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THE STUDY OF DEFICIENCY OF VITAMIN B12, MAGNESIUM AND SOME BIOCHEMICAL MARKERS IN PATIENTS WITH DIABETES MELLITUS

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THE STUDY OF DEFICIENCY OF VITAMIN B12, MAGNESIUM, AND SOME BIOCHEMICAL MARKERS IN PATIENTS WITH DIABETES MELLITUS

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January 2022

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ABSTRACT

THE STUDY OF DEFICIENCY OF VITAMIN B12, MAGNESIUM, AND SOME BIOCHEMICAL MARKERS IN PATIENTS WITH DIABETES MELLITUS

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Master of Science in Chemistry

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The goal of the present study is to identify the link between the deficiency in B12, magnesium deficiency, and several biochemical markers in diabetes mellitus people, testosterone changes, B12, and glucose in men with testosterone disorders. According to the results of the present study, it has been found that age is of great importance, as age increased, the incidence of diabetes increases. As for magnesium, it is significantly affected and had a link with diabetes, as well as vitamin B12. At the same time, there was a correlation between the diabetes test and changes in the lipid profile, but the most correlation was with total lipids. Adjustment for BMI at 45 years of age and average BMI significantly reduced the association between having a high body mass index (BMI) at age 25 and an increased risk of developing diabetes.

2022, 42 pages

Keywords: Diabetes mellitus, Vitamin B12, Some biochemical, Magnesium, Biochemical

ÖZET

DİABETES MELLİTUS, VİTAMİN B12, BAZI BİYOKİMYASAL, MAGNEZYUM, BİYOKİMYASAL

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Bu çalışmanın amacı, diyabet hastalarında B12 eksikliği, magnezyum eksikliği ve bazı biyokimyasal belirteçler ile testosteron bozukluğu olan diyabetli erkeklerde testosteron, B12, glukoz ve bazı biyokimyasal testlerdeki değişiklikler arasındaki bağlantıyı belirlemektir. Bu çalışmanın sonuçlarına göre, yaşın büyük önem taşıdığı, yaş arttıkça diyabet insidansının arttığı tespit edilmiştir. Magnezyum gelince, önemli ölçüde etkilenir ve diyabetin yanı sıra B12 vitamini ile bir bağlantısı vardır. Aynı zamanda diyabet testi ile lipit profilindeki değişiklikler arasında bir korelasyon vardı, ancak en fazla korelasyon toplam lipitlerle oldu. 25 yaşındaki BMI'nin diyabet riskiyle ilişkisi, 45 yaşında BMI ve ortalama BMI için ayarlama yapılmasıyla önemli ölçüde zayıflatılmıştır. Klinik ve biyokimyasal vitamin B12 eksikliği, hem tip 1 hem de 2 DM'li hastalarda oldukça yaygındır. Tip 1 ve tip 2 diyabetli hastalarda B12 vitamini eksikliği, B12 vitamini takviyesi ve optimal takviye dozu taramasına ilişkin gelecekteki büyük ve iyi tasarlanmış çalışmaların, diyabet klinik bakımında kılavuzların oluşturulmasına rehberlik etmesi garanti edilmektedir.

2022, 42 sayfa

Anahtar Kelimeler: Diabetes Mellitus, Vitamin B12, Bazı Biyokimyasal, Magnezyum, Biyokimyasal

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Abdulrahman Saleh Ibrahim IBRAHIM

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CONTENTS

LIST OF SYMBOLS

%	Percent
±	Plus minus
°C	Degrees Celcium
µg	Migrogram
µL	Microliter
g	Gram
h	Hour
kg	Kilogram
mg	Miligram
mL	Mililitter

LIST OF ABBREVIATIONS

BMI	Body mass index
CoA	Coenzyme A
DB	Diabetes mellitus
GAD	Glutamic acid decarboxylase
GAD	Glutamic acid decarboxylase
HbA1c	Hemoglobin A1C
Mg	Magnesium
MMA	Methylmalonic acid
MODY	Maturity onset diabetes of the young
RBS	Random blood sugar
T1DB	Tupe 2 Diabetes mellitus
T2DM	Tupe 2 Diabetes mellitus
TC-II	Transcobalamin –II

LIST OF FIGURES

LIST OF TABLES

1. INTRODUCTION

Vitamin is vital for DNA synthesis, appropriate hemopoesis, and brain health that have enough of the water-soluble vitamin B12 (cobalamin). It is mostly obtained from animal proteins and dairy products. The first stage in the metabolism of vitamin B12 is its release from animal sources, which is mediated by pepsin and stomach acid. Vitamin B12 in the diet binds to the salivary glands' R-protein after it is produced. To release vitamin B12, an alkaline medium and pancreatic proteases are used in the duodenum, where the R-protein is digested. The vitamin B12-IF complex is very tough to proteolytically break down. Upon binding to its receptor sites, this complex is absorbed. At this time, calcium is important for vitamin B12 absorption. Breakdown of the IF releases the intracellular vitamin B12 that was previously locked up. Transcobalamin – II (TC-II) is a protein carrier that binds to free vitamin B12 and is releases inside circulation. The combination, also known as holo TC-II, is readily absorbed by the liver, bone marrow, and other vital body cells. To put it another way, the liver is the main repository for most of your body's overall vitamin B12 supply (lam *et al.* 2014). Similarly, vitamin B12 affects the body in two ways: by making homocysteine into methionine and succinyl-Coenzyme A (CoA) from methylmalonyl coenzyme A (CoA). To help methylate homocysteine to methionine, vitamin B12 acts as a miner effect. In contrast, the methionine, in turn, activates to S-adenosyl-methionine, which subsequently transfers the amino. The methylation process will be disturbed of a physiologically significant vitamin B12 deficiency, resulting in a buildup of intracellular and serum homocysteine. Nutritional folate (methyl-tetrahydrofolate) is converted to active metabolic form of folate via this process (Carafoli and Krebs 2016).

1.1 Aim of Study

The purpose of this study is to determine whether or not there is a connection between low levels of testosterone, low levels of B12, low levels of glucose, and low levels of certain biological parameters in diabetic patients men who have testosterone disorders and low levels of B12, magnesium, and other nutrients in diabetes patients.

2. LITERATURE REVIEW

2.1 Diabetes Mellitus

Diabetes mellitus is a combination of the Greek terms diabetes, which means "to siphon," and mellitus, which means "sweet." The term "diabetes" was first used by. Mering and Minkowski identified the pancreas's involvement in the etiology of diabetes in 1889 (Fink *et al.* 2019). Untreated diabetes-related hyperglycemia may harm nerves, eyes, kidneys, and other organs. Type 1 diabetes, type 2, maturity-onset diabetes of the young (MODY), gestational diabetes, and neonatal diabetes are among the numerous subtypes of diabetes mellitus, as are endocrinopathies and the use of steroids as secondary causes. Insulin secretion problems (T1DM) and/or insulin resistance (T2DM) are common causes of both type 1 and type 2 diabetes mellitus (T2DM). In children and adolescents, T1DM is believed to develop, while in middle-aged and older adults, T2DM is considered to arise as a consequence of poor lifestyle and dietary choices in chronic hyperglycemia. T1DM and T2DM have distinct etiologies, presentations, and treatments according to the pathophysiology of each disease (Plows *et al.* 2018).

2.1.1 Type-1 diabetes mellitus

It is an autoimmune disease. This condition arises when the pancreas is assaulted by antibodies produced by the body. Because of the injury, the organ is unable to continue its normal function of manufacturing insulin. It's possible that this kind of diabetes is hereditary. It is also possible that it will develop as a result of abnormalities that occur inside the pancreas' cells. When the microscopic blood arteries in the body are damaged, this may lead to a variety of different health issues. In addition, those who have diabetes in the state have a higher risk of developing cardiovascular disease and stroke. Insulin pens use extremely small needles and use cartridges that are already loaded with insulin. This includes Jet injectors, which inject insulin into the skin by spraying it with high-pressure air; pumps, which supply insulin via a tube to an abdominal skin catheter; and syringes, which inject insulin manually into the body(Cole and Florez 2020).

2.1.2 Type-2 diabetes mellitus

The dependence of diabetes was the previous name for this kind of Non-insulin. However, during the last two decades, it has grown increasingly prevalent among children and adolescents, due to a rise in the number of overweight or obese young people. Approximately 90% of diabetics have type 2 diabetes. The pancreas generates insulin when a person has type 2 diabetes. In either case, the amount consumed is either inadequate or the body fails to use it correctly. It is most often present in adipose tissue, liver cells, and muscle cells. In general, type 2 diabetes is less severe than type 1 diabetes. The risk of severe health problems, especially in the vessels to supply the kidneys, nerves, and eyes, is high. Those who are obese have a greater chance of getting type 2 diabetes and its consequences (20% or more of their body weight is overweight compared to their height). The pancreas has to work harder to generate more insulin when a person is fat due to their high levels of insulin resistance. On the other hand, maintaining normal blood sugar levels is inadequate. Type 2 diabetes treatment include keeping a healthy body weight, consuming a balanced diet, and exercising. Furthermore, some people need medicine (Kleinert *et al.* 2018).

2.1.3 Diagnosis of DM

T1DM is typically reported based on a different origins and abnormally high blood glucose levels. Those with signs of diabetes or who is at risk of acquiring the disease should be evaluated (Chiefari *et al.* 2017). The A1C test is used to get a snapshot of the patient's blood sugar levels over the preceding three months. Blood sugar levels are tested an hour after drinking a sweet beverage during the glucose challenge test. One of the following methods is used to diagnose diabetes: A hemoglobin A1c level of at least 6.5 percent; A plasma glucose level of 11.1 mmol/L or 200 mg/dL or higher after two hours of a 75-g OGTT; A fasting plasma glucose level of 126 mg/dL (7.0 mmol/L) or higher (after going at least eight hours without feeding); Patients who are experiencing reactive hypoglycemia signs, such as polyuria, polydipsia, polyphagia, and weight loss, have a random plasma glucose level that is at least 11.1 molar ratio or more than 200 mg/dL. When a person's blood glucose level is abnormally high, they are diagnosed

with diabetes. Additionally, it is required to monitor blood glucose in those with diabetes-related illnesses such as recurrent infections or yeast (Nielsen and Keasling 2016).

To get an accurate reading of blood glucose levels, physicians often collect a blood sample from patients who have fasted overnight. However, blood samples obtained after individuals eat may be used. Although some increase of blood glucose levels is acceptable after a meal, the levels should not be too high. Additionally, doctors may determine the amount of a protein in the blood called hemoglobin A1C. Hemoglobin is the blood cell's crimson, oxygen-carrying component. When blood is subjected to prolonged elevations in blood glucose levels and glucose to produce glycosylated hemoglobin. The hemoglobin A1C level (given as the proportion of hemoglobin that is A1C) indicates long-term rather than abrupt fluctuations in blood glucose levels. When performed by a qualified laboratory, hemoglobin A1C measurements may be used to diagnose diabetes (Smith *et al.* H. 2018).

An oral glucose tolerance check is a type of blood test that may be used in a variety of circumstances, including screening pregnant women for gestational diabetes or evaluating older individuals with diabetic symptoms but acceptable fasting glucose levels. However, because of its complexity, it is seldom used for diabetes testing. Patients rest for the length of the test, then drink a specific solution containing a substantial, standard quantity of glucose after having their fasting blood glucose level measured. Additional blood samples are drawn and analyzed over the following two to three hours to determine whether the blood glucose level rises excessively. During regular medical exams, blood glucose levels are often tested. Because diabetes is so prevalent in later life, regular blood glucose monitoring is important for older individuals. Diabetes patients, especially those with type 2 diabetes, may be ignorant of their illness. Individuals who have one or more of these risk factors for diabetes should be checked every three years at the very least. Diabetes risk calculators are available online. Physicians suggest more regular screening tests, at least once a year, if the test results are borderline normal or abnormal (Niafar *et al.* 2015).

