

1.1 Introduction

The kidneys are two bean-shaped organs, each about the size of a fist. They are located just below the rib cage, one on each side of the spine. Every day, the two kidneys filter about 120 to 150 quarts of blood to produce about 1 to 2 quarts of urine, composed of wastes and extra fluid. The urine flows from the kidneys to the bladder through two thin tubes of muscle called ureters, one on each side of the bladder. In men the urethra is long, while in women it is short (Rockville *et al*, 2009). Renal failure is divided into acute renal failure (ARF) or chronic renal failure (CRF).

Acute renal failure is a sudden, sharp decline in renal function as a result of an acute toxic or hypoxic insult to the kidneys. Generally, acute renal failure occurs as a consequence of lower urinary tract obstruction or rupture of the urinary bladder. Chronic Renal Failure is a clinical syndrome that occurs when there is a gradual decline in renal function over time. (Michael *et al*, 2010). Dialysis is the process of removing waste products and excess fluid from the body. There are two types of dialysis: hemodialysis and peritoneal dialysis.

Trace elements or micronutrients are elements present in minute amounts in the body, many of which are essential in metabolism or for the manufacture of essential compounds, such as zinc (Zn), selenium (Se), iron(Fe), cobalt(Co), magnesium (Mg), manganese (Mn), chromium(Cr), copper(Cu).

Magnesium is one of the body's major electrolytes. It is the second most common intracellular cation in the body and plays a crucial role in deoxyribonucleic acid (DNA), and protein synthesis. In addition, magnesium

is a necessary cofactor for most enzymes in phosphorylation reactions and parathyroid hormone synthesis. (Mazidi *et al*, 2008)

Magnesium plays a key role in maintaining internal homeostasis through actions in the musculoskeletal, nervous, endocrine and cellular messenger systems, synthesis of carbohydrates, proteins, lipid and nucleic acid. Renal excretion is the major route of magnesium elimination from the body. A positive magnesium balance would be expected in renal failure. However, a compensatory decrease in tubular reabsorption is expected to operate to maintain adequate urinary magnesium excretion even when glomerular filtration rate is very low.

Patients with end-stage renal disease and those on dialysis have impaired regulatory mechanisms, predisposing them to disturbances in magnesium levels. The effects of high or low magnesium can have deleterious health outcomes, which impact on the co-morbidities and outcomes of chronic renal disease.(John *et al*, 2015)

Zinc is second to iron as the most abundant trace elements in the body (Robert *et al*, 2009), it is an essential mineral, it is required for over 300 enzymic reactions in the body.(Fox *et al*, 2001), transmission, and regulation of the expression of genetic information synthesize deoxyribonucleic acid(DNA), storage, , and action of peptide hormones and structural maintenance of chromatin and biomembranes. Zn is thus needed for healthy growth and development, protein and DNA synthesis, neuro-sensory functions, cell-mediated immunity, thyroid, and bone metabolism. (Coudray *et al*, 2005)

Also it is necessary to reproduction, and development of the epidermis and central nervous system(CNS), essential for wound healing , supports the childhood and pregnancy.

The changes in the levels of zinc absorption and excretion are the crucial mechanism for sustaining zinc homeostasis.

Lower zinc level in chronic kidney disease (CKD) patients is common and it might happen due to the increased urinary zinc excretion and the decreased intestinal zinc absorption. (Forough *et al*, 2014).

1.2 Rationale

Renal failure is a devastating medical , social and economic problem in Sudan and it is fatal unless treated properly.

According to the latest WHO data published in April 2011 Kidney Disease Deaths in Sudan reached 8,782 or 2.38% of total deaths, ranked the renal failure in 7th top 20 causes of death in Sudan.

Certain trace element and minerals are essential for normal metabolism of proteins, carbohydrates, and lipids. Any changes in elements may result in metabolic alteration , Zinc and magnesium have gained a lot of importance specially in case of renal failure

There is a strong association between hypozincaemia, hypermagnesaemia and renal failure which raises the question of whether this disturbance in zinc and magnesium levels are related to chronic kidney disease factors or to the hemodialysis treatment. Some researchers have assumed that there is a correlation between the serum zinc, magnesium and the severity of renal failure caused by glomerular lesions. So this study conducted to find out the disturbance of zinc and magnesium levels and their relation to renal failure .

1.3 Objectives

General objectives

To assess plasma levels of magnesium and zinc in Sudanese patients with renal failure under hemodialysis .

Specific objectives

- To determine the levels of magnesium and zinc in Sudanese patients with renal failure under hemodialysis compared to control group.
- To correlate between the duration of dialysis and plasma magnesium and zinc in patients with renal failure under hemodialysis.
- To correlate between age and plasma magnesium and zinc in patients with renal failure under hemodialysis.

2. Literature Review

2.1 The kidney

The kidneys are vital organs that perform a variety of important functions. The most prominent functions are removal of unwanted substances from plasma (both waste and surplus), homeostasis (maintenance of equilibrium) of the body's water, electrolyte and acid-base status, and participation in hormonal regulation. In the clinical laboratory, kidney function tests are used in assessment of renal disease, water balance, and acid-base disorders and in situations of trauma, head injury, surgery, and infectious disease. (Michael *et al*, 2005).

2.1.1 Renal Anatomy

The kidneys are paired, bean-shaped organs located retroperitoneally on either side of the spinal column. Macroscopically, a fibrous capsule of connective tissue encloses each kidney. When dissected longitudinally, two regions can be clearly discerned—an outer region called the cortex and an inner region called the medulla. It is a basin like cavity at the upper end of the ureter into which newly formed urine passes. The bilateral ureters are thick-walled canals, connecting the kidneys to the urinary bladder. Urine is temporarily stored in the bladder until voided from the body by way of the urethra. Functional units of the kidney that can only be seen microscopically. Each kidney contains approximately 1 million nephrons. Each nephron is a complex apparatus comprised of five basic parts expressed diagrammatically.

■ The glomerulus—a capillary tuft surrounded by the expanded end of a renal tubule known as Bowman's capsule. Each glomerulus is supplied by

an afferent arteriole carrying the blood in and an efferent arteriole carrying the blood out. The efferent arteriole branches into peritubular capillaries that supply the tubule.

- The proximal convoluted tubule—located in the cortex.
- The long loop of Henle—composed of the thin descending Limb, which spans the medulla, and the ascending limb, which is located in both the medulla and the cortex, composed of a region that is thin and then thick.
- The distal convoluted tubule—located in the cortex.
- The collecting duct—formed by two or more distal convoluted tubules as they pass back down through the cortex and the medulla to collect the urine that drains from each nephron. Collecting ducts eventually merge and empty their contents into the renal pelvis. The following section describes how each part of the nephron normally functions.(Michael et al, 2010).

