Introduction

Certain trace elements and minerals are essential for the normal metabolism of proteins, carbohydrate and lipids.It establishes that several trace element have great importance in a number of biological processes,mostly through their action as activators or inhibitors of enzymatic reaction by competing with other elements and proteins for binding sites, by influencing the permeability of cell on the blood vessel walls, on the blood pressure regulation centers, or on other systems related to cardiovascular function such as; the lipid and Carbohydrate metabolism(Bharot,*et al*1994,Zanger *et al*,1998).

Zinc and magnesium are reported to be essential elements and zinc and magnesium metabolism may play a role in the pathogenesis of diabetes and some of its complication .zinc is the most important of all trace elements involved in human metabolism, more than one hundred specific enzymes required zinc for the catalytic function. Zinc was first shown to be required for the growth of the mold(Barker, 1999).

Zinc has been demonstrated to be essential for the growth development and differentiation of all types of life, including microorganism plants and animals valley (1986) after iron is the second most abundant trace metal in human body an average70kg adult human contain2.3g of zinc, in most cases, the zinc ion is an essential cofactor for the observed biological

function of these metaloenzymes. furthermore, the biological function of zinc which are versatile and observed in many tissue, are most often associated with protein zinc is involved in the synthesis storage, secretion and conformation integrity of insulin monomers and that zinc assembles in a diametric form of storage and secretion as crystalline insulin (Chausmen 1998) low level of zinc may affect the ability of pancreatic enables cells responsible for the production and secretion of insulin

Magnesium is cofactor in numerous enzymatic reaction magnesium fulfils various intracellular physiological functions. Which has been found to be elevated in diabetic rats and mice. It was suggested that Mg is required for normal insulin synthesis and secretion.

In view of this fact, it is important to determine the essential trace elements concentration in biological samples of diabetes mellitus patients and to monitor and assess their impact in human health.

Among various biopsy material blood, scalp, hair, urine and other body fluids may be used as indicator for this purpose(Kung,Hoyer,*et al* 2005).

Trace elements and minerals are essential for normal metabolism of protein ,carbohydrates and lipids.There is a significant association between level of Mg, Zn and diabetes risk, any changes in magnesium and zincmay specify result in

diabetic alteration and may affect the physiology and action of insulin.

So our study shows the level of Mg and Zn in diabetic patients and to show the relation between of these elements and diabetes complication and to know the benefit role of the supplementation of Mg, Zn to the diabetic patients.

1. Literature Review:

1.1 DiabetesMellitus:

Diabetes mellitus is a group of metabolic diseases characterized by high blood glucose levels, which result from defects in insulin secretion, or action, or both. Diabetes mellitus, commonly referred to as Diabetes was first identified as aassociated with "sweet urine," and excessive muscle loss in the ancient world. Elevated levels ofblood glucose (hyperglycemia) lead to spillage of glucose into the urine, hence the term sweet urine. Normally, blood glucose levels are tightly controlled by insulin, a hormone produced by the pancreas. Insulin lowers the blood glucose level. When the blood glucose e1evats (for example, after eating food), insulin is released from the pancreas to normalize the glucose level. In patients with diabetes, the absence or insufficient production of insulin causes hyperglycemia. Diabetes is a chronic medical condition, meaning that although it can be controlled, it lasts lifetime.(*Barker , 1999*).

Over time, diabetes can lead to blindness, kidney failure, and nerve damage. These types of damage are the result of damage to small vessels, referred to as micro vascular disease. Diabetes is also an important factor in accelerating hardening and narrowing of the arteries (atherosclerosis), leading to strokes, coronary heart disease, and other large blood vessel

diseases. This is referred to as macro vascular disease. Diabetes affects approximately, 17 million people (about 8% of the population) in the United States. In addition, an estimated additional 12 millionpeople in the United States have diabetes and don't even know it. From an economic perspective, the total annual cost of diabetes in 1997 was estimated to be 98 billion dollars in,the United States. The per capita cost resulting from diabetes in 1997 amou1ted to $ 10.071. While healthcare costs for people without diabetes incurred a per capita cost of $2.699. During this same year, 13.9 million days of hospital stay were attributed to diabetes, while 30.3 million physician office visits were diabetes related Remember, these numbers reflect only the population in the United States. Globally, the statistics are staggering. Diabetes is the third leading cause of death in the United States after heart disease and cancer (*Kung HC, Hoyert DL, et al 2005*).

In 2002, the estimated prevalence of diabetes in the United States was (18.2 million people); approximately one quarter of cases were undiagnosed. More than 90% of cases of diabetes are type 2 diabetes mellitus. With increasing obesity in the population, an older population, and an increase in the population of higher-risk minority prevalence is increasing. Type 2 diabetes mellitus is less common in non-Western countries where the diet contains fewer calories and caloric expenditure on a daily basis is higher. However, as people in

these countries adopt Western lifestyles, weight gain and type 2 diabetes mellitus are becoming virtually epidemic. Diabetes mellitus is one of the leading causes of morbidity and mortality in the United States because of its role in the development of optic, renal, neuropathic, and cardiovascular disease. These complications, particularly cardiovascular disease (-50-75% of medical. .expenditures), are the major sources of expenses for patients with diabetes mellitus, Approximately two thirds of people with diabetes die from heart disease or stroke Men with diabetes face a 2-fold increased risk for coronary heart disease, and women have a 3- to 4-fold increased risk in 1994; 1 of every 7 health care dollars in the United States was spent on patients with diabetes mellitus. The 2002 estimate for direct medical costs due to diabetes in the United States was $92 billion, with another $40 billion in indirect costs. Approximately 20% of Medicare funds are spent on these patients.(Ammon, H. P. T.,*et al* , 1989)

Diabetes is the leading cause of blindness in working-age adults the United States, accounting for 12,000-24,000 newly blind persons every year. The National Eye Institute estimates that 90% of case of lost vision are preventable. Diabetes mellitus is the leading cause of end-stage renal disease (ESRD).

Accounting for 44% of new cases accordingto the Centers for Disease Control and Prevention (CDC). In 2001, 42,813

people began renal replacement therapy, and 142,963 people with diabetes were on dialysis or had received kidney transplant. Diabetes mellitus is the leading cause of non-traumatic lower limb amputations in the United States, with a 15- to 40-fold increase in risk compared to that of theno diabetic population. *In 2000-2001,* about 82,000 non-traumatic lower limb amputations were performed related to neuropathy and vasculopathy (*Abate . 1996*).

1.1.1 Types of diabetes mellitus:

World health organization guidelines recommended the following categories of diabetes:

(A)Primary diabetes mellitus:

- Type 1 diabetes.
- Type2 diabetes.

(B) Secondary diabetes mellitus:

- Other specific types of diabetes.

(C) Gestational diabetesmellitus. (Wharen*et al* 2007)

1.1.1.1 Type 1 diabetes mellitus (IDDM):

Type 1 diabetes was also called insulin dependent diabetes mellitus (IDDM), or juvenile onset diabetes mellitus.

It's characterized by inappropriate hyperglycemia due to pancreatic islet p-cell destruction causing an absolute deficiency of insulin secretion. Type1 constitutes on 10% of all diabetic and commonly occurs in childhood and adolescence.

In type 1 diabetes the pancreas undergoes an autoimmune attack by the body itself, and is rendered incapable of making insulin. Abnormal antibodies have been found inthe majority of patients with type 1 diabetes. The patient with type 1 diabetes must rely on insulin medication for surviva. (*Mallone, van Ender 2008*) and (*Savilahti, Ormala, et al 1999*).

1.1.1.2. Type 2 diabetes mellitus (NIDDM)

Characterized by hyperglycemia due to an individual's resistance toinsulin with an insulin secretary defect. This resistance result in a relative, not an absolute, insulin deficiency. Type 2 constitutes the majority of cases. Most patients in this type are obese or have increased percentage of body fats distribution in the abdominal region. This type of diabetes is associated with a stronggenetic predisposition with patient at an increased risk with an increase in age, obesity, lack of physical exercise, and often goes undiagnosed for several years. Characteristic usually include an adult onset of the disease and milder symptoms than in type 1, with ketoacidosis very seldom occurring. These patients are more likely to go into a hyperosmolar coma. And they are at an increase, risk of

developing macro vascular and micro vascular complications (*Fong, Aiello et al 2004*).

1.1.1.3. Gestational diabetes mellitus (GDM):

Is any degree of glucose intolerance with onset or first recognition during pregnancy. Causes of GDM includes metabolic and abnormal changes, thisdisease is an associated with an increased prenatal complications and an increaserisk for development of diabetes in later years.

1.1.1.4 Other specific types of diabetes:

Associated with certain condition (secondary) including genetic defect of p-cell function or insulin action, pancreatic diseases, diseases of endocrine origin, drugs or chemicals induced insulin receptor abnormalities and certain genetic syndromes. (Abbraira *et al* 1997)

1.1.2. Causes of diabetes mellitus:

Diabetes mellitus may be caused by:

An absolute insulin deficiency due to pancreatic disease (chronic pancreatitis, haemochromatosis, cystic fibrosis);

Relative, insulin deficiency, due to excessive growth hormone (acromegaly), gluco-corticoid secretion (Cushing syndrome), or increased plasma glucocorticoid concentrations due to administration of steroid. Drug such as thiazide diuretics.

1.1.3. Symptoms of diabetes mellitus:

The early symptoms of untreated diabetes are related to elevated blood sugar levels, and loss of glucose in the urine. High amounts of glucose the urine can cause increased urine output and lead to dehydration. Dehydration causesincreased thirst and water consumption. The inability of insulin to performnormal function has effects on protein, fat and carbohydrate metabolism. Insulin is an anabolic hormone, that is, one that encourages storage of fat and protein. A relative or absolute insulin deficiency eventually leads to weight loss despite an increase in appetite. Some untreated diabetic patients also complain of fatigue, nausea and vomiting. Patients with diabetes are prone to develop infections of the bladder, skin, and vaginal areas. Fluctuations in blood glucose levels can lead to blurred vision and extremely elevated glucose levelscan lead to lethargy and coma.