2.1.4 DM and metabolism

Diabetes mellitus is a set of metabolic diseases that impact carbohydrate metabolism and therefore are defined by high blood glucose levels (hyperglycemia). Type 1 diabetes is caused by inadequate insulin secretion, whereas type 2 diabetes is caused by cells that react inefficiently to insulin (type 2 diabetes). Insulin is necessary for blood glucose (sugar) transfer into cells. Diabetes is a primary cause of adult blindness and a substantial risk factor for cardiovascular disease (Harrington 2017). While type 1 diabetes is primarily hereditary, the majority of cases are caused by an autoimmune disease brought on by a virus, foreign protein, or environmental toxin. Although high blood sugar is a common symptom of diabetes, it is not caused by sugar or carbohydrate consumption. Insulin injections are used to treat type 1 diabetes, along with modest, evenly spaced meals and snacks to help distribute glucose consumption throughout the day and reduce blood glucose swings (DeFronzo *et al.* 2015).

Type 2 diabetes (also known as adult-onset or non-insulin-dependent diabetes) is the most prevalent form of the disease, accounting for 90–95 percent of cases. Although type 2 diabetes usually develops in middle life, it is becoming more common in youngsters, particularly obese children. Susceptibility to this kind of diabetes may not manifest until a person has an extremely high body fat percentage, particularly abdominal obesity. Weight loss is frequently helpful in reestablishing normal blood glucose control, and oral anti-diabetic medications may also be utilized. In high-risk people, lifestyle interventions (such as diet and exercise) are very successful in delaying or avoiding type 2 diabetes. Urbanization and the adoption of Western cuisine and practices have been found in migration studies to significantly increase the prevalence of type 2 diabetes. For example, the disorder is common among Arizona's Pima Indians, who are sedentary and eat a high-fat diet, but it is uncommon among a closely related group of Pimas who live a traditional lifestyle in a remote, mountainous region of Mexico, where they are physically active, have a healthy body weight, and eat a low-fat diet. Type 2 diabetes is a serious public health issue in the United States, particularly among Native Americans and other ethnic minorities. The incidence of type 2 diabetes

has risen in lockstep with the growth in obesity throughout the world (Kibirige and Mwebaze 2013).

2.1.5 The effect of DM on human

Generally diabetes affects our blood vessels and nerves, but it may manifest itself in any area of the body. However, some areas of our bodies are more severely impacted than others. Diabetic problems often emerge after many years of poorly managed diabetes. Complications are not a given and may be avoided or minimized by keeping strict management of the diabetes, blood pressure, and cholesterol. All of these may be mitigated by adhering to a nutritious diet, abstaining from tobacco and alcohol, and integrating regular physical exercise into the daily routine in order to maintain blood sugar levels within prescribed blood glucose ranges (Wolffenbuttel *et al.* 2019)

2.1.6 The effect of DM on man

It is a chronic illness in the body can be produced as small insulin or not effectively absorb it. Similarly, the body may not generate enough insulin or without properly absorb. While anybody may acquire the disease, it manifests itself in a number of ways that are specific to males (Capelli *et al.* 2019). Erectile dysfunction, vaginal thrush, and muscle mass loss may all occur as a result of the disease. This article discusses the distinctions between men's and women's diabetic symptoms. Numerous consequences of diabetes are comparable across sexes. Blood vessels and nerves are harmed by the disease. Up to 75% of men with diabetes have difficulty obtaining or keeping an erection. Nerves and blood arteries are critical components of the erection process, and injury to these systems may impair the penis's function. Men may get recurrent episodes of genital thrush, a yeast infection caused by a fungal fungus. Excess blood sugar is excreted in the urine. Yeast, on the other hand, thrives on sugar and is thus more prone to develop on the penis of a diabetic man. Genital thrush symptoms include redness, swelling, and itching around the penis's head, an offensive odor, a white-lumpy look to the penis's skin, and pain and discomfort during sex. Consistently elevated blood sugar levels may cause the body to use muscle and fat as fuel (Rizzo *et al.* 2016). This occurs

more often in males with type 1 diabetes and is characterized by decreased strength and muscular weakness.

2.1.7 The effect of DM on women

Diabetic, in general, doubles the risk of heart disease (the most frequent diabetes consequence) in women but just doubles it in males, and women have poorer outcomes after a heart attack. Women are also more likely to have additional consequences of diabetes, such as blindness, renal disease, and depression (Nakos *et al.* 2017). On the other hand, the mortality rate for males with diabetes decreased. This decline is attributable to advancements in diabetes therapy. However, the research shows that the mortality rate for diabetic women did not improve. Additionally, the gap in mortality rates between diabetic and non-diabetic women more than doubled. The results highlight the distinct ways in which men and women are affected by diabetes. The following reasons were given: Women are often treated less aggressively for cardiovascular risk factors and diabetes-related illnesses. Certain consequences of diabetes are more difficult to detect in women. Additionally, women often suffer from different types of cardiac disease than males. Some diabetic women are concerned about the safety of pregnancy. The good news is that women who have been diagnosed with type 1 or type 2 diabetes may be able to carry their babies to term. However, it is essential to manage the illness before to and during pregnancy in order to avoid complications (Paschou *et al.* 2018).

2.1.8 Treatment methods

Numerous therapies are available to assist in managing and treating diabetes. Because each person is unique, therapy will vary according to their unique requirements.If a patient has type 2 diabetes and the healthcare team advises initiating insulin, this does not imply the patient has been diagnosed it (Spence 2016). On the other hand, the test known as the glycated hemoglobin (A1C) test is often used to diagnose type 2 diabetes. This blood test reveals the amount of sugar that has been typically present in the blood over the course of the previous two to three months. An unannounced test of the

patient's blood sugar was carried out. Milligrams per deciliter (mg/dL) and millimoles per liter (mmol/L) are the units of measurement used to express the concentration of sugar in the blood. No matter when you last ate, if your blood glucose level is above 200 mg/dL (11.1 mmol/L), this is an indication that you have diabetes. This is especially true if you also experience signs and symptoms connected to diabetes, such as severe thirst and frequent urination. The oral check is used somewhat less often than the others, with the only exception of pregnancy. After fasting overnight, the patient must drink a sweet beverage. The levels of the sugar in the blood can be checked at regular intervals over the coming two hours. Those of younger than 45 years may have diabetes risk factors diagnosed with prediabetes, pregnant women, and children. To conclude, the primary aim of type 1 and type 2 diabetes treatment is to maintain normal blood sugar (glucose) levels with minimal dangerously low or high excursions. Insulin, exercise, and a type 1 diabetes diet are used to treat type 1 diabetes. Weight loss, adherence to a type 2 diabetic diet, and physical exercise are the most common treatments for type 2 diabetes (Oguntibeju 2019).

2.2 Vitamin B12

It's a liquid vitamin that may be obtained naturally in certain foods and as a complement in others. It is classified as both a dietary supplement and prescription medicine. Because vitamin B12 includes anatta, compounds that function similarly to it are called as "cobalamins" When methylcobalamin or 5-deoxyadenosylcobalamin is transformed to hydroxycobalamin or cyanocobalamin, two new forms emerge: hydroxycobalamin and cyanocobalamin. Vitamin B12 is covalently linked to haptocorrin, a salivary protein that binds to cobalamin. Additional vitamin B12 from the gastric food matrix, living keratin, is lacking in hydrochloric acid and stomach proteases. The resultant complex is absorbed from the distal ileum through receptor-mediated endocytosis (Duan and Liu 2019).

Vitamin B12 levels in the blood or plasma are often used to diagnose vitamin B12 deficiency. Although most labs define abnormal serum or plasma levels as less than 200 or 250 pg/mL (148 or 185 pkamol/L), the most sensitive biomarker of vitamin B12

insufficiency is methylmalonic acid (MMA), a metabolite linked to vitamin B12. Values higher than 0.271 mol/L indicate deficiency. MMA levels, on the other hand, rise with renal failure and are often greater in the elderly. Another indicator is plasma total homocysteine levels, which increase quickly when vitamin B12 E status deteriorates. However, because of its impact on other variables such as low folic acid levels and, more specifically, specific kidney function, a blood homocysteine level over 15 mol/L suggests vitamin B12 insufficiency. Experts suggest testing the patient's serum MMA level to confirm the diagnosis of vitamin B12 insufficiency continuously (Razzaque 2018).

2.2.1 Vitamin B12 source

It can be found naturally in animal-based foods including fish, poultry, meat and eggs. Meat, salmon, and cod, as well as milk and other dairy products and eggs, all contain vitamin B12. Breakfast cereals and nutritional yeasts are also easily available and bioavailable vitamin B12 sources. Milk contains about 0.44 mcg/L of the vitamin in women who consume more vitamin B12 than the RDA. Baby formulae supplied in the US must contain at least 0.15 micrograms of vitamin B12 per 100 kcal (Cui *et al.* 2017). Because absorption drops dramatically when intrinsic factor capacity is surpassed (at 1–2 mcg of vitamin B12), vitamin B12 bioavailability from food is expected to vary with vitamin B12 intake. Furthermore, bioavailability differs depending on the food supply. Vitamin B12 bioavailability, for example, have higher in dairy products than others. vitamin B12 bioavailability in dietary supplements appears to be roughly 50 percent higher than in food sources (Song *et al.* 2016).

2.2.2 The biochemical structure of Vit. B12

Vitamin B12 assists the production of red blood cells in. it has a complicated chemical structure, as shown in Figure 3, because it contains the metallic ion cobalt (Vitamin B12 comes in a variety of cobalamin forms, the most common of which being cyanocobalamin, which is used in vitamin supplements and pharmaceuticals. Both folic acid (folate) and Vitamin B12 work together to DNA. A lack of either component

results in disorganized DNA synthesis and, therefore, poor red blood cell formation. Vitamin is transported from the rumen to muscle and other tissues that are consumed by other animals including humans. Eggs, beef, and dairy products are all good sources of vitamin B12 (Humeau *et al.* 2018). Vitamin B12, on the other hand, refers to a particular class of cobalt-containing corrinoids that have biological action in humans. Cobalt is responsible for the vitamin's red hue. Corrinoids are also known as cobalamins. AdenosylCbl, methylCbl, and hydroxoCbl are the three major cobalamins found in humans and animals. Cbl in food is hydroxoCbl. The active coenzyme forms are adenosylCbl and methylCbl. AdenosylCbl is the main intracellular form in all tissues and is found in the mitochondria. MethylCbl is cytoplasmic in nature. While methylCbl is a small component of intracellular Cbl, it is the predominant form of Cbl in plasma and the type that is disproportionately decreased in Cbl shortage. CyanoCbl is a stable synthetic medicine that must be transformed to adenosylCbl or methylCbl before it can be used metabolically (Anbu *et al.* 2016).

2.2.3 Pathway

It is kept in abundance in the liver, which reduces the danger of deficiency. When vitamin B12 is not absorbed, for example, due to dietary insufficiency, malabsorption, or an intrinsic factor shortage, hepatic stores are exhausted, resulting in a deficiency. This exercise addresses the evaluation and treatment of vitamin B12 insufficiency and highlights the importance of improving care for affected individuals. Depending on underlying cause, vitamin B12 insufficiency can manifest in a variety of ways. According to research, a B12 deficiency affects roughly 1% to 2% of patients with anemia. According to other studies, between 18 percent and 20% of persons with clinical macrocytosis (defined as an MCV > 100) had a B12 deficiency. Regardless matter the reason, vitamin B12 insufficiency is more common among the elderly (Bauer 2013).