2.1.2Renal Functions

- Urine formation
- Regulatory function
 - Electrolytes homeostasis
 - Water homeostasis
 - Acid- base balance.
- Excretion of the waste products of protein metabolism
- Excretion of drugs and toxins
- Secretion of hormones

- Renin
- Erythropoietin
- 1,25-Dihydroxy vitamin D3
- Prostaglandins and thromboxanes.(Michael *et al*, 2010).

2.1.2.1 Assessment of renal function

- -Serum urea is a poor marker of renal function, because it varies significantly with hydration and diet, is not produced constantly and is reabsorbed by the kidney
- -Serum creatinine also has significant limitations. The level can remain within the normal range despite the loss of over 50% of renal function-GFR
- Plasma glucose: to detect undiagnosed diabetes or assess control of diabetes-
- Serum potassium is raised, serum sodium is usually normal, but may be low, serum bicarbonate is low, alkaline phosphatase is raised when bone disease develops, serum parathyroid hormone is rises progressively with declining renal function and dyslipidaemia is common in chronic renal disease.
- Hemoglobin falls with progressive chronic kidney disease , white cells and platelets are usually normal.(Levey *et al*, 2012)

2.1.3 Renal failure

2.1.3.1 Acute renal failure

Acute renal failure is a sudden, sharp decline in renal function as a result of an acute toxic or hypoxic insult to the kidneys, defined as occurring when the glomerular filtration rate (GFR) is reduced to less than 10 mL/minute. This syndrome is subdivided into three types, depending on the location of the precipitating defect.

- Prerenal failure: The defect lies in the blood supply before it reaches the kidney. Causes can include cardiovascular system failure and consequent hypovolemia.
- Primary renal failure: The defect involves the kidney. The most common cause is acute tubular necrosis; other causes include vascular obstructions/inflammations and glomerulonephritis.
- Postrenal failure: The defect lies in the urinary tract after it exits the kidney. Generally, acute renal failure occurs as a consequence of lower urinary tract obstruction or rupture of the urinary bladder.(Michael *et al*, 2010).

2.1.3.2 Chronic renal failure

Chronic kidney disease is the reduced ability of the kidney to carry out these functions in the long-term. This is most often caused by damage to the kidneys from other conditions, most commonly diabetes and high blood pressure. There is evidence that treatment can prevent or delay the progression of chronic kidney disease, reduce or prevent the development of complications, and reduce the risk of cardiovascular disease (CVD).

The Chronic renal failure is based on the presence of kidney damage (i.e. albuminuria) or decreased kidney function (i.e. glomerular filtration rate (GFR) <60 ml/minute per 1.73 m^2) for three months or more, irrespective of clinical diagnosis. (Levey *et al*, 2012).

2.1.3.3 Causes of renal failure

Poorly controlled diabetes, Poorly controlled high blood pressure, Chronic glomerulonephritis, Polycystic kidney disease, Reflux nephropathy (damage caused by urine backflow from the bladder into the ureters and kidney), Nephrotic syndrome, Alport's disease, Kidney stones, and Prostate disease. (Benjamin, 2011). Arteriopathic renal disease, Hypercalcaemia, Multisystem diseases with potential kidney involvement - eg, systemic lupus erythematosus (SLE), Neoplasms, and Myeloma. (Levey *et al*, 2012)

2.1.3.4 Risk factors of chronic kidney disease

Risk factors include:

- Cardiovascular disease (CVD)
- Proteinuria.
- Acute kidney injury (AKI)
- Hypertension.
- Diabetes.
- anaemia
- Smoking.
- Dyslipidemia.

- Elevated body mass index.
- Chronic use of non-steroidal anti-inflammatory drugs (NSAIDs).
- Untreated urinary outflow tract obstruction(Adeera,2001 Loretta *et al*, 2010).

2.1.3.5Symptoms of chronic renal failure

- Patients with chronic renal failure stages 1-3 (GFR >30 mL/min/1.73 m²) are generally asymptomatic. Typically, it is not until stages 4-5 (GFR < 30 mL/min/1.73 m²) that endocrine/metabolic derangements or disturbances in water or electrolyte balance become clinically manifestation.
- Lethargy
- Weakness
- Shortness of breath
- Generalized swelling (edema)
- Generalized weakness due to anemia
- Loss of appetite
- Fatigue
- Congestive heart failure
- Metabolic acidosis
- High blood potassium (hyperkalemia)

- Fatal heart rhythm disturbances (arrhythmias) including ventricular tachycardia and ventricular fibrillation.
- Rising urea levels in the blood (uremia) may lead to brain encephalopathy, pericarditis (inflammation of the heart lining), or low calcium blood levels (hypocalcemia). (Benjamin, 2015).

2.1.3.6 Classification of chronic kidney disease

Kidney function should be assessed using a combination of GFR and albumin:creatinine ratio (ACR) categories. Increased ACR and decreased ACR are associated with increased risk of adverse outcomes.

Stage 1

Slightly diminished function; kidney damage with normal or relatively high GFR (≥ 90 ml/min/1.73 m²).

Stage 2

Mild reduction in GFR (60–89 ml/min/1.73 m²) with kidney damage: Kidney damage is defined as pathological abnormalities or markers of damage, including abnormalities in blood or urine test or imaging studies.

Stage 3

Moderate reduction in GFR (30–59 ml/min/1.73 m²) British guidelines distinguish between stage 3A (GFR 45–59) and stage 3B (GFR 30–44) for purposes of screening and referral.

Stage 4

Severe reduction in GFR (15–29 ml/min/1.73 m²) Preparation for renal replacement therapy

Stage 5

Established kidney failure (GFR <15 ml/min/1.73 m²) permanent renal replacement therapy. (Waknine, 2012).

There is strong association between renal failure disease; GFR, glomerular filtration rate.

The available therapeutic options for ESRD are life-long, complex, and costly. These include kidney allograft transplantation, haemodialysis (HD), and peritoneal dialysis (PD). (Houshang *et al*, 2015)

2.1.4 Dialysis

Dialysis is defined as the diffusion of molecules in solution across a semipermeable membrane along an electrochemical concentration gradient. (Jonathan, *et al*, 2010)

2.1.4.1 Type of dialysis

There are two types of dialysis; 1) hemodialysis, and 2) peritoneal dialysis further broken down into two main types: continuous ambulatory peritoneal dialysis (CAPD) and automated peritoneal dialysis (APD).