1.1.4 Complications of diabetes mellitus:

1.1.4.1 Acute complications:

Diabetic keto acidosis:

Diabetic ketoacidosis (DKA) is an acute, dangerous complication in diabetes and is always a medical emergency. If left without prompt proper treatment, patients with diabetic ketoacidosis have substantial chance of death. DKA occurs more commonly in type1 diabetics during periods of extreme

hyperglycemia and insufficient insulin, though it can occur rarely in type2 diabetics. Sometimes patients with type1 diabetes mellitus present for the first time with DKA. Other times, patients with type1 diabetes have been sent into DKA because their glycaemic control has been upset byother factors which may include failure to institute proper insulin therapy or severe infection.(*Boussageon R, Bejan-Angoulvant et, al 2011*)

In situations where there is a severe deficiency in insulin, body switches to fat metabolism, a mechanism which actually exists to protect the organs during periods of starvation, as glucose is not available to be taken up due to the lack of insulin even though blood levels of glucose tare high. Ketones are produce from fats, partly because the brain can utilize ketones for energy as they can pass the blood-brain barrier. As the level of available glucose for the brain (and other organs) runs low due to the persistent low levels of insulin despite the rising levels of serum glucose as a byproduct of the fat metabolism more and more fats are metabolized releasing more and more ketones (acetone, acetoacetate and beta-hydroxybutyrate) and accumulation of these ketone bodies result in metabolic acidosis as pH buffers in the serum are used up. At the same time, as rising levels of glucose and ketones increase the osmolality of the serum, the hyperglycemic state initially encourages the patient's kidneys to producemore urine, causing the body to lose

water and electrolytes as potassium andphosphate, leading to dehydration and hypokalemia . (Buccolo, 1973)

Hypoglycemia:

Hypoglycemia in diabetic patients almost always arises as a result of poor management of the disease, either from too much orpoorly timed insulin or oral hypoglycemic drugs or too much exercise, not enough food, or poor timing of either. If blood glucose levels are low enough, the patient may become agitated, sweaty, and have many symptoms of sympathetic activation of the autonomic nervous system. Consciousness can be altered, or even lost, in extreme cases, leading to coma and/or seizures or even death and brain damage. Experienced diabetics can often recognize the symptomsearly and all diabetics should always carry something sugary to eat or drink as these symptoms can be rapidly reduced if treated early enough.(Corica *et al* 1999)

1.1.4.2 Long term complications:

Coronary heart disease:

The risk for CHD is 2-4 times greater in patients with diabetes than in individuals without diabetes. Conventional risk factors remain relevant and probably are more important in event reduction than glycemic control. Control of hypertension, aspirin therapy, and LDL-C lowering are vitally important in

reducing CHD risk. Epidemiologic studies suggest that the LDL-Q levels in patients with type.Diabetes mellitus should be less than or equal to 100 mg/Dl.

Cardiovascular disease is the leading cause ofmorbidity and mortality among persons with diabetes. In the United States in 1986, approximately 80,000 deaths from cardiovascular disease were associated with diabetes.

Hypertension, also a strong risk factor for cardiovascular disease, occurs two to three times more often in persons with diabetes than in persons without diabetes. The risk for cardiovascular disease increases linearlywith increases in blood pressure.

The precise relationship between hyperglycemia and atherosclerosis is also unknown. Among persons with diabetes, several concomitant conditions may affect the etiology of atherosclerosis: obesity, inactivity. hyperinsulinemia, abnormalities in platelet function, and defects in blood coagulation and flow.

Among persons with diabetes, part of the increased likelihood of cardiovascular disease appears to be a consequence of the increased frequency of risk factors. Yet diabetes itself is an independent risk factor for cardiovascular disease.

1.1.4.2.2 Retinopathy

Diabetic retinopathy is a progressive condition. In which, the tiny blood vessel in the eye weaken and develop small bulges that may burst and leak into the retina and into the gel-like fluid inside the eye called the vitreous gel.. Later,new fragile blood vessels grow on the surface of the retina that may break and bleed into the eye.(Eikelboom 1999)

1.1.4.2.4 Nephropathy

Diabetic nephropathy is a complication of diabetes that is caused by uncontrolled high blood sugar. High blood sugar damages the filtering system the kidneys, over time, the damage can lead to kidney failure. Diabetic nephropathy is the most common cause of kidney failure in the United States.(*Wahren J, Ekberg K, et, al 2007*).

Diagnosis of diabetes mellitus

1.1.5.1. Fasting and random plasma glucose test

The fasting blood glucose test is the preferred way to diagnose diabetes. It is easy to performand convenient. After the person has fasted overnight (at least 8 hours), a single sample of blood is drawn and sent to the laboratory for analysis.

Normal fasting plasma glucose levels are less than 100 milligram per deciliter (mg/dl). Fasting plasma glucose levels of more than 126 mg/dl on two or more testson different days

indicate diabetes14. A random blood glucose test can also be used to diagnose diabetes a blood glucose level of 200 mg/dl or higher indicates diabetes when fasting blood glucose stays above 100mg/dl, but in the range of 100-126mg/dl, this is known as impaired fasting glucose (IFG). While patients with IFG do not have the diagnosis of diabetes, this condition carrieswithit its own risks and concerns (*Wahren J, Ekberg K, et, al 2007*).

1.1.5.2. The oral glucose tolerance test:

Though not routinely used anymore, the oral glucose tolerance test (OGTT) is a gold standard for making the diagnosis of type2 diabetes. It is still commonly used for diagnosing gestational diabetes. With an oral glucose tolerance test, the person fasts overnight (at least eight but not more than 16 hours). Then first, the fasting plasma glucose is tested. After this test, the person receives 75 gramsofglucose (100 grams for pregnant women). Blood samples are taken at specific intervals to measure the blood glucose. (*Wahren J, Ekberg K, et, al 2007*)

For the test to give reliable results, the person must be in good health (not have any other illnesses, not even cold). Also, the person should be normally active (not lying down, for example, as an inpatient in a hospital) and should notbe taking medicines that could affect the blood glucose. For three days before the test, the person should have eaten a diet high in

carbohydrates (150- 200 grams per day). The morning of the test, the person should not smoke or drink coffee.

The classic oral glucose tolerance test measures blood glucose levels five times over a period of three hours. Some physicians simply get a baseline blood sample followed y a sample two hours after drinking the glucose solution. In a person without diabetes, the glucose levels rise and then fall quickly. In someone with diabetes, glucose levels rise higher than normal and fail to come back down as fast.

People with glucose levels between normal and diabetic have impaired glucose tolerance (IGT). People with impaired glucose tolerance do not have diabetes, but are at high risk for progressing to diabetes. Each year, 1-5% of people whose test results show impaired glucose tolerance actually eventually develop diabetes. Weight loss and exercise may help people with impaired glucose tolerance return their glucose levels to normal. In addition, some physicians advocate the use of medications, such as metformin (Glucophage), to help prevent/delay the onset of overt diabetes. Recent studies have shown that impaired glucose tolerance itself may be a risk factor for the development of heart disease. (*Wahren J, Ekberg K,et, al 2007*)

1.1.5.3 Evaluating the results of the oral g1ucose tolerance test:

Glucose tolerancetests may lead to one of the following diagnoses. Normal response: A person is said to have a normal response when the 2-hour glucose level is less than 140 mg/dl, and all values between fasting and 2 hours are less than200 mg/dl. Impaired glucose tolerance: A person is said to have impaired glucose tolerance when the fasting plasma g1ucos is less than 126 mg/dl andthe 2-hour glucose level is between 140 and 199 mg/dl. Diabetes: A person has diabetes when two diagnostic tests done on different days show that the blood glucose level is high. Gestational diabetes: A woman has gestational diabetes when she has any two of the following: a 100g OGTT, a fasting plasma glucose of more than 95 mg/dl, a l-hour glucose level of more than 180 mg/dl, a 2-hour glucose level of more than 1m55 g/dl, or a 3-hour glucose level of more than 140 mg/dl.

1.1.6 Patho physiology of diabetes mellitus:

Insulin is the principal hormone that regulates uptake of glucose from the blood into most cells (primarily muscle and fat cells, but not central nervous system cells). Therefore deficiency of insulin or the insensitivity of its receptors plays a central role in all forms of diabetes mellitus.

Humans are capable of digesting some carbohydrates, in particular those most common in food: starch, and some

disaccharides such as sucrose, are converted within a few hours to simpler forms most notably the monosaccharide glucose, the principal carbohydrate energy source used by the body. The most significant exceptions are fructose, most disaccharides (except sucrose andin some people lactose), and all more complex polysaccharides, with the outstanding exception of starch. The rest are passed on for processing by gut flora largely in the colon. Insulin is released into the blood by beta cells (13-cells), found in the Islets of Langerhans in the pancreas, in response to rising levels of blood glucose, typically after eating. Insulin is used by about two-thirds of the body's cells t absorb glucose from the blood for useas fuel, for conversion toother needed molecules, or for storage.

Insulin is also the principal control signal for conversion of glucose to glycogen for internal storage in liver and musclecells lowered glucose levels result both in the reduced release of insulin from the beta cells and in the reverse conversion of glycogen to glucose when, glucose levels fall. This is mainly controlled by the hormone glucagon which acts in the opposite manner to insulin. Glucose thus forcibly produced frominternal liver cell stores (as glycogen) re-enters the bloodstream; muscle cells lack the necessary export mechanism. Normally liver cells do this when the level of insulin is low (which normally correlates with low levels of blood glucose).

Higher insulin levels increase some anabolic ("building up") processes such as cell growth and duplication, protein synthesis, and fat storage. Insulin (or its lack) is the principal signal in converting many of the bidirectional processes of metabolism from a catabolic to an anabolic direction, and vice versa. In particular, a low insulin level is the trigger for entering or leaving ketosis (the fat burning metabolic phase).

If the amount of insulin available is insufficient, if cells respond poorly to the effects of insulin (insulin insensitivity or resistance), or if the insulin itself is defective, then glucose will not have its usual effect so that glucose will not be absorbed properly by those body cells that require it nor will it be stored appropriately in the liver and muscles. The net effect is persistent high levels of blood glucose, poor protein synthesis, and other metabolic derangements, such as acidosis.

When the glucose concentration in the blood is raised beyond its renal threshold (about 10 mmol/L, although this may be altered in certain conditions, such as pregnancy), re-absorption of glucose in the proximal renal tubuli is incomplete, and part of the glucose remains in the urine (glycosuria). This increases the osmotic pressure of the urine and inhibits re-absorption of water by the kidney, resulting in increased urine production (polyuria) and increased fluid loss. Lost blood volume will be replaced osmotically from water held in body

cells and other body compartments, causing dehydration and increased thirst.

1.1.7Glycated Hemoglobin:

A useful test that can be done i a doctor's clinic is the measurement of blood HbAlC (also called glycosylated hemoglobin, glycohemoglobin,hemoglobin Al and hemoglobin Al levels). This is the ratio of glycosylated red blood cells in relation to thetotal number of red blood cells. Persistent raised plasma glucose levels cause the proportion of these cells to go up. This is a test that measures the average amount of diabetic control ever a period of about 3 months (the average red blood cell lifetime), the non-diabetic, the Hb_{AlC} level ranges from 4.0-6.4%; patients with diabetes mellitus who manage to keep their Hb_{A1C}level below 7.0% are considered to have good glycaemic control (*McGance, et,al, 1995*). Some experts argue that a glycated hemoglobin test could e used for the diagnosis of diabetes mellitus, glycated hemoglobin levels are as highly correlated to adverse clinical outcomes (e.g., retinopathy)as are fasting plasma glucose or postprandial plasma glucose levels and are as reproducible as fasting plasma glucose levels. The major advantage ofmeasuring glycated hemoglobin is that the specimen can be collected without regard when the patient last ate (*Bry et.al, 2001*). Although was not specifically recommended by the National Diabetes Data Group as a

diagnostic test for diabetesmellitus, glycated hemoglobin may, in some cases, be used to diagnoses diabetes mellitus. The diagnosis of diabetes mellitus is made in the following fashion. A glycatedhemoglobin level of 1 percent above the reference laboratory's upper range ofnormal is consistent with diabetes mellitus and has a specificity of 98 percent. Peoplewith normal glycated hemoglobin levels (i.e., within the laboratory's published normal range) either do not have diabetes mellitus or have well- controlled diabetes mellitus (i.e., false-negative test). However, incorrectly diagnosing these persons as normal would not alter their treatment because exercise and diet are adequately controllingtheir blood glucose levels. People who are not diagnosed with diabetes mellitus 2nd who have near-normal glycated hemoglobin levels, (less than 1 percent above the normal range) may be advised of the high probability that they have diabetesmellitus be offered the same treatment as a person with mild diabetesmellitus(i.e., dietaryand exercise counseling), followed by repeat testing of glycated hemoglobin several months later. (*McCance, etal, 1997*).

