1.1 Introduction

Diabetes Mellitus (DM) is a chronic disease characterized by a disorder of the glucose metabolism associated with a reduced ability of tissues to respond to insulin (insulin resistance), DM causes high morbidity and mortality derived by chronic micro-vascular complications such as retinopathy, nephropathy, or neuropathy and macro-vascular complications such as ischemic cardiac problems, cerebral vascular accidents, peripheral vascular disorders. [1] Due to aging, accelerated population growth, urbanization and high prevalence of obesity and an inactive lifestyle, the number of people with diabetes is increasing globally at a rapid speed. The prevalence of DM worldwide was estimated at 4% in 1995 and is expected to rise to 5.4% by year 2025. [2]

DM is now one of the major health problems in Sudan resulting in 10% of all hospital admissions and mortality. A small population based study in 1993 of a sample of 1284 adult men, showed a prevalence of 3.4% of type 2 diabetes. [3] While annual health statistical report 2005, 2006, and 2007 of Federal Ministry of Health showed that, there is an increase in prevalence of DM. [4]

A number of studies report that there was an association between diabetes mellitus and alterations in the metabolism of several trace minerals. [5] Impaired insulin release, insulin resistance and glucose intolerance in experimental animals and humans with DM have been linked to a compromised status of copper and zinc. [6] Diabetes seems to be associated with numerous abnormalities of plasma trace elements and magnesium. [7]

The current study aim to estimate serum levels of zinc and magnesium among patients with type II Diabetes mellitus.

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1.2 Rationale

Trace elements are accepted as essential for optimal human health. Recently, there is accumulating evidence that the metabolism of these trace elements in particular zinc ,chromium and magnesium is altered in diabetes mellitus and these elements might have Specific roles in the pathogenesis progress of this disease. [7] It has been shown that the deficiencies of zinc and magnesium predispose a person to glucose intolerance and to promote the development diabetic complications such as cardiovascular disease, retinopathy and nephropathy. Several studies reported the deficiency of these two minerals in type II diabetes mellitus. These studies have been established on these problems in the western countries, but very few data is available from developing countries .Few published studies were found regarding the level of trace elements in Sudanese diabetic patients. In view of these facts it is important to determine the levels of trace elements among type II diabetics to clarify their status.

1.3. Objectives of the study

1.3.1 .General Objective

To evaluate serum zinc and magnesium level among type II Diabetes mellitus patients in Khartoum state.

1.3.2. Specific objective

1. To estimate serum levels of zinc and magnesium in diabetic patient and control group.

2. To compare the serum levels of zinc and magnesium between diabetic patient and control group,

3. To correlate between the duration of DM and serum levels of zinc and magnesium in the test group.

4. To correlate serum glucose level with zinc and magnesium levels in test group.

5. To correlate between the age and serum levels of zinc and magnesium in the test group.

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Chapter Two

2. Literature Review

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2.1 Diabetes mellitus

Diabetes mellitus is a syndrome characterized by chronic hyperglycemia and relative insulin deficiency, resistance or both. [8] Insulin enables cells to absorb glucose in order to turn it into energy. This causes glucose to accumulate in the blood leading to various potential complications. [9] It has been defined by the world Health Organization (WHO), on the basis of laboratory findings as a fasting venous plasma glucose concentration greater than 7.0 mmol/L (126 mg/dl) or greater than 11.1mmol/L (200 mg/dl) two hours after a carbohydrate meal or two hours after the oral ingestion of the equivalent of 75 g of glucose, even if the fasting concentration is normal. [10]

2.1.1 Glucose metabolism & regulation:

Glucose is a primary source of energy for humans. The nervous system, including the brain totally depends on glucose from the surrounding extracellular fluid (ECF) for energy. Nervous tissue cannot concentrate or store carbohydrates; therefore, it is critical to maintain a steady supply of glucose to the tissue. For this reason, the concentration of glucose in the ECF must be maintained in a narrow range. When the concentration falls below a certain level, the nervous tissues lose the primary energy source and are incapable of maintaining normal function. [11, 12] The principal organ of glucose homeostasis is the liver, which absorbs and stores glucose (as glycogen) in the post – absorptive state and releases it into the circulation between meals to match the rate of glucose utilization by peripheral tissues**.**About 200 g of glucose is produced and utilized each day**.** [9]

 The liver, pancreas, and other endocrine glands are all involved in controlling the blood glucose concentrations within a narrow range.

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During a brief fast, glucose is supplied to the ECF from the liver through glycogenolysis. When the fasting period is longer than one day, glucose is synthesized from other sources through gluconeogenesis. Control of blood glucose is under two major hormones: insulin and glucagon both produced by the pancreas. Their actions oppose each other. [11, 12]

Insulin is the major regulator of intermediary metabolism, although its actions are modified in many respects by other hormones, its actions in fasting state and postprandial states differ. In the fasting state its main action is to regulate glucose release by the liver, and in the postprandial state it additionally facilitates glucose uptake by fat and muscle. [9]

The effect of counter regulatory hormones (glucagon, epinephrine, cortisol, and growth hormone) is to cause greater production of glucose from the liver and less utilization of glucose in fat and muscle for a given level of insulin. [9]

  Both insulin deficiency and glycosuria are known to inhibit the tubularre-absorption of phosphate. This inhibition has previously been evaluatedeither in the fasted state or on a normal phosphate diet[13.]Glucagon is the primary hormone responsible for increasing glucose levels. It is synthesized by the α cells of islets of langerhans in the pancreas and released during stress and fasting states. When these cells detect a decrease in body glucose, they release glucagon. [11]

2.1.2 Classification of Diabetes mellitus.

2.1.2.1 Primary Diabetes mellitus:

Diabetes mellitus can be divided into two major types called type 1 and type 2. Type 1 diabetes is characterized by an absolute lack of insulin, while type 2 is a relative deficiency of insulin without loss of production. Historically, type 1 diabetes mellitus has been called juvenile onset diabetes. A name for type 1 diabetes that is used more often is insulin – dependent diabetes mellitus (IDDM).These descriptions inferred the early

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age of onset and dependence of these individuals on exogenous insulin for survival. [14]

Type 2 diabetes mellitus, with its relative lack of insulin, has been termed adult onset diabetes and non –insulin – dependent diabetes mellitus (NIDDM). [14]

2.1.2.1.1 Type 1 Diabetes mellitus:

Type 1 diabetes mellitus is characterized by loss of the insulin-producing beta cells of the islets of Langerhans in the pancreas, leading to insulin deficiency. This type can be further classified as immune-mediated or idiopathic. The majority of type 1 diabetes is of the immune-mediated nature, in which beta cell loss is a T-cell-mediated autoimmune attack. [12]

The epidemiology of type 1 diabetes is a disease resulting in insulin deficiency. It usually presents during childhood and has been suggested that many cases follow a viral infection which has destroyed the β-cells of the pancreatic islets. [10] Type 1 diabetes is common among Caucasians and African –Americans; it is uncommon in Asians, American Indians, African blacks, Inunits and other certain races. [14]

In western countries almost all patients have the immune-mediated form of the disease. Type 1 diabetes is prominent as a disease of childhood, reaching a peak incidence around the time of puberty, but can present at any age. A "slow-burning "variant with slower progression to insulin deficiency occurs in later life and is sometimes called latent autoimmune diabetes of adults (LADA). This may be difficult to distinguish from type 2 diabetes. The highest rates of type 1 diabetes in the world are seen in Finland and other northern European countries, with the exception of the island of Sardinia, which for un known reasons has the second highest rate in the world.The incidence of type 1 diabetes appears to be increasing in most populations. In Europe the annual increase is of the order of 3-4 %, and is most marked in children under the age of 5years. [9]

2.1.2.1.2 Type 2 Diabetes mellitus:

The epidemiology of type 2 diabetes is the most common form of diabetes. Typically this disease is characterized by an underproduction of insulin or insulin insensitivity at target tissues. Type 2 diabetes is three to four times as prevalent in people of African and Caribbean ancestry and four to seven times more prevalent in people of Hispanic American origin and in those from south Asia and Arabia living western lifestyles, than in white Europeans. Indolent well- fed populations are two to twenty times as likely to develop type2 diabetes as lean populations of the same race. [9] Diabetes type II is characterized by insulin resistance with relative insulin deficiency. This type accounts for 90% of all diabetic cases and commonly appears in adults so it's called adult- onset diabetes. In this type of diabetes, insulin is present in little amounts. Fatty acids are incorporated into triglycerides for release of very low –density lipoproteins. So they are at increased risk of developing macro vascular and micro vascular complications. [15]

Causes of type 2 Diabetes mellitus include the following:

I- Genetics:

Genetic factors may play a greater role in this disease than in type 1 diabetes. Concordance rates for type 2 diabetes in identical twins are 100%. Currently, a genetic marker has not been discovered for type 2 diabetes.[14] The genetic causes of some rare forms of type 2 diabetes have, however, emerged over the last 15 years. Dozens of mutations of the insulin receptor affect a tiny proportion of all type 2 patients.These usually cause type 2 diabetes with obesity, marked insulin resistance, hyper-androgen-ism in women and often an area of hyper-pigmented skin (acanthosisnigricans). [9]

