

Chapter one

Introduction

1.1 Back ground

Cancers are group of diseases that cause cells in the body to change and grow out of control most types of cancer cells eventually form a lump or mass called a tumor and are named after the part of the body where the tumor originates . Breast cancer begins in breast tissue, which is made up of glands for milk production, called lobules, and ducts that connect lobules to the nipple. The remainder of the breast is made up of fatty, connective, and lymphatic tissues. Many masses are benign, controllable ,and are not life-threatening.¹

Many breast cancers are start as in situ because they are confined within the ducts(Ductal carcinoma in situ) or lobules (lobular carcinoma in situ) of the breast . nearly all cancers at this stage can be cured . most cancerous breast tumors are invasive , or in filtrating. These cancers start in the lobules or ducts of the breast but have broken through the duct or glandular walls to invade the surrounding tissue of the breast.^{1,2}

Electrolytes are chemical in the body that regulate important physiological functions. Electrolyte imbalance cause a variety of symptoms That can be sever. Electrolyte imbalance is commonly caused by loss of body fluids through prolonged vomiting, diarrhea, sweating or

high fever. the most serious forms of Electrolytes imbalance in cancer patients include high blood calcium levels , called hyper Calcemia , or disorder called tumor lyses syndrome that result in electrolyte imbalance from killing of cancer cells. Both of these can be life threatening if not managed appropriately.³

1.2 Rational:

Breast cancer is the most common invasive cancer in women (the most common form of cancer is non – invasive non- melanoma skin cancers, non – invasive cancers are generally easily cured , cause very few deaths , and are routinely excluded from cancer statics).¹

Breast cancer comprises 22.9% of invasive cancers in women and 16% of all female cancer. In 2008, breast cancer caused 458.503 deaths World Wide (13.7% of cancer deaths in women and 6.0% of all cancer death for men and women to gather).⁴

There is association between breast cancer (which rich bone , pTHrp releasing cancer , chemotherapy and radiation therapy treatment of cancer) and electrolyte levels in body . That makes this type of research important in order to find a new tool for diagnosis , prevention , treatment and follow up.

Many studies were conducted to evaluate the plasma levels of electrolyte in breast cancer patient, but there were few published studies

in Sudan. This study done to assessment plasma levels of electrolyte in Sudanese women with breast cancer. In order to develop future prevention and follow up .

1.3 Objectives:

1.3 .1 General objectives :

- To assess of plasma electrolyte levels in Sudanese women with breast cancer .

1.3 .2 Specific objectives

- To compare plasma electrolyte levels in case and control group.
- To find correlation between electrolyte levels with age and duration of breast cancer.

Chapter Two

Literature Review

2.1 Normal anatomy and histology of the breast:

The breast consist of mammary glands, associated skin and connective tissues. it lies in the superficial fascia anterior to the pectoral muscle and the anterior thoracic wall . The base of each breast extends vertically from ribs 2 to 5 and transversely from the sternum to the mid auxiliary line. A layer of loose connective tissue (retro mammary space) separate the breast from the deep fascia and provide some degree of movement over underlying structures.^{1,5}

The mammary glands are modified apocrine sweat glands, classified as compound tubule-alveolar glands. It consists of 15 to 25 lobes radiating out from the nipple embedded in a mass of adipose tissue which is subdivided by collagenous septa . Each lobe is drained by its own lactiferous duct leading directly to the nipple. Immediately before opening on the surface, each duct is dilated to form lactiferous sinuses for milk storage. The nipple contains smooth muscle oriented in parallel to the lactiferous ducts . It covered by keratinized stratified squamous epithelium . The skin around nipple constitutes the areola, which contain sweat and sebaceous gland.⁶

Within lobe, the main duct branches repeatedly to form a number of terminal ducts each of which leads to lobule consisting of multiple acini. Each terminal duct and its associated lobule is called terminal duct lobular unit (TDLU). The ducts and acini are lined by two layers of cells; a luminal layer of epithelial cells and basal layer of myo-epithelial cells. In the larger ducts, the epithelial cells are columnar type and in small ducts and acini are cuboidal.⁷

2.2 Infections and inflammatory conditions:

2.2.1 Acute mastitis: almost all cases occur during lactation. The breast is vulnerable to bacterial infection because of the development of cracks and fissures in the nipples. Usually caused by staphylococcus aureus or less commonly streptococci. Localized infections often result in abscess and rarely lead to chronic mastitis with periductal inflammation, duct ectasia, and mononuclear infiltrates.⁸

2.2.2. Granulomatous mastitis: characterized by the presence of granulomatous reaction and giant cell formation. It is either caused by systemic granulomatous diseases e.g. sarcoidosis, or by infections e.g. mycobacterium tuberculosis.⁹

2.2.3 mammary duct ectasia: this affects extra lobular ducts. The most important histologic feature of this disorder is the dilatation of major ducts in the sub-areolar region. These ducts contain eosinophilic, granular secretion and foamy histocytes both within the duct epithelial and

lumen . the inspissated luminal secretions may undergo calcifications that may be the presenting sign in many patients . clinically it mimics carcinoma because scarring associated with periductal inflammation may cause retraction of the nipple. However , there is no evidence in the literature indicating that mammary duct ectasia is associated with an increased risk for breast cancer.⁸

2.2.4 Fat necrosis : Is a benign non suppurative inflammatory process of adipose tissue . It occurs secondary to accidental or surgical trauma or it may be associated with carcinoma or any lesion that provokes suppurative or necrotic degeneration , such as mammary duct ectasia, and . to lesser extent fibrocystic disease with large cyst formation . Few cases associated with therapeutic radiation of the breast. Clinically and mammo graphically it mimics breast carcinoma with hard irregular mass, skin retraction. Erythema , and skin thickness .