2.1.4.2 Hemodialysis

Hemodialysis is the most common form of treatment for end-stage renal disease (ESRD), and is associated with considerable morbidity and mortality due to accelerated cardiovascular disease and infection. involves using an artificial kidney, known as a hemodialyzer, to remove waste and chemicals from the blood. It accesses the blood through a minor surgical procedure in the arm or leg, or through a plastic tube in the neck called a catheter.

Hemodialysis removes uremic toxins primarily by allowing equilibration of plasma and dialysate across a semi-permeable membrane. Dialysate is created by adding carefully regulated quantities of biologically essential ions such as potassium, sodium, bicarbonate, and calcium to water that has been treated to reduce solutes to very low levels. The dialysate concentration of other substances such as trace elements is not routinely manipulated. Substances that have lower concentrations in dialysate than in blood tend to be removed by dialysis. Although this is appropriate in the case of uremic toxins, it may lead to depletion of biologically essential substances. Besides the potential for ongoing removal of trace elements by dialysis, hemodialysis patients are at risk for low dietary intake of such substances due to uremia-related anorexia and dietary restrictions (Marcello et al, 2009). Hemodialysis patients are exposed to very high volumes (>300 liters/week) of dialysate. Dialysis treatments normally occur three times a week and last a few hours at a time. Most commonly, patients travel to an outpatient center to have dialysis, but home dialysis therapy is becoming an option for some. Outpatient dialysis is available on some cruise ships. They are equipped with dialysis machines with trained health care professionals ready to care for those with kidney failure while traveling. (Benjamin, 2015).

2.1.4.3Dialyzer

The dialyzer is a large canister containing thousands of small fibers through which blood is passed. Dialysis solution, the cleansing fluid, is pumped around these fibers. The fibers allow wastes and extra fluids to pass from blood into the solution, which carries them away. The dialyzer is sometimes called an artificial kidney

2.1.4.4 Peritoneal dialysis

Uses the lining of the abdominal cavity as the dialysis filter to rid the body of waste and to balance electrolyte levels. A catheter is placed in the abdominal cavity through the abdominal wall by a surgeon, and it is expected to remain in place for the long-term. The dialysis solution is then dripped in through the catheter and left in the abdominal cavity for a few hours and then is drained out. In that time, waste products leech from the blood flowing through the lining of the abdomen (peritoneum), and attach themselves to the fluid that has been instilled by the catheters. Often, patients instill the dialysate fluid before bedtime, and drain it in the morning. (Benjamin, 2015).

The two main types of peritoneal dialysis are:

- continuous ambulatory peritoneal dialysis (CAPD)
- automated peritoneal dialysis (APD).

2.2 Magnesium

Magnesium is the fourth most abundant cation in the body and it is the second most intracellular cation after potassium. Magnesium ions regulate over 300 biochemical reactions in the body through their role as enzyme co-factors. it is an important cation in the body The central role of magnesium within the chlorophyll and as cofactor for enzymes in the 12-transphosphorylation reaction in photosynthesis makes it probably the most inorganic element in the production of foods and fossil fuel Magnesium is absorbed in the ileum and excreted in stool and urine. The minimum daily requirement of magnesium is 300-350 mg, or 15 mmol; this amount is easily obtainable with a normal daily intake of fruits, seeds, and vegetables because

magnesium is a component of chlorophyll and is present in high concentrations in all green plants. (Mazidi et al, 2008)

The total body magnesium content is approximately 25 g (1.03 mol), of which about 55% resides in the skeleton. One third skeletal magnesium is exchangeable and is thought to serve as reservoir for maintaining extracellular magnesium concentration. about 45% of magnesium is intracellular (Carl et al, 2006)

2.2.1 Physiological role of magnesium in the body

The body of most animals contains ~0.4 g magnesium/kg. The total magnesium content of the human body is reported to be ~20 mmol/kg of fat-free tissue. In other words, total magnesium in the average 70 kg adult with 20% (w/w) fat is ~1000 to 1120 mmol or ~24 g. These values should be interpreted with caution, however, as analytical methods differ considerably throughout the years. In comparison, the body content of calcium is ~1000 g (i.e. 42 times greater than the body content of magnesium). (Wilhelm et al, 2012)

Magnesium is an important element for several physiologic processes in humans, as the maintenance of bone health and the well functioning of the nervous system, energy metabolism and the synthesis of proteins DNA and RNA. The magnesium storage in healthy adults amounts to 24 g (60–65%) (2000 mEq), magnesium is mainly stored at the bone level and to a lesser extent into skeletal muscles (25–30%) and in other soft tissues (Mg) magnesium tissues (10–15%). Only 5–10% of the intracellular free, while the remaining is bound to proteins, citrates, RNA and DNA. Consequently, circulating Mg represents only 1% of the total body

contents, ranging between 0.62 and 1.02 mmol/L, where the 60% of this circulates as the biologically active free cation, while the remaining 40% is protein bound or complexed as salts. A neutral balance of Mg requires a daily intake of 0.5–0.7 mEq/Kg of Mg, which is mainly present in .cereals, nuts, legumes and green vegetables

Intracellular magnesium concentrations range from 5 to 20 mmol/L ; 1–5% is ionized, the remainder is bound to proteins, negatively charged molecules and adenosine triphosphate (ATP) . Extracellular magnesium accounts for ~1% of total body magnesium which is primarily found in serum and red blood cells (RBCs)

Serum magnesium can—just like calcium—be categorized into three fractions. It is either free/ionized, bound to protein or complexed with anions such as phosphate, bicarbonate and citrate or sulphate Of the three fractions in plasma, however, ionized magnesium has the greatest biological activity . (Wilhelm et al,2012)

Magnesium is primarily found within the cell where it acts as a counter ion for the energy-rich ATP and nuclear acids. Magnesium is a cofactor in >300 enzymatic reactions .Magnesium critically stabilizes enzymes, including many ATP-generating reactions .ATP is required universally for glucose utilization, synthesis of fat, proteins, nucleic acids and coenzymes, muscle contraction, methyl group transfer and many other processes, and interference with magnesium metabolism

also influences these functions . Thus, one should keep in mind that ATP metabolism, muscle contraction and relaxation, normal neurological function and release of neurotransmitters are all magnesium dependent. It is also important to note that magnesium contributes to the regulation of vascular tone, heart rhythm, platelet-activated thrombosis and bone formation . (Wilhelm *et al*,2012)

2.2.2Clinical Consequences of Alterations in Magnesium Balance

2.2.2.1Hypomagnesemia.

Hypomagnesemia is usually defined as serum magnesium<0.7 mmol/L, 1.4 mEq/L, or 1.7 mg/dl. Biochemical hypomagnesemia is common, with a prevalence of up to 15% in the general population and up to 65% in patients in the intensive care units.