Individuals with some mutations or deletions of mitochondrial DNA develop type 2 diabetes or impaired glucose tolerance, often associated

with rare neurological syndromes. A rare variant of type 2 diabetes is referred to as maturity –onset diabetes of the young (MODY). This is dominantly inherited. [9]

II- Environmental factors ((early and late)):

A strong association has been noted between weight at birth and at 12 months of age and glucose intolerance later in life, particularly in those who gain excess weight as adults. The concept is that poor nutrition early in life impairs beta-cell development and function, predisposing to diabetes in later life. Low birth weight has also been shown to predispose to heart disease and hypertension in later life. [9] Diet factor may play an important role in the development of type 2 diabetes. Obesity, especially of the abdominal viscera, is common in individuals with type 2 diabetes.Changes in the normal substrate concentrations delivered to the liver, adipose tissue, and skeletal muscle are thought to affect the normal functioning of the insulin receptor. [14]

III- Immunological factors:

There is no evidence of immune involvement in the pathogenesis of type 2 diabetes, but as noted earlier a proportion of late – onset patients carry islet auto-antibodies-ICA and GAD- at diagnosis and these are more likely to progress to insulin therapy. Such cases are probably type 1 diabetes masquerading as type 2 diabetes. [9]

2.1.2.2 Secondary Diabetes mellitus.

Diabetes mellitus associated with other conditions include:

I- Absolute insulin deficiency due to pancreatic disease

(Chronic pancreatitis, haemochromatosis, cystic fibrosis).

II- insulin deficiency due to excessive growth hormone (acromegaly), glucocorticoid secretion (Cushing's syndrome) or increased plasma glucocorticoid concentrations due to administration of steroids.

III- Drugs such as thiazide diuretics and corticosteroid therapy. [9, 10]

2.1.2.2.2 Gestational Diabetes mellitus:

Gestational diabetes occurs temporarily during pregnancy in women with an inherited predisposition, over weight; family history of diabetes. [16]

Gestational diabetes mellitus (GDM) resembles type 2 diabetes in several respects, involving a combination of relatively inadequate insulin secretion and responsiveness. It occurs in about 2–5% of all pregnancies and may improve or disappear after delivery. Gestational diabetes is fully treatable, but requires careful medical supervision throughout the pregnancy. About 20–50% of affected women develop type 2 diabetes later in life. [17]

2.1.3 Pathophysiology of Diabetes mellitus:

In both type 1 and type 2 diabetes mellitus, individual will be hyperglycemic, which can be severe. Glucosuria can also occur after the renal tubular transporter system for glucose becomes saturated. This happens when the glucose concentration of plasma exceeds roughly 180mg/dl in an Individual with normal renal function and urine output. [14] As hepatic glucose overproduction continues, the plasma glucose concentration reaches a plateau around 300 to 500 mg /dl (17 to 28 mmol /l) provided renal output is maintained. [14] The individual with type 1 has higher tendency to produce ketones. Type 2 diabetes patients seldom generate ketones, but instead have a greater tendency to develop hypersmolar non ketotic states.The difference in glucagon and insulin concentrations in these two groups appears to be responsible for the generation of ketones through increased B- knoops oxidation. In type 1, there is an absence of insulin with an excess of glucagon. This permits gluconeogenesis and lipolysis to occur. In NIDDM, insulin is present as is (sometimes) hyper-insulinemia therefore glucagon is attenuated. Fatty acid oxidation is inhibited in type 2 diabetes. [14] This causes fatty acids to

be incorporated into triglycerides for release as very low density lipoproteins (VLDL). [14]

2.1.3.1 Clinical presentation of diabetes:

Acute and sub acute presentations often overlap.

(i) Acute presentation:

Polyuria due to the osmotic diuresis that results when blood glucose levels exceed the renal threshold.

Thirst due to the resulting loss of fluid and electrolytes. Weight loss due to fluid depletion and the accelerated break down of fat and muscle secondary to insulin deficiency.

Ketoacidosis may be the presenting feature if these early symptomsare not recognized and treated in a type 1 diabetes patients**.** [14]

(ii) Sub acute presentation:

The clinical onset may be over several months or years, particularly in older patients. Thirst, polyuria and weight loss are usual features, but medical attention is sought for such symptoms as lack of energy, visual blurring ((owing to glucose – induced changes in refraction)), or pruritus vulvae or balanitis that is due to Candida infection. [14]

2.1.5 Complications of Diabetes.

(A) Acute metabolic complications:

Patients with diabetes mellitus may develop one of the several metabolic complications; these include diabetic ketoacidosis and hyperosmolar non-ketotic coma. [18]

(B) Long-term complications:

Vascular disease is a common complication of diabetes mellitus.

I-Macrovascular disease: Due to abnormalities of large vessels, may present as coronary artery, cerebrovascular or peripheral vascular insufficiency**.** The condition is probably related to alterations in lipid metabolism. [10]

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II-Microvascular disease: Due to abnormalities of small blood vessels particularly affects the retina ((diabetic retinopathy)) and kidney, the incidence of both may be related to in adequate glucose control. [10]

(i)Diabetic eye disease: Diabetes can affect the eyes in a number of ways. The most Common and characteristic form of involvement is diabetic retinopathy. [9]

 Diabetes has been the most common cause of blindness in the population as a whole up to the age of 65years. [9] Other forms of eye disease may also occur.

 (ii) Diabetic kidney: The kidney may be damaged by diabetes in three main ways:Glomerular damage, Ischaemia resulting from hypertrophy of afferent and efferent arterioles, and ascending infection. [9]

(iii)Ischaemiclesions: Arteriolar lesions, with hypertrophy and hyalinization of the vessels, can occur in patients with diabetes. The appearances are similar to those of hypertensive disease and lead to ischemic damage to the kidneys. [9]

(iv)Infective lesions: Urinary tract infections are relatively more common in women with diabetes, but this does not apply to men. Ascending infection may occur because of bladder stasis resulting from autonomic neuropathy, and infections more easily become established in damaged renal tissue. [9]

(v) Diabetic neuropathy:

Diabetes can damage peripheral nervous tissue in a number of ways .The vascular hypothesis postulates occlusion of the vasa nervorum as the prime cause. This seems likely in isolated mononeuropathies, but the diffuse symmetrical nature of the common forms of neuropathy implies a metabolic cause. Since hyperglycaemia leads to increased formation of sorbitol and fructose in Schwann cells, accumulation of these sugars may disrupt function and structure. [9]

The complications that cause excess death in early -onset patients are mainly related to diabetic nephropathy, but there is also a considerable excess cardiovascular mortality. Heart disease, peripheral vascular disease and stroke are the major causes of death in patients over the age of 50 years. [9]

2.2 Trace elements

In analytical chemistry, a trace element is an element in a sample that has an average concentration of less than 100 parts per million measured in atomic count or less than 100 micrograms per gram.In biochemistry, a trace element is a dietary mineral that is needed in very minute quantities for the proper growth, development, and physiology of the organism. [19] .Trace element, also called Micronutrient, in biology, any chemical element required by living organisms in minute amounts, usually as part of a vital enzyme, a cell-produced catalytic protein. [19]

2.2.1 Zinc

The discovery of a variety of zinc- related clinical disorders have directly demonstrated the importance of zinc in human nutrition. It is second to iron as the most abundant trace element in the body. [20]

2.2.1.1 Chemistry of zinc

Zinc (atomic number 30, relative atomic mass 65.39) is a particularly stable ion. Zinc has fast ligand exchange kinetics and flexible coordination geometry, and is a good electron acceptor (strong Lewis acid), with no redox reactions. There is a hypothesis that zinc ions, present in the cytoplasm at10 mol/L and in equilibrium with numerous zinc metalloenzymes and transcription factors, act as "master hormone," particularly in relation to cell division and growth. [20]

2.2.1.2 Dietary sources of zinc

Zinc is widely distributed in food mainly bound to proteins. The bioavailability of dietary zinc us dependent upon the digestion of these proteins to release zinc and allow it to bind to peptides, amino acids, phosphate, and ligands within the intestinal tract. The most available dietary sources of zinc are red meat and fish. Wheat germ and whole bran are good sources, but their zinc content is reduced by miling and food processing. The median intake for men in the United States is about 14mg/day and for women 9mg/day. [20]

2.2.1.3 Absorption, transport, metabolism and excretion of zinc

Regulation of the net intestinal uptake of zinc is by control of absorption efficiency and usually ranges from 20% to 50% of the dietary content. At an intake of 12.2mg zinc per day, the fractional absorption is 26%, but at the very low intake of 0.23mg zinc per day this has been shown to increase to 100%.interaction with other dietary constituent, such as phytate, fiber, calcium, and iron, reduce the net absorption of zinc.Iron at supplemental dosages (up to 65mg/day) may decrease zinc absorption so that pregnant and lactating women taking iron require zinc supplementation. [20]

Absorbed zinc is transported to the liver where active incorporation into metalloenzymes and plasma proteins occurs. About 80% of plasma zinc is associated with albumin and most of the rest tightly bound in the high molecular proteins α_2 -macroglobulin. The zinc on albumin is in equilibrium with plasma amino acids (mostly histidin and cysteine) and this small (<1%) ultrafilterable fraction may be important in cellular uptake mechanisms[20].Total adult body content of zinc is about 2 to 2.5g and the metal is present in the cells of all metabolically active tissue and