Histologically it is characterized by a nuclear fat cells often surrounded by Gaint foamy phagocytic histocytes.¹⁰

2.3. fibrocystic changes (FCCs): constitute the most frequent benign disorder of the breast . such changes generally affect premenopausal women between 20 and 50 years of age . the most common presenting symptoms are breast pain and tender nodularities in breasts . although, the exact pathogenesis in not clear , hormonal imbalance , particularly estrogen predominance over progesterone, seems to play an important

role in its development . FCCs classified in to : non proliferative lesions, proliferative lesions without a typical , and proliferative lesions with a typical (atypical hyperplasia).¹⁰

Non-proliferative lesion include cysts , papillary apocrine change , epithelial-related calcifications , mild epithelial hyperplasia , as well as non-sclerosing adenosis , and pre-ductal fibrosis . Proliferative lesions with a typical include moderate or florid ductal hyperplasia of the usual type, sclerosing adenosis , radial scar , and intra-ductal papilloma or papillomatosis . proliferative lesion with a typical include atypical and lobular hyperplasia . Women with non proliferative lesion on breast biopsy have no elevation in breast cancer risk, where , as women with proliferative disease without atypical and women with atypical ductal or lobular hyperplasia have a greater breast cancer risk, with relative risks ranging from 1.3-1.9 and 3.9 – 13.0 respectively .¹¹

2.4 Benign Tumors :

2.4.1. Fibro adenoma : is the most common lesion of the breast , it occurs in 25% of asymptomatic women . It is usually a disease of early reproductive life ; the peak incidence between the ages of 15 and 35 years. The lesion is hormone – dependent that lactates during pregnancy and involves along with the rest of the breast in pre menopause . A direct association has been noted between oral contraceptive use before age 20 and the risk of fibro adenoma , the Epstein – Barr virus might

play a causative role in the development of this tumor in immune suppressed patients. Fibro adenoma present as highly mobile, firm, non-tender and often palpable breast mass. Although, most frequently unilateral in 20% of cases , multiple lesions occur in the same breast or bilaterally. It consist of proliferation of epithelial and mesenchymal elements . Most fibro adenoma behave in benign fashion. However , malignant transformation occurs in 0.1% of cases commonly to lobular carcinoma in situ. ¹²

2.4.2 Lipoma : is a benign , usually solitary tumor composed of mature fat cells . It is occasionally difficult to distinguish lipoma from other conditions.

Clinically, thus causing diagnostic and therapeutic challenges- clinically, it present as a well – circumscribed, smooth or tabulated mass that is soft and usually non tender. F.N.A biopsy of these lesions reveals fat cells with or without normal epithelial cells usually both mammography and ultrasound scanning give negative results unless the tumor is large. ¹³

2.4.3 Adenoma : is pure epithelial neoplasm of breast . This lesion is divided into tubular , lactating, apocrine , and ductal adenoma . Except for lactating and tubular adenomas, these lesions are uncommon . ¹³

Lactating adenoma is the most prevalent breast mass during pregnancy and puerperium. It present as a solitary or multiple discrete, palpable , freely movable breast mass than tends to be small (< 3cm) .

It is characterized by hyperplastic lobules in which proliferated acini are lined by actively secreting cuboidal cells.¹⁴

Tubular adenoma present as solitary, well circumscribed, firm mass. It may resemble the appearance of non – calcified fibro-adenoma radiographically. Histological, tightly packed tubular of acinar structures that are very regular in size and shape are seen in a sparsely cellular stroma. Micro calcifications inside dilated acini have been described.¹⁵

2.5 Breast cancer:

With 1 million new cases in the world each year, breast cancer is the commonest malignancy in women and comprises 18% of all female cancers. It is estimated that 192,370 women were diagnosed with and 40,170 women died of breast cancer in 2009. Based on rates from 2004-2006, 12.08 of women born today will be diagnosed with cancer of breast at some time during their life time, this number can also be expressed as 1 in 8 women will be diagnosed with cancer of breast during their life time. In Sudan, according to radiation and isotopes cancer Khartoum (RICK) records from 2000 to 2007 there are 4892 cases of breast cancer, of whom 193 were male (3.9%) and 4699 were female (96.1%).¹⁶

2.5.1 Risk factors :

Age : besides being a female, age is most important factor for breast cancer. The incidence and death rates increase with age.

Age at menarche: women who reach menarche when younger than 12 years have 20% increase risk compared to women who reach menarche when more than 14 years .¹⁷

First birth: those with first full-term pregnancy at younger than 20 have half the risk of nulliparous or over the age of 35 women at their first birth. It is hypothesized that pregnancy result in terminal differentiation of epithelial cells, removing them from the potential pool of cancer precursor.¹⁷

Family history and genetic predisposition: women with a family history of breast or ovarian cancer in their first degree relatives are at increased risk of developing breast cancer . Thirteen percent of cases have one affected first – degree relatives, and only 1% have two or more . About 5-10% of cases attributed to Inherited mutation or alteration in auto-somal dominant genes ; BRCA₁ , and BRCA₂ both acts as tumor suppressor genes BRCA1 mutation are estimated to have 57% risk for developing breast cancer by age of 70 years . the corresponding risk for BRCA2 is 49%. BRCA1 associated breast cancers are poorly differentiated and don't express hormone receptor or over express HER2/neu . Genetic susceptibility due to other genes is less common, it accounts for less than 10% of hereditary breast cancer. Cell cycle check point kinase gene (CHEK2) important in repair of DNA damage , account for 5% of familial cases p53 gene mutation occurs in 19-57% of sporadic

breast carcinoma mutation of PTEN gene confers a 25% to 50 Lifetime risk of breast cancer .¹⁸

2.5.2 Signs and symptoms:

Although, widespread use of screening mammography has increased the number of breast cancers found before they cause any symptoms, some breast cancers are non found by mammography , either because the test was not done or because even under ideal conditions mammography cannot found find every breast cancer . the most common sign of breast cancer is a new lump or mass . A mass that is painless, hard, and has irregular edges is more likely to be cancerous, but some rare cancers are tender, soft, and rounded. Other signs of breast cancer include a generalized swelling of part of abreast, skin irritation or dimpling , nipple pain or retraction , redness or scariness of the nipple or breast skin , or discharge. Some- times a breast cancer can spread to underarm lymph nodes that are obviously enlarged, even before the original tumor in the breast tissue is large enough to be felt. ¹⁹

2.5.3 Diagnosis :

The triple test is recommended approach for the investigation of palpable and impalpable breast lesions . it comprised of clinical examination and medical history , imaging, and biopsy or fine needle aspiration cytology. It has 99.6% sensitivity and specificity of 93%.¹⁴