Vitamin B12 interacts with R-factor, a protein produced by healthy people's salivary glands. Pancreatic enzymes separate B12 from R-factor once the mixture reaches the small intestine. It is bind to an intrinsic factor glycoprotein produced by gastric parietal

cells. Vitamin B12 and intrinsic factor may then attach to ileal receptors, allowing B12 to be absorbed more easily. Once absorbed, vitamin B12 participates in critical metabolic pathways for neurologic and hematologic functions. Regardless of the cause, a failure to absorb B12 can lead to deficits (Villarroel *et al.* 2014). The enzyme Methyl-THF is transformed to THF as a byproduct of this process, which is then turned to intermediates needed for the production of pyrimidine nucleotides in DNA. The homocysteine can increase and causes DNA synthesis to be delayed and the development of megaloblastic anemia. Anemia manifests itself as weariness and pallor, which are common symptoms of vitamin B12 deficiency. Reduced DNA synthesis has a negative impact on other rapidly proliferating cell lines, such as polymorphonuclear leukocytes (PMNs). As a result, a lack of vitamin B12 is connected to hypersegmented neutrophil development (Blaine *et al.* 2015).

2.2.4 Vitamin B12 with DM

Vitamin B-12 is nedded for the functioning of the neurological and blood cells. Vitamin B-12 is best obtained via food. This critical nutrient is present in red meat, fish, poultry, and dairy products. There are other ways to acquire a deficit. For instance, having diabetes mellitus increases the chance of developing a B-12 deficit, which may be a side effect of metformin. According to a 2009 research, 22% of individuals with type 2 diabetes had insufficient B-12. The study's findings indicate that metformin may have contributed to the deficit.It's tempting to dismiss them as trivial grievances. However, inadequate B-12 may eventually result in more serious issues. Certain vegetarian meals, such as morning cereals and energy bars, may include B-12 (Carafoli and Krebs 2016).

2.3 Trace Element

Trace elements have long been thought to be therapeutic agents for metabolic illnesses such prediabetes [insulin resistance, obesity, metabolic syndrome] and diabetes. Clarifying the cellular targets and areas of action of trace elements has renewed interest in their therapeutic potential, in tandem of the cellular and molecular mechanisms underlying or aggravating these metabolic diseases. Because of the importance of these

activities in glucose homeostasis and insulin sensitivity, researchers looked into stimulating insulin receptor signaling (chromium), antioxidant characteristics (selenium, zinc), and phosphatase inhibition (vanadium). Glycemic dysregulation and insulin resistance are assumed to be caused by abnormalities in insulin receptor/postreceptor signaling, while the exact causes are unknown. As a result of an imbalance in the formation and scavenging of free radicals by distinct antioxidant systems, prediabetic conditions, and even more so frank diabetes, are characterized by inflammation [cytokines] and oxidative stress. Furthermore, these assist with each other to promote and develop insulin resistance and other diseases (Nielsen and Keasling 2016). A link between diabetes and trace elements has been discovered in numerous investigations. Numerous studies have revealed that the metabolisms of various minerals differ. It has been discovered that certain trace minerals boost insulin activity. According to studies, trace elements may enhance insulin action by activating insulin receptor sites, functioning as cofactors or components of glucose metabolism enzyme systems, increasing insulin sensitivity, and acting as antioxidants to prevent tissue peroxidation (Plows *et al.* 2018).

The most prominent characteristic of diabetes metabolism is an excessively high blood glucose level. Zinc's role in cellular metabolism is determined by its enzymatic affinity and the formation of a zinc-enzyme complex or metallo-enzyme. Diabetes in people and animals results in a deficiency of this critical trace element. Zinc is necessary for the production and storage of insulin, and insulin is secreted in the form of zinc crystals. It protects insulin's structural integrity. In immune system, Zinc plays a critical role in DM and can link of the deficiency causes. Magnesium is required for the phosphorylation of glucose and its metabolism. Its absence is linked to insulin resistance and other complications. The link between diabetes mellitus and hypomagnesaemia is strong, owing to hypomagnesaemia's broad effect on diabetic management. It is unknown if trace element insufficiency causes illness or whether disease occurs as a result of trace element deficiency. Although it is widely accepted that tight metabolic management prevents the onset of late problems in it. DM type-2 and metabolic syndrome are both significant risk factors for atherosclerosis and its consequences. Due to the importance

of the trace elements in this study, the following sections will discuss in details the Magnesium, Magnesium with DM, and Calcium (Cole and Florez 2020).

2.3.1 Magnesium

Magnesium is an element that is necessary for the organism to operate correctly. It aids in the maintenance of healthy blood pressure, strong bones, and a steady heartbeat.According to the research, many people do not consume enough magnesium-rich foods. Adults who consume less magnesium than recommended have a higher chance of inflammatory markers being raised. Furthermore, a the deficiency found as a cause for osteoporosis. Research suggests that consume high-magnesium-meal and minerals to assist in prehypertension avoid developing hypertension. Figure 5 depicts the chemical structure of magnesium (DeFronzo *et al.* 2015). Magnesium is also used to treat a range of disorders, such as pre-eclampsia and severe asthma attacks, intravenously or by injection. Magnesium is also found in a variety of antacids and laxatives. Magnesium supplements are sometimes recommended by health care professionals to those who are suffering from specific diseases. Low magnesium levels have also been linked to PPIs, a prominent type of acid reflux medication (Chiefari *et al.* 2017). Magnesium is one of the seven microminerals that are required for life. These microminerals must be ingested in large amounts each day, at least 100 milligrams (mg). Iron and zinc, which are microminerals, are also required, though in smaller amounts. A link between diabetes and trace elements has been discovered in numerous investigations. Numerous studies have revealed that the metabolisms of various minerals differ. It has been discovered that certain trace minerals boost insulin activity. According to studies, trace elements may enhance insulin action by activating insulin receptor sites, functioning as cofactors or components of glucose metabolism enzyme systems, increasing insulin sensitivity, and acting as antioxidants to prevent tissue peroxidation (Stabler 2013).

2.3.2 Magnesium and DM

Magnesium insufficiency has been related to insulin resistance, affect in the occurring diabetes, according to studies. However, boosting the magnesium intake has been found to potentially reduce the chance of acquiring chronic illness. Consuming 100 mg of magnesium daily via the consumption of magnesium-rich foods may reduce the incidence of diabetes by 15%. The researchers emphasized that more research is necessary before suggesting magnesium supplementation to prevent diabetes (Smith *et al.* 2018).

Although fewer studies have examined the connection between type 2 diabetes and magnesium after the illness has been identified, they found that individuals with the disease who are magnesium deficient may be more prone to develop complications, such as heart health problems. Studies show that the kidneys are critical organs responsible for maintaining a magnesium equilibrium. However, individuals with diabetes lose a significant quantity of magnesium via their urine. People with diabetes are more likely to be magnesium deficient, particularly if their blood sugars are uncontrolled and high, since their bodies may be excreting magnesium along with excess sugars in the urine.

DM2 is usually linked along a change in magnesium status. The deficiency was found to be more connected in the patients, particularly those with poorly managed glycemic profiles, those with a longer duration of the illness, and those with micro- and macrovascular chronic sequelae (Kleinert *et al.* 2018). Magnesium insufficiency may exist in the absence of hypomagnesemia. When hypomagnesemia is evident, it is often symptomatic of a significant systemic Mg deficiency. Individuals with normal total serum magnesium may have a decrease of intracellular and/or ionized plasma magnesium. However, the majority of research in the literature examined total blood magnesium concentrations rather than free, ionized (bioactive), or intracellular magnesium concentrations, making it difficult to link Mg deficiency with illness (Wolffenbuttel *et al.* 2019).

2.3.3 Calcium

it is a mineral that is essential for the functioning of the bodies of all living things. It is the mineral that is found in the greatest quantities in the body and is essential to the formation of bones. In addition to this, maintaining a healthy connection between the brain and the rest of the body is very necessary. It has a role in the movement of muscles as well as the operation of the heart and lungs. It is found in many different foods in its natural state, and some food manufacturers also add it to certain products. Alongside calcium, due to the fact that this vitamin facilitates the body's absorption of calcium. It may be gained by sun exposure, the use of fish oil, and enriched dairy products (Rizzo *et al.* 2016). This page discusses why the body need calcium, which foods contain it, what happens if the body does not have enough, and the benefits and drawbacks of supplementation. Calcium, the body's most abundant mineral, is naturally present in certain foods, incorporated into some medications. İt is responsible for a significant percentage of the architecture that allows for correct physical movement by preserving the stiffness, strength, and flexibility of tissue. There is a possibility of its presence in the extracellular fluid, the vascular system, and in other tissues. It is a very tiny ionized pool. Active transport and passive diffusion are the two mechanisms that allow calcium to be absorbed from the gastrointestinal mucosa after it has been ingested via food or dietary supplements. When calcium intake is low, active transport is responsible for the bulk of the absorption process; however, when calcium intake is high, passive diffusion is responsible for an increasing proportion of the absorption process (Chromiński and Gryko 2013).

The skeleton store almost all of the body's calcium (98 percent) and act as a calcium reservoir and source to keep calcium homeostasis in check. Bone remodeling is necessary to maintain bone size throughout development, heal injury, maintain appropriate serum calcium levels, and offer a supply of other minerals (Capelli *et al.* 2019). The body has about 26 to 30 g of iron at birth. This quantity rapidly increases after birth, reaching about 1,200 g for females and 1,400 g for men at maturity. Male levels stay stable, while female levels begin to decline after menopause as a consequence of increased bone remodeling caused by reduced estrogen production. Calcium absorption is inversely proportionate to calcium intake. At a dosage of 200 mg per day, calcium is absorbed approximately 45 percent of the time, but just 15 percent at

a dose of 2,000 mg per day. Additionally, calcium absorption from food may be influenced by age. In babies and early children, who need significant quantities of calcium to form bone, net calcium absorption may be as high as 60%, but it drops to approximately 25% at maturity and continues to diminish with age (Nakos *et al.* 2017). Calcium levels in the blood or plasma may be tested; serum calcium levels in healthy individuals usually range from 8.8 to 10.4 mg/dL (2.2 to 2.6 mmol/L). Due to their strict homeostatic regulation, serum levels, on the other hand, do not reflect nutritional status. The quantity of ionized (or free) calcium in the blood, which is the biologically active form, also determines calcium status. In healthy individuals, ionized calcium levels usually range between 4.6 and 5.3 mg/dL (1.15 and 1.33 mmol/L). Because the skeleton stores almost all of the body's calcium, dual x-ray absorptiometry measurements of bone mineral density may be used to track a person's calcium status over time (Nabiyouni *et al.* 2018).

2.3.4 Calcium and DM

In pancreatic cells, cytosolic calcium is required for insulin production. Calcium channels are present in the cytoplasm, endoplasmic reticulum, and mitochondria, and they all play a role in maintaining intracellular calcium homeostasis. Changes in the expression of calcium-associated proteins and the calcium channel were found in an experimental type 1 DM. A variety of features of calcium transport were investigated . the levels are all elevated in a mouse model of type 1 diabetes. Its expression in cellular calcium-channel calcium is decreasing, whereas PMCA expression is rising, indicating that intracellular calcium levels are progressively reduced. These results indicate that calcium metabolism is regulated by a number of calcium channels in the model.. Streptozotocin treatment may induce ER stress and cell death in pancreatic cells. They are endoplasmic reticulum chaperone proteins that assist in protein folding. Stress may be caused by dysregulation of ER calcium transporters including SERCA and IP3R. The ER is implicated in the disturbance of calcium homeostasis caused by streptozotocin. SERCA2a and SERCA2b are involved in calcium transport from the cytosol to the endoplasmic reticulum. SERCA2b expression was significantly decreased after treatment, while streptozotocin enhanced IP3R expression. The expression of these ER

calcium-channel transporters indicates that the ER calcium pool has been depleted, indicating that streptozotocin treatment has depleted the ER calcium pool, resulting in ER stress.