1.2. Trace Elements

For the past four years the World Health Organization and Atomic Energy - Agency have been jointly coordinating investigations at an international level on the role possibly played by stable trace elements in the aetiology of cardiovascular diseases, and the use of nuclear techniques in studying these elements.

Cardiovascular diseases (particularly atherosclerosis and ischaemic heart disease) are the major epidemic of modern times in the technologically advanced countries, where they account for roughly 50% of all causes of death.The incidence of these diseases is characterized by two alarming trends. Firstly, it seem to be related to the economic and technological development of a given country or group of countries among the so-called “primitive” population groups which are relatively unexposed to technological civilization, coronary heart disease is practically non- existent. Secondly, it is constantly increasing, the highest proportional increase being in younger and more productive strata of the population. In addition, within the same country, thereare significant differences in cardiovascular mortality rates according to geographical location.

It is well established that several trace elements are of great importance in a number of biological processes, mostly through their action as activators or inhibitors of enzymatic

reactions, by competing other elements andproteins for binding sites by influencing the permeability of cell, on the blood vessel walls, on the blood pressure regulating centres; or on other systems related to cardiovascular function such as e.g. the lipid and carbohydrate metabolism.

Man -made alterations of the environment, such as the use of fertilizes, food additives, foodprocessing and canning, treatment and softening of drinking water, and thepollution of air and water, may bring about changes in the mineral balance and, as a consequence, in some biological functions, including the cardio circulatory function. In recent years, epidemiological, clinical, pathological and experimental evidence has accumulated which justified undertaking deeper studies of trace elements in cardiovascular diseases. (*Patt, and Mstrom, 2010*)

1.2.1. Magnesium

Magnesium is the fourth most common cation in the body and the second most common intracellular cation after potassium. The central role of magnesium within the molecule andas a cofactor for the enzymes in the l2-transphosphorylation reactions inphotosynthesis makes it probably the most important inorganic element in the productionof food and fossil fuel 17.

In addition, it has a fundamental role as a cofactor in more than 320 enzymatic reactions involving energy metabolism and nucleic acid synthesis2.(Romani, *et al* 1999)

1.2.1.1. Biological Function of Magnesium

Until recently, the function of magnesium in biological processes was largely ignored to the point where it was described as the ‘forgotten’ ion. In recent years, there has been an explosion of interest in the physiological and therapeutic properties of this essential element. It is involved in several processes, including hormone receptor binding and gating of calcium channels, Trans-membrane ion flux, regulation ofadenylate cyclase, muscle contraction and neuronal activity, control of vascular tone,cardiac excitability and neurotransmitter release 17.

Magnesium increasesthe body’s ability to utilize calcium, phosphorus, sodium, potassium, vitamins C, E and B complex6.

From a physiological perspective, magnesium is primarily regarded as a calcium antagonist, as most of itsactions are linked to calcium. Calciumis an ideal agent for fast signal transduction and cell activation as cytosolic freecalcium is only 1/10,000 of the corresponding extracellular species, traditionally called ionized calciuml7.

Magnesium, on the other hand, having a slight gradient over the plasma, plays the complementary role of a more long-term regulatory element. Alterations ofintracellular or extracellular magnesium concentration may affect cell function through its effect on calcium handling.

Most of the intracellular magnesium is located within the mitochondria metabolically active the cell is, the higher is its magnesium content.

Levels of magnesium in the plasma of healthy people are remarkably constant,being on an average of 1.7— 2.4 m/dl (0,7-1.0 mmol /l).(Raccah *et al* 1994)

1.2.1.2. Absorption and regulation of magnesium.

From recent studies, it has been observed that magnesium absorption in humans occurs uniformly throughout the small intestine. Reports have shownan inversecurvilinear relationship between intake of magnesium and fractional absorption, which ranges from 65% absorption at low intake to 11% absorption at high intake. Clinically, this suggests that treating magnesium deficiency with oral supplementation may requirean extended period.

Plasma magnesium is carefully regulated within the narrow range of 1.7 - 2.4 mg/dl (0.7 - 1.0 mmol/l). In contrast to the tight hormonal control of concentrations ofcalcium in the blood, kidney is the primary regulator of magnesium balance.

Normal intake of magnesium is approximately 300 mg per day, and about one-third of the intake is absorbed by the gastrointestinal tract. Renal magnesium handling is essentially afiltration- re-absorption process, even though magnesium

secretion has been suggested. Eighty percent of serum magnesium is ultra-filterable out of which 70 to 80% is ionized.

Over a24 h period, 3500mg of magnesium is filtered: in humans only 3 - 4% of this amount is excreted in the urine, or about 100 to 150 mg per day; an amount equal to that absorbed by the gastrointestinal tract each day18.

About 20 - 30 % of the filtered magnesium is reabsorbed along the proximaltubule which is less than that of sodium, potassium or calcium. The thick ascending limb of the *Henle's loop* is now known to be the major site of magnesium reabsorptionin the renal tubule (50 - 60%), and the principal locus of renal control of magnesium excretion. The status of body magnesium balance is determined by the renal excretionof magnesium.

The most striking change in renal magnesium handling occurs in response to alterations in plasma magnesium concentration.

Recent evidences suggest that the cells within the distal tubule, and possibly the thick ascending limb of *Henle*; are capable of adapting to magnesium and calciumavailability through receptors that sense the concentration of these cations. Thus when the magnesium status s sub-optimal, these receptors sense the need for magnesium retention and cause more re-absorption.

Renal magnesium wasting may be either primary, due renal defect or secondary, representing the, response of the kidney in a normal manner to a variety ofsystemic and local factors that increase magnesium losses.

Several drugs, particularly diuretics, thiazides, cisplatin, gentamycin and cyclosporine cause magnesium lossinto urine by inhibiting magnesium reabsorption in the kidneys.

Lipid-lowering drug treatment in type-2 diabetic patients has recently been added to that list 19

1.2.1.3. Hormonal modulation of magnesium

Despite early proposals for the existence of a specific hormonal control of magnesium homeostasis, no singleendocrine factor that controls circulatingor urinary magnesiumhas been identified. Among many extensive and excellent review dealing with magnesium homeostasis, one describes magnesium as the body's 'orphan ion', because of an apparent lack of a specific endocrine control similar to that existing for calcium, sodium and potassium, A number of hormones, including parathyroid hormone and calcitonin, vitamin D insulin, glucagon, antidiuretic hormone, aldosterone and sex steroids have been reported to influence magnesium balance, notwithstanding the possibility that these may not be primary regulators of magnesium homeostasis 20.

Recent observation suggest that these hormones act through a common second messenger, adenosine 3¢, 5¢ cyclic mono-phosphate to enhance magnesium transport and modulate magnesium excretion at that nephron site 20.

1.2.1.4. Magnesium deficiency

Magnesium deficiency is common and multifactorial. Numerous research reports and clinical commentaries regarding magnesium deficiency have appeared in recent years. Magnesium deficit can be categorized into two types: magnesium deficiency and magnesium depletion.

Dietary amounts of magnesium are marginal in the whole population and little alteration in magnesium intake may increase the prevalence of magnesium deficiency. Magnesium depletion may be due to dysregulation of factors controlling magnesium status: intestinal hypo-absorption of magnesium, reduced uptake and mobilization of bone magnesium, sometimes urinary leakage,hyperadrenoglucocorticism by decreased adaptability to stress; insulin resistance and adrenergic hypo-receptivity. Magnesium deficiency in aging largely results from various pathologies and treatment to elderly person, i.e. diabetes mellitus and use of hypermagnesuric diuretics. Osmotic dieresis caused by glucosuria(as in diabetes mellitus), mannitol and urea results in urinary magnesium wasting.

It has been suggested that aging, stress and various disease states may increase magnesium requirement 21.

Magnesium deficiency has been demonstrated in 7- 11% of hospitalized patients and is found tocoexist in up to 400% of patients with other electrolyte abnormalities, particularly hypokcalemia and to a lesser extent, hyponatremia the co-existence of secondaryelectrolyte abnormalities plays a key role in the clinical features of magnesium depletion. Among the endocrine and metabolic disorders associated with magnesium deficiency, diabetes mellitus is the most common. Although hypomagnesemia reliably indicates magnesium deficiency, a normal plasma magnesium concentration does not exclude magnesium depletion. Alternative methods for the estimation of body magnesium store include direct measurement of intracellular magnesium using erythrocytes or tissue magnesium.

Deficiency of magnesium is closely linked to abnormalities in calcium and potassium metabolism. A fundamental interaction between magnesium and other ions seems to occur at the cellular level. Intracellular calcium concentrations are controlled within narrow limits, with transient increases rapidly returning to normal levels.

The release of intracellular calcium plays a key role in many cell functions, both basic (cell division and gene

expression) and specialized (excitation, contraction and secretion). A common pathway for the release of intracellular calcium from many stimuli such as hormones, growth factors and neurotransmitters is photosholipase C activation and hydrolysis of phosphatidylinositol 4,5 -biphosphate into inositol 1,4,5-triphosphate(IP3). IP3 acts by binding to the transmembrane IP3 receptor, causing opening of a calcium channel, which is part of the same molecule 22. Magnesium acts as a non-competitive inhibitor of the IP3-gated calcium channel and of IP3 binding. Therefore, it may be considered as an intracellular calcium antagonist acting at IP3- sensitive calcium-release channels.

It is well known that patients with moderate- to-severe magnesium deficiency commonly have accompanying hypocalcemia, which has been shown to secondary toimpaired secretion of parathyroid hormone as well as skeletal andrenal resistance to the action of parathyroid hormone 22. Altered serum calcium and magnesiumconcentrations result in increased neuromuscular hyperexcitability, which is responsiveonly to magnesium therapy.

In addition to interactions with calcium, magnesium has a marked effect on the regulation of transmembrane sodium and potassium movement, as suggested by (*Baraetal ,22*).

Experimental observations to date support the view that magnesium and potassium metabolism are closely linked. Concentrations of magnesium and potassium have been inversely correlated. The mechanism behind this interrelationship may be the magnesium dependency of the activity of Na+, K+ ATP ase, a physical influence per se by low magnesium concentration on the cellular membrane leadingto leakage of potassium, and/or interaction between magnesium and the secretion of aldosterone.

1.2.1.5. Magnesium deficiency in diabetes

Magnesium ion has a fundamental role in carbohydrate metabolism in general, and in the action of insulin in particular23.

