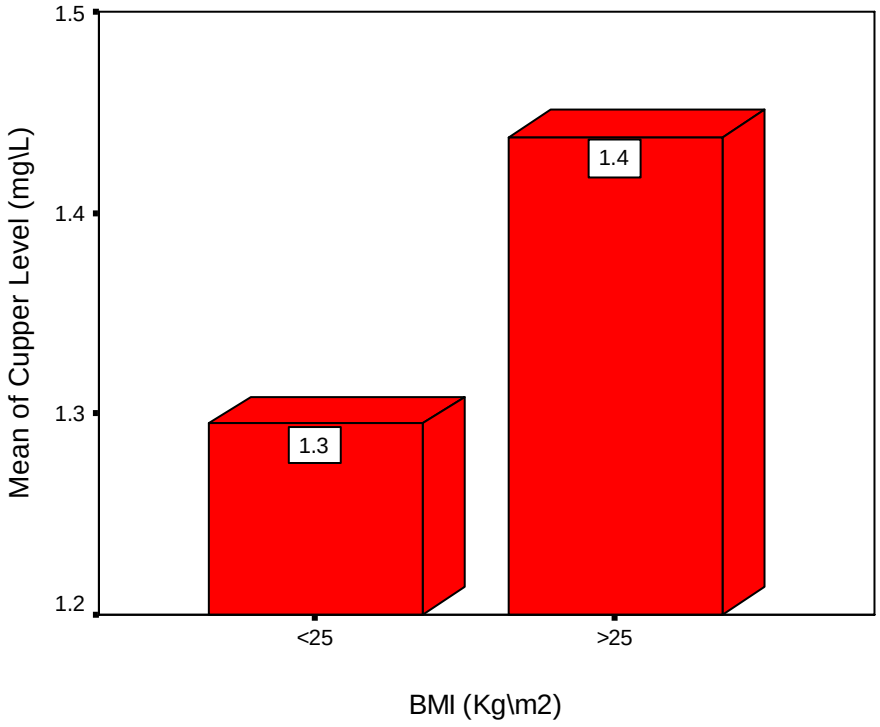


Figure 3.5 showed mean of cupper levels in patients classified according to BMI < 25 , with mean of patients with BMI > 25 , result express as (M ± SD) , (*P*-value < 0.05) = (0.167).

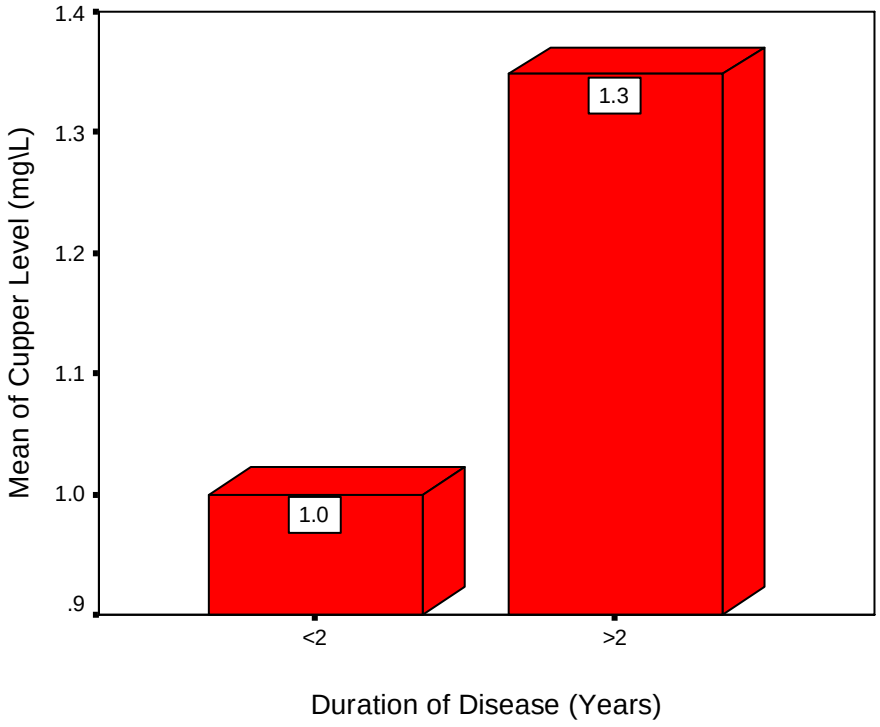


Figure 3.6 showed mean of cupper levels in patients classified according to duration of disease for < 2 years, with mean of patients with duration of disease for > 2 years, result express as (M ± SD), (*P*-value 0.312).