2.5.3.1 Breast physical exam: is a careful manual examination of the breasts by a health professional this exam can help find lumps that women may miss with their own self- exams . About 20 % of the time , breast cancers are found only by physical exam and not seen on a mammogram.¹⁹

2.5.3.2 Imaging test :

Mammograms are probably the most important tool used to screen, diagnose, evaluate and follow breast cancer. It is safe and reasonably accurate. A mammogram is an X- ray photograph of the breast. CT scan (computerized tomography scan) is an X-ray technique that gives information about the body's internal organs in 2-dimensional slices . CT scans are not used routinely to evaluate the breast. It used to assess the spread of cancer into the chest wall . this helps determine whether or not the cancer can be removed with mastectomy .Also to examine other part of the body where breast cancer can spread . such as the lymph nodes , lungs , liver , and brain . Magnetic resonance imaging (MRI) is a technology that used magnets and radio waves to produce detailed cross- sectional images of the inside of the body . Breast MRI has a number of different uses for breast cancer , including : screening high – risk women , gathering more information about an area of suspicion found on a mammogram and monitoring for recurrence after treatment.²⁰

2.5.3.3 Biopsy :

Is a small operation done to remove tissue from area of cancer in the body . Different techniques can be used to perform biopsy:

- It can be done by placing a needle through the skin into the breast to remove the tissue sample: or it can involve a minor surgical procedure, in which the surgeon cuts through the skin to remove some or all of the suspicious tissue . In cases where the lump cannot be felt. Imaging tools used to guide the needle to the right location . This is called ultra sound – guided biopsy when ultra sound is used or stereotactic needle biopsy when mammogram is used .²⁰

The principal aims for FNA cytology slide preparation is to make a thin smear that is not subject to crush artifact , and to allow rapid air-drying of air – dried slides and rapid fixation of wet- fixed slides . the reliability of FNA cytology depends on the skills of the aspirator , his cytology depends on the skills of the aspirator , histological type of the lesion , age of the patient , and size of the lesion . The advantages of FNA include quicker procedure, does not require local anathetic, less traumatic and relatively inexpensive. While the disadvantages are : require training in the preparation of quality smears , considerable cytology expertise is required for interpretation , inappropriate for the assessment of micro calcifications, also does not enable distinguish between carcinoma in situ and invasive carcinoma.²⁰

2.5.3.4 Immune Histo Chemistry (IHC):

shows whether or not the cancer cells have HER2 receptors and/ or hormone receptors on their surface . It plays a critical role in treatment planning .ER and PR positive cancers tend to have a better outlook than cancers without these receptors, because they are much more likely to respond to hormone treatment. About 2 out of 3 breast cancers have one of these receptors . about 1 out of 5 breast cancers have too much of HER2/neu protein . these cancers tend to grow and spread faster than other breast cancers.^{19,20}

2.5.3.5 Staging:

is the process of finding out the wide spread of cancer at the time it is found . It is important factor in choosing the treatment . The most common system used to describe the stages of breast cancer is the TNM system . This system takes in to account the tumor size and spread (T) , whether the cancer has spread to lymph nodes (N), and whether it has spread to distant organs (M) (M for metastasis). Numbers after the T,N and M give details about the cancer . It is expressed as Roman numeral . After stage 0 (carcinoma in situ), the other stages are I through IV .As a rule , the lower the number , such as stage IV (4) means a more advanced cancer.^{19,20}

2.5.4 Treatment :

The earlier breast cancer is found the better the chances that treatment will work. The size of a breast cancer and how far it has spread are the most important factors in predicting the outlook for the patient.²⁰

2.5.4.1 Surgery:

Is usually the first line of attack against breast cancer. Surgery options include : lumpectomy , also known as breast conserving surgery, is the removal of only the tumor and small a mound of surrounding tissue . Mastectomy is the removal of all of the breast tissue . lymph node removal , or axillary lymph node dissection, can take place during lumpectomy and mastectomy ,if the biopsy shows that breast cancer has spread outside the milk duct. Breast reconstruction is the rebuilding of the breast after mastectomy and some- times lumpectomy. Reconstruction can take place at the some- times as cancer removing surgery, or months to years later.²⁰

2.5.4.2 Chemotherapy treatment:

uses medicine to weaken and destroy cancer cells in the body, including cells at the original cancer site and any cancer cells that may have spread to another part of the body .It destroys the cells or stopping them from dividing. Chemotherapy is used to treat: early – stage invasive breast cancer to get rid of any cancer cells that may be left

behind after surgery and to reduce the risk of cancer coming back .Also in advanced – stage breast cancer to destroy the cancer cells as much as possible . In some cases , chemotherapy is given before surgery to shrink the cancer . Some- times chemotherapy medicines are given in combination , this known as chemotherapy regimens e.g AT: Adriamycin and taxotere. Chemotherapy have systemic side effect because it destroys normal cells in blood , intestinal tract, nose , nails , vagina and hair. ²⁰

2.5.4.3 Radiation Therapy:

Is a highly targeted, effective way to destroy cancer cells in breast that may stick around after surgery. radiation can reduce the risk of breast cancer recurrence by about 70% . It is relatively easy to tolerate and the side effects are limited to the treated area . It uses a special kind of high- energy beam to damage cancer by damaging cell's DNA. Radiotherapy has an important role in treating stage 0 through stage III of breast cancer. There are two main types of radiation: External radiation is the most common type, typically given after lumpectomy.²¹

2.5.4.4. Hormonal therapy :

Is systemic treatment for hormone-receptor Positive breast cancers. About 80 % breast cancers are estrogen – receptor positive 65% of them are also progesterone – receptor positive . 13% of breast cancers are estrogen receptor positive and progesterone – receptor negative . and about 2% of breast cancers are positive for both . Hormonal therapy medicines treat breast cancers in two ways: by lowering the amount of the hormone estrogen in the body ERDs (estrogen receptor down regulators) eg Faslodex. Or by blocking the action of estrogen on breasts cancer cells : this group includes: aromatase inhibitors (Arimidex, Aromasin. And Femava) and selective Estrogen Receptor Modulators (Tamoxifen). In some cases, the ovaries and fallopian tubes may be surgically removed to treat hormone – receptor – positive breast cancer or as a preventive measure for women at very high risk of breast cancer , the ovaries also may be shut down temporarily using medication.²¹