Magnesium is a cofactor in the glucose transporting mechanism of the cell membrane and various enzymes, in carbohydrate oxidation. Cellular magnesium seems to play an important role in glucose metabolism as it is a critical cofactor for the activities of various enzymes involved in glucose oxidation and may play a role in the release of insulin Magnesium is involved at multiple levels in insulin secretion, binding and activity 23. .It is also involved in many phosphorylation reactions and is a cofactor for ATPase and adenylate cyclase enzymes. Magnesium deficiency has recently been proposed as a novel factor implicated in the pathogenesis

of diabetic complications. Hypomagnesemia can be both a consequence and a cause of diabetic complications.

Linkage between magnesium deficiency and insulin resistance, carbohydrate intolerance, accelerated atherosclerosis, dyslipidemia, hypertension and adverse outcomes inprgnancies complicating diabetes have been observed or postulated30. Recognizing the signs of diabetes- associated magnesium deficiency is importantbecause the deficiency can occur long beforeit is reflected by serum values.

Diabetes mellitus has been suggested to be the most common metabolic disorder associated with, magnesium deficiency, having 25 to 39% prevalence. Hypomagnesemia in diabetes represents secondary magnesium depletion, whichrequires more or less specific correction of the different perturbations of the control mechanisms of magnesium deficit that are involved with diabetes. The mechanism responsible for magnesium deficiency in patients with diabetes is not completely known. Osmotic diuresis clearly accounts for a portion of the magnesium loss. It isbelieved that glycosuria which accompanies the diabetic state, impairs renal tubular reabsorption of magnesium from the glomerular filtrate. Magnesium is reabsorbed principally in the proximal tubule (30%) and thick ascending loop of Henle (65%), with minimal resorption (1-5%) in the distal convulated tubule.

Hypomagnesemiaresults specifically from a reduction in tubular absorption of magnesium, as recentlysuggested by Garland. Renal magnesium handling may be modulated by glucose andinsulin even in non-diabetic individuals, where the administration of insulin with or without glucose increases urinary magnesium excretion rate 24. A rise in the urinary magnesium excretion rates in diabetic patients with increasing insulin dosage has been reported despite maintenance of serum levels, suggesting the effect of insulin on renal magnesium handling Dietary magnesium intake may also be a factor in deficiency, as the individualsdo not consume the fully-recommended daily allowance for magnesium. Glucose itself is a crucial part of cellular ion homeostasis, increasing intracellular calcium and decreasing intracellular magnesium. Recent evidencessuggest that insulin can increase free magnesium entry into the cell. Furthermore, in the state of insulinresistance, insulin-inducted entry of magnesium is also impaired. Glycemic control in patients with type-2 diabetes, however, may not correct low magnesium concentration, suggesting that othe factors may regulate magnesium levels in diabetic patients 25.

The existence magnesium of a close relationship between metabolic control and impaired magnesium balance was confirmed by *Fujii et, al* who observed that a marked depletion in plasma and erythrocyte magnesium levels was particularly evident indiabetic patients whit advanced retinopathy and poor

diabetic control. The relationshipbetween magnesium and glucose metabolism is supported by a recent epidemiological study showing that deficient magnesium intake is a risk factor for the development of type-2 diabetes independent of age, body mass index, alcohol intake and family history of diabetes.

The influence of magnesium on cell membrane AT-Pase activity and consequently on intracellular Ca^{+2} , Na^{+} , K^{+} metabolism may also play a role in diabetic complications.

The cellular abnormalities of diabetes mellitus may be related to altered transmembrane transport systems.

Most Na^{+} extrusion and K^{+} influx are dependent upon the Na^{+}, K^{+} pump whose biochemical expression is the ouabain-sensitive Na^{+}, K^{+} ATPase. Na^{+}, K^{+} ATPase necessary for maintaining intracellular potassium concentration; is a magnesium-dependent enzyme Na^{+}, K^{+} ATPase activity has been reported low in various tissues of animals with streptozotocin-induced diabetes and in the erythrocytes of type1 diabetic patients. It has been reported that this impaired enzyme activity plays a role in the pathogenesis of diabetic polyneuropathy 26.

Clinical and experimental studies have documented decreasedCa^{+2}. ATPase activity and intracellular Ca^{+2}accumulation in human and experimental diabetes mellitus.

The ATP- fuelled, Mg^{2+}dependent Ca^{2+}pump inthe plasma membrane is involved inmaintaining low intracellular Ca^{2+} concentration in intact cells, by pumping $Ca^{2+}$from the cytosolic component to the extracellular space26.

1.2.1.6. Hypomagnesemia and diabetes

Studies in experimental animals demonstrated that magnesium supplementation could retard or prevent the induction of insulin resistance and diabetes mellitus, while a magnesium deficit can predispose to .hyperglycemia.

Moles and*McMullen* have shown that low plasma magnesium concentrations may contribute to insulin resistance. The relationship between intracellular magnesiumand insulin action is also supported by: negative correlation between the integrated insulin response after glucose loading and erythrocyte-free magnesium concentration.The results of the prospective study of *DeValket dl*, provided the support for an association between plasma concentration and developmentof progression ofretinopathy in insulin using patients; but whether the plasma magnesium concentrationis a causative factor or a marker remains to be determined. *McNaiet al.* reported thatdiabetes-induced damage to the eyes is more likely to occur in magnesium-deficientpatients with insulin dependent diabetes mellitus (IDDM) and suggested hypomagnesemia as a possible risk factor in the development and progress of

diabeticretinopathy. In pregnant women with IDDM who are magnesium deficient, lack of magnesium may even account for the highrate of spontaneous abortion and birth defects associated with IDDM 27.

Recent data indicate that hypomagnesemia may be linked to the development ofdiabetic complications via reduction in the rate of anositol transport and subsequent intracellular depletion. Grafton and Baxter have suggested that hypomagnesemia leads to reduction of inositol transport and subsequent inositol depletion that might enhance the development of diabetic complications.

Despite the evident role of hypomagnesemia in the outcome of diabetic morbidity, little clinical emphasis has been placed on the long-term treatment of hypomagnesemia in diabetes.

Magnesiumhas been, reported to be mainly intracellular, and its intracellular uptake is stimulated by insulin, although the cellular physiology is not fullyunderstood.

In healthy, subjects, insulin have been shown to stimulate erythrocyte magnesium uptake Studies have demonstrated that insulin regulates the intracellular magnesiumconcentration bystimulating the plasma membrane AT-Pase pump. Intracellular magnesium deficiency may be the consequence of insulin resistance, but may alsoworsen this condition. The

studies done by *Rosolova et al*indicated that. Insulinmediated glucose disposal was decreased in non-diabetic subjects designated as having a low plasma magnesium concentration, than subjects with a high magnesium concentration. They also proposed that the association between a low plasma magnesium concentration and insulin resistance is not primary but is related to abnormalities, of other cations, for example, a low plasma calcium concentration. A study to explore further the link between magnesium deficiency and insulin resistance, hypertension and cardiovascular disease looked at the effect of diet lacking in magnesium (<0.5 mmol/day) on insulin resistance in non-diabetics 28.

The result showed that diet-induced magnesium deficiency leads to decreased insulin sensitivity in lean non-diabetics. In addition, urinary thromboxane concentration increased after magnesium deficiency, again pointing towards. hypomagnesemia as a common factor in insulin resistance and vascular disease.

Humphries et al. 29 reported a clear association between the lowest consumption of dietary magnesium and the highest degree of insulin resistance among non-diabetic subjects. *Dominguez et al. 30* confirmed this observation, finding hat among both normotensive and hypertenive subjects, higher magnesium level corresponded to agreater degree of sensitivity

to insulin. *Lefebvre and Scheen* 31, in their evaluation of the role of magnesium in glucose metabolism, concluded that magnesium deficiency results in impaired insulin secretion, while magnesium replacement restores insulin secretion. Furthermore, experimental magnesium deficiency reduces tissue sensitivityto insulin.

Insulin resistance is a common finding in elderly people. Moreover, insulinresistance per se has also been associated with low extracellular plasma and intracellular erythrocyte magnesium content. At the sometime, a close relationship between insulin, glucose homeostasis and intracellular magnesium has also been demonstrated to occur. Studies have shown that insulin inducesopposite changes in plasma and erythrocyte magnesium concentration in normal men and that dietary magnesium supplements can improve both insulin response and action in aged, non-insulin dependent diabetic patients. Aging is also associated with an impaired glucose handling and a low intracellular magnesium concentration, probably the consequence of an insulin-resistant state. There are more complex interrelationships between intracellular magnesium deficiency and insulin action. In particular, intracellular magnesium deficiency impairs the function of many rate-limiting, magnesium dependent glycolytic enzymes utilizing high-energy phosphate bonds. As recently reviewed by *Jackson 33*, an age-related reduction in the activity of numerous

enzymes such as hexokinase type-II. and hosphofructokinase seems to occur in magnesium deficiency.

Diabetes patients tend to have low magnesium levels. Double-blind research that supplanting with magnesium overcomes this problem. Magnesium lead to improved insulin production in elderly people with NIDDM60. Elders without diabetes can also produce more insulin because of magnesium supplements, according to some, but not all studies. Insulin requirements are lower in people with IDDM, who are supplementedwith magnesium. In a study in type-1 diabetic patients oral replacement withmagnesium in the skeletal muscle42.

This was associated with decreased insulin requirements but no reduction in glycisylated hemoglobin level insiabitic patients.

In a double-blind, randomized crossover study, Paolisso and colleagues 33 investigated the effect of magnesiumsupplementation in elderly subjects with insulin resistance on the handling of glucose following an-intravenous glucose load and an euglycemic hyperinsulinemic clamp procedure. Magnesium pidolate at 4.5 g per day (15.8 mmol/day), for four weeks significantly improved insulin action, oxidative-glucose metabolism, increased erythrocyte

magnesium concentration and decreased erythrocyte membrane micro-viscosity.

1.2.1.7. Magnesium deficiency and oxidative stress

Diabetes mellitus has been shown to be a state of increased free radical activity and is associated with higher prevalence of atherosclerotic disease and cardiovascular mortality. Lipid peroxidation of cellular structures, a consequence f free radical activity, is thought to play an important role in aging, arthrosclerosis and late diabetic complications. By-products of lipid peroxidation are increased in diabetes mellitus 34. Mechanisms that contribute to the formation of free radicals in diabetes mellitus may include non-enzymatic and auto-oxidative glycosylation, the levels of inflammatory mediators and the status of antioxidant defence.

In recent year there has been a growing interest in magnesium and its correlation with the development, of various age-related diseases, viz hypertension, diabetes nellitus, cardiovascular diseases, atherosclerosis, myocardial damage and cardiac arrhythmias through free radical oxidation of cellular components. There is a large volume of literature suggesting that magnesium deficit contributes to the ageing process and vulnerability to age-related diseases 35. Mammalian tissues contain numerous defences against oxidative stress, some of which have been shown to be compromised during magnesium

deficiency *Weglicki et al*. have proposed that during magnesium deficiency, natural antioxidant defences present in mammalian tissues against oxidative stress may be compromised. Our recent studies have also demonstrated that magnesium deficiency is associated with increased oxidative stressthrough reduction in plasma antioxidants and increased lipid peroxidation 36.

Free radical oxidation of cellular components is a well-established mechanism of cellular injury in many of the above-mentioned age-related diseases..

