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BIOCHEMICAL COMPARATIVE STUDY FOR THE ROLE OF VITAMIN B12 IN TYPE2 DIABETIC PATIENTS WITH DYSLIPIDEMIA

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By Taghreed Kadhim Fadaam ALMOTLAK

November 2022

We certify that we have read this thesis and that in our opinion it is fully adequate, in scope and in quality, as a thesis for the degree of Master of Science

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ABSTRACT

BIOCHEMICAL COMPARATIVE STUDY FOR THE ROLE OF VITAMIN B12 IN TYPE2 DIABETIC PATIENTS WITH DYSLIPIDEMIA

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Master of Science in Chemistry

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One of the main risk factors for cardiovascular disease in people with diabetes mellitus is dyslipidemia. This study aimed to determine the role of vitamin B12 in diabetic patients with dyslipidemia. The study included 160 subjects divided into four groups: 40 of them were healthy, 40 had good diabetes control, 40 had fair diabetes control, while the last 40 were poor in diabetes control. All participants were tested for FBG, HbA1C, TG, CHOL, HDL and Vitamin B12. The parameters were statistically studied to find out the mean and the significant difference compared to the control group. The difference between the studied groups of patients was also studied, and the correlation coefficient was determined to know the correlation between the studied parameters. The levels of FBG, HbA1C, TG and CHOL increased in the fair-control group, and the level of HDL and vitamin B12 decreased. These levels were more dangerous in the poor control group, while the levels were similar or close to the control group in the good controlled group. The study achieved a clear positive correlation between HbA1C and TG (r=0.503), CHOL (r=0.459), while a negative correlation was observed between TG and HDL (r=-0.612), Vit. B12 (r='0.595) with a significant difference of ($P<0.01$). When compared with triglycerides, all other metrics clearly diverged.

2022, 47 pages

Keywords: Atherogenic index, Cobalamin, Diabetes, Dyslipidemia, HBA1C

ÖZET

DİSLİPIDEMİ OLAN TİP 2 DİYABETİK HASTALARDA B12 VİTAMİNİNİN ROLÜ İÇİN BİYOKİMYASAL KARŞILAŞTIRMALI ÇALIŞMA

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Kasım 2022

Diabetes mellituslu kişilerde kardiyovasküler hastalık için ana risk faktörlerinden biri dislipidemidir. Bu çalışma, dislipidemili diyabetik hastalarda B12 vitamininin rolünü belirlemeyi amaçladı. 160 denek dört gruba ayrıldı: 40'ı sağlıklı, 40'ı iyi diyabet kontrolüne sahipti, 40'ı orta düzeyde diyabet kontrolüne sahipti ve son 40'ı diyabet kontrolünde zayıftı. Tüm katılımcılar FBG, HbA1C, TG, CHOL, HDL ve Vitamin B12 için test edildi. Kontrol grubuna kıyasla ortalama ve anlamlı farkı bulmak için parametreler istatistiksel olarak incelenmiştir. Çalışılan hasta grupları arasındaki fark da incelendi ve çalışılan parametreler arasındaki korelasyonu bilmek için korelasyon katsayısı belirlendi. Adil kontrol grubunda FBG, HbA1C, TG ve CHOL seviyeleri artarken, HDL ve vitamin B12 seviyeleri azaldı. Bu seviyeler kötü kontrol grubunda daha tehlikeliyken, iyi kontrol grubunda seviyeler kontrol grubuna yakın veya benzerdi. Çalışmada HbA1C ile TG (r=0,503), CHOL (r=0,459) arasında net bir pozitif korelasyon elde edildi. TG ile HDL arasında negatif korelasyon (r=-0,612) gözlenirken, Vit. B12 (r='0,595) ile (P<0,01) anlamlı bir fark. Trigliseritlerle karşılaştırıldığında, diğer tüm ölçümler açıkça birbirinden ayrıldı.