2.3.4.5 Targeted cancer therapies :

Are treatments that target specific characteristics of cancer cells, such as a protein that allows the cancer cells to grow in abnormal way . It is less likely than chemo therapy to harm normal cells . Some targeted therapies are antibodies; these types are called immune targeted therapies. Currently there are 3 targeted therapies available : Herceptin

works against HER₂ positive breast cancers by blocking the ability of the cancer cells to receive chemical signals that tell the cells to grow.²²

- Tykerb works against HER2- positive breast cancers by blocking certain proteins that can cause uncontrolled cell growth. Avastin works by blocking the growth of new blood vessels that cancer cells depend on to grow.^{20,21}

2.6 Electrolytes:

Electrolytes are ions capable of carrying an electric charge. They are classified as anions or cations based on the type of charge they carry. These names were determined based on how the ion migrates in an electric field. Anions have a negative charge and move toward the anode, whereas cations migrate in the direction of the cathode because of their positive charge.²²

The numerous processes in which electrolytes are an essential component are volume and osmotic regulation (Na, CL, K), myocardial rhythm and contractility (K, Mg, Ca): co-factors in enzyme activation (Mg, Ca, Zn) regulation of ATPase ion pumps (Mg); acid – base balance (HCO₃, K, CL); blood coagulation (Ca, Mg); neuromuscular excitability (K, Ca, Mg) and the production and use of ATP from glucose (Mg, Po₄).^{3,23}

2.6.1 Sodium:

is the major cation of extracellular fluid representing almost one – half the osmotic strength of plasma . it is therefore plays a central role in maintaining the normal distribution of water and osmotic pressure in the extracellular fluid . Acute changes in serum sodium will produce free water shifts into and out of the vascular space until osmolality equilibrates . The body of an adult contains approximately 4000 mmol of sodium , 70% of which is freely exchangeable , the remainder being complexed in bone . The majority of the exchangeable sodium is extracellular : normal E.C.F sodium concentration is 135-145 mmol/L while that of ICF is only 4-10 mmol/L . Most cell membranes are relatively impermeable to sodium but some leakage in to cells occurs and the gradient is maintained by active pumping of sodium from the ICF to the ECF by Na^+ , K^+ -ATP ase.^{22,23}

2.6.1.1 Sodium Homoeostasis :

The normal daily diet contains 8-15g (130-260mmol) of sodium chloride which absorbed nearly completely from the gastro intestinal tract . the body requires only 1-2 mmol /day the kidneys which are the ultimate regulators of the amount of Na^+ (and thus water) in the body excrete the excess.²²

Sodium initially is filtered freely by the glomeruli , then 70% to 80% of the filtered sodium load is reabsorbed actively in the proximal tubules , with Cl^- and water passively following an iso – osmotic and electrically

neutral manner . Another 20% to 25% is reabsorbed in the loop of Henle along with Cl^- and more water.²⁴

In the distal tubules , interaction of the adrenal cortical hormone aldosterone with the coupled $\text{Na}^+ - \text{K}^+$ and $\text{Na}^+ \text{H}^+$ exchange systems result directly in the re absorption of Na^+ ,and indirectly of Cl^- from the remaining 5% to 10% of the filtered load . The regulation of this latter fraction of filtered Na^+ determines the amount of Na^+ excreted in the urine .²⁵

TABLE 2.1: Hypo - natremia related to blood volume 22

<p style="text-align: center;">hypo volumic – hypo natremia)</p> <ul style="list-style-type: none"> • Decrease in total body water <ul style="list-style-type: none"> - dehydration , - over diuresis ketonurei - vomiting and diarrhea. <p style="text-align: center;">(normo volumic_ hypo natremia)</p> <ul style="list-style-type: none"> • Near normal total body water <ul style="list-style-type: none"> - Syndrome of inappropriate anti- diuretic hormone secretion (SIADH) - Hypothyroidism - Addison disease. <p style="text-align: center;">(hyper volumic_ hyponatremia)</p> <ul style="list-style-type: none"> • An increase in total body water <ul style="list-style-type: none"> - renal failure - congestive heart failure - Nephritic syndrome and - Liver cirrhosis .

Table 2.2 :Hyper natremia related to extra cellular volume 22

If extra cellular volume is low
<ul style="list-style-type: none">- Water lost due to Burns- Excessive sweating- Diuretics .
If extra cellular volume is normal ,
<ul style="list-style-type: none">- diabetes.• If extra cellular volume is high<ul style="list-style-type: none">-Hyperaldosteronism- Cushings syndrome- Sodium bicarbonate ingestion.

Table 2.3: References values for sodium 22

Adults	136-145mmol/L
Children (1-16)	136-145 mmol/L
Full-term infants	133-142 mmol/L
Premature infants	132-140 mmol/L

2.6.2 Potassium (K^+)

Is the major positive cation within cells. The total potassium of 70kg body weight man accounts about 3500 mmol, 98% of this is intracellular since most intracellular potassium is within muscle cells.

Potassium is one of the most important electrolytes, since it is needed for neuro-muscular activity and myocardial contractility.²²

Therefore both severe hypokalemia and hyperkalemia are dangerous. Potassium is important in muscles for glycogen synthesis and maintains the intracellular content of muscles and properties of cell membrane. Potassium in association with sodium plays a primary role in maintenance of intracellular fluid volume and pressure. Also potassium acts as activator for the enzyme sodium-potassium activated adenosine triphosphate ($Na^+ K^+$ ATPase) which is present in large amount.²³

In active tissues such as nerve, kidneys and epithelial potassium levels are mainly controlled by the steroid hormone aldosterone.²³

2.6.2.1 Potassium re-absorption:

Potassium reabsorption occurs by two mechanisms

1. Active reabsorption in the proximal tubules almost completely conserves potassium.
2. Exchange with sodium is stimulated by aldosterone. Hydrogen competes with potassium for this exchange the amount of (K^+) is

directly related to aldosterone secretions and sodium re absorption . Small increase body potassium directly stimulated the adrenal cortex to release aldosterone and thus (k+) secretion enhanced in the absence of aldosterone, (k+) secretion stops and re absorption occur. This show that (k+) re absorption taking place all the time but it is usually masked by the normally greater amount secreted .^{3,26}