Early studies by ul Hassan and Lehninger 37 established the requirement of magnesium for thebiosynthesis of ascorbic acid, in vitro by rat liver microsomes. Ascorbate may recycle tocopherol from the relatively stable tocopherol radical at the lipid interface and also function as a key aqueous-phase antioxidant in its own right.

Glutathione (GSH), a thiol containing tripetide is present in the plasma andintracellularly in the reduced state. It has antioxidant properties to inhibit free radical formation, and functions more generally as a redox buffer. GSH is also a cofactor for many enzymes such as glutathione peroxidase, which catalyses detoxification of intracellular peroxides. Recent evidences suggest that GSH may also be important in blood pressure and glucose homeostasis, consistent with the

involvement of free radicals in both essential hypertension and diabetes mellitus 38.

Magnesium is an obligatory cofactor in the enzyme reaction of GSH synthesis and in all biosynthetic enzyme reactions involving ATPase, and magnesium deficiency has been are reported to inhabit biosynthesis of GSH. *Hsu et al. 39* investigated the concentrationof GSH in erythrocytes and other target tissues of magnesium-deficient, Magnesium- reported and their respective pair-fed control animals. Their data clearly showed decreased magnesium levels in plasma and a reduction of GSH concentration in erythrocytes studies by *Barhagallo et al*. have demonstrated that GSH acts in vivo and in vitro to enhance intracellular magnesium content.

The observed significant and.independent positive relationship in vivo among intracellular magnesium content, GSH/GSSG ratios and insulin disposal, suggestingthe role of magnesium in mediating the effects of glutathioneon peripheral insulinaction.

Vitamin E has, been proposed to be the major lipid-soluble chain-breaking antioxidant which protects the biological membrane from lipid peroxidation. It isone of the, most effective scavengers of freeradicals,and recent studies indicate that vitamin E administration offers significant protection against the pro-xidant influence of magnesium deficiency and

prevents the occurrence of enhanced cart post-ischemic injury following magnesium deficiency. Moreover; a decrease in tissue vitamin E content following long-term magnesium deficiency in rats has been reported 40.

1.3 Zinc

The importance of zinc for normal growth and survival of plants and animals was recognized a long time ago. Yet the existence of its deficiency in humans was doubtedbecause of the element's ubiquitous distribution in the environment and the lack of obvious clinical signs of deficiency. Nevertheless, evidence of human deficiency began to emerge during the l960s, when cases of zinc-responsive dwarfism and delayed sexual maturation were first reported in Egyptian adolescents 45. Since then, a number of intervention trials have been carried out to assess the impact of zinc supplementation, particularly in low incomepopulations who are likely to suffer from zinc deficiency 46. Results of these studies have shown, that zinc supplementation increases growth among stunted children and reduces the prevalence of common childhood infections.

1.3.1. Biological Functions of Zinc

Zinc is the most ubiquitous of all trace elements involved in human metabolism. More than one hundred specific enzymes require zinc for their catalytic function 47. If zinc is removed from the catalytic site, activity is lost; replacement of zinc

restores activity. Zinc participates in all major biochemical pathways and plays multiple roles in the perpetuation of genetic material, including transcription of DNA, translation of RNA, and ultimately cell division. When the supply of dietary zinc is insufficient to support these fuctions, biochemical abnormalities and clinical signs may develop. Studies in individuals with acrodermatitis enteropathica, a genetic disorder with zinc malabsorption resulting in severe deficiency, have provided much insight into thefunctional outcomes of zinc deficiency 48. These include impairments of dermal, gastrointestinal, neurologic and immunologic system.

Zinc affects both non-specific and specific immune functions In terms of non-specific immunity; it affects the integrity of epithelial barrier and function of neutrophils, monocytes and macrophages. With regard to specific immunity, both lymphopenia and declined lymphocyte function occur in zinc deficiency. Although most of these effects are derived from experimental animals, studies in human subjects have also shown that altered zinc status can affect immune competence. For example, elderly subjects who received supplemental zinc demonstratedimprovement in delayed cutaneous hypersensitivity, number of circulating T cells and serum IgG antibody response to tetanus toxoid. In other studies of experimentally induced mild zinc deficiency among adults, a reduction in serum thymulin and specific subpopulations of

lymphocytes occurred during zinc depletion, and these returned to normal levels following zinc repletion 49. Although specific links between altered immunity and - different infections are not well understood, changes in immune functions are clinically important because decreased rates of infections have been observed following zinc supplementation in community based studies.

1.3.2. Zinc Metabolism

Zinc is released from food as fee ions during digestion. These liberated ions may then bind to endogenously secreted ligands before their transport into the enterocytes in the duodenum and jejunum 47. 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 carriers absorbed zinc directly to the liver, and then released into systemic circulation for delivery to other tissues. About 70% of 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.

Loss of zinc through gastrointestinal tract accounts for approximately half of all zinc eliminated from the body.

Considerable amount of zinc is secreted through the biliary and intestinal secretions, but most of it is reabsorbed and this process is an important point of urine of zinc balance. Other routes of zinc excretion include the urineand surface losses (desquamated skin, hair, sweat).

1.3.3. Human Requirements

Since the mid-1990s, the World Health Organization/Food and Agriculture Organization International Atomic Energy Association (WHO/FAO/LAEA) and the Food and Nutrition Board (FNB) of the Institute of Medicine (IOM) have convened expert committees to develop estimates of human zinc requirements and dietary intakes needed to satisfy these requirements 48-49. For most age groups, he committees used a factorial method to estimate the average physiological requirement, which is defined as the amount of zinc that must be absorbed to offset the amount of zinc lost from both intestinal and non-intestinal sites. In growing children and pregnant women, the amount of zinc retained in newly accrued tissues is added to the requirements, and in lactating women the zinc secreted in breast milk is added. The estimates of requirements developed by the WHO committees are based on limited studies conducted insubjects who had very low zinc intakes. The FNB/IOM reviewed a larger number of studies conducted at varyinglevels of intakes. The reference body weights used by

the WHO are based on the NCHS growth data, while the FNB/IOM committee applied, reference body weights that are suitable for the North American population. The estimates of requirements derived by the two committees are different for these reasons (Table 1) More recently, the International Zinc Nutrition Consultative Group (IZiNCG) reviewed the methods adopted by these committees and revised the estimates of zinc requirement and recommended dietary intake 50.

1.3.4. Dietary Sources of Zinc and Bioavailability

Zinc occurs in a wide variety of foods, but is found in highest concentrations in animal sources, particularly beef, pork, poultry and fish, and in lesser amounts in eggs and dairy products. Zinc content is relatively high in nuts, legume and whole grain cereals and is lower in fruits and vegetables.

Dietary factors can alter the proportion of zinc that is available for absorption in the intestine by as much as ten-fold. Most of the available information specific dietary factors on zinc absorption has been derived from studies measuring absorption from single test meals. Though these data may not reflect the true proportion of zinc absorbed from meals consumed over the whole day they are useful to identify the factors that affect zinc absorption. The dietary components that have a substantial impact onthe absorption of zinc are phytate and calcium, which inhabit zinc absorption, and protein, which

enhances the absorption 51. Phytate content is high in cereal grains, nuts, and legumes. It is a strong chelator of minerals including zinc. Because phytate cannot bedigested and absorbed, minerals boundtophytate also pass through the intestines unabsorbed. The inhibitory effect of calcium may result from the formation of insoluble calcium-zinc-phytate complexes in the intestinaltract. Both the total amount and the type of protein in the diet influence zinc absorption. Increasing protein intake results in a higher absorption of zinc. Animal proteinsuch as meat and egg protein enhances absorption of zinc while casein may have inhibitory effect.

1.3.5. Dietary Reference Intakes

The two committees charged with developing dietary reference value, WHO/FAO/IAEA and FNB/IOM, estimated percent absorption of dietary zinc based on data available from absorption studies48-49. Two types of study designs have been used to estimate dietary zinc absorption single test meal and total-diet studies. Using data from both types of studies, the WHOcommittee derived the estimates of zinc absorption for different types of diets; 50% from highly refined diet, 30% from mixed refined diets and 15% from unrefined cereal diets. The FNB/IOM committee selected data from only total diet studies, which provide more valid estimates, of zinc absorption. These studies, using labeled meals, are able to measure true zinc absorption by making correction for intestinal losses of endogenous zinc. But the committee used only studies conducted in American males and the diet types included mixed diets as well as semi-purified formula diets, resulting in higher estimates of absorption (40%). These types of diets do not represent typical diets consumed by most populations. The IZiNCG has recently revised the estimates of zinc absorption using studies with different types of diets, but excluded those that used formula diets. The absorption estimates with mixed refined vegetarian diets are 26% for men and 34% for women. The corresponding figures with unrefined cereal diets are 18% for men and 25% for women.

The estimates for absorption are now applied to the physiological requirements for absorbed zinc derive average requirement and recommended dietary allowances (RDA) for zinc. Estimated average requirement (EAR) represents the mean dietary requirement, or the dietary intake level at which. 50% of individuals would meet their physiological requirement. The RD is set at two standard deviations (SD).above the EAR, considering the individual variation of requirement. At this level of intake, almost all individuals meet their requirements. The revised estimates of EAR and RDA developed by IZiNCG for the purpose of international application are presented inTable2.

Objectives:

1. To assess the level of Mg and Zn in plasma, in Sudanese diabetic patients
2. To estimate the level of glucose. RBG.
3. Correlate between the level of this trace element and glysimic control.

Chapter Two

Materials and methods

2.1 Study design

This is a cross sectional, control and hospital case based study.

Study area

This study is conducted during (Jan- Jul 2013), Jaber Abo- Alaz Hospital and Khartoum Hospital Khartoum Sudan.

Target population:

The study includs type II diabetic (male and females) Sudanese patients

2.2 Data collection:

Clinical data were obtained from the participated diabetic patients through questionnaire made for the purpose of this. The questionnaire include age,gender and duration of disease including approval of health authorities was obtained, to out this study participants were notified and excused before taken sample and tire importance and the benefit of the outcome of this study were explained to them.

The study sample are 150 persons arrange in to two groups. Diabetic patients Group (1) (N=1 00) and group 2 nondiabetic apparently healthy subjects (N50) will be serve as control.

2.3 Sample collection:

Blood samples were taken from all participants (both diabetic + control) in a tubes containing anti-coagulant and processed using centrifugation and the clear serum obtained used for the estimation of blood glucose magnesium and zinc elements.

2.4. Methods

2.4.1 Estimation of blood glucose:

The estimation of blood glucose was done by the enzymatic colorimetric using Glucose oxidase / peroxidase method.

Principle of the method

Glucose in the sample originates, by means of coupled reaction described below a coloured complex that can be measured by spectrophotometry .

$\beta - D$ glucose $= H_2O + O_2$ GOD $\rightarrow$ D- Gluconate $+ H_2O_2$

4 amino antipyrine + phenol H2O $\rightarrow$ Quinoneimine $+ H_2O$

Reagent concentration:

Buffer consist of 150 mmol u/I phosphate buffer (pH= 7.5) mmol/1 phenol.

Enzyme reagent consist of 1200 u/I GOD 660 U/I POD and 0.4 mmol/1 4- aminoantipyrnie.

Standard glucose = 100 mg/dI

The working reagent was prepared by mixing the buffer and the enzyme reagents.