Calcium channels are present in the cytoplasm, endoplasmic reticulum, and mitochondria, and they all play a role in maintaining intracellular calcium homeostasis. Changes in the expression of calcium-associated proteins and the calcium channel were found in an experimental type 1 diabetic mellitus (T1DM) mouse. A variety of features of calcium transport have been investigated. For example, Cav1.2 expression in cellular calcium-channel calcium is decreasing, whereas PMCA expression is rising, indicating that intracellular calcium levels are progressively reduced. These results indicate that calcium metabolism is regulated by a number of calcium channels in the T1DM model. The ER quality control genes CALR and CANX are likewise suppressed by streptozotocin. Streptozotocin treatment may induce ER stress and cell death in pancreatic cells. CALR and CANX are endoplasmic reticulum chaperone proteins that assist in protein folding (ER). Stress in the ER may be caused by dysregulation of ER calcium transporters including SERCA and IP3R. The ER is implicated in the disturbance of calcium homeostasis caused by streptozotocin. SERCA2a and SERCA2b are involved in calcium transport from the cytosol to the endoplasmic reticulum. SERCA2b expression was significantly decreased after treatment, while streptozotocin enhanced IP3R expression. The expression of these ER calcium-channel transporters indicates that the ER calcium pool has been depleted, indicating that streptozotocin treatment has depleted the ER calcium pool, resulting in ER stress. Diabetes patients are more likely to develop acute renal failure owing to volume depletion, as previously mentioned. Patients with diabetes, for example, are more prone to have low parathyroid hormone levels (PTH). Calcium homeostasis is disrupted, which leads to renal failure. Calcium deficiency is a common complication of diabetes mellitus. In pancreatic cells, type 1 diabetes mellitus is absent, and calcium levels in the intracellular and intra-endoplasmic reticulum are low (ER). Type 2 diabetes lowers intra-ER calcium levels, resulting in ER stress (Spence 2016).

2.4 Some DM tests

Diabetes is diagnosed and treated with a blood test to determine the glucose level. To assess the blood glucose level, three methods are available: the fasting, the random, and A1C. The accuracy of the fasting plasma glucose test is maximized when it is carried out first thing in the morning following an overnight fast of eight hours (nothing to eat or drink except sips of water). Plasma glucose test at random: This test does not need any kind of fasting, so it may be done whenever you choose. A person's average blood glucose level for the previous two to three months may be determined via a test called the A1c test, which is also known as the HbA1C test or the glycated hemoglobin test. This test is used to detect the amount of glucose that is connected to hemoglobin, which is the protein in red blood cells that carries oxygen (Oguntibeju 2019). The oral glucose tolerance test is used to measure the amount of blood glucose in the blood following an overnight fast. Drink a sugary beverage after that. Then, during the first, second, and third hours, blood glucose levels are monitored every hour (Edelmann *et al.* 2016). The A1C test is performed to measure the average amount of sugar that has been present in the blood over the course of the preceding two or three months. Values of A1C that are less than or equal to 5.7 percent are deemed normal; levels that fall between 5.7 and 6.4 percent are categorized as prediabetic; and levels that are more than or equal to 6.5 percent are categorized as diabetic. A blood sample is taken before and after an overnight fast to evaluate the amount of glucose in the patient's blood (Duan and Liu 2019).

3. MATERIALS AND METHODS

3.1 Material

3.1.1 Instrument and Material

The materials, methods, Kits, and chemical material which are important and used in virto during the experimental are distributed in Table 3.1 and Table 3.2

Table 3.1 The intromen and Materials that used in vitro and its company

No	Instrument and Material	Company
1	Cobas C111	Rouch
2	Cobas E411	Rouch
3	Spectrophotometer	China
4	Automatic Pipette	China
5	White tube	China
6	Gel Tube	China
7	Yellow - Blue Tip	China
8	Incubator	China

Table 3.2 Kits and chemical material which uses in vitro

No	Kits	Use
1	Vitamin B12 kit	Measurement of Vitamin D
2	Mg kit	Measurement of Mg
3	Testeostrone kit	Measurement of testosterone
4	Randum Blood Sugar kit	Measurement of RBS
5	Total cholestrol kit	Measurement of Tc
6	Triglyceride kit	Measurement of Tg
7	HDL kit	Measurement of HDL
8	Calcium kit	Measurement of Ca
9	C-RP kit	Measurement of C-RP

3.2 Methods

3.2.1 Vitamin B12 - E4638

Vitamin B12 is a cofactor in the process of DNA creation, and it is also involved in the metabolism of fatty acids and amino acids. It is necessary for the production of myelin

and the maturation of growing red blood cells in the bone marrow, both of which are dependent on its presence. The Vitamin B12 ELISA kit that is offered by BioVision is a competitive ELISA assay that is designed for the quantitative detection of Vit-B12 in cell culture supernatants, serum, and plasma. The quantity of vitamin B12 that was extracted from the samples is directly related to the amount of color that is present.

Table 3.3 Sumary of some important Kit detials

Cat. +Size	**E4638-100**
Size	100 assays
Kit Summary	• Detection method- Absorbance (450 nm) • Species reactivity- Universal • Application- quantitative measurement of Vitamin B12 in serum, plasma, tissue homogenates and other biological fluids.
Detection Method	Absorbance (450 nm)
Species Reactivity	Universal
Applications	This ELISA kit is used for quantitative measurement of Vitamin B12 in serum, plasma, tissue homogenates and other biological fluids.
Features & Benefits	• Easy, convenient and time-saving method to measure the level of Vitamin B12 in serum, plasma, tissue homogenates, culture supernatants and other biological fluids. • Detection Range: 0.781 - 50 ng/mL • Sensitivity: < 0.469 ng/mL Assay Precision: Intra-Assay: CV < 8%; Inter-Assay: CV < 10% No significant cross-reactivity or interference was observed.

Kit Components

• Micro ELISA Plate

•Lyophilized Standard

• Sample / Standard dilution buffer

• Biotin- detection antibody (Concentrated)

• Antibody dilution buffer

• HRP-Streptavidin Conjugate (SABC)

• SABC dilution buffer

• TMB substrate

• Stop Solution

• Wash buffer (25X)

• Plate sealers

3.2.2 Magnesium assay Kits - orb390813

Magnesium assay kits are available from several suppliers to measure levels of magnesium in biological samples. Magnesium is involved in many biological functions, including enzyme activity, nucleic acid stability, and cellular signaling. Deviations in normal levels of magnesium are associated with various metabolic and endocrine disorders. Some magnesium assays employ a glycerol kinase-linked reaction to generate an intense change in color, which is directly proportional to Mg^{2+} concentration. Other assays oxidize magnesium ions to react with a chromogen. Magnesium assay kits can be useful in enzyme studies and in detecting metabolic imbalances. Visit the magnesium assay kit suppliers for more details, including kit components.

3.3 Statistics

ANOVA one way test were conducted for all study samples using the SPSS program, version 23. It was also using the same program to find the correlation between the biochemical paramaters.

4. RESULTS AND DISCUSSION

The case study was carried out on 120 patients suffering from type 2 diabetes between the months of September 2021 and April 2022. The patients were split into two groups: group A consisted of 55 individuals suffering from type 1 diabetes mellitus, and group B included 110 individuals suffering from type 2 diabetes mellitus. A comparison was made between the patients and a control group consisting of fifty individuals who were in good health. We are going to investigate whether or not there is a connection between the lack of vitamin B12, magnesium, and certain biochemical parameters in diabetic patients and the shifts in testosterone, B 12, glucose, and certain biochemical tests associated with testosterone disorders in diabetic men.

4.1 Age

In our study, the mean of age in group B, C and total was (30.5 ± 8.44, 48.9 ± 7.99 and 45.5 ± 14.4 respectively) which showed a significant difference when compared to group A (30.5 ± 8.44). The minimum was (21.00, 39.00 and 40.00 respectively) and maximum (47.00, 61.00 and 77.00 respectively) as shown in the Table 4.1 and Figure 4.1. The results of the age association test with vitamin B12 test indicated r= 0.320^{*} at P <0.05.

Table 4.1 The mean for age in all study groups

Variables	Age M±SD	Minimum	Maximum	P value
Group A n=50	30.5 ± 8.44	21.00	47.00	0.003
Group B n=55	48.9 ± 7.99	39.00	61.00	0.003
Group C n=55	57.1 ± 11.1	40.00	77.00	0.003
Total n=160	45.5 ± 14.4	21.00	77.00	

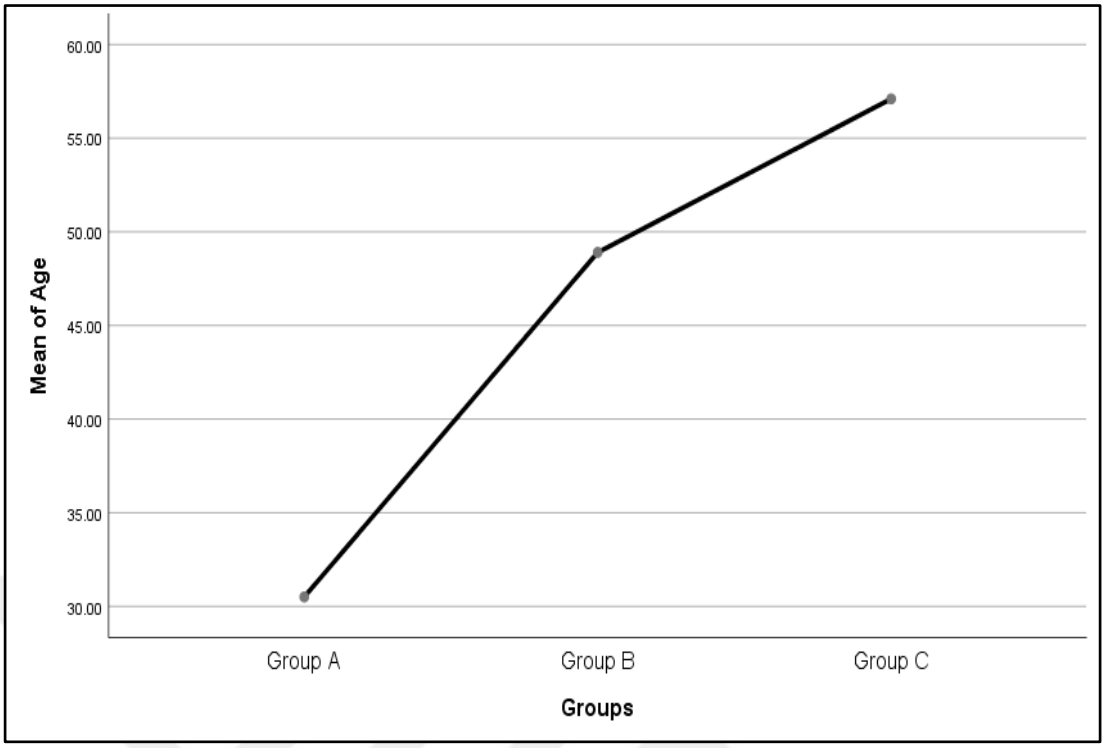

Figure 4.1 The mean for age in all study groups

4.2 Weight

In the present study, the mean of weight in group B, C, and total was (67.1 ± 10.0, 64.1 ± 8.93 and 71.9 ± 9.10) which showed a significant difference when compared to group A (84.5 ± 8.38). The minimum was (74.00, 69.00 and 66.00 respectively) and maximum (102.00, 99.00 and 96.00 respectively) as shown in the Table 4.2 and Figure 4.2. The results of the weight association test with vitamin B12 test indicated r= 0.030 at P <0.05.