Magnesium deficiency has been demonstrated in 7-11% of hospitalized patients and is found to coexist in up to 40% of patients with other electrolytes abnormalities , particularly hypokalemia which is mediated by stimulation of the renal outer medullary potassium (ROMK) channel resulting in increased potassium

excretion. and to lesser extend hyponatremia or hypocalcemia secondary to impaired PTH release and PTH resistance.. Secondary electrolytes abnormalities plays a key role in clinical feature of magnesium depletion. Among the endocrine and metabolic disorders associated with magnesium deficiency.

Hypomagnesemia can be secondary to impaired intestinal magnesium

absorption or increased urinary magnesium excretion secondary to various hormones or drugs that inhibit magnesium reabsorption. At the clinical level, the assessment of magnesium stores and cause of magnesium deficiency continues to be a real challenge.

Simultaneous measurements of serum and urine magnesium may help differentiate the cause of hypomagnesemia.

Although proton pump inhibitors most likely cause impaired intestinal magnesium absorption, most of the other drugs associated with hypomagnesemia impair renal tubular magnesium reabsorption by direct or indirect inhibition of magnesium reabsorption in the thick ascending limb or the distal convoluted tubule .

Clinical manifestations of hypomagnesemia include weakness and fatigue, muscle cramps, tetany, numbness, seizures, increase blood pressure which can predispose to cardiac arrhythmias and sudden death. (Wilhelm *et al*, 2012)

The causes of magnesium depletion and hypomagnesemia are decreased gastrointestinal (GI) absorption and increased renal loss. Decreased GI absorption is frequently due to diarrhea, malabsorption, and inadequate dietary intake. Common causes of excessive urinary loss are diuresis due to alcohol, glycosuria, and loop diuretics.

Medical conditions putting persons at high risk for hypomagnesemia are alcoholism, congestive heart failure, diabetes, chronic diarrhea, hypokalemia, hypocalcemia, and malnutrition.(David *et al*, 2005)

2.2.2.2Hypermagnesemia.

Hypermagnesemia is caused by ingestion and increased intestinal absorption of Epsom salts and magnesium-containing cathartics, antacids, laxative abuse, and enemas. In addition, overzealous intravenous or intramuscular injection of magnesium for treatment of preeclampsia can also result in hypermagnesemia.

Hypermagnesemia is associated with nausea, vomiting, neurologic impairment, hypotension, and electrocardiography changes .

At higher levels due to intoxication, complete heart block, respiratory paralysis, coma, and shock can occur.

Maintenance of normal serum levels of calcium, phosphorus, and magnesium depends on a complex interplay between absorption from the gut, exchange from bone stores, and renal regulation. Renal reabsorption of calcium, phosphorus, and magnesium occurs in several different parts of the nephron and involves a number of channels, transporters, and paracellular pathways, some of which remain to be defined. The Kidneys are important and the main organ involved in magnesium homeostasis. Magnesium absorption occurs primarily in the proximal tubule and thick ascending limb of the loop of Henle. Kidney is very effective in excreting excess magnesium. Therefore, hypermagnesemia is very rare and symptomatic hypermagnesemia is even rarer in absences of renal insufficiency. This case illustrates the potential for severe hypermagnesemia in patients with normal renal function and the importance of a complete history. Recognition of Hypermagnesemia and its effects is critical to institution of appropriate therapy and prevention of its lethal effects(Mazidi *et al*, 2008)

The importance of the kidney in maintaining normal calcium, phosphorus, and magnesium homeostasis can be seen in renal failure in which abnormalities of calcium, phosphorus, and magnesium levels are very common clinical findings. (Wilhelm *et al*, 2012)

2.3Zinc

The importance of zinc for normal growth and survival of plant and animals was recognized a long time ago. Yet the existence of its deficiency in human was doubted because element's ubiquitous distribution in the environment and the lack of obvious clinical signs of deficiency. zinc deficiency in humans is now known to be an important malnutrition problem world-wide. It is more prevalent in areas of high cereal and low animal food consumption (Nazanin et al, 2013). The diet may not necessarily be low in zinc, but its bio-availability plays a major role in its absorption.

Phytic acid is the main known inhibitor of zinc. Compared to adults, infants, children, adolescents, pregnant, and lactating women have increased requirements for zinc and thus, are at increased risk of zinc depletion. Nevertheless, evidence of human deficiency began to emerge during 1960s, when case of zinc-responsive dwarfism and delayed sexual maturation were first reported in in Egyptian adolescent 45. Since then a number of intervention trial have been carried out to assess the impact of zinc supplementation particularly in low-income population who are likely to suffer from zinc deficiency . 46 results of these studies have shown that zinc supplementation reduces the prevalence of common childhood infections.

2.3.1Absorption of zinc

Zinc is absorbed in the small intestine by a carrier-mediated mechanism. Under normal physiologic conditions, transport processes of uptake are not saturated. The fraction of zinc absorbed is difficult to determine because zinc is also secreted into the gut. Zinc administered in aqueous solutions to fasting subjects is absorbed efficiently (60-70%), whereas absorption from solid diets is less efficient and varies depending on zinc content and diet composition. Generally, 33% is accepted as the average zinc absorption in humans). More recent studies have suggested different absorption rates for different population groups based on their type of diet and phytate: Zinc molar ratio. Zinc absorption is concentration dependent and increases with increasing dietary zinc up to a maximum rate. In addition, zinc status may influence zinc absorption. Zinc-deprived humans absorb this element with increased efficiency, whereas humans on a high-zinc diet show a reduced efficiency of absorption. Zinc is released from food as free ions during digestion. These liberated ions may then bind to endogenously secreted ligands before their transport into the enterocytes in the duodenum and jejunum. Specific transport proteins may facilitate the passage of zinc across the cell membrane into the portal circulation. With high intakes, zinc is also absorbed through a passive paracellular route. The portal system carries absorbed zinc directly to the liver, and then released into systemic circulation for delivery to other tissues. About 70% of the zinc in circulation is bound to albumin, and any condition that alters serum albumin concentration can have a secondary effect on serum zinc levels. Although, serum zinc represents only 0.1% of the whole body zinc, the circulating zinc turns over rapidly to meet tissue needs. (Nazanin *et al*, 2013)

2.3.2 Psychological functions of zinc

About 90% of the total brain Zinc is tightly bound to metalloproteins.

Zinc In the adult brain is located in the cerebral cortex, the 'thinking' part of the brain. This region includes the hippocampus, which is assumed to play a role in episodic memory and spatial ability, and the amygdala or 'feeling' part of the brain. Zinc is found in the presynaptic vesicles of glutamatergic neurons, which use glutamate as a transmitter. The role of Zinc in these neurons is controversial but may include participation in the storage, release and uptake of glutamate, and modulation of glutamate receptors.