Table 2.4 :Causes of hypo- kalemia 22

<p>GIT Loss</p> <ul style="list-style-type: none"> - vomiting and diarrhea - gastric section - intestinal tumor - malabsorption -cancer therapy(chemotherapy- radiation therapy) <p>Renal Loss</p> <ul style="list-style-type: none"> - Diuretic use . - Renal artery stenosis. - Hyper aldosteronism . - Hypo kalemic periodic paralysis . - Cushing's syndrome . <p>Cellular shift</p> <p>Insulin over dose</p>

Table 2.5 :Causes of hyper-kalemia 22

Decreases renal excretion <ul style="list-style-type: none">- Renal failure- Addison's disease .- Hypo aldsteronism- Diuretics
Cellular shift <ul style="list-style-type: none">- Red blood cells destruction .- Metabolic or respiratory acidosis .- Transfusion of haemolyzed blood .- Hyper kalemic periodic paralysis .

Table 2.6: Reference values for potassium 22

Adults	3.5 – 5.7 mmol /L
Children	3.4 – 4.7 mmol /L
Infant	4.1 – 5.3 mmol /L
Neonates	3.7 – 5.9 mmol /L

2.6. 3 Calcium

Calcium is a soft gray alkaline earth metal which is essential for living organisms particularly in cell physiology , where movement of the calcium ion Ca^{++} into and out of the cytoplasm , functions as signal for many cellular processes .As a major material used in mineralization of bones and shells , calcium is the most abundant metal by mass in many animals.²⁷

Calcium is an important component of a healthy diet and a mineral necessary for life – it plays an important role in building strong denser bones and teeth . the rest of the calcium in the body has other important roles such as neuro transmitters and muscle contraction.²⁷

2.6.3.1 Regulation of Calcium:

Three hormones are known to regulate blood calcium by altering their secretion rate in response to changes in ionized calcium. These hormones are PTH , vitamin D, and Calcitonin.²⁸

-PTH : secretion in to blood is stimulated by decrease in ionized calcium and conversely . PTH exert three major effects on both bone and kidney. In the bone , PTH activates bone desorption and release calcium by increasing tubular re absorption of calcium ions, Also stimulate renal production of active vitamin D.²⁹

2.6.3.2 Distribution of body calcium:

More than 99% of calcium in the body is part of bone. The remaining 1% is mostly in the blood and other ECF. Very little is in the cytosol of most cells.²⁹

Calcium in blood is distributed among several forms. About 45% circulate as free calcium ions 40% is bound to proteins, mostly albumin, and 15% is bound to anions such as bicarbonate and phosphate.²⁹

2.6.3.3 Calcium disorders:

Although both total calcium and ionized calcium measurements are available ionized calcium is usually a more sensitive and specific marker for calcium disorders.^{29,30}

Table 2.7 : Causes of hypo-calcaemia 23

- | |
|--|
| <ul style="list-style-type: none">- Primary hypo- parathyroidism.- Vitamin D deficiency.- Parathyroid gland aplasia, destruction or removal.- Hypo- magnesemia .- Hypo- albuminemia .- Renal failure .- Pseudo hypo- parathyroid's . |
|--|

Table 2.8 :Causes of hyper-calcaemia 23

<ul style="list-style-type: none">- Primary hyper- parathyroid's .- Renal failure.- Benign familial hypo-calciuria- Malignancy- Prolonged immobilization
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Table 2.9: Reference ranges for calcium 23

Total calcium :

Child:	8.8-10.8 mg/dl (2.2-2.7 mmol/L)
Adult :	8.6-10.0 mg/dl (2.15- 2.50mmol/L)
Urine (24hour):	100-300/day (2.5-7.5mmol/day)

2.6.4 Phosphate

Compounds of phosphate are every where's in living cells and participate in many of the most biological processes. The genetic material deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) are complex phosphodiesteres. Most coenzyme are esters of phosphoric or pyro phosphoric acid. The most important reservoirs of biochemical energy are ATP, creatine phosphate and phosphor enol pyrvate.^{29,30}

The body contains about 17mol (530g) of phosphate. of this 87% is present in the bones the reminder in all cells and soft tissues.³⁰

2.6.4.1 Regulation of phosphate:

Phosphate in the blood may be absorbed in the intestine from dietary sources released from cells in to blood , and lest from bone. In healthy individuals , all these processes are relatively constant and easily regulated by renal excretion or re absorption of phosphate.³⁰ Disturbances to any of these processes can alter phosphate concentration in the blood; however, the loss of regulation by kidneys will have the most profound effect. Although over factors such as vitamin D, calcitonin , growth hormone , and acid – base status can affect renal regulation of phosphate, the most important factor is PTH, which over all lowers blood concentration by inter-casing renal excretion.³⁰

Vitamin D act to increase phosphate in the blood by increasing both phosphate absorption in the intestine and phosphate re absorption in the kidneys.³⁰

Growth hormone, which helps regulation of skeletal growth, can increase blood phosphate by decrease renal excretion.³¹

Table 2.10:Causes of hypo- phosphatemia 22

- Respiratory alkalosis .
- Hyper- para thyroidism .
- Renal tubular defects .
- Decreased net intestinal phosphate absorption .
- Vitamin D deficiency.
- Use of antacid bind phosphate.
- Nutritional recovery syndrome .

Table 2.11:Causes of hyper-phosphatemia 22

- Renal insufficiency .
- Hypo- thyroidism .
- Increase phosphate intake .
- Tumor – lysis syndrome .
- A metabolic or respiratory acidosis .
- Hemolysis .
- Vitamin D intoxication .