Sample : heparmized plasma

Procedure :

The reagent was brought to room temperature and labeled test tubes were prepared as follows:

	Blank	Standard	Test
Working reagent	1000μL	1000μL	1000μL
Standard	-	10μL	-
Plasma	-	-	10μL

The tubes were mixed thoroughly and incubated for 13 minutes at 37C then the absorbance (A) of sample (As) were measured against the blank at 520 nm.

Calculation:

$\frac{As\ x\ 100}{Ast}$ mg/dl glucose

2.4.2 Estimation of magnesium:

Magnesium was estimated in serum, heparinized plasma using atomic absorption (mirco automation)

Principle :

Magnesium forms a coloured complex when reacts with Magon sulfonate in alkaline solution. The intensity of the colour formed is proportional to the magnesium concentration in the sample.

Procedure:

1- assay conditions:

Wavelength : 546 mm Temperature 37 ˚C / 15 – 25 ˚C

2- The instrument was adjusted to zero with distilled water.

A volume of I ml or (1000 Mm) of WR yxlidle Blue (0 mmoL/L) + thioglycolic acid (0.7 mmoLL) +Dimethyl sulfoxide DMSO (3000 MMoL/L) was added to tube then added to about I0 mL from sample then I was mixed and incubated for 5 min at room temperature. Then the absorbance of sample was read, immediately in 3 to 5 seconds.

Calculation

From 1.6 to 2.5 mg/dL s .0.66 mmoL/L is a normal result, up or low this is up normal result.

2.4.3 Estimation of zinc

Zinc in the sample was estimated by coulometer using serum according to Bio System reaction method.

Principle

Direct colorimetric test without derprotenization of the sample. End point increase, at pH 8.6 in a buffered media . Zinc reacts with the specific complex is t5-13-PAPS, from a stable coloured complex. The colour intensity proportional to the amount of zinc present in the ample.

Procedure

1- Assay conditions.

Wavelength : 560 nm (550 – 580)

Cuvette : 1 " m light path

Temperature : 25- 30 37 ˚C

2- The instrument was adjusted to zero distilled water.

3- A volume of 1 mL or (1000 Mm) from W.R was added to 50 Mm from sample and mixed and the absorbance of the samples was read (A1) against the blank. Then added to 100 Mm was mixed and then the absorbance (A2) of sample and standard were read against blank.

Calculations

(A2 – A1) sample x 200 (standard cons)

(A2- A1) stander.

Test result:

From 68 – 107 Mg/dL s 10.4 – 164 Mol/L it a normal range up or low than this is up normal range.

Reference range is approximately 64. Hb AiC.

2.4.4 Quality control

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

2.4.5 Statistical analysis :

Data obtained from this study was analyzed using statistical package for the social sciences (SPSS). The mean and the standard deviation of serum levels in both diabetic and control were obtained, T. test was used for comparison P. value equal or less than 0.05 considered as significant (Sendecor.G.W. and WC. Cochran, (1989) .

Chapter Three

Results

In this study trace elements, magnesium (Mg^{+2}) and Zinc (Zn^{+2}), were measured in the blood of (100) diabetic patients of type II (NIDDM), as well as in the blood of 50 volunteers non diabetic persons selected to serve as control.

Primary observation and findings from the specially designed questionnaire revealed that nearly all the 100 diabetic patients and the 50 apparently healthy diabetic control subjects were matched in ages. Diabetic patients were of ages between 20 years to above sixty years, while control subjects were of an age between 20 to above sixty years (table-1). No significance difference between two means of age groups.

On the other hand gender distributed as males in control were 22 (44%), females were 28 (56%), while males in diabetic patients were 54 (54%) and females were 46 (46%) (table-)

3.1. Biochemical investigation

3.1.1 Random blood glucose

Table (2) shows the mean plasma glucose levels. Control group maintained normal blood glucose concentration of a mean of 133.5 mg/dl, while in diabetic patients blood glucose was (241.5 mg/dl), there was an expected significance difference between two groups ($p = 0.000$).

3.1.2 Serum magnesium

Table (2) shows blood magnesium concentration, Control group magnesium value was (22.47 mg/d)l, while diabetic patients was (17.92mg/dl) there was a significant difference between two groups

3.1.3 Serum zinc

Table (3) shows blood zinc concentration, Control group zinc value was (0.67mg/d), while diabetic patients was (0.51 mg/dl) there was a significant difference between two groups

2.4 Biochemical results according to gender

Tables show the status of the biochemical results of diabetic and control males.

Blood glucose level of diabetic males was higher than control group (p=0.00) there was a significant difference between two groups although Blood glucose level of diabetic females (p=0.00) Blood magnesium level of diabetic males was lower than control group (p=0.00) there was a significant difference between two groups while blood magnesium level of diabetic females had mild decrease than control group, so there was no significant difference between two groups (p=0.4l7).

Blood zinc level of diabetic males was lower than control group (p0.00) there was a significant difference between two groups. Blood zinc level of diabetic females was lower than control (p=0.01) a significant difference between two groups.

2.5Biochemical parameters according to age

Tables show the status of the biochemical parameter, measured blood glucose level of diabetic at age(20-30) was higher than control (p=0.02)there was significant different, and blood glucose level of diabetic at age(31-40) was higher than control (p=0.00)there was significant different, and blood glucose level of diabetic at age(41-50) was higher than control (p0.00)there was significant different, and blood glucose level of diabetic at age(51-60) was higher than control (p=0.00)there was significant different and blood glucose level of diabetic at age(above 60) was higher than control (p=0.00)there was significant different between two groups.

Blood magnesium level of diabetic at age (20-30) was moderate lower than control (p=0.877) there was no significant different between two groups. Blood magnesium level of diabetic at age (31-40) was moderate lower than control (p0.l22) there was no significant different between two groups. Blood magnesium levelof diabetic at age (31-40) was moderate lower than control (p=0.122) there was no significant different between two groups.

Blood magnesium level of diabetic at age (41-50) was lower than control (p=0.0l2) there was significant different between two groups. Blood magnesium level of diabetic at age (51-60) was moderate lower than control (p=0.073) there was no significant different between two groups. Blood magnesium

level of diabetic at age (51-60) was moderate lower than control (p0.124.) there was no significant different between two groups.

Blood zinc level of diabetic at age (20-30) was moderate lower than control (p=0.4.97) there was no significant different between two groups. Blood zinc level of diabetic at age (31-40) was lower than control (p=0.00) there was significant different between two groups. Blood zinc level of diabetic at age (4 1-50) was moderate lower than control (p=0.5) there was no significant different between two groups. Blood zinc level of diabetic at age (51-60) was moderate lower than control (p=0344) there was no significant different between two groups. Blood zinc level of diabetic at age (above 60) was moderate lower than control (p0.344) there was no significant different between two groups.

2.6 Biochemical results according to duration of disease

Table (12) shows the status of the biochemical results of blood glucose level of diabetic patients and control according to their duration of disease.

Blood glucose level of group (1) of duration of disease (1-10) years was higher than control (p=0.000), (group 2) of (>10 years)disease was higher than control group (p=0.00), comparing group (1) with group (2) mg and Zn there was no significant difference between two groups so duration of disease has no effect on mentioned minerals.

Table (1)Study groups age, number and gender

Parameters	Control	Diabetic patients
Number	50	100
Age	20 – 75	25- 80
Gender Male Female	 22 (44%) 28 (56%)	 54 (54%) 46 (46%)

Table (2) Blood glucose concentration of control and diabetic patients

Study groups	**No**	**Mean mg (mg/dl) ∓ standard deviation**	**p. value**
Diabetic patients	**100**	**241.5± 20.3**	
Control	**50**	**133.25± 15.6**	**0.00**

Table (3) Concentration of serum magnesium (Mg) in control and diabetic patients

Study groups	No	Mean mg (mg/dl) ∓ standard deviation	p. value
Diabetic patients	**100**	**17.92± 6.12**	
Control	**50**	**22.47± 7.93**	**0.01**

Table (4) Concentration of serum zinc in control and diabetic patients

Study groups	No	Mean mg (mg/dl) ∓ standard deviation	p. value
Diabetic patients	100	0.51±0.19	
Control	50	0.67±0.22	0.05

Table (5): Correlation between blood glucose, magnesium and zinc among control and patients (male)

Groups		No	Mean± St. deviation	*P value*
Glucose	control	26	134.54±17.36	
	patients	53	239.23±77.28	0.00
Magnesium	control	26	23.59±7.93	
	patients	53	16.05±6.36	0.00
Zinc	control	26	.68±.22	
	patients	53	.52±.23	0.05

p≤0.05 means significant difference between two groups

Table (6):Correlation between blood glucose, magnesium and zinc among control and patients (Female)

Groups		No	Mean± Std.Deviation	*p- value*
Glucose	control	23	133.96±14.84	
	patients	47	244.83±82.o	0.00
Magnesium	control	23	21.35±0.48	
	patients	47	19.78±8.28	0.467
Zinc	control	23	.67±.19	
	patients	47	.50±.17	0.01

p≤0.05 means significant difference between two groups\

Table (7) : Correlation between blood glucose, magnesium and zinc with age (20-30) to diabetic patients

Groups		No	Mean± Std.Deviation	*p- value*
Glucose	20 to 30patientt	4	303.0000±73.26	
	20 to 30control	12	140.0000±15.70	0.02
Magnesium	20 to 30patient	4	21.4625±8.413	
	20 to 30control	12	22.3279±11.71	0.877
Zinc	20 to 30patient	4	.6425±0.24	
	20 to 30control	11	.7374±0.15	0.497

Table (8) : Correlation between blood glucose, magnesium and zinc with age (31-40) to diabetic patients

Groups		No	Mean± standard Deviation	P- value
Glucose	31 to40 patient	19	249.3272.62±	
	31 to 40 control	16	130.69±18.95	0.00
Magnesium	31 to 40 patient	19	17.42±6.15	
	31 to 40 control	16	20.98±6.96	0.122
Zinc	31 to 40 patient	19	.41±0.15	
	31 to 40 control	16	.72±.24	0.00

P=patients, c= control

Table (9) : Correlation between blood glucose, magnesium and zinc with age (41-50) to diabetic patients

Groups		No	Mean± standard deviation	*p- value*
Glucose	41 to 50 patient	20	228.05±64.71	
	41-50control	9	133.22±20.43	0.000
Magnesium	41 to 50patient	20	18.29±7.59	
	41-50control	9	24.58±4.41	0.010
Zinc	41 to 50patient	20	55±.29	
	41-50control	9	.60±.21	0.565

Table (10): Correlation between blood glucose, magnesium and zinc of control and diabetic patients with age (51-60)

Groups		No	Mean± standard deviation	*p- value*
Glucose	Patients (51-60)	28	245.68±.89.1	
	Control	9	133.56±10.30	0.00
Magnesium	Patients (51-60)	28	16.38±7.56	
	Control	9	23.31±9.60	0.073
Zinc	Patients (51-60)	28	.51±.19	
	Control	9	.59±.21	0.344

Table (11) : Correlation between blood glucose, magnesium and zinc with age (>60) to diabetic patients