Zinc can act as a neuromodulator or neurotransmitter . As Zinc deprivation may influence brain Zn homeostasis, it is an important nutrient for the brain function . Evidence from the available literature suggests that both deficiency and excess of Zn may have profound positive and negative consequences, respectively, on human behaviour.

Serum Zinc concentrations have been associated with impaired cognitive function in older individuals . Research has shown that certain micronutrients, including Zinc, are significantly depleted in depressed patients, and Zinc depletion has also been implicated in mood disorders. Furthermore, older studies of human subjects reported that Zinc deficient individuals have declined taste acuity which can be restored by Zinc supplementation however, the literature on this topic appears contradictory. Zinc deficiency has also been identified as a possible contributor to loss of appetite and anorexia , by inhibiting the release of neuropeptide Y (NPY), which is required for receptor activation. Indeed, neuropeptide Y (NPY) regulates a wide variety of physiologic functions and it is also known to act as an orexigen (a stimulator of food intake) (Coudray *et al*, 2005)

2. 3.3Zinc toxicity

Excessive zinc intake will eventually affect the balance and proper ratios to numerous other important nutrients that may include iron, calcium, selenium, nickel, phosphorus, copper, as well as Vitamin A, B1,C, and others.

Long term overdosing on zinc may also cause, or contribute to gastrointestinal problems, hair loss, anemia, loss of libido, impotence, prostatitis, ovarian cysts, menstrual problems, depressed immune functions, muscle spasms, sciatica, renal tubular necrosis / interstitial nephritis, dizziness and vomiting, among others.

Zinc toxicity may also (in doses > 80 mg/day) decrease levels of HDL-cholesterol and white blood cells. Impaired cholesterol metabolism may also result from excess intake of zinc supplements.

The upper limit of safety for zinc established by the Food and Nutrition Board of the Institute of Medicine is 40 milligrams daily for adults.

2.3.4Zinc deficiency

Zinc is one of the essential trace elements and, as such, a member of one of the major subgroups of the micronutrients that have attained such prominence in human nutrition and health.

Clinical presentation of deficiency disease is varied , non specific and related to the degree and duration of depletion . sings and symptoms include

increased incidence of infection , alteration in immune function , diarrhea , altered cognition , defects in carbohydrate use , reproductive teratogenesis , skin lesions and alopecia and other adverse clinical outcomes (Carl e *et al*, 2006)

2.3.5 Individuals at risk of zinc deficiency

Individuals with chronic renal disease, Premature and low-birth-weight infants, Older breast-fed infants and toddlers with inadequate intake of zinc-rich complementary foods, Children and adolescents, Pregnant and lactating (breast-feeding) women, especially adolescents, Patients receiving total parenteral nutrition (intravenous feedings), Malnourished individuals, including those with protein-energy malnutrition and anorexia nervosa, Individuals with severe or persistent diarrhea, Individuals with malabsorption syndromes, including celiac disease and short bowel syndrome, Individuals with inflammatory bowel disease, including Crohn's disease and ulcerative colitis, Alcoholics and those with alcoholic liver disease who have increased urinary zinc excretion and low liver zinc levels, Individuals with chronic renal disease, Individuals with sickle cell anemia, Individuals who use medications that decrease intestinal zinc absorption, increases zinc excretion, or impair zinc utilization, and older adults (65 years and older) and Strict vegetarians: The requirement for dietary zinc may be as much as 50% greater for strict vegetarians whose major food staples are grains and legumes, because high levels of phytic acid in these foods reduce zinc absorption (Jane ,2001)

2.3.6 Blood zinc and chronic renal failure

Hypoalbuminemia is common in patients with chronic renal insufficiency, and in patients with end stage renal disease (ESRD) treated with peritoneal and hemodialysis. The causes of the decreased plasma zinc concentration in these patients are not completely understood, but poor nutritional zinc intake, diets associated with attempts to prevent the progression of renal failure, prescribed mineral supplements and medications, hypoalbuminemia, diminished gastrointestinal zinc absorption, increased urinary zinc excretion and redistribution of zinc into the intracellular fluid may all affect extracellular fluid zinc concentration and total body zinc stores in patients with chronic renal insufficiency. Zinc intake is probably the most important determinant of total body zinc status and plasma zinc concentration in these patients. The extent of true zinc depletion in this population is unknown, but it is likely to be an important problem.

Patients with ESRD treated with hemodialysis may have similar reasons for developing hypoalbuminemia, although it is unlikely that urinary losses play an important role in the development of negative zinc balance in this group. Although there is little evidence that hemodialysis results in net zinc losses, treatment effects may result in the redistribution of zinc from the extracellular fluid, resulting in the development of hypoalbuminemia. Because such patients appear to be susceptible to the development of negative zinc balance due to gastrointestinal zinc losses, it would seem that they require increased levels of zinc intake. The similarity of the symptoms of zinc deficiency and uremia have prompted several studies of the effects of zinc supplementation on taste acuity, sexual, immune and neurologic function in hemodialysis patients, with some positive results.

Patients with ESRD treated with CAPD may have hypozincemia as well, for reasons similar to those suggested in patients treated with hemodialysis. Despite protein losses in the peritoneal dialysate, net influx of zinc occurs during peritoneal dialysis.(Paul, 1989)

3.Materials and Methods

3.1 Materials

3.1.1 Study approach

A quantitative method was used to measure magnesium, and zinc in Sudanese patients with renal failure under hemodialysis in Khartoum state, during the period from May to September 2015.

3.1.2 Study design

This is a case control study.

3.1.3 Study area

This study was conducted in Sudanese Kidney Transplanted Association hospital and Alnaw teaching hospital in Khartoum state.

3.1.4 Target population:

The study included patients with renal failure under hemodialysis.

3.1.5 Inclusion and Exclusion criteria:

Sudanese male and female patients with renal failure under hemodialysis a test group and apparently healthy volunteers and as control group were

included while patient with liver diseases, inflammatory diseases, malabsorption syndromes, sickle cell disease hepatitis positive were excluded.

3.1.6 Sample size

A total of 60 patients with renal failure were enrolled in this study, and 30 apparently healthy volunteers (age and sex matched with the test group) were included to serve as control.

3.1.7 Ethical consideration

A consent was taken regarding acceptance to participate in the study and reassurance of confidentiality. Before the specimen was collected, the donor knew that this specimen was collected for research purpose.

3.1.8 Data collection

The Clinical data were obtained from history, clinical examination and hospital follow up records and were recorded on a questionnaire sheet.