Clinical manifestation of hyper - phosphatemia includes Tetany and seizures due to hypo- calcemia .³¹

Table 2.12: Reference ranges for phosphate 22

Neonate :	4.5 – 9.0 mg/dl (1.45 – 2.9 mmol/L)
Child :	4.5- 5.5 0 mg/dl (1.45 – 1.78mmol/L)
Adult :	2.7-4.50 mg/dl (0.87- 1.45mmol/L)
Urine(24 hours)	0.4 – 1.3 g/day.(13-42mmol/ day)

2.7 Background Studies

The effect of breast cancer on electrolyte levels was not performed in Sudan but there are studies were performed in other countries.

A studies were performed by NI Usoro (2010) in Nigeria explain hyper calcimia in breast cancer and no effect on phosphate level, AA Onitilo (2007), in Mexico also take about hyper calcimia in metastatic breast cancer. GL backburn (1997)discuss the effect breast cancer on electrolyte imbalance. Aj Shaik (2011) Japan treat hypo natremia of malignancy. SM Moe (2008), W Wulaningsih (2013) Jaban , Sp Kantrow (2012) in UK and B Garder (1992)take about side effects of cancer therapies lead to sodium and potassium loss. E Shane (2006) in UK take about emergency of metastatic cancer hyper calcimia .

Chapter Three

Material and Methods

3.1 Materials

3.1.1 Study design

This is case – control study .

3.1.2 Study area :

The study was done in radiation and isotope Khartoum center.

3.1.3 Study period

This study was carried during the period from March to June 2014.

3.1.4 Target population :

The study included Sudanese females with breast cancer.

3.1.5 Study population and sample size :

This study was included 50 females breast cancer patients and 50 healthy females as control group both were group matched for age .

3.1.6 Inclusion criteria:

Sudanese females breast cancer patient were included in this study as a case group, and healthy as control group.

3.1.7 Exclusion criteria:

Those non health or using a medication in a test or control group.

3.1.8 Ethical consideration :

* Permission of this study was obtained from the local authorities in the area of the study.

* All samples were taken are used for research purposes only .

3.1.9 Data collection:

Interviews were done to all study groups to obtain the clinical data and to provide health education – clinical assessments were done by a medical doctor.

A questionnaire was specifically designed to obtain information which helps in either including or excluding criteria of individuals in the study.

3.1- 10 Sample collection :

2.5 ml of venous blood was collected from each volunteer using local skin antiseptic (70% ethanol) , disposable plastic syringe poured in Heparin containers , then sample were centrifuged at 3000 rpm to obtain heparin zed plasma.

3.2 Methods

3.2.1 Measurement of sodium and potassium:

The method used was Emission flame photometer.

3.2.1.1 Principle of flame photometer.

Was used compressed air diluted plasma was sprayed as fine mist droplets (neublised) in to a known luminous gas flame which become colored by the characteristic emission of sodium or potassium metallic ions present in the sample.²²

3.2.1.2 Reagents :

* Standard (sodium , potassium).

* Control – normal.

* Control pathological .

* De ionized water .

3.2.1.3 procedure :

Samples were diluted 1:200 by manual dilution .

1. 0.2 ml of sample / standard / control) were added to 19.8ml de ionized water in container .
2. The device was pre washed by de ionized water for 5 min.
3. Fuel knob opened and flame ignited.

4. Zero was adjusted by blank knob with de ionized water .
5. The standard inserted and adjusted at (140mmol/L) for sodium and (5.0 mmol / L) for potassium .
6. Step (4) and (5) were repeated till de ionized water given zero and standard given 140 mmol/L for Na^+ , 5.0 mmol/L for K^+ .The results was obtain directly from the display in mmol/ L .
7. Post washing had been done by putting of the flame and allow the device to work by de ionized water only for 3 min .
8. The gas supply was cut off.
9. The electrical current was stop.

2.2.2 Measurement of calcium :

3.2.2.1 Principle:

Calcium in the sample reacts with methyl thymol blue in alkaline medium to form a colored complex that can be measured by spectro photometry , hydroxyl quinoline is included in the reagent to avoid magnesium interference .²²

3.2.2.2 Reagent composition :

* Potassium cyanide 7.7mmol/L, ethanol amine 1.5mol/L (reagent A)

* Methyl thymol blue 0.1 mmol /L

Hydrochloric acid 10 m mol/ L, hydroxyl quinoline 17m mol/L (reagent 8)²²

3.2.2.3 Standard:

* Calcium standard 10 mg/dl (2.5 m mol/L)

3.2.2.4 Reagent preparation and stability .

- Standard was provided ready to use
- Working reagent Equal volumes of reagent A and B were mixed gently. Stable for 2 days at 2.8°C

3.2.2.5 Linearity range:

This method is linear up to calcium concentration 15mg/dl (3.75 m mol/L).

3.2.2.6 Procedure

1. In labeled test tubes

the following were pipette:

	Blank	Standard	Test
Sample			0.01ml
Calcium standard		0.01ml	
Working reagent	1.0 ml	1.0 ml	1.0 ml

2 The tubes were mixed thoroughly and let to stand for 2minutes at room temperature.

3 The absorbance (A) of the standard and the sample were read at 610nm against the blank . The color is stable at least I heure .

3.2.2.7 Calculations :

$$\text{calcium mg/dl} = \frac{\text{absrobabce of test}}{\text{absorbance of standard}} \times 10\text{mgldl}$$

3.2.3 Measurement of phosphorus:

3.2.3.1 Principle:

Inorganic phosphorus reacts with molybdcic acid forming a phosphor-molybdcic complex.

It's subsequent reduction in alkaline medium originates a blue molybdenum color

The intensity of color formed is proportional to the inorganic phosphrus concentration in the sample.²³

R₁ molybdcic (molybdate – Boorate 1.21 mmol/L

+ (H₂SO₄)

R₂ Catalyzer : 1,2 phenylene di amine 2.59 mmol/L

3.2.3.3 Standard :

Phosphrus aqueous primary standard 5mg/Dl

3.2.3.4 Reagent preparation and stability :

- working reagent : equal volumes of R₁ and R₂ were mixed .

Stable for 24hrs at 2-8°C, protected from light.