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organs. About 55% of the total is found in muscle and approximately 30% in bone. Red cell zinc concentration is about 10 times higher than in plasma, due to the large amounts of carbonic anhydrase. [20]

Zinc binding to the metal-regulatory transcription factor 1 (MTF1) activates metallothionein (Mt) expression. This multifunctional, low molecular weight protein (9000 to 10,000 Da) has a high content of cysteine and reversibly binds zinc. Mt is important in intracellular zinc trafficking and helps to maintain intracellular zinc concentrations. Hepatic synthesis of Mt is induced by interleukin-1, Interlekin-6 and glucocorticoids in response to infection, trauma, and other stressors. Fecal excretion includes both unabsorbed dietary zinc and zinc resecreted into the gut. Urine output of zinc is normally only about 0.5 mg/day, but increases greatly during catabolic illness and ketosis. The release of intracellular contents from skeletal muscle has been established as the source of the excess urinary zinc. [21]

2.2.1.4 Functions of zinc

More than 300 zinc metalloenzymes occur in all six categories of enzyme systems, important examples in human tissue include carbonic anhydrase, alkaline phosphatase, RNA and DNA polymerases, thymidine kinase carboxpeptidases, and alcohol dehydrogenase. The key roles of zinc in protein and nucleic acid synthesis explain the failure of growth and impaired wound healing observed in individuals with zinc deficiency.Proteins from domains able to bind tetrahedral zinc atoms by coordination with histidin and cysteine to form folded structures that are known as "zinc fingers." These have important roles in gene expression by acting as DNA-binding transcription factors and play a key role in developmental biology and also in the regulation of steroid, thyroid, and other hormone synthesis. [20]

2.2.1.5 Deficiency of zinc

As might be expected from the multiple biochemical functions of zinc, the clinical presentation of deficiency disease is varied, nonspecific, and related to degree and duration of the depletion. Signs and symptoms include depressed growth with stunting, increased incidence of infection, possibly related to alteration in immune function, diarrhea, skin lesion, and alopecia. [20]

2.2.1.6 Effects on growth

Dietary zinc deficiency is prevalent in countries worldwide where a cereal-based diet high in phytate and fiber, but low in animal proteins, is common. In children, reduced growth and other developmental abnormalities are reversible by zinc supplementation. Zinc in human breast milk is efficiency absorbed because of the presence of factors such as picolinate and citrate. [20]

2.2.1.7 Parental Nutrition

Some patients requiring intravenous feeding after surgery are likely to be significantly zinc depleted because of poor oral intake before and after surgery. They may also have increased zinc losses from intestinal tract via diarrhea and in urine from catabolism of muscles during periods of negative nitrogen balance. [21]

2.2.1.8 Infectious disease

Zinc depletion impairs immunity and has a direct on gastrointestinal tract, which increases the severity of infections. A review of controlled trails of zinc supplementation of children in low-income countries found significant clinical benefits in cases of persistent diarrhea and respiratory diseases. Interaction with vitamin A is important because in population at risk of zinc and vitamin A deficiency, provision of zinc alone increases in

the incidence of respiratory infection, but when vitamin A is also added, respiratory infections are decreased. [20]

2.2.1.9 Subclinical effects of zinc deficiency

When zinc deficiency is not server enough to cause clinical signs and symptoms, it may still have a subclinical effect on immune function, the synthesis and action of hormones, and neurological function. [20]

2.2.1.10 Immune function

Patients with zinc deficiency in the middle ⁻east were known to die before the age of 25 because of various infections and parasitic disease. In zinc deficiency, there is a reduction in the activity of serum thymulin, the thymus-specific hormone that is involved in t-cells function, and an imbalance develops between Th1 and Th2 helper cells. The lytic activity of natural killer cells also decreases. These complex changes result in an impairment of cells mediated immunity. [20]

2.2.1.11 Hormones synthesis

Zinc has a role in the synthesis and actions of hormones, via zinc transcription factors. Zinc depletion is associated with low circulating concentrations of testosterone, free T4, insulin-like growth factors (IGF)-I, and thymulin. Both plasma IGF-I and growth velocity increased in zinc-supplementation children. [20]

2.2.1.12 Neurological effects

Sever zinc deficiency is known to affect mental well-being, with varying degrees of confusion and depression being consistent with zinc enzymes having important activity in brain development and function. [20]

2.2.1.13 Zinc toxicity

Zinc toxicity can occur in both acute and chronic forms. Acute adverse effects of high zinc intake include nausea, vomiting, loss of appetite, abdominal cramps, diarrhea, and headaches.[20.]One case report cited severe nausea and vomiting within 30 minutes of ingesting 4 g of zinc

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gluconate (570 mg elemental zinc)[22].Intakes of 150–450 mg of zinc per day have been associated with such chronic effects as low copper status, altered iron function, reduced immune function, and reduced levels of high-density lipoproteins[23].Reductions in a copper-containing enzyme, a marker of copper status, have been reported with even moderately high zinc intakes of approximately 60 mg/day for up to 10 weeks. [24]

2.2.1.14 Zinc and diabetes mellitus

Zinc is an important essential mineral in human nutrition with a wide range of biological functions. Zinc fulfills catalytic, structural, or regulatory roles in more than 200 zinc-requiring metalloenzymes. [25]The interaction of zinc with insulin induces conformational changes and enhances binding to the insulin receptor, [26] With regard to glucose metabolism, zinc is a co-factor of several keyenzymes. Zinc is an activator of fructose-1-6-bisphosphate aldolase, and an inhibitor of fructose-1-6-biphosphatase. [26] Zinc can also exert antioxidant activity, and is a cofactor in Cu-Zn SOD, a major antioxidant enzyme There is accumulating evidence that the metabolism of zinc is altered inInsulin dependent diabetes mellitus and that zinc might have specific roles in the pathogenesis and progress of this disease. Increased urinary loss of zinc is a commonly encountered feature of diabetes some studies has reported zinc deficiency along with alterations in zinc metabolism in patients with diabetes. [27, 28, 29]

In another study it was found that diabetes can alter copper, zinc and lipid peroxidation. Plasma copper was higher and plasma zinc and plasma peroxide concentrations were lower in diabetic than in control subjects. [30] Consequently, considering the possible modulating effects of zinc on insulin sensitivity and its antioxidant functions, it was postulated that a restored Zn status in individuals with type 2 DM might counteract the deleterious effects of oxidative stress and help to prevent complications

associated with diabetes. [31] .In patients with type II diabetes mellitus, these patients had decreased serum zinc concentrations, because there presence of male absorption of zinc which leads to hyperzincuria. [32] In diabetes, zinc is decreased, copper execration increased and SODactivity decreased. [33] Therefore it was postulated that elevated levels of Cu-Zn SOD, elicit a protective effect against diabetes. [34]Another study it was found that the amounts of copper was increased but there were no significant alternation in levels of serum zinc in DM. [35] The amounts of Cu-Zn SOD activity was found reduced in diabetic patient, the copper and zinc status of these diabetic patients was reduced, providing further evidence of a role for these antioxidant trace elements in this disease. [36] Because zinc can exert a number of indirect antioxidant functions, ahypothesis in humans that increased zinc intake will protect against oxidant stress in persons with tendencies for both moderate zinc deficiency and high oxidant stress. [27]

2.2.2 Magnesium

Is the fourth most abundant cation in the body and second most abundant intracellular ion The average human body (70 kg) contains 1 mole (24 g) of Magnesium . Approximately 53% of the body Magnesium is found in bone, 46% in muscle and other organs and soft tissue, and less than 1% is present in serum and red blood cells. Of the Magnesium present in serum, about one third is bound to protein, primarily albumin. Of the remaining two thirds, 61% exists in the free or ionized state and about 5% is complexed with other ions, such as phosphate and citrate. Similar to calcium, it is the free ion that is physiologically active in the body. [37]

2.2.2.1Sources of magnesium

In nature magnesium is existent as magnesium carbonate, calcium magnesium carbonate, calcium magnesium cilicate, calcium magnesium hydroxide, magnesium sulfate and magnesium chloride. [37] In food green

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vegetables good sources of magnesium, some legumes (beans and peas),nuts ,seed ,and whole unrefined grains are also good sources of magnesium ,tap water can be a source of magnesium, but the amount is varies according to the water supply. [39]

2.2.2.2Homeostasis magnesium

Approximately 50 percent of total body magnesium is found in bone . The other half is found in predominantly inside cells of body tissues organs. Only 1 percent of magnesium is found in blood . it may exist as a protein –bound complex ,or free cation. [40]

An average dietary intake is around 15 mmol per day which generally meets the recommended dietary intake, children and pregnant or lactating women have higher requirements. [41] Magnesium is absorbed in the upper intestine .The major proportion of ingested magnesium (60–70 percent) is not absorbed but excreted in the feces Intestinal absorption, together with an effective renal regulation of urinary excretion allows homeostasis of magnesium over a wide range of dietary intake. [42]

2.2.2.3 Functions of magnesium

Magnesium is the most abundant cation , second only to potassium in the intracellular compartment and to calcium in bone tissues .In physiological situations ,magnesium is involved in metabolism of fat , amino acids and sugar. [42]

Magnesium is a cofactor for more than 300 enzyme systems, including some 100 systems that either produce or use MgATP. [44] Magnesium helps maintain normal muscle and nerve function and supports a healthy immune system .Also magnesium keeps heart rhythm steady, helps regulate blood sugar levels and promotes normal blood pressure. [45]

2.2.2.4 Hypomagnesaemia

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Magnesium is essential for DNA duplication and repair and Magnesium deficiency favors DNA mutations leading to carcinogenesis. Hypomagnesaemiais most frequently observed in hospitalized individuals in intensive care units or those receiving diuretic therapy or digitalis therapy. These patients most likely have an overall tissue depletion of magnesium as a result of severe illness or loss, which leads to low serum levels. Hypomagnesaemia is rare in nonhospitalized individuals. Metabolic disorders are associated with hypomagnesaemia. Studies have indicated that approximately 40% of hospitalized patients with hypokalemia are also hypomagnesaemia. In addition, 20%–30% of patients with hyponatremia, hypocalcemia, or hypophosphatemia are also hypomagnesaemia. [37]

2.2.2.5 Hypermagnesaemia

Hypermagnesaemia is observed less frequently than hypomagnesemia. The most common is renal failure. The most severe elevations are usually a result of the combined effects of decreased renal function and increased intake of commonly prescribed magnesium containing medications.