Table 4.2 The mean of weight for all study groups

Variables	Weight M±SD	Minimum	Maximum	P value
Group A n=50	84.5 ± 8.38	74.00	102.00	0.035
Group B n=55	67.1 ± 10.0	69.00	99.00	0.035
Group C n=55	64.1 ± 8.93	66.00	96.00	0.035
Total n=160	71.9 ± 9.10	69.00	102.00	

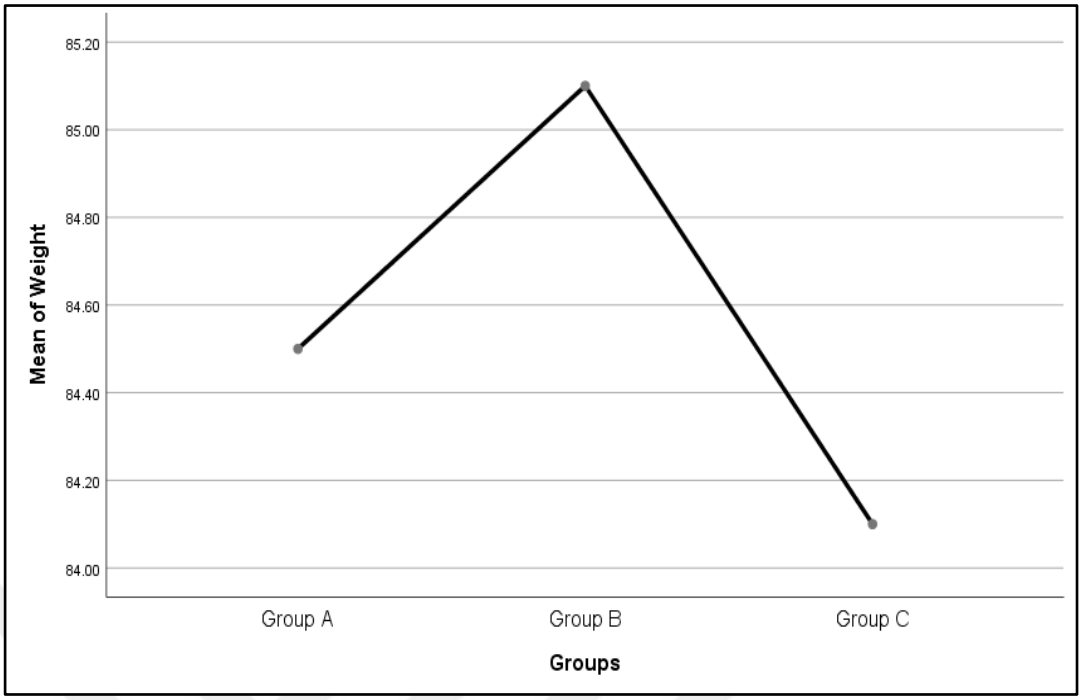

Figure 4.2 The mean of weight for all study groups

4.3 Magnesium (Mg)

In our study, the mean of magnesium in group B and C was (1.31 ± 0.45, 1.90 ± 0.40) which only group B showed a significant difference when compared to group A (1.87 ± 0.28). The minimum was (1.48, 0.29 and 1.53 respectively) and maximum (2.45, 1.85 and 2.96 respectively) as shown in the Table 4.3 and Figure 4.3. The results of the Mg association test with vitamin B12 test indicated r= 0.557^{**} at P <0.05.

Table 4.3 The mean of magnesium for all study groups

Variables	Age M±SD	Minimum	Maximum	P value
Group A n=50	1.87 ± 0.28	1.48	2.45	0.041
Group B n=55	1.31 ± 0.45	0.29	1.85	0.041
Group C n=55	1.90 ± 0.40	1.53	2.96	0.041
Total n=160	1.69 ± 0.46	0.29	2.96	

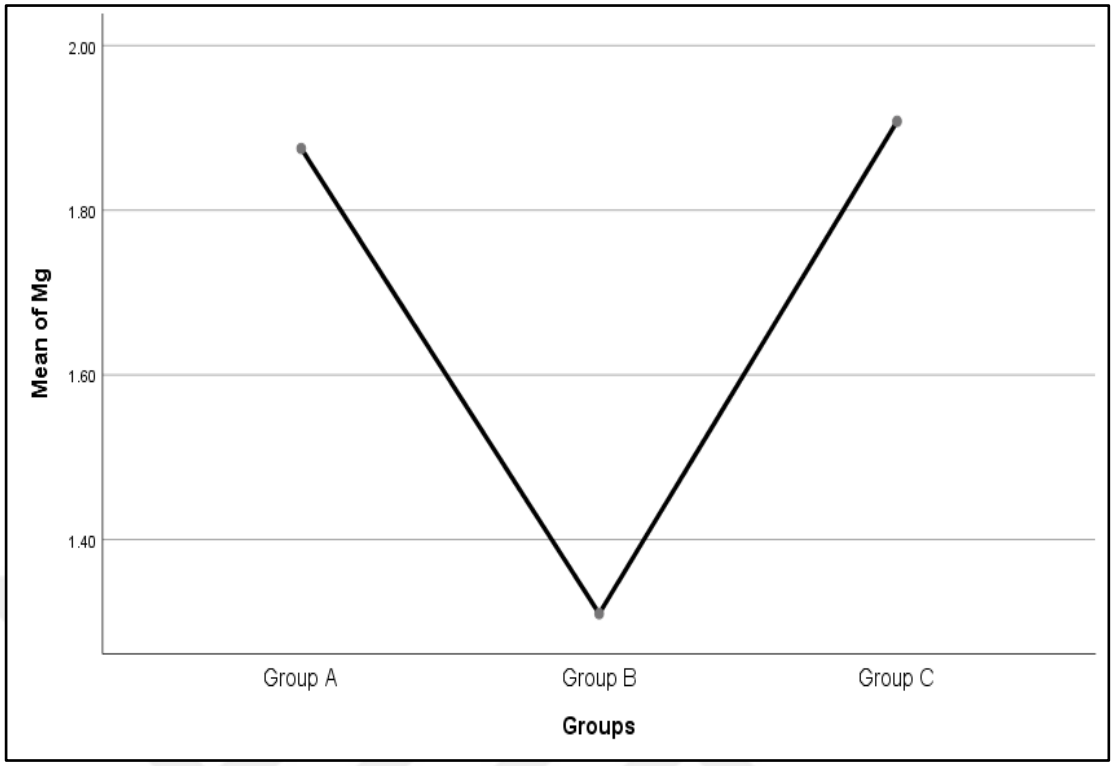

Figure 4.3 The mean of magnesium for all study groups

4.4 Vitamin B12

Herein, the mean of vitamin B12 in group B and C was (271.8 ± 38.5, 387.1 ± 64.0) which showed a significant difference when compared to group A (432.8 ± 107). The minimum was (295.00, 208.00 and 284.00 respectively) and maximum (648.00, 319.00 and 486.00 respectively) as shown in the Table 4.4 and Figure 4.4.

Table 4.4 The mean of vitamin B12 for all study groups

Variables	Age M±SD	Minimum	Maximum	P value
Group A n=50	432.8 ± 107	295.00	648.00	0.014
Group B n=55	271.8 ± 38.5	208.00	319.00	0.014
Group C n=55	387.1 ± 64.0	284.00	486.00	0.014
Total n=160	363.9 ± 100	208.00	648.00	

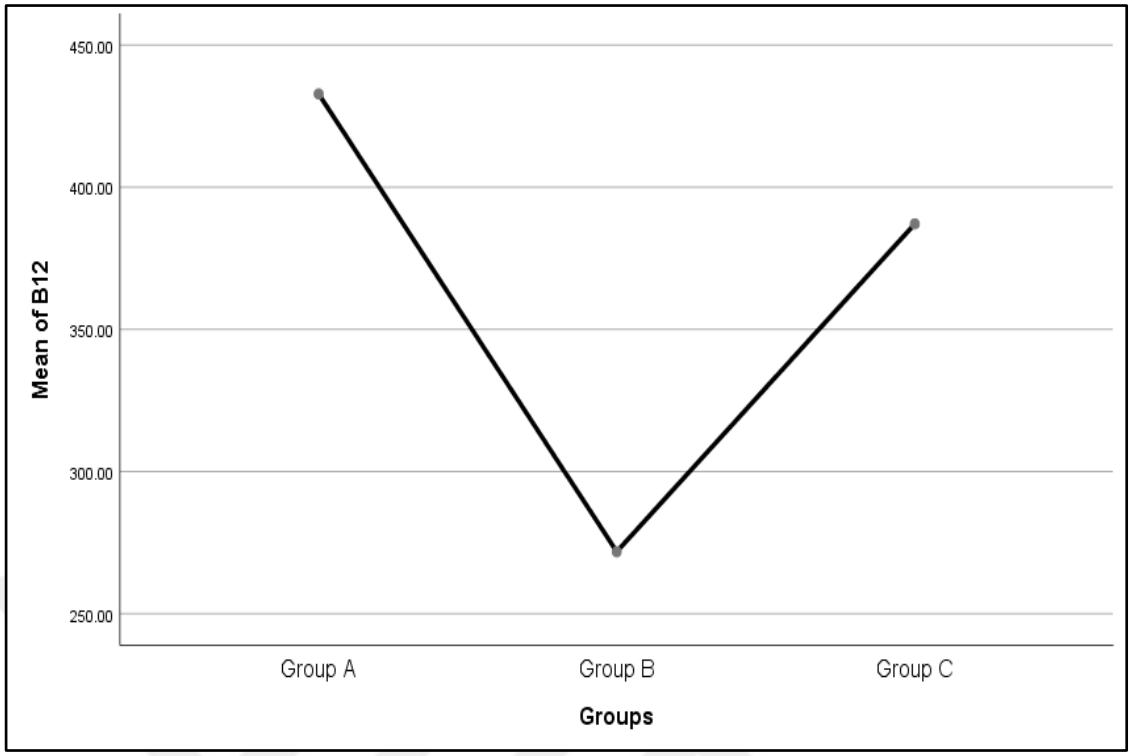

Figure 4.4 The mean of vitamin B12 for all study groups

4.5 Testosterone

The mean of testosterone in group B and C was (219.3 ± 76.1, 308.8 ± 97.5) which showed a significant difference when compared to group A (531.1 ± 144). The minimum was (274.00, 117.00 and 203.00 respectively) and maximum (794.00, 364.00 and 490.00 respectively) as shown in the Table 4.5 and Figure 4.5. The results of the testosterone association test with vitamin B12 test indicated r= 0.300* at P <0.05.

Table 4.5 The mean of testosterone for all study groups

Variables	Age M±SD	Minimum	Maximum	P value
Group A n=50	531.1 ± 144	274.00	794.00	0.001
Group B n=55	219.3 ± 76.1	117.00	364.00	0.001
Group C n=55	308.8 ± 97.5	203.00	490.00	0.001
Total n=160	353.1 ± 211.7	274.00	794.00	