Groups		No	aMen± standard deviation	*p- value*
Glucose	above 60(patient)	24	245.25±86.75	
	above 60(control)	5	134.40±7.47	0.00
Magnesium	above 60(patient)	24	17.10±7.57	
	above 60(control)	5	22.70±6.23	0.124
Zinc	above 60(patient)	24	1.73±6.01	
	above 60(control)	5	.71±.17	0.413

Table (13): Correlation between blood glucose, magnesium and zinc with duration of disease to diabetic patients

groups		N	aMen± standard deviation	*p- value*
glucose	1 to10years(patient)	79	79.60±8.96	
	above 10 years(control)	23	79.30±16.53	0.399
magnesium	1 to 10years(patient)	79	6.88±.77	
	above 10 years(control)	23	8.73±1.81974	0.815
zinc	1 to10years(patient)	78	.22±.02	
	Above 10 years(control)	23	.18±.04	0.519

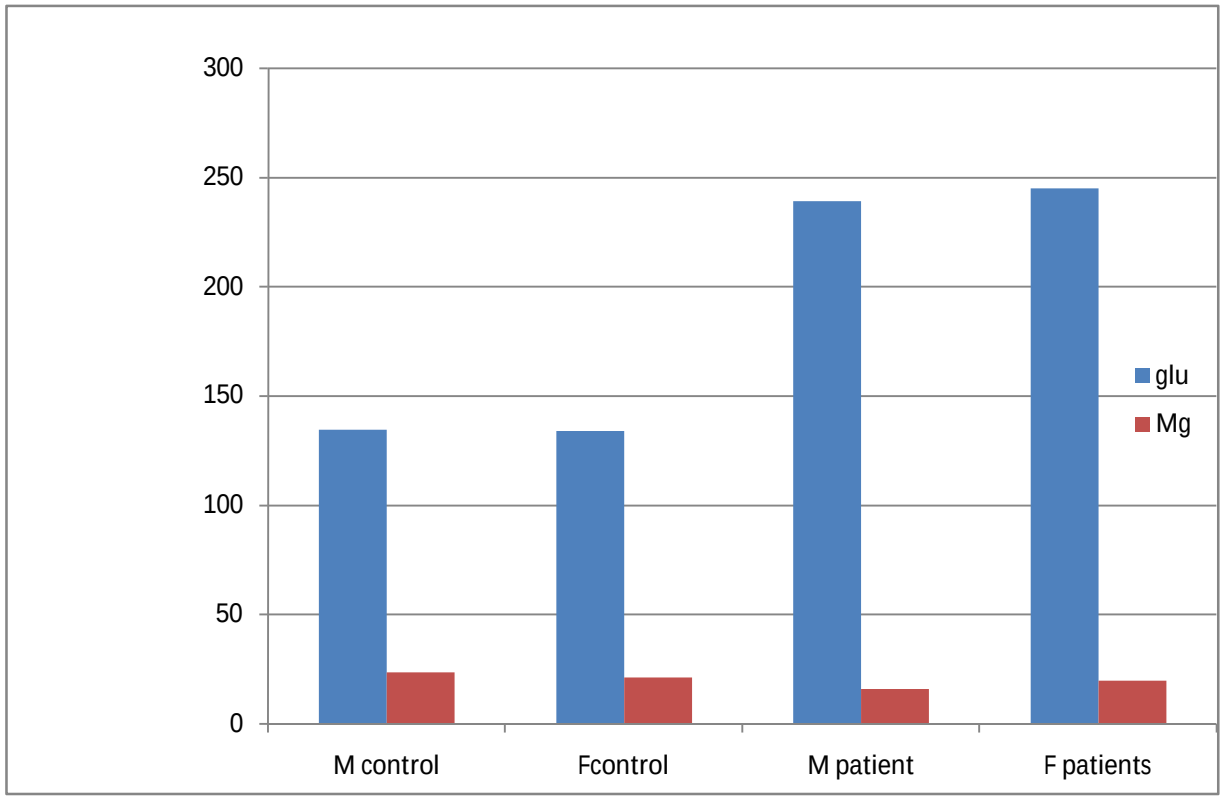

Figure (1): Concentration of serum glucose and magnesium of male and female diabetic patient

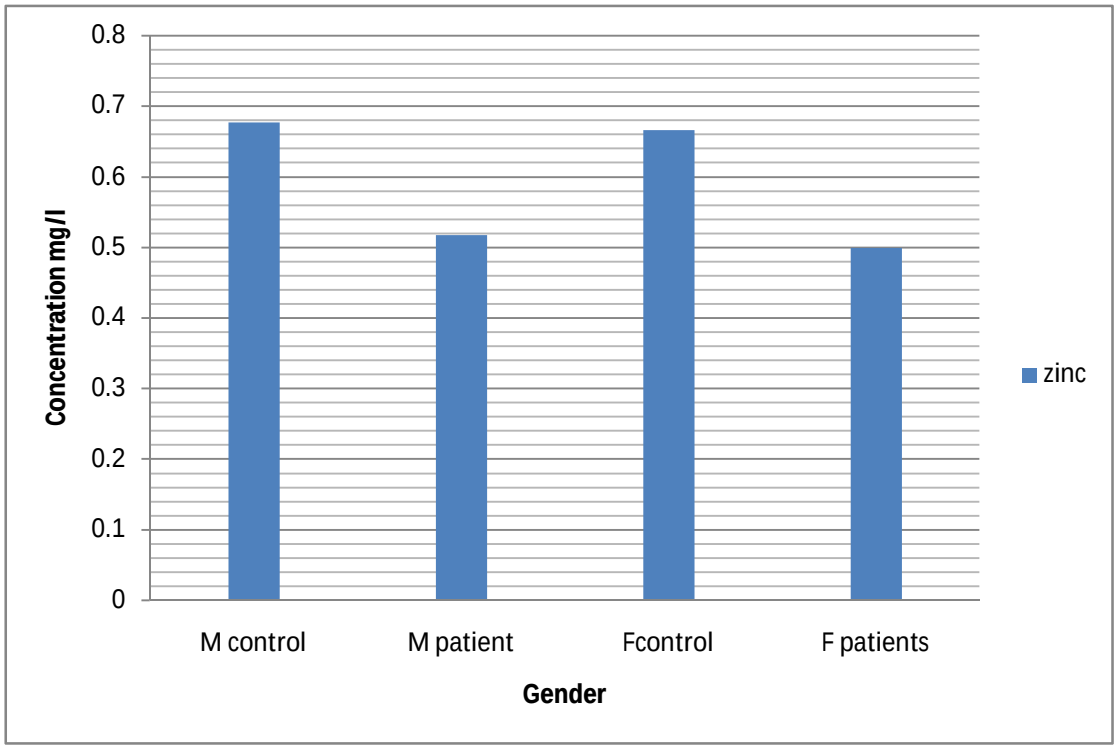

Figure (2): Concentration of serum of zinc of male and female diabetic patients

4- Discussion

In this study, zinc levels were found to be reduced in the serum of the diabetic patients compared with control. A significant reduction was observed (p. value = 0.01), and the zinc level reduced about 23.88% was observed from control value. These result in line with the previously reported finding of (Golik et al, 1993, Zarger et al, 202, Maroof and Al Sharbatti, 2007.

Results obtained from this study agreed with several large observational studies including the atherosclerosis risk in commonalties (ARIC) study have demonstrated strong sectional association between low serum magnesium and zinc levels and type —II diabetes (Poolssg and Rayssm, 1995, Ma et al, 1995). The results also agreed with previous in vitro studies done by Hwang and his colleges (1993) who demonstrated that there was an effect of magnesium and zinc on the secretion of insulin by the pancreas and on the responsiveness to insulin by peripheral tissues.

The establishment of an association between magnesium and zinc levels and risk of diabetes might suggest dietary or pharmacological measures to prevent type-II diabetes.

Preliminary investigations and findings obtained from specially designed questionnaire revealed that the majority of diabetic patients participated in this study were of an average ages of about 52 years, while control subjects were of about 49 years. It is well documented that Non-Insulin dependent diabetes

mellitus (NIDDM) MDDM category is characterized by adult onset and account for approximately 90% of all diabetes patients (Michael and Janet, 202, Elmahdi *et al* , 1991). Moreover, the relative frequency of NTDDM in relation to age has been demonstrated by many author (Gunnid, 1991). Harris et al, (1994) reported that NIDDM is rare under the age of 25 years.

(Elmahdi *et al* 1991) reported that in Sudan the majority of diabetic patients developing NIDDM at the age between 40-50 years. The prevalence rate of patients participated in this study was found to be lower than the reported prevalence of rate of other European countries (David et al, 1986, Gamble and Taylor, 1993) the lower age prevalence rate of Sudanese diabetic patients may be related to less longevity in their society, and it has been claimed that the incidence of NIDDM increase with age (Michael and Janet, 2000).

Sex distribution in diabetic patients of this study revealed that predominate in both study groups (control and diabetic patients). 55% were females in normal control and diabetic groups respectively. Other studies reported that higher incidence and prevalence rate of NIDDM were observed within females than in males. (Harris et al, 1994).

It appeared that control subjects maintained adequate level of random glucose of 133.25 mg/dl, but diabetic patients showed value of highly elevated level of 241.5 mg/dL. High statistically

difference between the two values at p< 0.5 (p. value = 0.000) of significance glycosylated haemoglobin.

Magnesium concentration in control group was of an average value of 22.47 ± 9.93mg/dl , while the level diabetic patients was 17.92 ± 6.12 mg/dl. A significant reduction of Mg++ level of about from control was obtained (p. value 0.011 at p < 0.05. the reduced levels of magnesium in diabetic patients was also reported by other workers. For example , (Song et al, 2004) claimed that hypomagnesemia was a feature of type-II diabetes , and supported a protective role of higher magnesium intake reducing the risk of developing type-II diabetes specially in overweight women.

Nadler *et al* (1992) evaluated the level of magnesium in 22 NIDDM patients, reported that diabetic patients of type-II diabetes, have intracellular magnesium deficiency and that magnesium deficiency may be a key factor in leading to enhance platelet reactivity in type-II diabetes and magnesium supplementation may prevail new therapeutic approach to reducing vascular disease in patients with diabetes.

Many studies suggested that low serum magnesium level might lead to type-II diabetes and association between dietary magnesium intake and plasma insulin levels in risk of type-II diabetes (Such Nack *et al* 1992, Song et al 2004, Remevo et al 2008).

Zinc was also investigated in this study. Zinc is involved in many biological processes, that include catalysts , stabilization of cell membrane and regulation of gene expression (Sammon, 2002).

The risk factors for diabetic mellitus were established and proposed. The potential effect of most inorganic nutrients especially zinc, have received limited attention (Truseul, 2003). In this present study we examined the levels of zinc in serum of diabetic patients, since few study death with evaluation of serum zinc levels in diabetes, but most of the study cited by the biological.

Literature dealt with the investigation of dietary zinc intake and the risk of type-II dealt diabetes and the effect of zinc supplementation on glycemic control in diabetic patients. The physiological significance of zinc is reflected in the diverse range of symptoms which is observed in zinc deficiency (Prosad, 1983) thus the potential exists for zinc to influence many metabolic function and to impact a range of disease including diabetes remains the leading cause of death in western countries.