3.1.9 Sample collection and processing

Antiseptic was done for the skin (70%), 3 ml of venous blood was collected from the forearm of each patient and directly into centrifuge tube which contained heparin anticoagulant for plasma preparation. plasma was separated from blood cells after centrifugation for 5 minutes at 5000 r.p.m at room temperature and the plasma were used immediately for estimation of zinc and magnesium.

3.1.10 Requirements

Sterile needle

70% alcohol, Cotton

Plain and heparin containers

Constant temperature

Cuvette, Test tubes.

Distilled water

Atomic Absorption spectrophotometer Model 210 VGP Buck Scientific

Cobas c 311 autoanalyzer.

Automatic pipette

Blue and yellow tip.

Centrifuge.

3.2Methods

3.2.1 Estimation of Zinc:

Zinc was estimated by Atomic Absorption spectrophotometer

3.2.1.1 Principle of the reaction

The basic principle is that light is passed through a collection of atoms. If the wavelength of the light has energy corresponding to the energy difference between two energy levels in the atoms, a portion of the light will be absorbed. The relationship between the concentration of atoms, the distance the light travels through the collection of atoms, and the portion of the light absorbed is given by the Beer-Lambert law.

3.2.1.2 Procedure:

Appendix II

3.2.1.3 Calculation:

Zinc (mg/l) = (A° sample/ A° stander) * conc. Stander* DF

A =absorbance

Conc= concentration

DF = dilution factor

3.2.1.4Reference values:

Serum or plasma

0.5-1.2 mg/l

50-120 µg/dl (Jon V,1996)

3.2.2.Estimation of magnesium

Magnesium was estimated in heparinized plasma using cobas c 311 autoanalyzer.

3.2.2.1Principle of reaction

- Colorimetric endpoint method

In alkaline solution, magnesium forms a purple complex with xylidyl blue, diazonium salt. The magnesium concentration is measured photometrically via the decrease in the xylidyl blue absorbance.

3.2.2.2Prucedure

Appendix III

3.2.2.3Calculation

Roche/Hitachi cobas c systems automatically calculate the analyte concentration of each sample.

Conversion factors:

$$\text{mmol/L} \times 2.43 = \text{mg/dL}$$

$$\text{mg/dL} \times 0.411 = \text{mmol/L}$$

$$\text{mval/L} \times 0.5 = \text{mmol/L}$$

$$\text{mval/L} \times 1.22 = \text{mg/dL}$$

$$\text{mval/L} = \text{mEq/L}$$

3.2.2.4 Reference value

1.7-2.4 mg/dl (0.66 to 1.07 mmol/L) (Carl *et al*, 2006)

3.2. 3 Quality control

The accuracy and precision of all methods used in this study were checked commercially prepared control sample before its application for the measurement of test and control samples.

3.2. 4 Statistical analysis

Data obtained from this study was analyzed using statistical package for the social sciences (SPSS11.5). The mean and standard deviation of plasma levels in both hemodialysis patients and control were obtained, T.test was used for comparison P.value equal or less than 0.05 considered a significant. Pearson regression analysis was used to assess correlation between Mg^{+2} and Zn^{+2} and body mass index and duration of hemodialysis.

4.Results

The results of the biochemical determinant plasma levels of zinc and magnesium in patients with renal failure under hemodialysis are given in tables and figures:

Tables (4-1) represents the mean of the levels of plasma zinc and magnesium in both of study group.

The plasma level of zinc was significant decreased in patients with renal failure compared to control group, (mean \pm SD: $.24033 \pm .075514$ versus $.77267 \pm .200154$ mg/l $p=0.00$) .

The plasma level of magnesium were significant increased in patient with renal failure compared to control group, (mean \pm SD: $2.7295 \pm .49397$ versus $1.8400 \pm .13025$ mg/dl $p=0.00$) .

Figure (4-1): a scatter plot shows the correlation between zinc level and duration of dialysis. Showed weak negative correlation (significant) ($r= -0.378$, $p\text{-value}=0.003$).

Figure (4-2): a scatter plot shows the correlation between magnesium level and duration of dialysis. Showed weak negative correlation and (significant) ($r= -0.358$, $p\text{-value}=0.005$).

Figure (4-3): a scatter plot shows the correlation between magnesium level and age. showed weak negative correlation (insignificant)($r = -0.050$, $p\text{-value} = 0.703$)

Figure (4-4): a scatter plot shows the correlation between zinc level and age. showed weak negative correlation (insignificant)($r = -0.170$, $p\text{-value} = 0.193$)

Table (4-1)

Comparison of the mean of plasma levels of zinc and magnesium in patients with renal failure under hemodialysis group and control group:-

p-value	Control mean \pm SD N=40	Case mean \pm SD N=60	Variable
0.00	200154. \pm 77267.	.24033 \pm .075514	Zinc (mg/l)
0.00	13025. \pm 1.8400	2.7295 \pm .49397	Magnesium (mg/dl)

- T independent test.
- Results given mean \pm SD.
- P-value ≤ 0.05 consider significant.

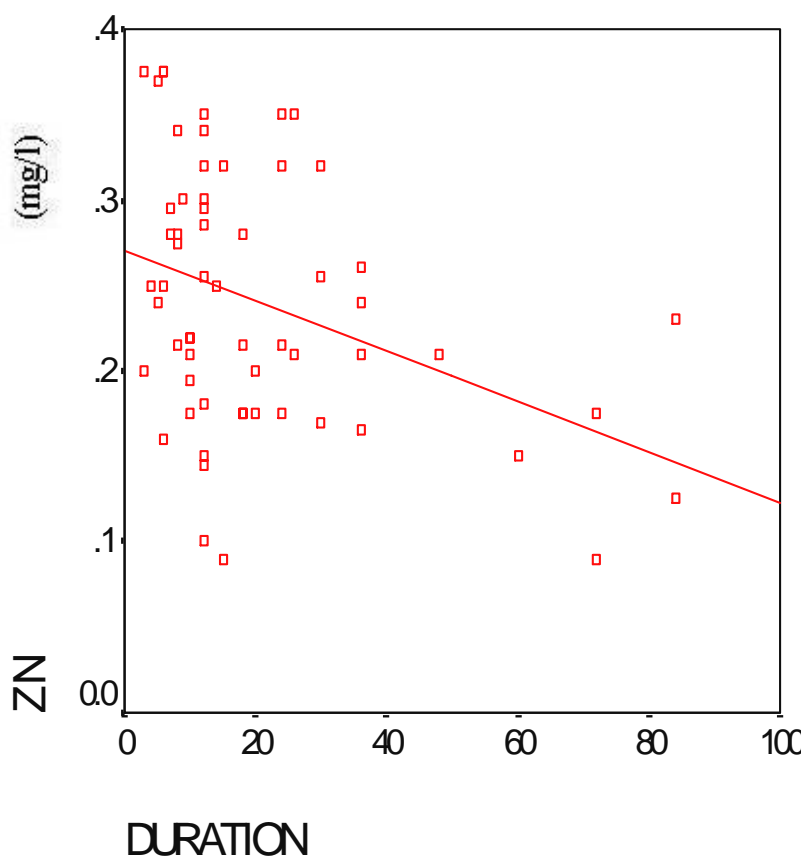


Figure (4-1): correlation between zinc level (mg/l) and duration of dialysis: ($r = -0.378$, $p\text{-value} = 0.003$).