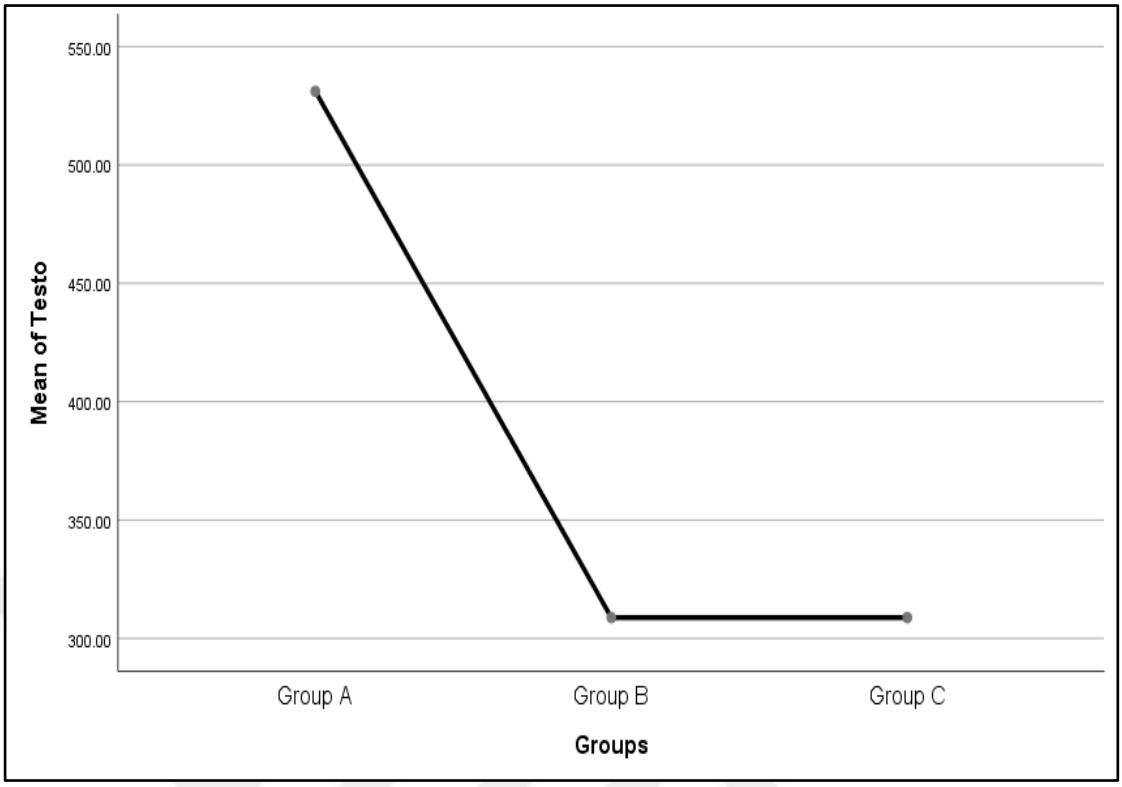

Figure 4.5 The mean of testosterone for all study groups

4.6 Random Blood Sugar (RBS)

In study, the mean of RBS in group B, C and total was (274.1 ± 55.2, 214.3 ± 38.4, 88.5 ±191.2, 191.2 ± 88.5 respectively) which showed a significant difference when compared to group A (85.4 ± 9.44). The minimum was (71.00, 188.00 and 175.00 respectively) and maximum (101.00, 385.00 and 296.00 respectively) as shown in the Table (4.6) and Figure (4.6). The results of the RBS association test with vitamin B12 test indicated r= 0.583^{**} at P <0.05.

Table 4.6 The mean of RBS in all study groups

Variables	Age M±SD	Minimum	Maximum	P value
Group A n=50	85.4 ± 9.44	71.00	101.00	
Group B n=55	274.1 ± 55.2	188.00	385.00	
Group C n=55	214.3 ± 38.4	175.00	296.00	
Total n=160	191.2 ± 88.5	74.00	385.00	

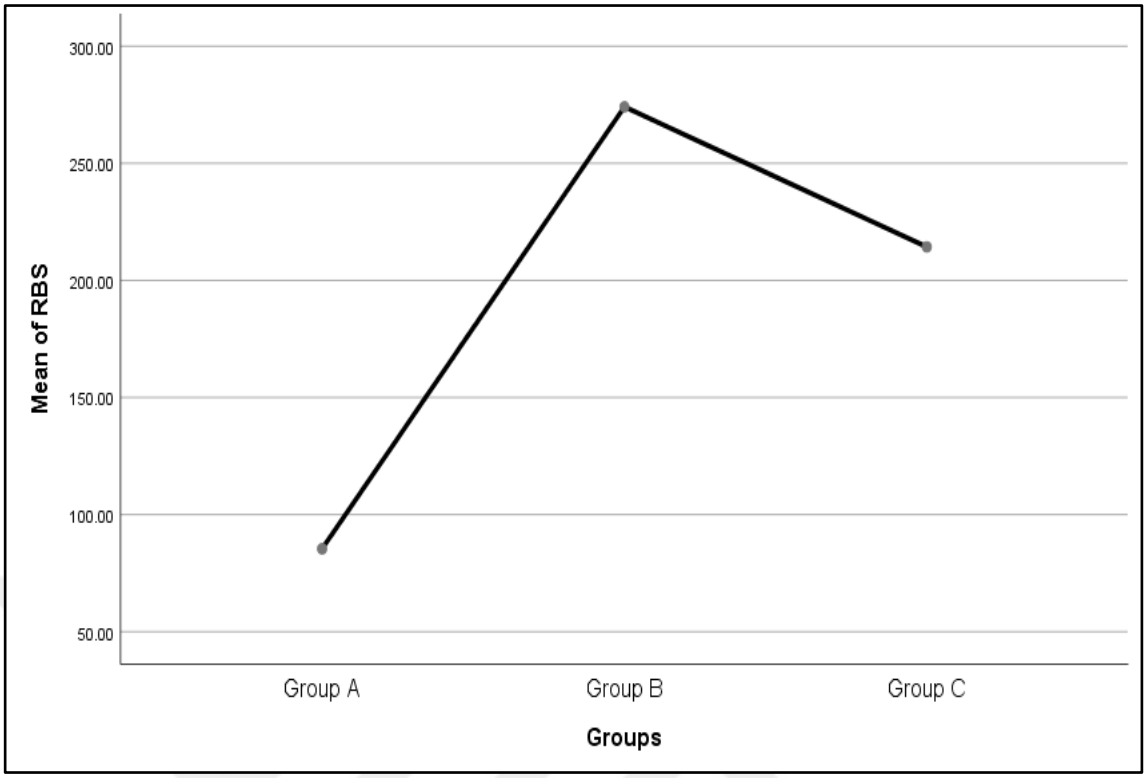

Figure 4.6 The mean of RBS in all study groups

4.7 Total Cholesterol (TC)

In our study, the mean of total cholesterol in group B, C and total was (156.5 ± 18.4, 166.9 ± 14.8, 166.0 ± 23.1 respectively) which showed a significant difference when compared to group A (174.6 ± 31.4). The minimum was (137.00, 131.00 and 144.00 respectively) and maximum (239.00, 197.00 and 195.00 respectively) as shown in the Table 4.7 and Figure 4.7. The results of the total cholesterol association test with vitamin B12 test indicated r= 0.203* at P <0.05.

Table 4.7 The mean of total cholesterol for all study groups

Variables	Age M±SD	Minimum	Maximum	P value
Group A n=50	174.6 ± 31.4	137.00	239.00	0.016
Group B n=55	156.5 ± 18.4	131.00	197.00	0.016
Group C n=55	166.9 ± 14.8	144.00	195.00	0.016
Total n=160	166.0 ± 23.1	137.00	239.00	

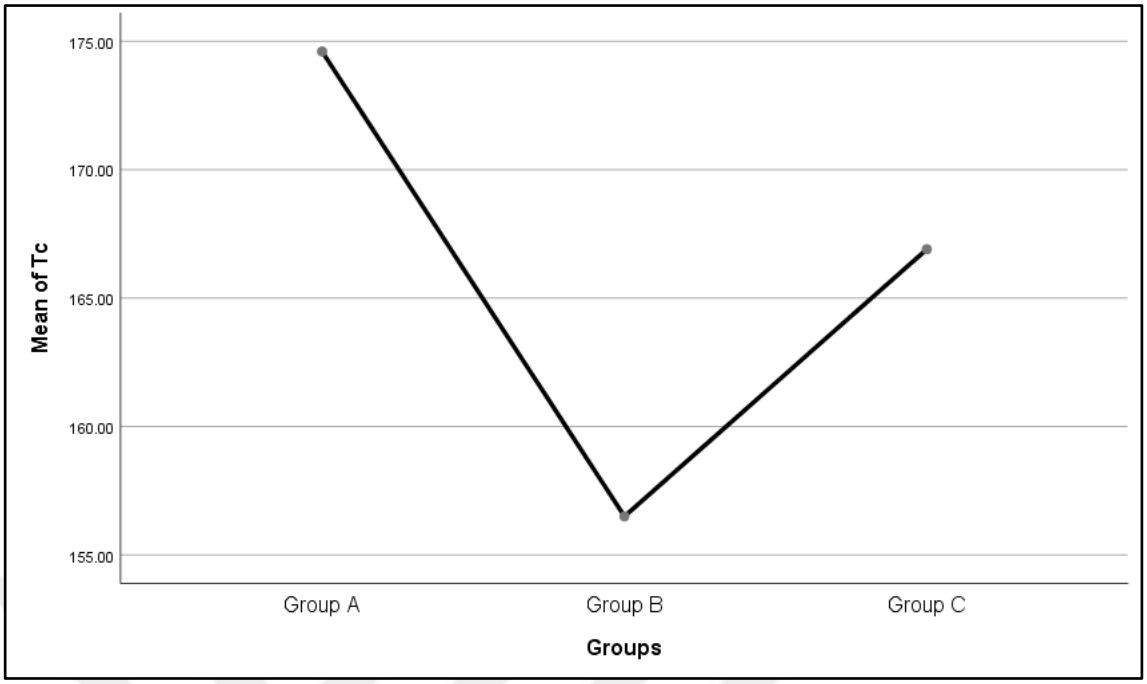

Figure 4.7 The mean of total cholesterol for all study groups

4.8 Triglyceride

In our study, the mean of triglyceride in group B, C and total was (80.9 ± 12.2, 88.3 ± 6.18, 84.4 ± 10.9 respectively) which showed a non-significant difference when compared to group A (84.1 ± 12.8). The minimum was (66.00, 58.00 and 77.00 respectively) and maximum (103.00, 95.00 and 96.00 respectively) as shown in the Table 4.8 and Figure 4.8. The results of the triglyceride association test with vitamin B12 test indicated r= 0.133 at P <0.05.

Table 4.8 The mean of triglyceride for all study groups

Variables	Age M±SD	Minimum	Maximum	P value
Group A n=50	84.1 ± 12.8	66.00	103.00	0.061
Group B n=55	80.9 ± 12.2	58.00	95.00	0.061
Group C n=55	88.3 ± 6.18	77.00	96.00	0.061
Total n=160	84.4 ± 10.9	58.00	103.00	

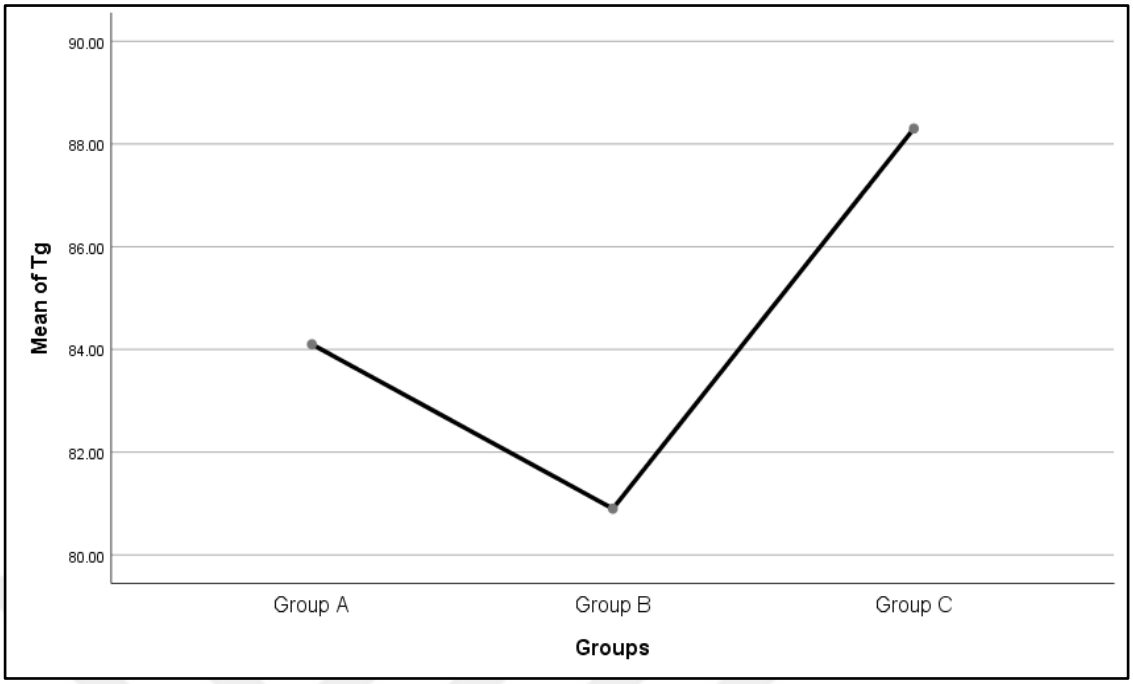

Figure 4.8 The mean of triglyceride for all study groups

4.9 High Density Liproprotein (HDL)

In our study, the mean of HDL in group B, C and total was (47.9 ± 8.45, 59.1 ± 10.9 and 50.8 ± 10.6 respectively) which showed a non-significant difference when compared to group A (45.5 ± 7.59). The minimum was (35.00, 36.00 and 46.00 respectively) and maximum (58.00, 64.00 and 77.00 respectively) as shown in the Table 4.9 and Figure 4.9. The results of the HDL association test with vitamin B12 test indicated r= 0.017 at P <0.05.