Tasneem et al (2007) compared the levels of essential trace elements including chromium (Cr), coper (Cu) , iron(Fe), manganese (Mn) and zinc (Zn) in the biological samples (whole blood, urine, and hair) of patients having diabetes mellitus type --II concluded that mean value of zinc were significantly

reduction blood and hair sample of diabetic patients compared with control subjects of both gender and the urinary level of zinc were found to be higher in diabetic patients than in the urine of control. Other epidemiological studies also reported decreased plasma and intracellular zinc concentration in conjunction with increase urinary secretion in diabetic patients (Singh et al, 1998).

The earlier available data on zinc status in patients with diabetes mellitus are controversial. The controversy arises because the current methods to characterize marginal and sub-optimum zinc nutrition, such as the measurement of zinc in serum, plasma, and red blood cells (RBC), granulocytes, platelets lymphocytes, hair, nail and urine, do not provide consistent results (Walsh et al, 1994).

Heto *et al* 2000 studied the zinc kinetics, in IDDM patients concluded that no evidence of zinc deficiency or-h changing ,a kinetics parameters of zinc in patients with type-I diabetes following a venous, zinc tolerance test. Our results concerning the level of zinc in diabetic, patient€ are. in line with other findings of (Al-Maroct and Al-Shaatti (2007)) in Iraq, Yoon (2008 in Korea, Golik et al (1993 and Havivii(1989,).

Anetor et al (2008) investigated the atheragenic implication and magnesium in Nigerians with type-11 diabetes, concluded: that both Mg and: Zn levels were significantly reduced, probably suggesting lower antioxidant status. The implication is the

greater susceptibility in LDL- cholesterol oxidation and the attendant risk of development of premature coronary head disease is reduced.

Conclusion

From the results and findings of this study, we may conclude that:

1. Diabetic patients participated in this study presented with poor glycemic control e.g. with high blood glucose level.
2. Magnesium level in the blood of diabetic patients was much reduced compared with the levels in control non diabetic , a reduction of about of 22% was attained.
3. Zinc level in the blood of diabetic patients was also reduced compared with control and a reduction of about 17% was observed.
4. Gender had no effects on the level of these trace elements, males and females maintained nearly the same levels of these trace elements in diabetic patients.

Recommendations

1. Appropriate studies concerning the supplementation of trace elements magnesium and zinc to diabetic patients to compensate the complication which might be arise with the deficiency of this trace elements in the diabetic patient should be carried out.
2. More attention must paid to improve the zinc and magnesium status and glycemic control of diabetic
3. patients.
4. A support to the recommendations to increase consumption of major source of magnesium such as whole grains , nuts, and green leafy vegetable must be encourage.
5. More attention is needed to improve the zinc and magnesium status among Sudanese diabetic patients, especially whose activity pattern tend to be sedentary and increasing physical exercise.
6. Appropriate nutrition education is urgently required for diabetic patients as well as general adult population to attain dietary adequacy and increase the amount of their physical activities.
7. Farther investigation is needed to elucidate the relation between trace elements, magnesium and zinc supplementation and glycaemic control before firm conclusion to be drawn for this relation.

References:

1. Abate N. Insulin resistance and obesity. The role of fat distribution Patten. Diabetes Care Mar; 1996, (3: 292-4) .
2. Abbraira, c, Colwell, J, Nurgall, F, cardiovascular events and correlated in the diabetes mellitus 1997, Jan 27, 52(2) 181-8.
3. Ammon, H. P. T., Klumpp, S., Fub, A., Verspol. F. L., Jaecke, H., Wendel, A. and Muller, P., Dia .etologia, 1989, 32, 797—800.
4. Barker DJ. The fetal origins of type 2 diabetes myelitis. Ann Intern Med. Fe 16 1999: 130 (4 pt 1) : 322-4
5. Beroavanny, I., Waas, I. and Ebrlich. B. E.. Nature 1991, 351,751-754.
6. Bhandari N Substantial reduction in severe diarrheal morbidity by daily zinc supplementation in young north Indian children. Pediatrics, 2002: 109: e86.
7. Bousageon R, Bejan – Angoulvant, T Saddatian – Elahi M, Lafont's, Bergeonea C, Kassai, B, Erpeldirger , S, Wright JM, Gueyffier, F, Cornu C" Effect of intensive glucose lowering treatment on all cause morality, cardiovascular death, and microvascular events in type 2 diabetes : meta-analysis of randomized controlled trials (2011).

8. Briefel RR J Nutr. Zinc intake of US population: findings from the third National Health and Nutrition Survey l988-1994.; 130: 1367 S.
9. Brown KH. Am J Clin Nufr Effect of supplemental zinc on the groh and serum zinc co4ent.rations Of: pre-pubertal children: a .meta-analysis. of randomized, controlled trial, 2002.375: 1062.
10. Buccolo G. Quantitative determination of serum triglycerides by use of enzimes Clin Chem, 1973,19 (5) : 476-482.
11. Corica, F., Allegra, A and Lentile, R, Am. J. Hyepertens., 1999, 12, 128—136.
12. Cousins RI ILSI Press. Zinc. In: Present knowledge in Nutrition Ed. Ziegler EE. Filer LJ. Washington DC.1996.
13. Domingu, L. J., Bdrbagallo, M., Sowers, I. R. and ReSnick, L. M.. J. Endocrinol. Mttab., 1998, 83, 4402—4407.
14. Durlàch, , DurHch v:, Bac, P., Rayssiguier, Y. Bara, M. and Bara, A. G., Maes. Res., 1993, 6, 37—394.
15. Eikelboom J.E, Ann Intern Med,1999, 131:363-75.
16. Fawcett,W. J., Haxby, E. J. and Male, D:A,, Br. Anaesth.1999, 83 302-320.
17. Fong DS, Alellp LP, Ferris FL 3rd Klen R Diabetic retinopathy , Diabetes Care,2004: 27: 2540 – 2553
18. Forsmire GJ Am J Cin Nutr zinc toxicity,1990; 51: 225.

19. Freeland -Graves. E Am J Clin Nutr, effect of zinc supplementation on plasma high –density lipoprotein cholesterol and zinc. 198235: 988.
20. Garfinkel, D., Magnesium, 1988, 7, 249—261.
21. Garg, M. C., Ojha, S. and Bansal, D. D., Indian J. Exp. Biol, 1996, 34, 26266.
22. Guther, T. Ho1leg1; V, Voam, I., isch, G. and Classen,H. G., Magnes. Bull, 1992, 14, 88—93.
23. Haenni, A., OhaJ, M. and Lithell, H., Metabolism, 2001, 50, 1147- 151.
24. Hans, C.P, Chaudhary D. P. and Bans, D. D. Indian J. Exp. Biol., 2002, 40; 1275—1279.
25. Hassan, M. and Lehningêr, A. L. J. Biol. Chem., 1956, 223, 123—138.
26. Hotz C and Brown KR BZ Assessment of the risk of zinc deficiency in populations and options for its control. Food Nutr; 2004,25 : 99S.
27. Hsu, J. M.Rubenstein, B.. and Paleker, A. G., ibid, 1982, 112,488-496.
28. Humphries, S.; Kusbnei, H. and Faller,.B1Ain, J:.Hynertens., 1999, 12, 747—756.
29. Kaplan. Tg1ycerides. Clin Chem The C.V. Mosby Co. St Louis. Toronto. Princeton; 437 and Lipids 1984,1194-1206.

30. Kaplan L . Glucose. Kaplan A tal Clin Chém The CV. 4sby Co. St Louis. Toronto. Princeton; 1984,1032-1036.
31. Lefebvre, f.J. and Scheen, A. J. Clin. Invest. Med., 1995,18, 340—347.
32. Lind T. Community based randomized controlled trial of iron and zinc supplementation in Indonesian infants: interactions between iron and zinc. Am J Clin Nur; 2003,77:883.
33. Lonneredal B. J Nutr. Deitar factors influencing zinc absorption; 2000, 130: 1378 S.
34. Mahely RWJlipids Res; 1984, 25: 1277 – 1294.
35. Mallone R, vaa Ender P. T cells in the pathogenesis of type 1 diabetes Curr Diab Rep; 2008: 8: 101-106.
36. Meiattini F. The 4-hydroxybenzoate /4-aminophenazeone , chromogenic system. Clin Chem;, 1978. 24 (12): 2161-2165.
37. Mimou, F. Miodovink, M. and Tsang, R.C. Obstet. Gneco1., 1987, 70, 85-89.
38. Nadler, J. L., Buchanan, T., Natarajan, R.. Atonipillai. I., Bergman, R. and Rude, R, K., Hypertension, 1993, 21, 1024—1029.
39. Naito H K1984 High-density Lipoprotem (HDL) cholesterol Kaplan A et al Clin Chem The C V Mosby Co St Louis Toronto Princeton, 1207-121 and 437

40. Naito H.k Cholesterol, Kaplan A et al. Clin Chem The C.V. Mosby Co. St Louis . Toronto. Princeton; 1984,1194-11206 and 437.
41. National Academy Press,. Food and Nutrition Board IOM. Dietar reference intake of vitamin A, vitamin K, arsenic , borom, chromium copper, iodine, iron, manganese, molybdenum, nickel, silicon and zinc. Washington DC.,2002.
42. National Vital Statistics Reports Available from http://www.cdc.gov/nchs/ data/nvsr/nvsr56/nvsr56_010.pdf 2008; 56(10).
43. Okada M Low-density lipoprotein cholesterol can be chemically measured J. Lab. Clin. Mad.; 1998,132, 195-201.
44. Orimo, H. and Ouchi, Y.,. Ann N, Y. Acad. ci., 1990, 598, 44 464.
45. Paolisso, G., Sganbafo S. and Gambardella, A., Am. J. Clin. Nufr., 1992, 55. 1161-1167
46. Parasad AS JAMA Hypocupremia induced by zinc therapy in adults; 1978. 240: 2166.
47. Prasad AS Am J Clin Nutre Discovery of human zinc deficiency and studies in an experimental model.; 1991. 53: 403.
48. Prasad AS J Clin Invest 988 J Clin Invest Serum thymulin in human zinc deficiency,1988, 82: 1202.

49. Raccah, D., annct, M. F Issautier, D: and Vae, F. Dio. Med.1994, 20,271—274.
50. Romani, A , Marf1la, C and Laksmanan, M , Thyod, 1999 , 513-519
51. Rude, R. K., Postgrad. Med., 1992, 92 21.7—224.
52. Savillahit E, Ormala, T. Sukkonen, T, Sandini-Poajavouri U, Kantele JM, Arato A, Ilonen J, Akerblom HK(1999) Jjuna of Patients with insulin dependent diabetes mellitus (IDDM); 116-70-77.
53. Shills, M. E., nu. Rev. Nutr., 1988 8, 42960.
54. Van Wouwe Jp Eur J Pediatr Clinical and laboratory diagnosis of acroderatitis enterophahica' 1989. 149: 2
55. Weglicki,W. B., Freedman, A. M., Bloom, S., Atrakchi: A. H., Cassidy, M. M., Dickens, B. F. fid Mak, I. 1., Am. J Cardiovasc. Pathol., 1992, 110, 169—173.
56. Wharen j, Elberg Km Jornvall H "C-peptide is bioactive peptide" Diabetologia,2007,50(3) 503-9.