2022, 47 sayfa

Anahtar Kelimeler: Aterojenik indeks, Kobalamin, Diyabet, Dislipidemi, HBA1C

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Taghreed Kadhim Fadaam ALMOTLAK
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CONTENTS

LIST OF SYMBOLS

%	Percent
±	Plus-minus
°C	Degrees Celsius
cm	Centimeter
dL	Deciliter
g	Gram
kg	Kilogram
L	Liter
m^2	Square meters
mg	Milligram
mL	Milliliters
mmol	Millimoles
ng	Nanogram
nm	Nanometer

LIST OF ABBREVIATIONS

A	Absorbance
ADMA	Asymmetric dimethylarginine
ANOVA	Analysis of variance
CBC	Complete blood counts
CDC	Centers for disease control
EIZA	Enzyme immunoassay
ERK	Extracellular signal-regulated kinase

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1. INTRODUCTION

Diabetes mellitus, more often known as diabetes, is a dangerous, long-term (or "chronic") disorder that manifests as elevated blood glucose levels when the body is unable to create any, sufficient amounts of, or utilize the insulin that is produced (Federation 2021). Diabetes is a significant and expanding global health concern. Impairment in glucose tolerance was expected to affect 7.5% (374 million) people worldwide in 2019, and it is anticipated that this number will increase to 8.0% (454 million) by 2030 and 8.6% (548 million) by 2045. By 2045, there will be 693 million people worldwide who have diabetes (Saeedi *et al.* 2019). Around 79% of diabetics reside in low- and middle-income nations, where there will be a sharp rise in cases over the next 22 years of 13.3% and 13.9% in the Middle East and North Africa region, respectively (Cho *et al.* 2018).

There are three primary categories of diabetes: (1) insulin-dependent diabetes mellitus(T1DM), or type 1 diabetes mellitus, inability to secrete insulin is a defining feature, (2) non-insulin-dependent diabetes mellitus, or type 2 diabetes mellitus (T2DM); and gestational DM (American Diabetes Association 2022). Around 90% of diabetics worldwide have T2DM, which is mostly linked to being overweight and inactive (Alhaji 2022).

Unhealthy blood lipid (fat) levels of one or more lipid types are referred to as dyslipidemia. Three primary lipid types are present in blood: Triglycerides, low-density lipoprotein (LDL), and high-density lipoprotein (HDL) (Yuan *et al.* 2021). The two forms of dyslipidemia are primary and secondary. Secondary dyslipidemia is acquired, meaning it resulted from other conditions like obesity or diabetes, whereas primary dyslipidemia is inherited (Yanai and Yoshida 2021). A recognized indicator of endothelial dysfunction and cardiovascular risk in diabetes is dyslipidemia. Dyslipidemia is highly prevalent among diabetic population particularly in those with poorly controlled diabetes. This calls for early and universal screening of lipid profile (Shahwan *et al*. 2019).

A crucial vitamin is vitamin B12, which is present in many foods from animals. It contributes to the synthesis of DNA, hematopoiesis, and neurological processes. In addition to insufficient uptake and malabsorption, its deficit can be seen with metformin therapy, obesity, insulin resistance, and type 2 diabetes (Ho *et al*. 2014). A major global health issue is obesity. Comorbidities include insulin resistance, type 2 diabetes, hypertension, dyslipidemia, non-alcoholic fatty liver disease, and cardiovascular disease are frequently present with it. Some researchers have revealed that obese individuals have reduced vitamin B12 levels (MacFarlane *et al.*2011)

In this work, we will try to find the possible relations between serum vitamin B12 levels and each one of the parameters relevant to control hyperglycemia such as fasting blood glucose and glycosylated hemoglobin. Besides, to shed a light on the possible relations between serum vitamin B12 levels and lipid profile as well as an atherogenic index to provide information about its role in diabetic dyslipidemia

1.1 Objectives of The Study

To evaluate the realtionship between vitamin B12 defeciencey and Diabetes mellitus.

1.1.1 Specific objectives

- Examine the Serum vitamin B12 levels analyzed in connection to fasting blood glucose, glycosylated hemoglobin, and other indicators important in the management of hyperglycemia.

- Furthermore, to elucidate its potential significance in diabetic dyslipidemia by illuminating the connections between serum Vitamin B12 levels and lipid profile and an atherogenic index.