CHAPTER FOUR

4. Discussion, Conclusion and Recommendation

4.1 Discussion

Cupper is a trace mineral which is vital for the functioning of numerous cellular processes, is critical for growth, and may play an important role in cancer etiology and outcome. Considerable evidence indicates that cupper increase risk of several cancers, including breast cancer. [17]

This is a quantitative, descriptive, cross-sectional study conducted in the Radiation & Isotopes Centre Khartoum (RICK), located in Khartoum state, during the period from March 2013 to march 2014, aim to evaluate plasma cupper levels among breast cancer patients.

A total number of sixty women with breast cancer were enrolled in this study, they regularly visit the Radiation & Isotopes Centre Khartoum (RICK) for routine follow up, and in addition to thirty apparently health individual women were volunteers shared in this study as a control group.

The results of frequency showed that, 19 out of 60 (32%) in age 40 and less, 29 out of 60 (48%) in age between 41 and 60, and 12 out of 60 (20%) in age 61 and more. This finding revealed high prevalence of breast cancer among postmenopausal women.

In this study patients with breast cancer revealed significant difference in the mean plasma of cupper when compared with mean concentration in control group (*P*-value 0.000). The present study finding agreed with a study done by M.K.J. Siddiqui who uses Atomic Absorption Spectrometry to determine the potential of changes in trace elements concentration as a diagnostic marker and/or its etiological involvement in breast cancer. , Cupper level was significantly higher ($P < 0.05$) in tumor of benign cases when compared with those of their respective normal tumor free breast tissue. [20] Another study by Burson reported that, the level of Cu, Zn, and Se were significantly lower in breast cancer patients as compared to their controls. [30]

The results of the present study showed significant difference in mean age of patients less than 45 years when compared with mean of patients who are more than 45 years (*P*-value 0.009), study of Ralph J. Coates[2], show no appreciable effect of age, sex, race, occupational status, cigarette smoking, family history of cancer, alcohol consumption, and, among females, use of exogenous hormones. [31] While Syyed Mohammad reported that serum copper was strongly associated with age The highest mean copper

concentrations were found in the sub-population with an age between 50-59 years (*P* <0.01). [32]

The results of present study provide experiential evidence that there was significant difference in mean of patients who have less than 3 children when compared with mean of patients who have more than 3 children (*P*-value 0.003), this finding agree with Julie. Napieralski that number of children and a woman age at first birth influence in breast cancer risk (*P*-value0.04). [33]

the study showed no significant difference in mean of patients with Body mass index (BMI) less than 25 when compared with mean of patients with body mass index (BMI) more than 25 (*P*-value 0.167), this in contrast with the study done by Omer, the levels of serum cupper rise with the BMI. In fact, 58.3% of the obese that have a BMI > or = 40 kg/m2 show a high concentration of serum copper although only 5% of obese with BMI < 40 kg/m2 show this high concentration (*P*-value < 0.001). [34]

In the present study there was no significant difference in mean of patients who have the disease for less than 2 years when compare with mean of patients who have the disease for more than 2 years (*P*-value 0.312). Epidemiologic studies have shown an inverse association in which serum copper levels were found to be increased significantly in stage II, III and IV breast cancer patients, when compared with controls. But in stage I patients the serum copper levels were not altered significantly (*P*-value 0.12). [35]

4.2 Conclusion

From this study we concluded that plasma cupper levels increase in Sudanese breast cancer women compared with control.

In addition to that plasma cupper level increased proportionally with age of the patients and plasma cupper level decreased inversely with parity.

4.3 Recommendation

-Periodic checkup of plasma cupper levels in order to discover early increased of plasma cupper.

-Efforts of competent authorities to availability of instrument and its component, quality control, periodic checkup and repair, training and courses to provide more professional personals know how to deal with the atomic absorption spectroscopy and provide confirmed and approved results.

-Further studies recommended in order making cupper one of the trace elements that can be easily and routinely estimated for diagnosis of breast cancer.

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