3.2.3.5 Linearity range:

This method is linear up to phosphate concentration 15mg/dl

3.2.3.6 Procedure:

1. Instrument was adjusted to zero with distilled water.
2. Into to test tubes pipe He

	Blank	Standard	Test
Phosphrus standard		50μ.L	
Sample			50μL
Working reagent	1.5ml	1.5ml	1.5ml

- 4 Mix , incubate at room temperature for 10min and read against blank at 710 n.m .the clauer is stable for 2hours

3.2.3.7 Calculation :

$$\text{Phosphrus mg/dL} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 5.0\text{mg/dL}$$

Chapter Four

Result

Fifty breast cancer patients and fifty healthy women's as controls were enrolled in this study.

Plasma sodium:

Table 4-1 : Show a significant reduction in the mean of sodium in the test group when compared with control group (p. value :0.00) Table 2 and Figure 4-5 :Show weak negative correlation with duration of the disease (p value = 0.01) (r = -0.25) and no correlation with age (p. value = 0.87).

Plasma potassium:

Table 4-1 : Show a significant reduction in the mean of potassium in the test group when compared with control group (p. value = 0.00) , Table 4-2 and Figure 4-6 :Show weak negative correlation with duration of the disease (p value = 0.00) (r = -0.26) , and no correlation with age (p .value = 0.48).

Plasma Calcium:

Table 4-1 : Show a significant raised in the mean of calcium in the test group when compared with control group (P. value = 0.00),Table4-2 and Figure 4-7:Shows weak positive correlation with duration of the disease (P. value = 0.00) (r= 0.47), and no correlation with age (value = 0.10).

Plasma phosphorus:

Table 4-1 : Show no significant difference between the mean of phosphate in case when compared with control (p. value = 0.74) ,Table4-2 : Show no correlation with duration of disease and age (P. value = 0.722) (p .value = 0.09) respectively.

Table 4.1: mean of plasma electrolytes in women with breast cancer

Parameter	Control Mean \pm SD n=50	Case Mean \pm SD n=50	p.value
Sodium	140 \pm 4.5mmol/L	136 \pm 4. 6 mmol/L	0.00
Potassium	4 +0.59 mmol/L	3.5 \pm 0.59 mmol/L	0.00
Calcium	9.1 \pm 0.56mg/dl	10.7 \pm 1.4 mg/dl	0.00
Phosphate	3.36 \pm 0.53 mg/dl	3.41 \pm 0.78mg/dl	0.74

- Independent sample T- test was used
- P. value considered significant at ≤ 0.05

Table 2: correlation between plasma electrolytes in women with breast cancer with age and duration of breast cancer

Parameter	Age	Duration of breast cancer
Sodium	p. value = 0.87 r= -0.016	p. value = 0.01 r= -0.25
Potassium	p. value = 0.481 r= 0.071	p. value = 0.000 r= - 0.26
Calcium	p. value = 0.101 r=0.165	p. value = 0.00 r=0.47
Phosphate	p. value = 0.722 r= -0.036	p. value = 0.09 r= -0.170