Table 4.9 The mean of HDL in all study groups

Variables	Age M±SD	Minimum	Maximum	**P value**
Group A n=50	45.5 ± 7.59	35.00	58.00	0.104
Group B n=55	47.9 ± 8.45	36.00	64.00	0.104
Group C n=55	59.1 ± 10.9	46.00	77.00	0.104
Total n=160	50.8 ± 10.6	35.00	77.00	

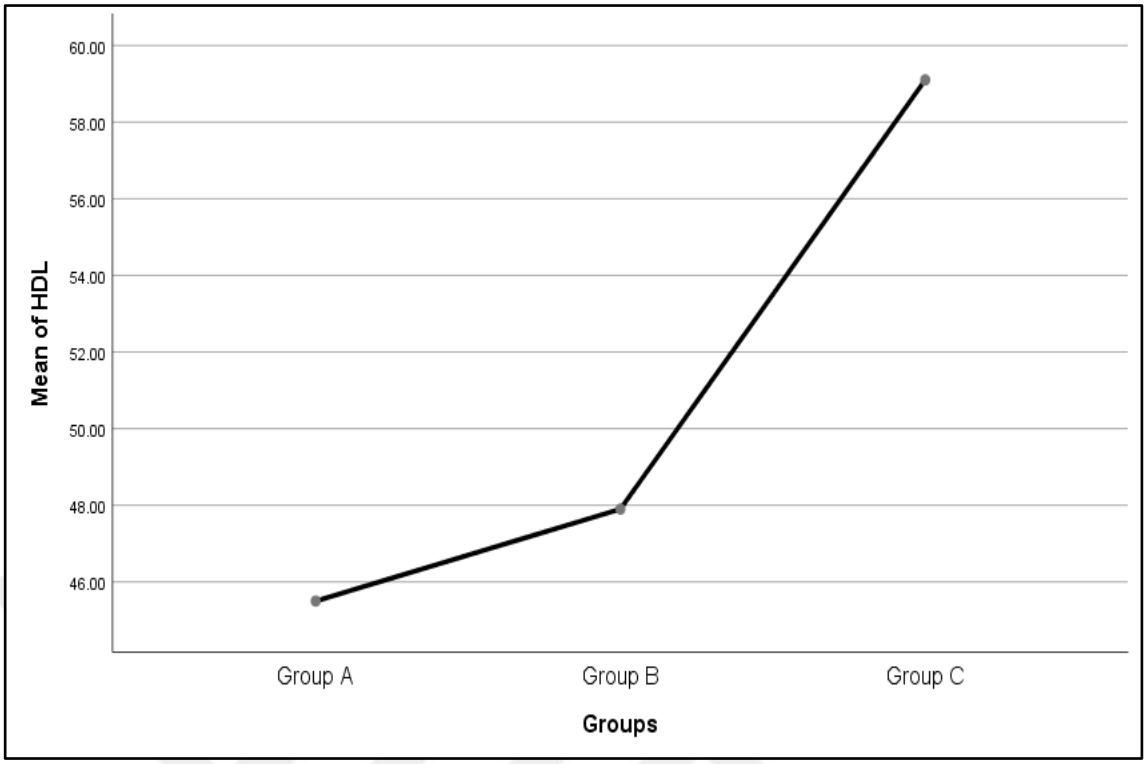

Figure 4.9 The mean of HDL in all study groups

4.10 Low Density Liproprotein (LDL)

In our study, the mean of LDL in group B, C and total was (124.7 ± 16.9, 112.4 ± 13.4 and 111.5 ± 10.7 respectively) which showed a non-significant difference when compared to group A (97.6 ± 11.7). The minimum was (84.00, 104.00 and 98.00 respectively) and maximum (113.00, 152.00 and 137.00 respectively) as shown in the Table 4.10 and Figure 4.10. The results of the LDL association test with vitamin B12 test indicated r= 0.057 at P <0.05.

Table 4.10 The mean of LDL for all study groups

Variables	Age M±SD	Minimum	Maximum	P value
Group A n=50	97.6 ± 11.7	84.00	113.00	0.639
Group B n=55	124.7 ± 16.9	104.80	152.60	0.639
Group C n=55	112.4 ± 13.4	98.20	137.00	0.639
Total n=160	111.5 ± 13.7	84.00	137.60	

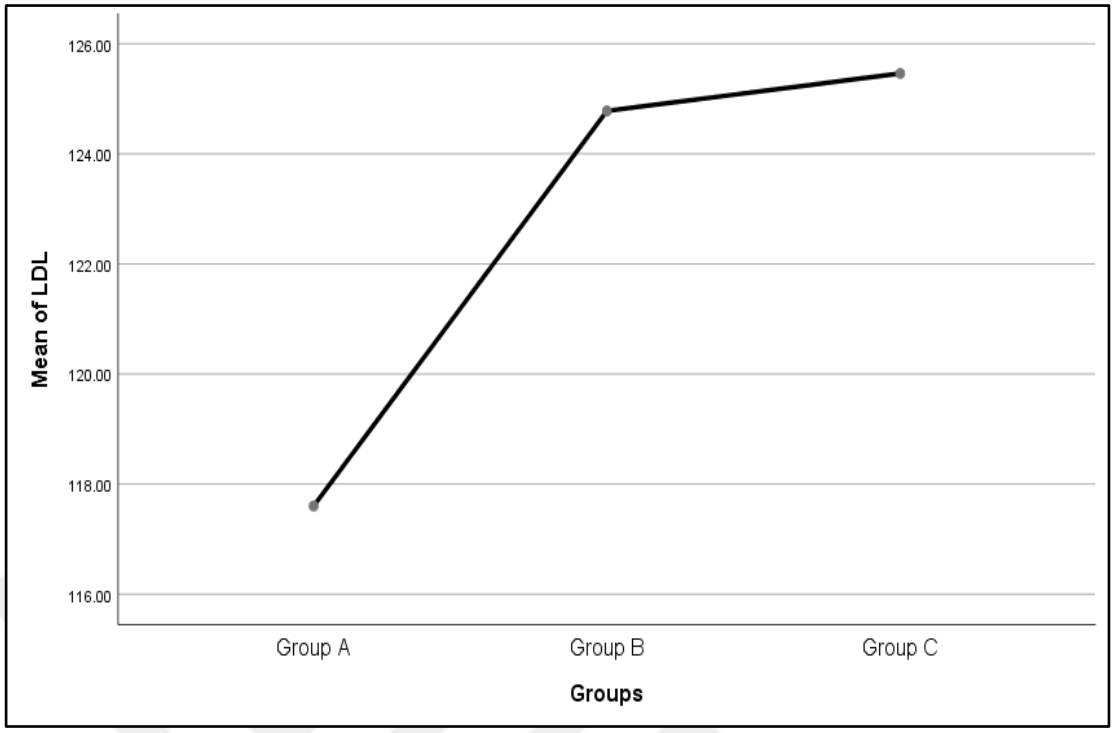

Figure 4.10 The mean of LDL for all study groups

4.11 Very Low Density Liproprotein (VLDL)

In our study, the mean of VLDL in group B, C and total was (17.1 ± 2.44, 16.6 ± 1.23 and 16.8 ± 2.18 respectively) which showed a non-significant difference when compared to group A (15.8 ± 2.57). The minimum was (13.2, 15.4 and 11.6 respectively) and maximum (20.6, 19.00 and 19.2 respectively) as shown in the Table 4.11 and Figure 4.11. The results of the VLDL association test with vitamin B12 test indicated r= 0.133 at P <0.05.

Table 4.11 The mean of VLDL for all study groups

Variables	Age M±SD	Minimum	Maximum	P value
Group A n=50	15.8 ± 2.57	13.20	20.60	0.094
Group B n=55	17.1 ± 2.44	11.60	19.00	0.094
Group C n=55	16.6 ± 1.23	15.40	19.20	0.094
Total n=160	16.8 ± 2.18	11.60	20.60	

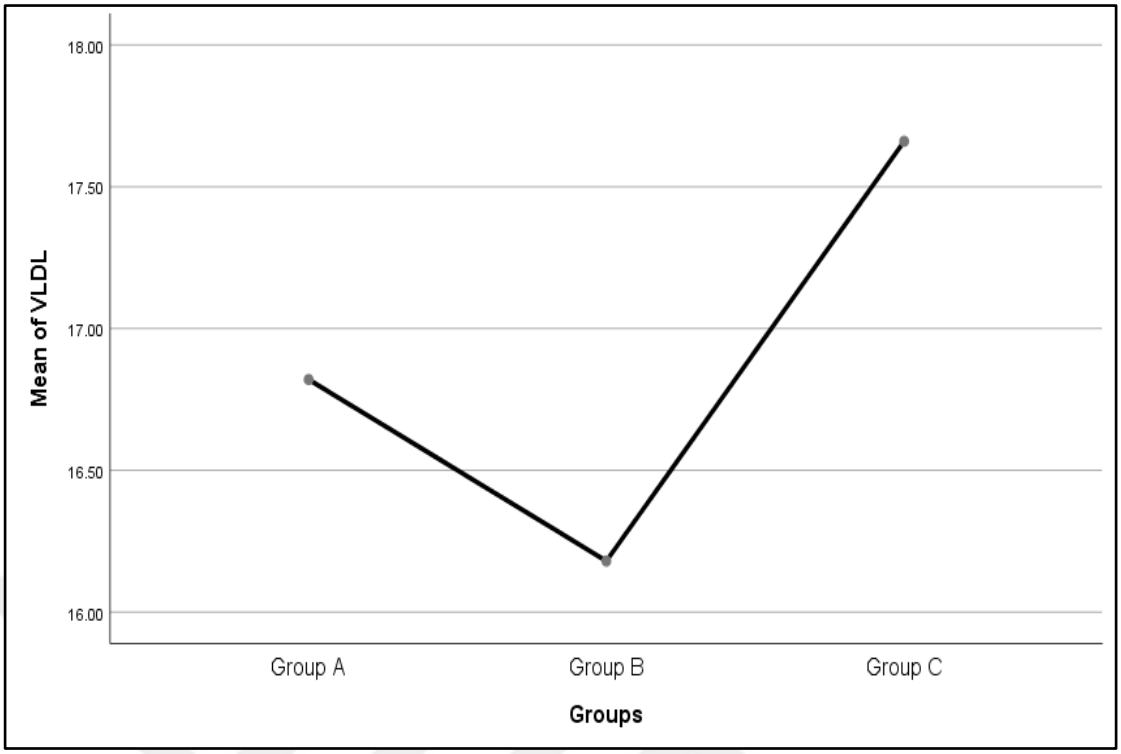

Figure 4.11 The mean of VLDL for all study groups

4.12 Calcium

In our study, the mean of calcium in group B, C and total was (7.85 ± 1.01, 8.98 ± 0.92 and 8.90 ± 0.96 respectively) which showed a non-significant difference when compared to group A (8.18 ± 1.04). The minimum was (7.48, 6.38 and 7.84 respectively) and maximum (10.4, 9.50 and 10.8 respectively) as shown in the Table 4.12 and Figure 4.12. The results of the calcium association test with vitamin B12 test indicated r= 0.077 at P <0.05.