Hypermagnesemia has been associated with several endocrine disorders. Thyroxine and growth hormone cause a decrease in tubular reabsorption of magnesium and a deficiency of either hormone may cause a moderate elevation in serum magnesium Adrenal insufficiency may cause a mild elevation as a result of decreased renal excretion of magnesium. Dehydration can cause a pseudohypermagnesemia, which can be corrected with rehydration. Because of increased bone loss, mild serum magnesium elevations can occur in individuals with multiple myeloma or bone metastases. [37]

2.2.2.6 Magnesium and diabetes:

Magnesium plays an important role in CHO metabolism .It may influence the release and activity of insulin, it is involved on multiple levels in insulin secretion, binding and activity and is critical factor of mant enzymes in CHO metabolism. [46] Extra-and intracellular alterations of magnesium metabolism have been identified in clinical status characterized by insulin resistance, such as metabolic syndrome, hypertension, altered glucose tolerance and type 2 diabetes. [47]

Materials and Methods

3.1 Study design

This study is descriptive case control study

3.2 Study area

Jabber Abu AI-iz Diabetic health center and Saad Rashwan Medical center-Khartoum

3.3 Study population

The present study included 108 individuals, categorized into 2 groups.

The first group includes 45 normal subjects considered as apparently healthy by clinical examination and with no history of any disease . Their age ranged between 35-70 years. Interviews were performed with a protested and validated questionnaire (Appendix)The second group includes 63 patients with type II diabetes. Their age ranged between 35-70 years

3.4 Inclusion criteria

Study subjects were type II diabetes mellitus patients who diagnosed before, according to WHO criteria

3.5 Exclusion criteria

Type 1 Diabetes mellitus patients were excluded. Also type 2 diabetes mellitus with renal failure were excluded.

3.6 Ethical consideration

Permission of this study was obtained from the medical authorities in the area of the study. The objectives of the study were explained to all individual participating in the study. Participation was applied after explanation and taking oral consent

3.7 Sampling

Venous blood samples (5ml) were obtained from all patients and controls.Patients had fasted from 8 p.m to 8 a.m. Specimens were collected atstandardized time to minimize any effect of diurnal variation. Sterile disposable plastic syringes were used. The sample left to clot and

the serum was separated by centrifugation. The serum was used for determination of zinc by using atomic absorption spectroscopy; also serum samples were used for determination of serum magnesium and glucose.

3.8 Methodology

3.8.1.Magnesium

Magnesium was measured by xylidyl blue method.

Principle

Magnesium ions react with xylidyl blue in alkaline medium to form a water soluble purple-red cheiate, the color density of which is proportional to the concentration of magnesium in the sample. Calcium is excluded from the reaction by complexing with EGTA.(Appendix)

Sample material

The recommended sample is serum.

Test procedur

All reagents and samples were allowed to come to room temperature, then 1ml of colour reagent was pipetted in blank , standard and sample tube 10µl of (distilled water, standard, and sample) was pipetted in reagent blank , standard, and sample tube respectively. Then tubes were mixed well and incubated for 5 minute at room temperature. The final absorbance of the sample and stander was measured against the reagent blank.Then result was calculated as following(A sample/A stander) (Magnesium stander concentration)

Linearity

The linearly of this method is to 2.00 mmol/l (4.9mg/dl). Samples with higher concentration should be diluted 1+1 with distilled water and reassayed. Multiply the result byb2.

Sensitivity

The minimum detectable level has been determined as 0.07 mmol/l.

Normal value

1.7-2.7mg/dl. [12]

3.8.2. Glucose

Glucose was measured in the samples using commercially aviable kits Biosystem Glucose oxidase method

Principle

In the reaction, the glucose is oxidized to D-gluconate by the glucose oxidiase with the formation of hydrogen peroxide. In the presence of peroxidase, a mixture of phenol and 4-aminoantipyrine is oxidized by hydrogen peroxide to form a red quinoneimine dye, which is proportional to the concentration of glucose in the sample.(Appendix)

Procedure

All reagents, samples and controls were brought to room temperature before starting the test.

Serum samples free of hemolysis were used because any hemolysis will give false low result because the enzymes released will cause consumption of glucose .Also catalase liberated from RBCs will compete with peroxidase for Hydrogen peroxide, giving untrue results The test was carried out as follows:

1mL of colour reagent was pipetted in blank , standard and sample tube and10µl of (distilled water, standard, and sample) was pipetted in reagent blank , standard, and sample tube respectively. Then tubes were mixed well and incubated for 10 minute at room temperature.The final absorbance of the sample and stander was measured against the reagent blank.

Calculation

The concentration was calculated according to the following formula

A sample/ A standard × C standard=mg/dL

Normal range values

Adult 70-105 mg/dL

Children 60-110 mg/dL

Newborn 40-60 mg/dL. [12]

3.8.3. Zinc

Zinc levels were determined by an atomic absorption spectrophotometry

Principle

While a sample is being aspirated into a flame, a light-beam is directed through the flame into a monochromator and onto a detector that measures the amount of light absorbed by the atomized element in the flame. A source lamp composed of the element of interest is used because each element has its own characteristic wavelength. This makes the method relatively free from spectral or radiation interferences. The amount of energy at the characteristic wavelength absorbed in the flame is proportional to the concentration of the element in the sample over a limited concentration range. Most atomic absorption instruments are also equipped for operation in an emission mode.

Reagents

Metal-free water was essentially used for the preparation of all reagents. Hydrochloric acid and nitric acid, HNO3 , (analytical grade) was used for standard preparation and for digestion methods.

Preparation of standards

Standard solutions of known metal concentrations were prepared in water with a matrix similar to the tested samples. Standards were bracket the expected sample concentration and fall within the method's working range. Very dilute standards having concentrations of at least 0.05 mg L-1 were prepared daily from fresh standard stock solutions. If sample digestion is used, standards should becarried through the same digestion

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procedures. The standard stock solutions described below have a concentration of 1000 mg L-1 .

Zinc: 1.000 g zinc metal was dissolved in 20 ml 1:1 HCl and diluted up to 1,000 ml with water.

Procedure

It is not possible to provide an operating procedure that would be correct for all atomic absorption spectrophotometers because of differences between models of instrument. The manufacturer's operating manual should be followed. A general procedure contains three components as described below.

Adjustment of the Instrument:

1. A hollow cathode lamp for the desired element was installed in the instrument and the wavelength dial was set to the appropriate setting for the element.

2. The slit width was set according to the manufacturers suggested value for the element being measured.

3. The instrument then turned on and the lamp current was adjusted to the level suggested by the manufacturer.

4. The instrument then warmed up, 10 - 20 minutes, and current readjusted asnecessary.

5. The wavelength dial was adjusted until optimum energy gain is obtained.

6. The lamp was aligned in accordance with the directions in the operating manual.

7. The suitable burner head was installed and its position was adjusted.

8. The air was then turned on and its flow was adjusted to the rate recommendedto give maximum sensitivity for the metal being measured.

9. Acetylene then turned on and its flow was adjusted to recommended rate, then ignited and the flame was allowed a few minutes to stabilize.

10. A blank of deionized water that has been given the same treatment and acid concentration as the standards and samples was aspirated and the reading adjusted to zero.

11. A Standard solution was aspirated and the aspiration rate was adjusted to obtain maximum sensitivity.

12. The burner was adjusted horizontally and vertically to obtain maximum response.

Preparation of the calibration curve

1. At least five concentrations of each metal ion standard solutions were selected to perform a calibration curve. There should be one concentration greater and one less than that expected in the sample(s).

2. A blank was aspirated and adjusted to the zero value.

3. Each standard was aspirated in turn into the flame and the absorbance was recorded.

4. A calibration curve was performed by plotting the absorbance of the standards against their concentrations. This step is not necessary for instruments with direct concentration readout.

Analysis of samples

Lamps were changed and the procedure repeated for each element.