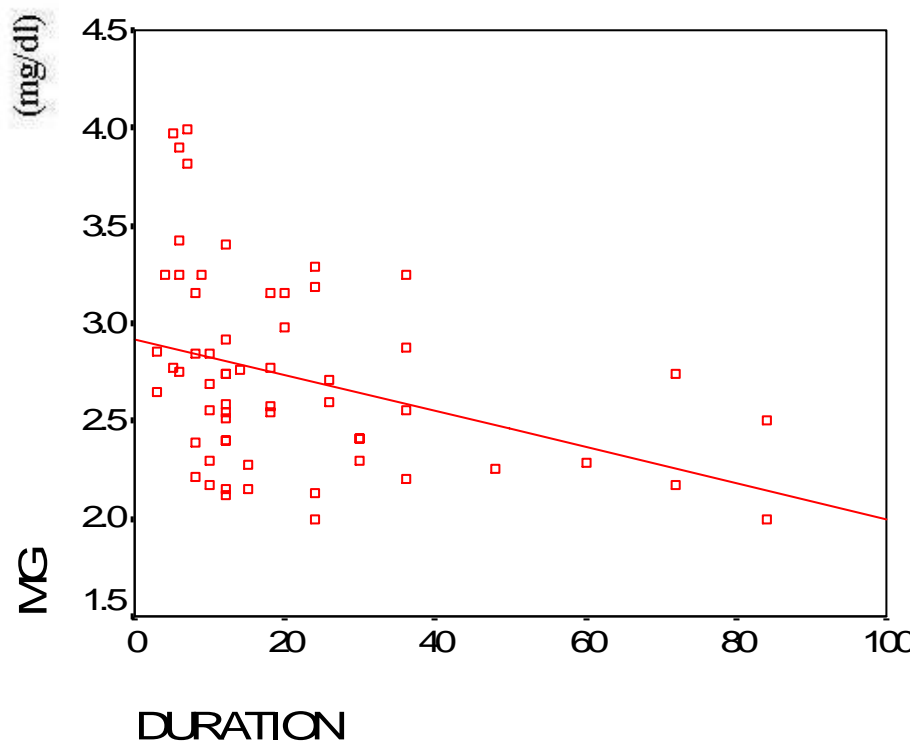


Figure (4-2): correlation between magnesium level (mg/dl) and duration of dialysis: ($r = -0.358$, $p\text{-value} = 0.005$).

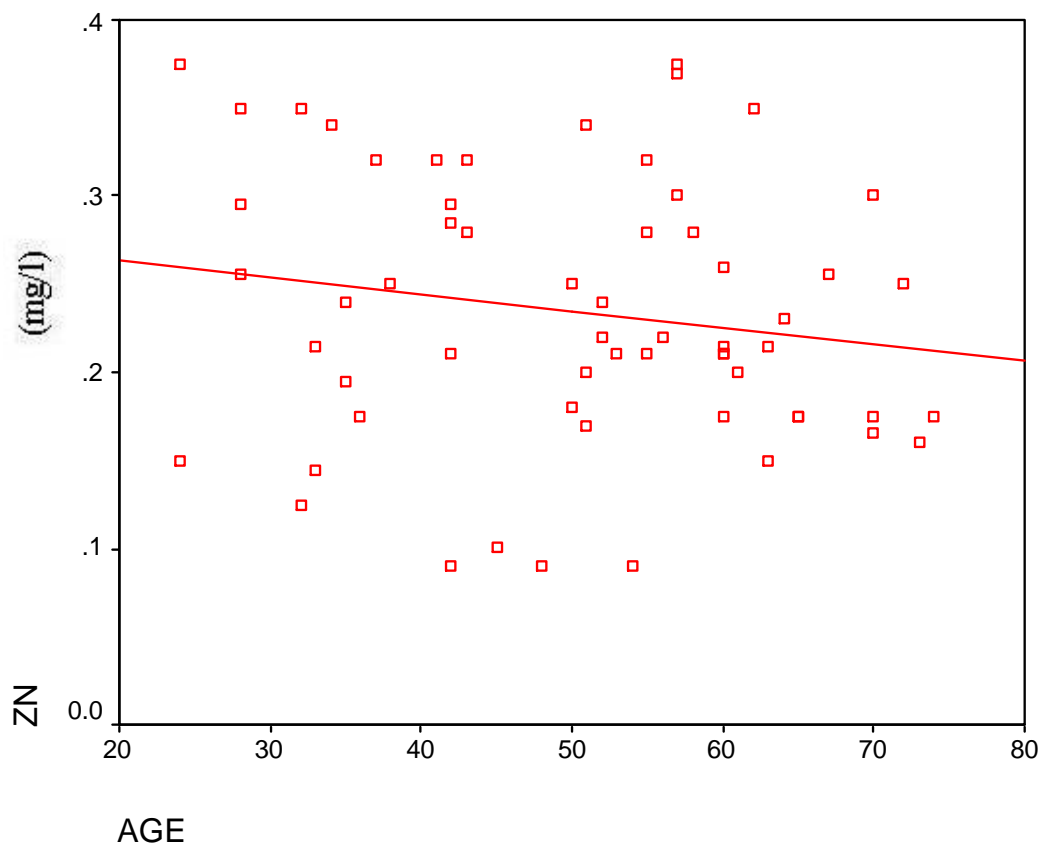


Figure (4-4): Correlation between zinc level(mg/l) and age (years). showed weak negative correlation (insignificant)($r = -0.170$, $p\text{-value} = 0.193$)