2. LITERATURE REVIEW

2.1 Diabetes

A collection of metabolic illnesses with hyperglycemia in the absence of treatment are referred to as diabetes. The diverse aetiopathology encompasses problems in the metabolism of carbohydrates, fats, and proteins as well as errors in insulin secretion, insulin action, or both. Diabetes increases the likelihood of developing a number of illnesses, such as heart disease, peripheral artery disease, and cerebrovascular disease, as well as obesity, cataracts, erectile dysfunction, and nonalcoholic fatty liver disease. They also have a higher chance of contracting some infectious diseases, such tuberculosis (WHO 2019).

2.2 Classification of Diabetes

Over the past century, understanding diabetes has seen enormous changes. Diabetes had no organized classification prior to the 1960s. The first World Health Organization report on the classification of diabetes was released in 1965 by an Expert Committee on Diabetes Mellitus (WHO. 1965). One of the first attempts at an international consensus on the classification of diabetes was represented by this report.

2.2.1 Type 1 diabetes mellitus

Type 1 diabetes mellitus (T1DM), commonly referred to as autoimmune diabetes, is a chronic condition that causes hyperglycemia due to an inability to produce enough insulin as a result of pancreatic cell loss (Katsarou *et al*. 2017).

2.2.2 Type 2 diabetes mellitus

The most typical kind has varying degrees of beta-cell malfunction and insulin resistance; it is frequently linked to being overweight or obese. Insulin resistance (IR)

and an inadequate level of insulin secretion are the outcomes of the interplay of hereditary and environmental variables that leads to type 2 diabetes mellitus (DM2). Candidates for the condition include genes that encode the insulin-related enzymes or protein factors insulin receptor substrate-1 (IRS-1), phosphoinositide 3-kinase (PI-3 K), calpain 10, and transcription factor 7-like 2. Android aging, obesity, glucotoxicity, and lipotoxicity are environmental variables that support T2DM (Durruty and Sanhueza 2019). About 90% of all instances of diabetes are Type 2 diabetes mellitus (T2DM). Insulin resistance is the term used to describe the reduced insulin response in T2DM. Since insulin is useless in this condition, the body produces more insulin to maintain glucose homeostasis at first, but over time, this diminishes, leading to T2DM. T2DM is most frequently diagnosed in those over the age of 45. Nevertheless, it is becoming more common in kids, teenagers, and young adults as a result of increased obesity rates, inactivity rates, and calorie-dense diets (Goyal and Jialal 2022).

2.2.3 Gestational diabetes mellitus

During pregnancy, glucose intolerance either starts or is first seen, which is what is referred to as gestational diabetes mellitus (GDM). Over 200,000 pregnancies are affected by GDM each year, or 7% of all pregnancies. The prevalence could be between 1 and 14% depending on the population sample and diagnostic standards (Setji *et al*. 2016).

2.2.4 Latent autoimmune diabetes of adults

LADA refers to a diagnosis of type 2 diabetes made after the age of 35 in a patient who also has an islet autoantibody consistent with type 1 diabetes. It's critical to recognize autoimmune diabetes because the prognosis and recommended treatment vary. The current definition of LADA, however, recognizes a population with clinical and genetic characteristics that lie somewhere between those of conventional type 1 and type 2 diabetes (Jones *et al*. 2021).

2.2.5 Maturity-onset diabetes of the young

The most prevalent type of monogenic diabetes caused by a single gene mutation is MODY. Mild hyperglycemia, autosomal dominant inheritance, early development of diabetes (before age 25), insulin resistance, and maintenance of endogenous insulin secretion are its defining characteristics. There are currently 14 MODY subtypes known, each of which differs in incidence, clinical characteristics, the severity of the diabetes and its consequences, and how well it responds to treatment. Because of the clinical similarities, the high expense of genetic testing, and the lack of awareness, it is challenging to distinguish between this type of diabetes and type 1 or type 2 diabetes mellitus. Thousands of patients aren't getting the right care as a result. With an accurate diagnosis, therapeutic management and treatment approaches that are different from those utilized for type 1 and type 2 diabetes can be used more effectively (Tshivhase *et al.* 2021).