1. The nebulizer was rinsed by aspirating with water containing 1.5 ml HNO3 per liter. The blank was atomized and set to the zero value.

2. Samples were atomized and there absorbance were determined.

Calculations

Determination appropriate of the concentration of each metal ion, were based on the calibration curves. Results for trace elements were calculated in g L-1 while, in mg L-1 for the more common metals.

Concentrations may be read directly from instruments with a direct readout capability. If a sample has been diluted, appropriate dilution factor were applied. The recommended wavelength 213.8 nm

3.9.Quality Control

The use of standard to calculate results to obtain accuracy independent of the system and instrument used. To ensure adequate quality control (QC), each run included a set of controls.

3.10. Data processing and analysis

Data were collected manually in a master sheet and analysis was performed using Statistical packages for social sciences program (SPSS version 15), using independent t test and Pearson correlation.

4. Result

Serum levels of the trace elements, magnesium and zinc, were measured in the control group and diabetic group. Magnesium (Mg) mean concentration was significantly lower than control group (P value = 0.000), also mean concentration of zinc (Zn) was significantly lower than control group (P value = 0.047) and glucose significantly higher in test group (P- value= 0.000, table (4.1).

Duration of diabetes mellitus was not affect on Zn, Mg and glucose levels, figure (4.1), (4.2) and (4.3).

The age showed no relation with Zn and glucose levels, figure (4.4), (4.6)

While showed weak negative correlation with Mg levels, figure (4.5)

Figure (4.7) and (4.8) showed negative relation between glucose level with Zn and Mg.

Table (4.1): Comparison between serum levels of Zinc (mg/L), Magnesium (mg/d) and Glucose (mg/dl) of test and control group.