Table 4.12 The mean of calcium for all study groups

Variables	Age M±SD	Minimum	Maximum	P value
Group A n=50	8.18 ± 1.04	7.48	10.40	0.304
Group B n=55	7.85 ± 1.01	6.38	9.50	0.304
Group C n=55	8.98 ± 0.92	7.84	10.80	0.304
Total n=160	8.90 ± 0.96	7.38	10.80	

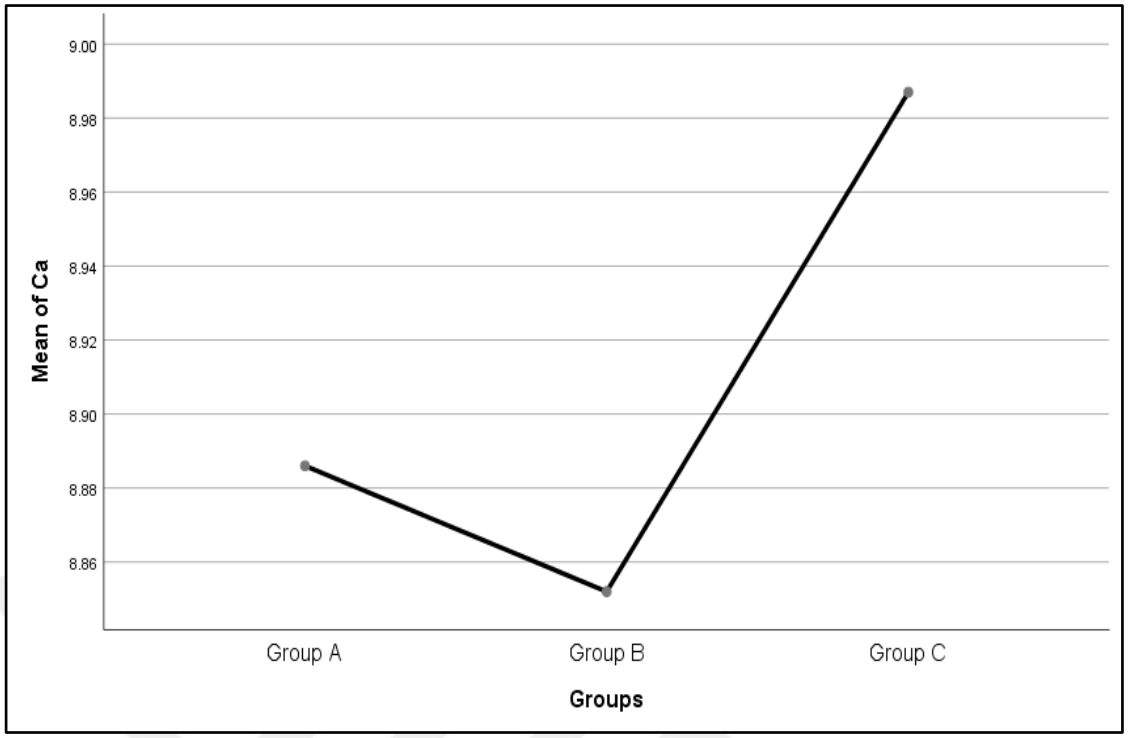

Figure 4.12 The mean of calcium for all study groups

4.13 C-Reactive Protein (C-RP)

In our study, the mean of C-RP in group B and C was (2.68 ± 0.29, 1.91 ± 0.24 and 2.17 ± 0.23 respectively) which showed a non-significant difference when compared to group A (1.92 ± 0.21). The minimum was (0.58, 0.11 and 0.37 respectively) and maximum (3.65, 4.69 and 3.68 respectively) as shown in the Table 4.12 and Figure 4.12. The results of the C-RP association test with vitamin B12 test indicated r= 0.028 at P <0.05.

Table 4.13 The mean of C-RP for all study groups

Variables	Age M±SD	Minimum	Maximum	P value
Group A n=50	1.92 ± 0.21	0.58	3.65	0.061
Group B n=55	2.68 ± 0.29	0.11	4.69	0.061
Group C n=55	1.91 ± 0.24	0.37	3.68	0.061
Total n=160	2.17 ± 0.23	0.11	3.99	

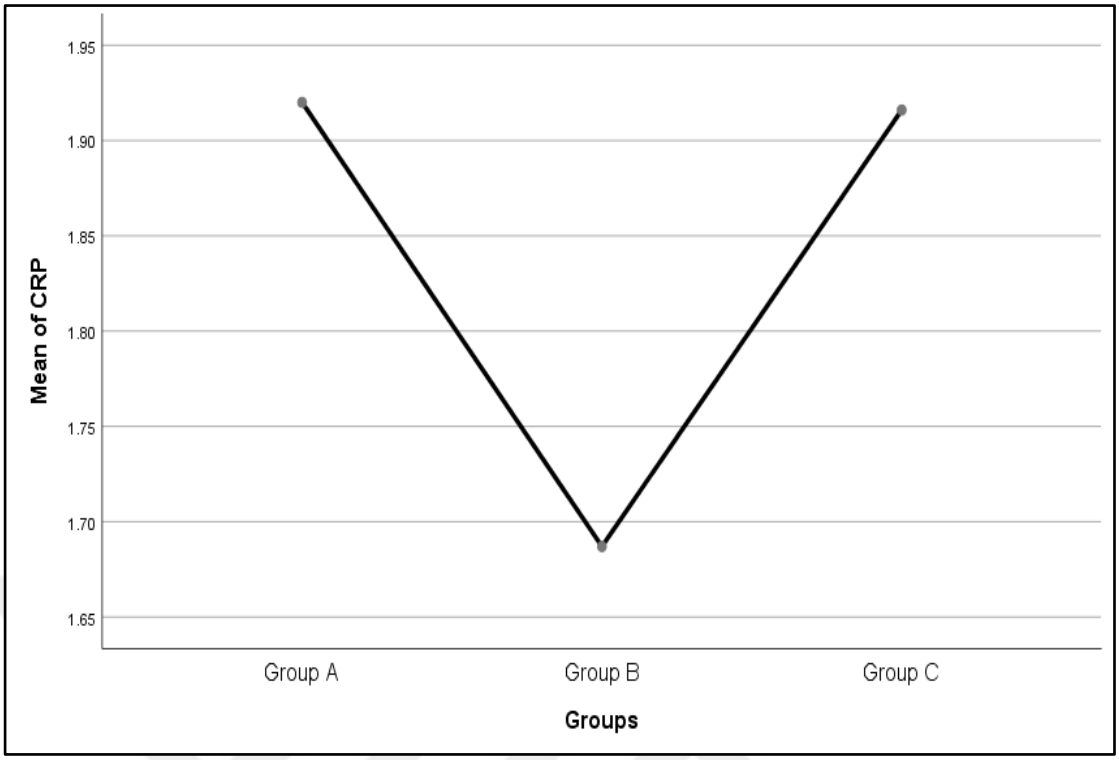

Figure 4.13 The mean of C-RP for all study groups

4.14 Vitamin B12

The mean levels of vitamin B12 in groups B and C, which exhibited a substantial value in comparison to group A and are shown in Table 4.13, are revealed to have been significantly higher than those in group A.

Table 4.14 The Correlation table between vitamin B12 and some chemical tests

Variables	**r**	**P value**
Vitamin B12 - Age	0.320*	< 0.05
Vitamin B12 - Weight	0.030	> 0.05
Vitamin B12 - Mg	0.557**	< 0.05
Vitamin B12 - Testeo	0.300	> 0.05
Vitamin B12 - RBS	0.583**	< 0.05
Vitamin B12 - Tc	0.203*	< 0.05
Vitamin B12 - Tg	0.133	> 0.05
Vitamin B12 - HDL	0.017	> 0.05
Vitamin B12 - LDL	0.057	> 0.05
Vitamin B12 - VLDL	0.133	> 0.05
Vitamin B12 - Calcium	0.077	> 0.05
Vitamin B12 - C-RP	0.028	> 0.05

5. CONCLUSION AND RECOMMENDATION

In our study, the aim was to evaluate the levels of vitamin B12 in diabetes mellitus and the extent to which its levels are affected in this disease. Age was of great importance, as the age increased, the incidence of diabetes increased, as well as the increase in vitamin B12 deficiency. As for magnesium, it was significantly affected and had a link with diabetes, as well as vitamin B12. At the same time, there was a correlation between diabetes test and changes in the lipid profile, but the most correlation was with total lipids. Body mass indexes (BMIs; calculated as weight in kilograms divided by the square of height in meters) at 25, 35, and 45 years of age were all strongly associated with diabetes risk (relative risks for overweight [BMI> or =25.0] vs. not overweight, >3.0; all Ps.05), as were maximum and average BMI to 49 years of age. This was found in previous research after simultaneous adjustment was made for age, physical activity, and a lifetime maternal history of diabetes Adjustment for BMI at 45 years of age and average BMI had a significant attenuating effect on the association between BMI at 25 years of age and risk of diabetes. However, this relationship was independent of weight change, weight variability, or maximum BMI. When it comes to men, having a high body mass index (BMI) at 25 years of age is a reliable indicator of whether or not they will develop diabetes during middle age. This is primarily due to the association between having a high BMI at 45 years of age and having a high average BMI until 49 years of age (Brancati et al. 1999). There was a correlation between adult-onset diabetes and both a person's birth weight and its percentile for gestational age. Even if a woman has a normal BMI now, she should still be concerned about the possibility of developing diabetes since she was born with a low birth weight (Katanoda et al. 2017). Vitamin B12 deficiency, both clinically and biochemically, is quite common in people diagnosed with either type 1 or type 2 diabetes. For the purpose of assisting in the formulation of guidelines in diabetes clinical care, the conduct of future studies that are both extensive and carefully designed on the subject of screening for vitamin B12 deficiency, vitamin B12 supplementation, and the optimal supplementation dose among diabetic patients of types 1 and 2 is warranted (Kibirige and Mwebaze 2013). Metformin usage for longer periods of time and at greater doses is linked to lower levels of vitamin B12 in the blood. Therefore, regular assessment of vitamin B12 levels in patients with type 2

diabetes who are taking a high dosage of metformin and those who have been using metformin for an extended period of time can aid in identifying people who might benefit from vitamin B12 supplements (Akinlade et al. 2015). The findings of this investigation were consistent with our own. When compared to the diabetic group that did not receive oral B12 supplementation, the prevalence of vitamin B12 deficiency was shown to be greater in the control group. It is usual practice to neglect older patients with or without diabetes who have low levels of vitamin B12 in their blood. There was no correlation between the prevalence of diabetes mellitus and a decrease in BMD in the elderly. In addition, there is no significant correlation between blood levels of vitamin B12 and bone mineral density (BMD) in diabetics (Amer et al. 2015). Metformin medication in instances is related with a greater incidence of vitamin B12 insufficiency, the severity of which rises with the length of diabetes and the amount of metformin (Kim et al. 2019). When compared to non-diabetics, diabetics have a greatly increased chance of developing atherosclerosis, and the clinical relevance of hyperlipidemia in diabetics should be considered to be much more severe. Hypertriglyceridemia is the most common form of improper fat metabolism in diabetes. This is caused by an increase in triglyceride-carrying lipoproteins, such as chylomicrons and very low-density lipoproteins. In patients with type I diabetes, the most important determinant in the development of hypertriglyceridemia is a reduction in the clearance of chylomicrons and an impairment in the degradation of VLDL, both of which are induced by a reduction in the activity of the lipoprotein lipase (Biesenbach 1989).

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