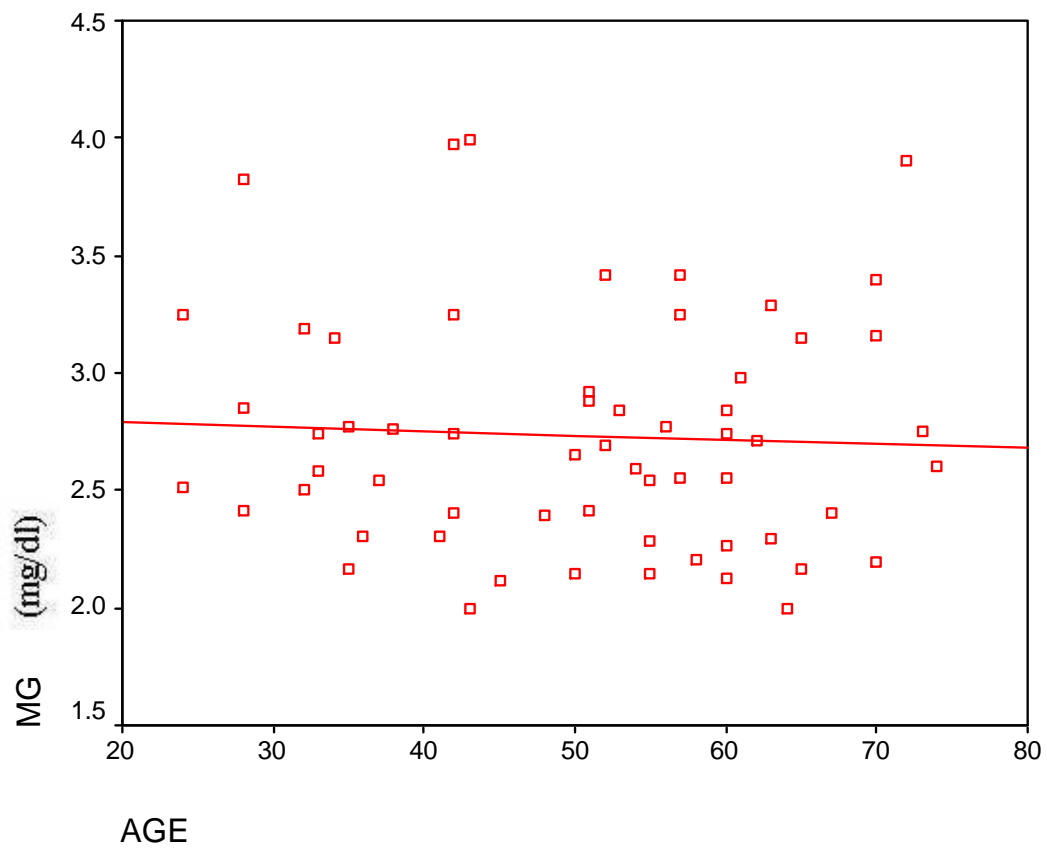


Figure (4-3): Correlation between magnesium level(mg/dl) and age (years). showed weak negative correlation (insignificant)($r = -0.050$, $p\text{-value} = 0.703$)

5.discussion , conclusion and recommendation

5.1 Discussion:

Kidney failure is a condition in which the kidneys fail to remove metabolic end product from the blood, so when kidney failure is reached the end stage it must need dialysis. (Benjamin, 2015).

This study was conducted to assess plasma levels of zinc and magnesium in patients with renal failure under hemodialysis.

In this study the levels of zinc was significant lowered in patients with renal failure under dialysis compared to control group(p-value 0.00). This result agreed with result carried by many authors (Cristina *et al*, 2009 Simin *et al*, 2010 Rajashri *et al*, 2013 ,Itir *et al*, 2015), Which found serum level of zinc in patients with renal failure under hemodialysis; was decreased, they were hypothesized, in chronic renal failure, derangements in zinc homeostasis may be found due to altered protein metabolism, malabsorption of microelements in the gastrointestinal tract, disturbed renal excretion and expected faulty cellular and tissue redistribution.

In this study the comparison of level of magnesium between case and control showed that significant increased in the levels of magnesium in patients with renal failure under hemodialysis when compared with control.(p-value 0.00)

The result agreed with study carried by (Baradaran *et al*, 2004 Mohamoud 2013). which found that serum level of magnesium in patients with renal failure on hemodialysis; were increased because renal excretion is the major route of magnesium elimination from the body and a positive magnesium

balance would be expected under conditions of renal insufficiency. However, a compensatory decrease in tubular reabsorption is operating to maintain an adequate urinary magnesium excretion even when glomerular filtration rates are very low.

Also the result agreed with another result done by (John *et al*, 2012) , which found that serum level of magnesium in patients with renal failure; were increased due to the renal excretion of magnesium is so powerfully adaptable, impairment of renal function has long been recognized as a frequent prerequisite for the development of hypermagnesaemia. renal function further deteriorates to chronic kidney disease (CKD) Stages 4 and 5, the quantitative excretion of magnesium tends to decrease, and cannot be compensated any longer by an increased fractional excretion of magnesium.

Also the result agreed with another result done by (Cecile *et al*, 2013) which found Hemodialysis patients have tendency to become hypermagnesemic.

In this study results showed , there was no significant difference between zinc and magnesium according to gender

The findings of this study showed no correlation between age and plasma zinc concentration (insignificant)($r=-0.170$ p.value=0.193) This result agreed with study done by (Simin *et al*; 2010) which found significant inverse correlation between zinc and age.

The findings of this study showed no correlation between age and plasma magnesium concentration (insignificant)($r=-0.050$ p-value=0.703) This result

Also In this study as appeared in figures (4-1 &4-2), which showed no correlation between zinc, magnesium and duration of dialysis zinc (p-value=0.003). magnesium (p-value=0.005). (significant negative correlation).

This result agreed with result carried by (Hamid *et al*, 2008).which found no correlation between duration of dialysis and magnesium.

This result agreed with study done by(Anees *et al*, 2011) by whose showed that insignificant correlation between magnesium and zinc and duration of dialysis.

5. 2 Conclusion

From the results and findings of this study ,it is concluded the following:

- Zinc is significantly decreased in the blood of hemodialysis patients with renal failure.
- Magnesium is significant increased in the blood of hemodialysis patients with renal failure.
- No correlation between duration of disease and concentration of zinc and magnesium in patients with renal failure under hemodialysis.
- No correlation between age and concentration of both zinc and magnesium in patients with renal failure under hemodialysis.

5.3 Recommendation

It is recommended that:

- Serum calcium, alkaline phosphatase and parathyroid hormone (PTH) should be done with magnesium to link between bone disease and renal failure.
- Serum zinc dependant metalloenzymes should be done for example super oxide dismutase (SOD) and zinc to link oxidative stress, inflammation and renal failure.
- Serum zinc can be done for relation to erythropoietin dose on renal failure patients under hemodialysis.
- Further studies should be done to investigate the link between zinc status and clinical outcomes in patients with renal failure under dialysis, in whom the risk of infection is dramatically elevated compared with people with normal kidney function.

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