Variables	Test group (n=63)	Control group (n=45)	P-value
Zinc (mg/L)	0.457 ± 0.11	0.496 ± 0.07	0.047
Magnesium(mg/d)	1.943 ± 0.28	2.291 ± 0.27	0.000
Glucose (mg/dl)	181 ± 84.4	86 ± 12.7	0.000
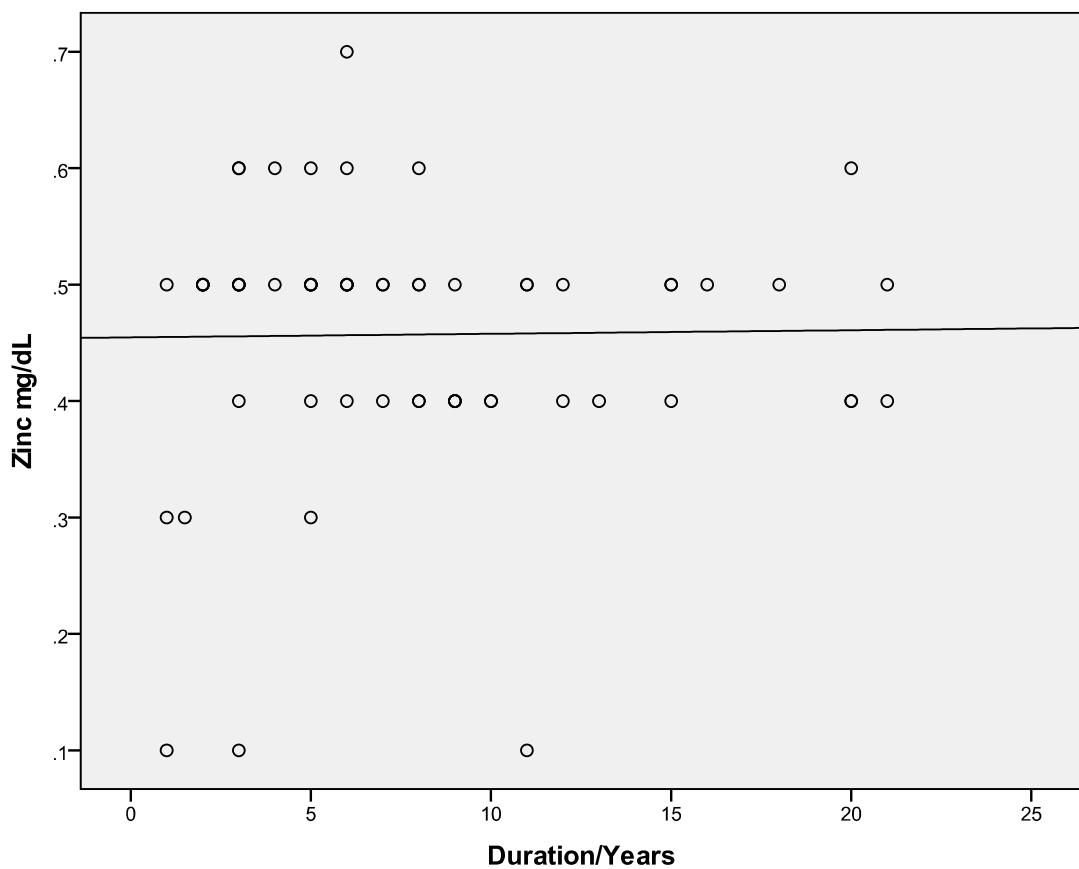
Figure (4.2.1) A scatter plot of the correlation between serum level of zinc (mg/L) and duration (year)   (r=0.015, P-value=0.907)
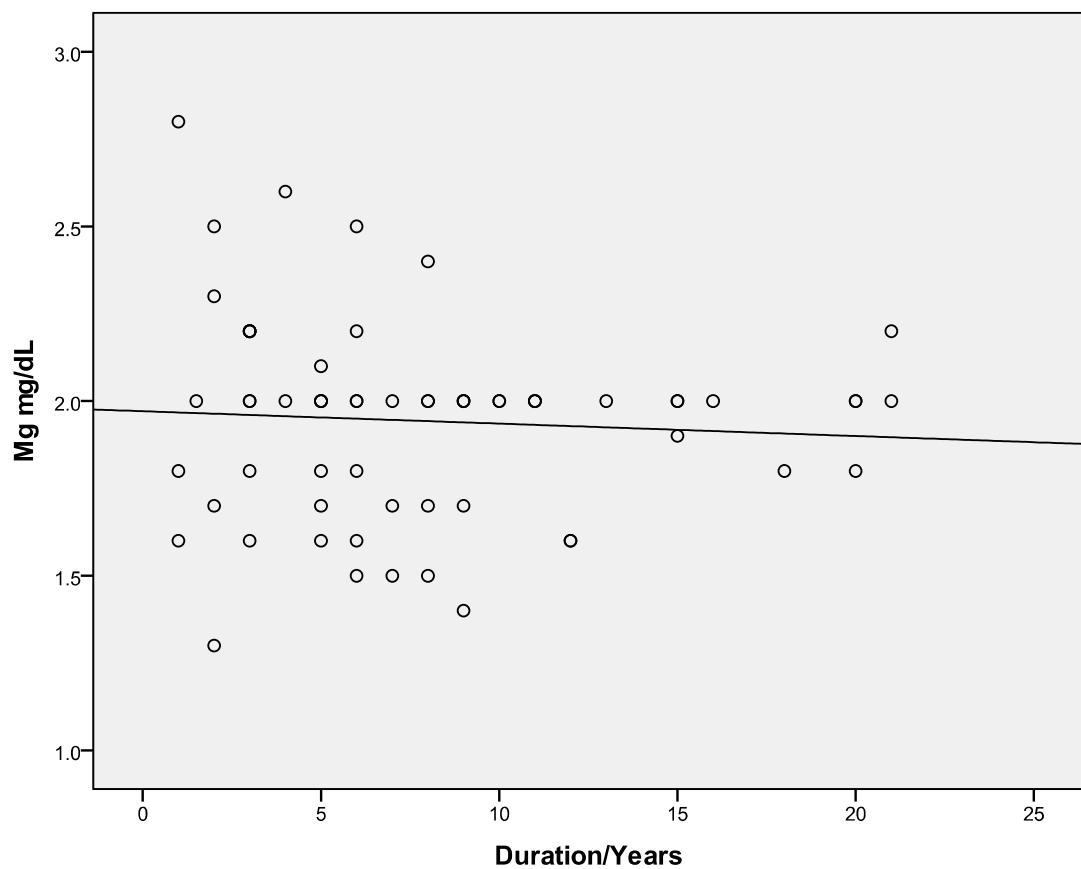
Figure (4.2.2) A scatter plot of the correlation between magnesium (mg/dl) and duration (years) (r=-0.068, P-value=0.599)
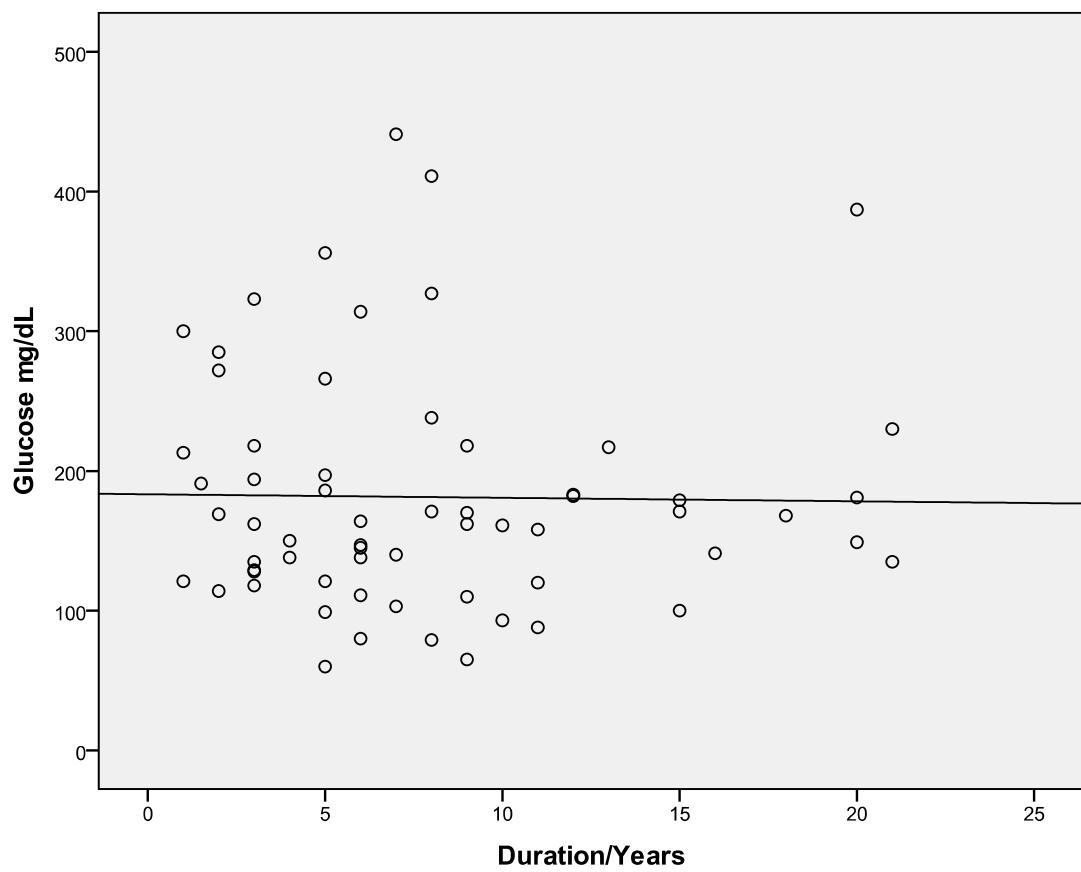
Figure (4.2.3) A scatter plot of the correlation between glucose (mg/dl) and duration (year) (r=-0.016, P-value=0.899)
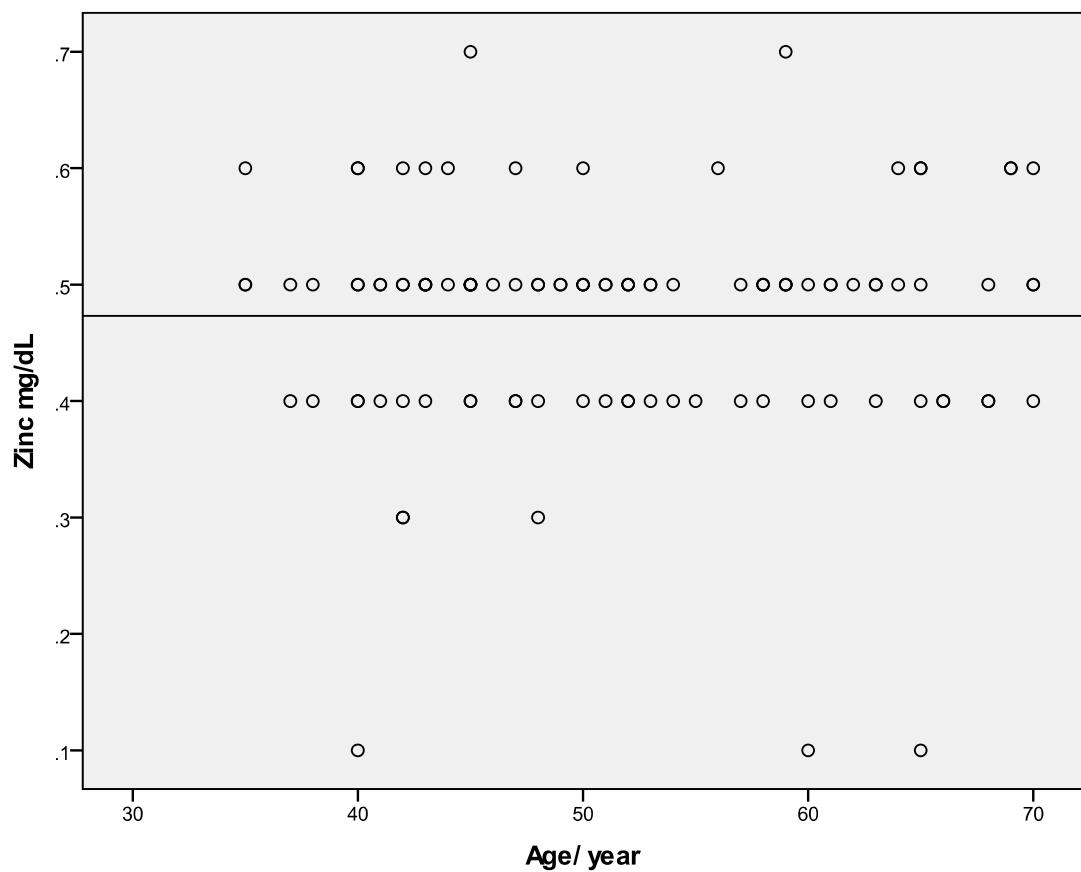