-
- P. value considered significant at ≤ 0.05

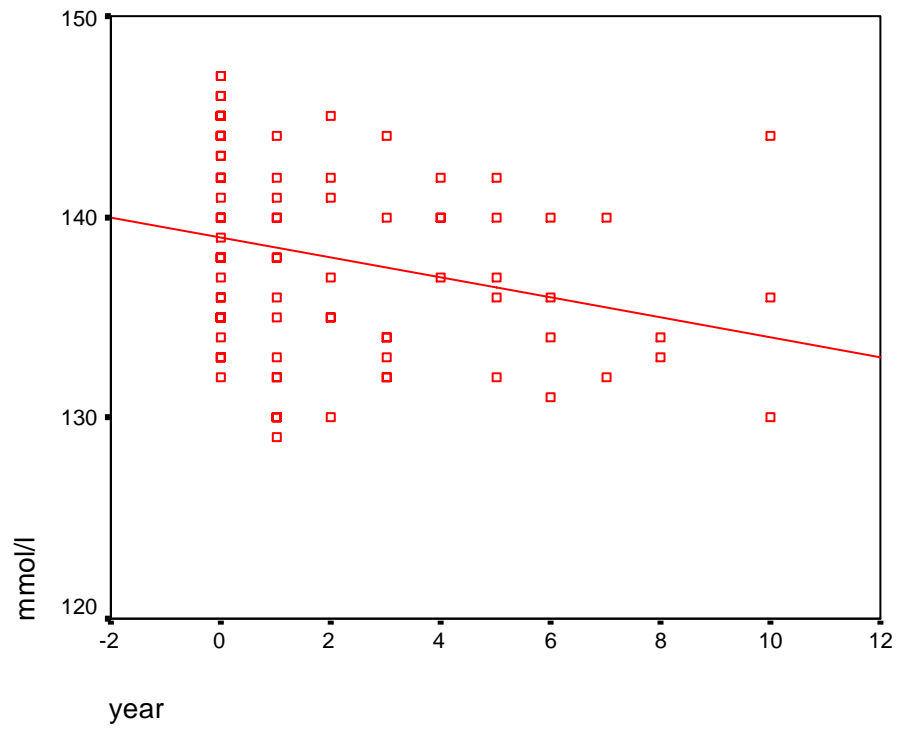


Figure 4.5 : Scatter of Sodium – Duration of breast cancer

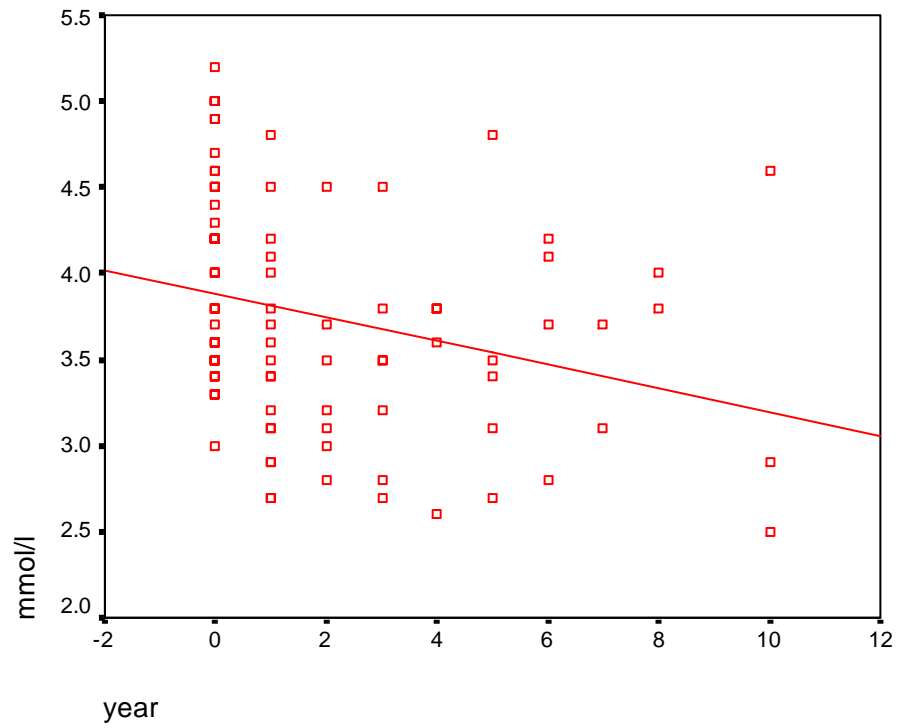


Figure 4.6 : Scatter of potassium – Duration of breast cancer

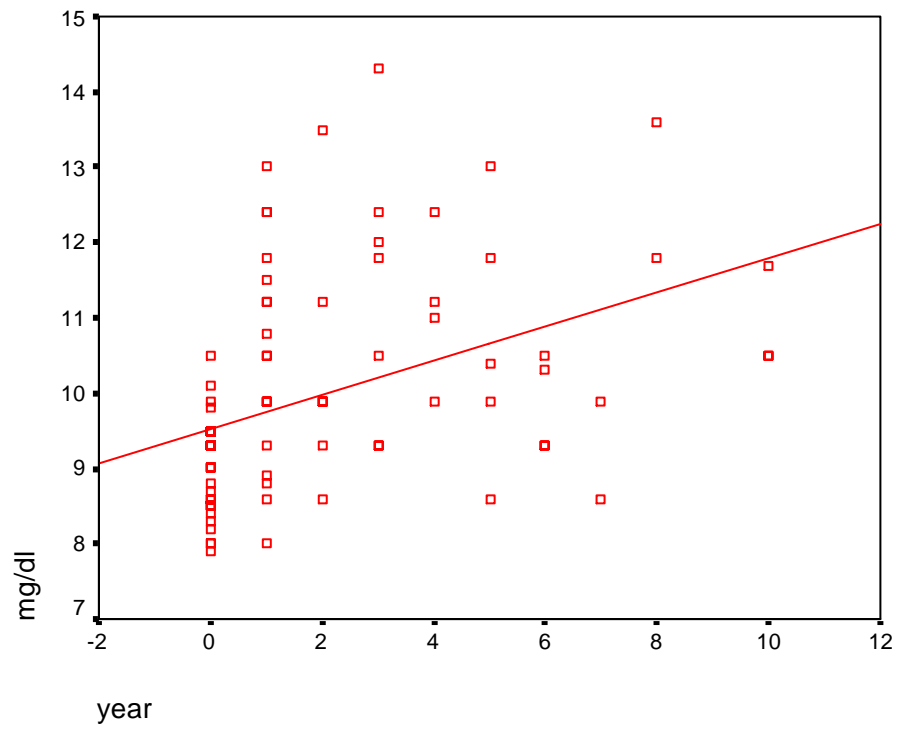


Figure 4.6 : Scatter of calcium – Duration of breast cancer

Chapter five

Discussion, Conclusion and Recommendations

5.1 Discussions:

This study was carried to investigate the plasma levels of sodium ,potassium, calcium and phosphorus in breast cancer patient.

In the present study, plasma sodium and potassium are found to be significantly reduced in patient with breast cancer, these results agree with other studies include W wulaningsih (2013), SP Kantrow(2012),SM Moe(2008) and B Garder (1992). This reduction may be due to loss of them in diarrhea and vomiting occur as aside effects of chemo therapies and radiation therapy in some patients. Which lead to cell death in the GIT, blood , hair and nails .^{20,21}

In this study, plasma calcium found to be significantly increase in breast cancer patient , this result agree with NI Uoro (2010) ,AA Onitilo (2007) and GL Blackburn (1997) . The hyper-calcemia in breast cancer has been attributed in part in to esteolytic bone metastases , the skeletal invasion and destruction by tumor induced by tumor- production of various cytokines such as transforming- growth factor: a'(TGF-O') , tumor necrosis factor o'), interleukin-1 and interleukin2, leads to increasing bone osteolysis and modification of the re-absorption ,excretion and re-sorption of calcium and phosphate ion.^{27,28}

Alteration in humeral regulation of calcium resulting from production of (pTHrp) has also been implicated in tumor associated hyper-calcemia, plasma concentration of (pTHrp) is rarely elevated in healthy individual . (pTHrp) interact with parathyroid hormone receptors on cell membranes, activating adenyl- cyclase which trigger and increase in cyclic AMP production and increase intracellular calcium. These actions are responsible for increasing bone demineralization and elevated serum calcium concentration, decreasing re-absorption of phosphate in the proximal renal tubules, increasing calcium re-absorption in the distal tubules.^{29,30}

In the current study, plasma phosphorus showed no significant difference in breast cancer patient. That means it's not effect by breast cancer, this result agree with NI Uoro (2010).³⁰

5.2 Conclusion:

According to the results of this study:

1. Plasma sodium is significantly reduced in breast cancer patients has weak negative correlation with duration of the disease and no correlation with age.
2. Plasma potassium is significantly reduced in breast cancer has weak negative correlation with duration of the disease, and no correlation with age.

3. Plasma calcium is significantly increase in breast cancer have weak positive correlation with duration of the disease and no correlation with age.
4. Plasma phosphorus is not significantly changed in breast cancer and has no correlation with duration of disease and age.

5.3 Recommendations:

1. Measurement of plasma sodium, potassium and calcium should be done regularly in patient with breast cancers.
2. Breast cancer patients may require sodium, potassium supplement to compensate the loss.
3. More studies are needed to clarify the effect of breast cancer in electrolytes imbalance .

Chapter Six

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