**Figure (4.4) A scatter plot of the correlation between zinc (mg/L) and age (year)   (r=0.000, P-value=0.997)**
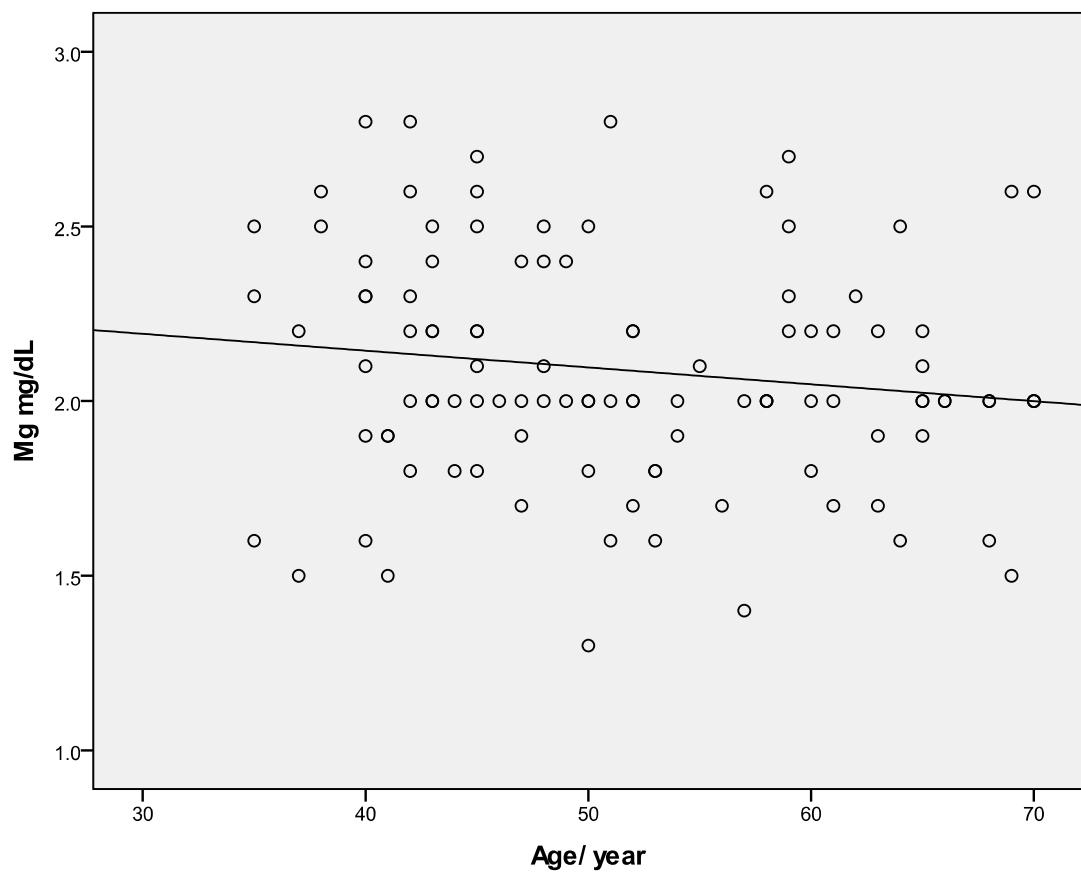
Figure (4.5) A scatter plot of the correlation between magnesium (mg/dl) and age (year)(r=-0.145, P-value=0.0134)
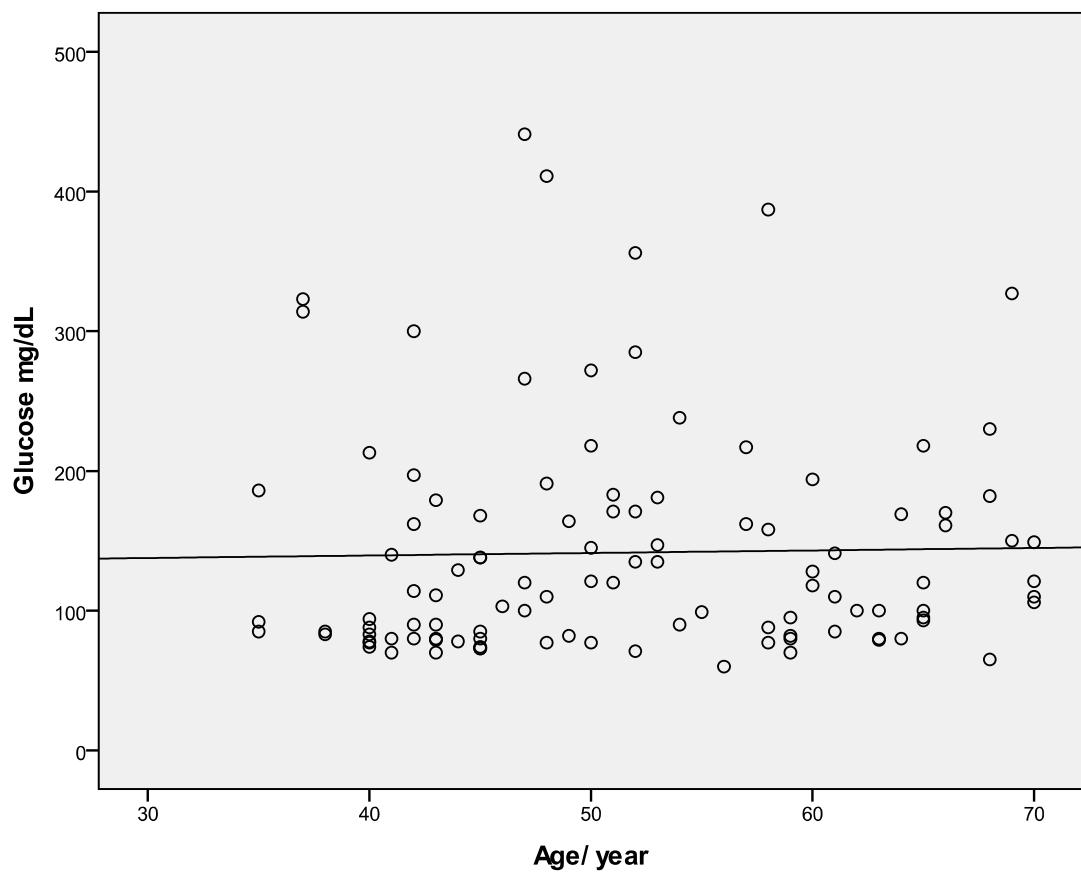
Figure (4.6) A scatter plot of the correlation between glucose (mg/dl) and age (year)   (r=0.022 P-value=0.821)
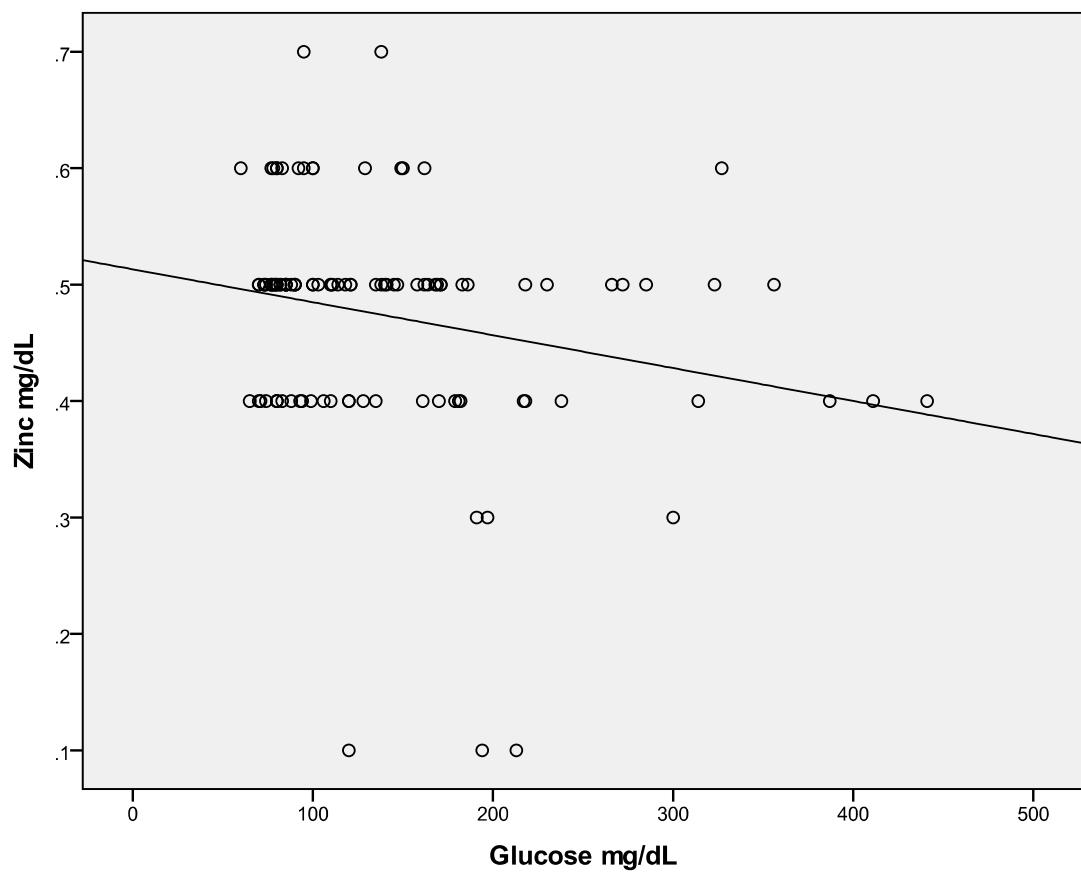
**Figure (4.7) A scatter plot of the correlation between zinc (mg/L) and glucose (mg/dl)   (r=-0.229, P-value=0.017)**
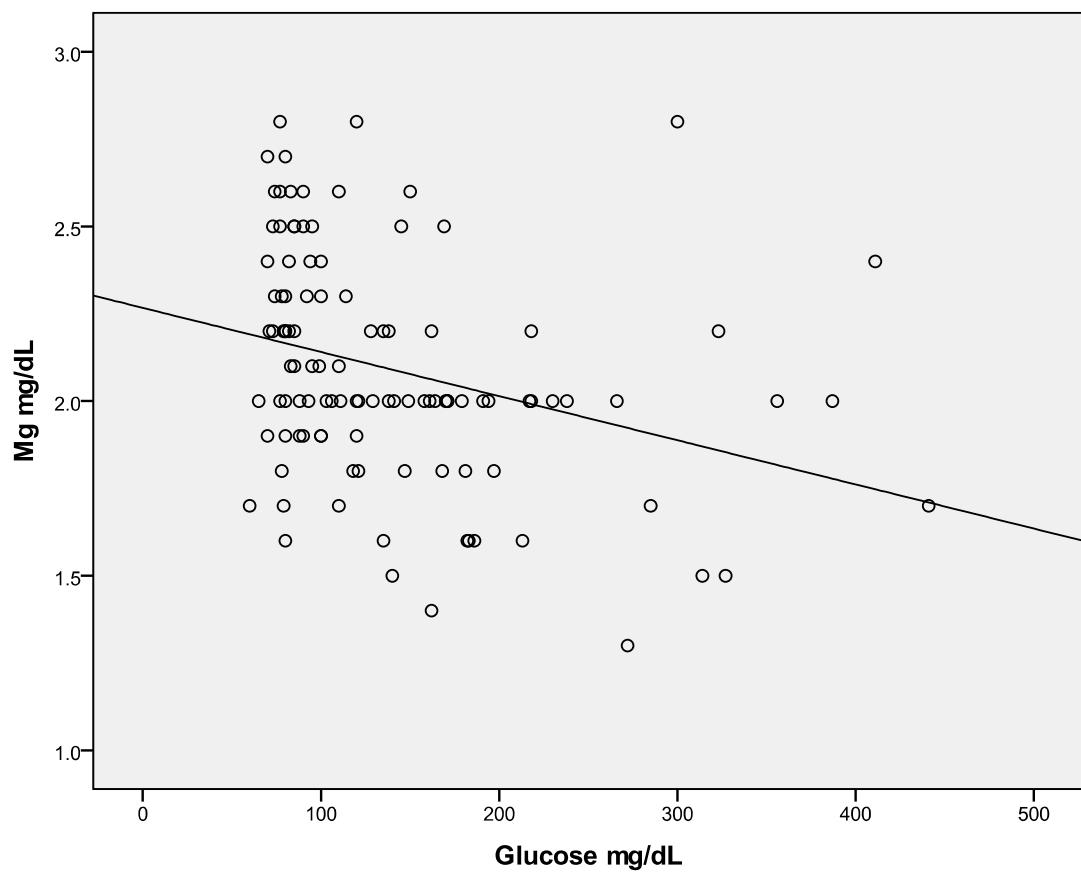
Figure (4.8) A scatter plot of the correlation between magnesium (mg/dl) and glucose (mg/dl)   (r=-0.309, P-value=0.001)

5.1 Discussion

In recent years, chronic diseases such as diabetes have been shown to be a major cause of death worldwide and there is accumulating evidence that the metabolism of these trace elements in particular zinc ,chromium and magnesium is altered in diabetes mellitus and these elements might have Specific roles in the pathogenesis progress of this disease. The present study was designed to evaluate serum levels of zinc and magnesium in diabetic Type II patients and control group. This study was conducted on 108 individuals categorized into 2 groups, 45 normal subjects considered as control group and 63 patients with type II diabetes, age and gender of test group are matched with control group.

Our study observed that mean serum zinc and magnesium level were significantly decreased in diabetics Type II patients when compared with control subjects, similar observations are reported, that significantly lower serum zinc level in diabetics than in control subjects.[1,46, 47, 48]

In contrast to these results , no difference in plasma magnesium level between control subjects and diabetic patients. [50] [52]

In this study, glucose showed significant negative correlation with serum zinc (r=-0.229 and P.value 0.017) and magnesium (r=-0.309 and P.value 0.000), this agree with study who reported that, there was negative correlation of blood glucose level with Zn, and Mg levels. [51]

In this study, there was no significant correlation between serum zinc and magnesium with duration of diabetes. [52]

In this study, there was no relation between serum zinc and age (r = 0.000 and P.value = 0.997), these agree with who stated that, there was no relation between serum zinc and age in diabetic patients.

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5.2 Conclusion

This study concludes that:

1- The serum levels of zinc and magnesium are decrease in patients with type II diabetes mellitus

2- There was weak negative correlation between serum levels of zinc and plasma level of glucose.

3- There was weak negative correlation between serum levels of magnesium and plasma level of glucose

4- Age showed no correlation with zinc and glucose ,but weak negative correlation with magnesium

5- Duration of disease showed no correlation with zinc, magnesium and glucoses

5.3 Recommendations

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

1- Periodic checkup for magnesium and zinc levels to delay complication associate with low levels

2- Diabetic patients should be advised to increase amount of food that contain magnesium and zinc

3- Blood sugar should be kept within the reference value in diabetics, to avoid the complication.

4- Generally, health education, diet control and exercise are important factors in lowering body weight especially in obese patients so as to achieve good control of diabetes mellitus

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Appendix Three
Mesurement of plasma zinc

Method

The estimation of zinc was carried by atomic absorption spectroscopy device using analytical method for atomic absorption spectroscopy that principally based on the technique makes use of absorption spectrometry to assess the concentration of an analyte in a sample. It requires standards with known analyte content to establish the relation between the measured absorbance and the analyte concentration and relies therefore on Beer's-Lambert law. In short, the electrons of the atoms in the atomizer can be promoted to higher orbital's (excited state) for a short period of time (nanoseconds) by absorbing a defined quantity of energy (radiation of a given wavelength). This amount of energy, i.e., wavelength, is specific to a particular electron transition in a particular element. In general, each wavelength corresponds to only one element, and the width of an absorption line is only of the order of a few picometers (pm), which gives the technique its elemental selectivity. The radiation flux without a sample and with a sample in the atomizer is measured using a detector, and the ratio between the two values (the absorbance) is converted to analyte concentration or mass using Beer's-Lambert law.
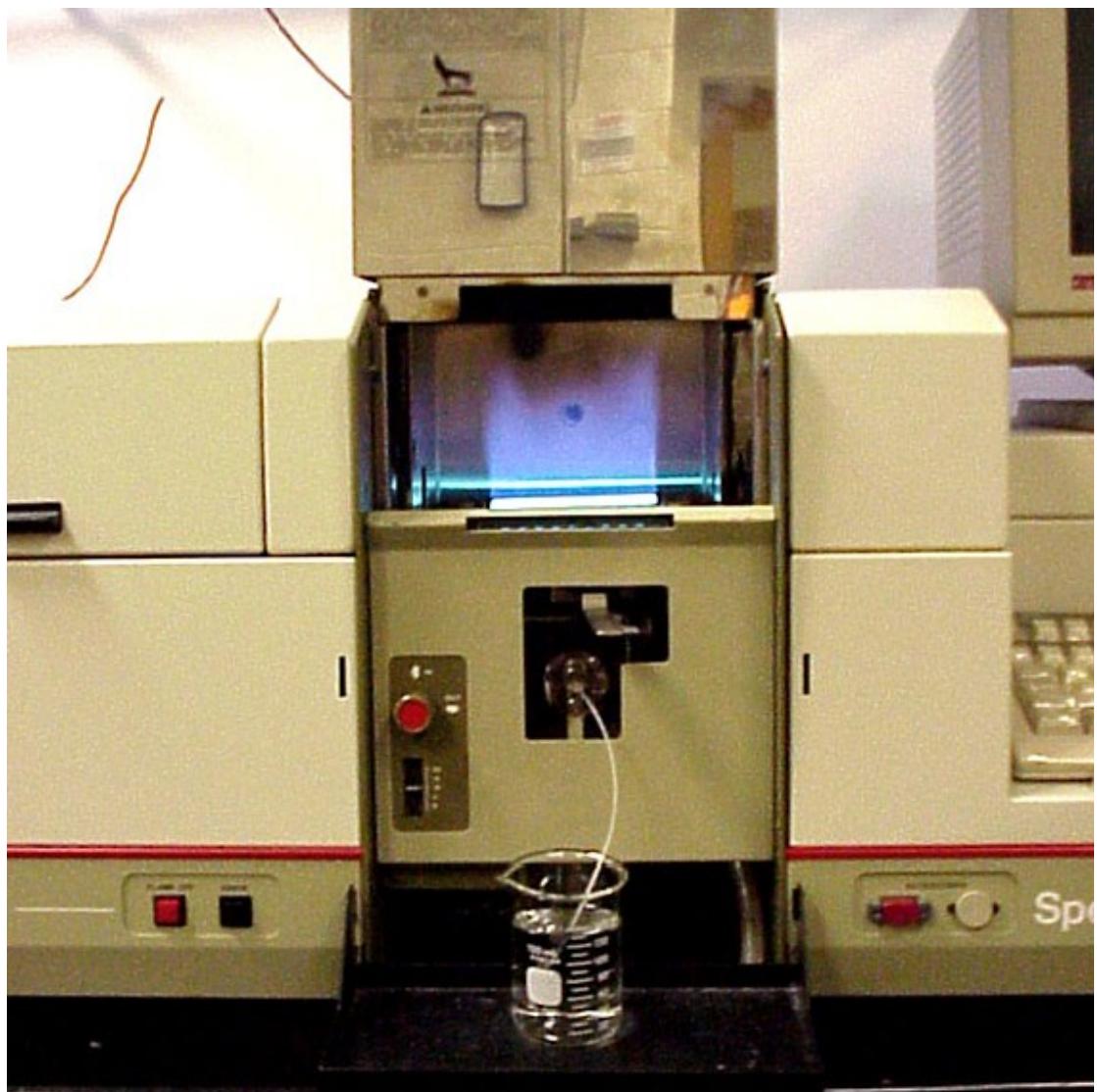
Procedure:

 Plasma samples dilute 1:5 with deionized water.

Establish instrumental and gas-flow settings and aspiration rate precisely, to optimize signal and minimize background noise. The instrumental settings shown in the table below apply to the instrument we used in this study. Once the aspiration rate is optimized with 10-mL aliquots of water, lock the nebulizer flow adjustment in place. Aspirate glycerol/water solution (5/95 by vol) into the luminescent flame and set the baseline to read 0.000 ±0.001 absorbance *(A).* Take a baseline reading before and after each sample and reset the baseline as required.[48]

- **Instrumental arrangements used for plasma zinc analysis:[48]**

Instrument settings		
Wave length	213.8	
slit	0.7	
Mode	Absorbance,1.0-s reading	
Lamp current	15mA	
Gain	Midscale	
Lamp focus	Grazing burner head	
Burner height	7-7.5 units	
flame	Luminescent, fuel rich	
Gas-flow settings	Pressure (ib/in^2)	Flowmeter
Air	30	54
Acetylene	9	38
Aspiration rates, water,ml/min	6.0±0.1	

- **Standard atomic absorption conditions for zinc:**

Wavelength (nm)	Slit (nm)	Relative Noise	Characteristic Concentration (mg/L)	Characteristic Concentration Check (mg/L)	Linear Range (mg/L)
231.9	0.7	1.0	0.018	1.0	1.0

307.6	0.7	0.38	79.0	3500.0	____

- **Standard flame emission conditions for zinc:**

flame	Slit (nm)	Wave length (nm)
Nitrous oxide – acetylene[45]	0.2	213.9

 Advantages of atomic absorption spectroscopy:

The advantages of atomic absorption spectroscopy include:

Inexpensive (equipment, day-to-day running), high sample throughput, easy to use and high precision.[47]

Disadvantages of atomic absorption spectroscopy:

The disadvantages of atomic absorption spectroscopy include:

Only solutions can be analyzed, relatively large sample quantities required(1–2mL) ,less sensitivity (compared to graphite furnace), problems with refractory elements.