2.3 Diabetic Dyslipidemia

Both type 1 and type 2 diabetic people are more likely to develop cardiovascular disease. Disturbances in plasma lipoprotein synthesis and clearance are among the metabolic disorders that frequently accompany diabetes. Additionally, the emergence of dyslipidemia can be a sign that diabetes is on the horizon. Low high density lipoprotein (HDL), elevated triglycerides, and postprandial lipemia make up the defining pattern of diabetic dyslipidemia. The type 2 diabetic population is the one most likely to exhibit this pattern, which may be a manageable risk factor for developing cardiovascular disease later on (Dixit *et al*. 2014).

2.4 The Decades-long Evolution of Diagnostic Criteria

Over the past few decades, recommendations for diagnosing diabetes have also changed. In 1979, the US Diabetes Data Group examined epidemiological studies and found a correlation between blood glucose levels and complications like nephropathy

and retinopathy, which led them to set diagnostic blood glucose targets (National Dıabetes Data Group 1979). 140 mg/dL was set as the diagnostic blood sugar level. In 1980, the WHO also endorsed this (WHO 1980). The ADA expert group changed the diagnosis criteria in 1997, lowering the threshold for fasting plasma glucose to 126 mg/dL (The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus 1997). Although there is no definitive advice regarding the best diagnostic test for diabetes, FPG is still widely regarded as the best test. New diagnostic standards for diabetes were established by the ADA, referring to FBG levels between 110 and 125 mg/dL (Rivellese *et al*. 2007). In the latest update of a group of scientific institutions, including the ADA, in a unified meeting to set standards for metabolic syndrome, they unanimously agreed that the value of FBG does not exceed 100 mg /dL, otherwise it will become among the standards of the syndrome (Alhameed 2022).

2.5 Warning signs of Diabetes

Increased glucose from the blood accumulates in the kidney tubules, attracting large amounts of water by osmosis. Urine output increases from around 1.8L seen in healthy people to typically over 3L, in those with diabetes. Patients produce urine containing large amounts of glucose. Normally, glucose is reabsorbed from the kidney tubules back into the blood; in diabetes, the amount overwhelms reabsorption mechanisms. Producing large amounts of urine rapidly leads to dehydration, triggering thirst that is difficult to satisfy (Knight and Nigam 2017).

Although there may be large amounts of sugar in the blood, it does not reach the cells to be used as an energy source. Effectively, patients are in a state of starvation, which triggers the release of hunger hormones such as cortisol and ghrelin. Patient's will experience an increase in their appetite, but many will crave and eat sugary and starchy foods, exacerbating their condition (Fargion *et al*. 2005).

With no sugar to fuel metabolism, the body uses other molecules such as proteins and fats. Breaking down fat (lipolysis) leads to the generation of ketones such as acetone, which can be used in metabolism. Ketones are mildly acidic, but reduce blood pH if

they accumulate in large amounts, resulting in ketoacidosis. Unless treated quickly, this medical emergency can lead to coma and death; it can present as increased breathing rate (Kussmaul breathing) and a fruity smell on the breath, and in the sweat and urine. It may also manifest as general abdominal pain, decreased appetite and nausea and vomiting (Gupta 2021).

The metabolism of fat and protein in diabetes can lead to significant and unintentional weight loss, particularly in type 1 diabetes. Glucose is the primary energy source for muscles; the lack of glucose uptake leads to fatigue, which is often compounded by the sleep interruptions. Blurred vision and 'black spots' in the field of view may indicate damage to the lens and retina (Knight and Nigam 2017).

2.6 Epidemiology

Due to its high prevalence and rising numbers, diabetes is becoming a disease of concern on a global scale. By 2035, there will be 592 million people with diabetes, up from 382 million in 2013 (Guariguata *et al*. 2014). An increase of patients of this magnitude entails a significant disease burden for the individual, the population, and the entire health care system.

CVD is the main factor in the morbidity and death of type 2 diabetes patients. The American Heart Association estimates that at least 68% of diabetics aged 65 or older pass away from heart disease, while 16% pass away from stroke. Additionally, persons with diabetes have a two to four times higher risk of developing CVD than adults without the disease. As a result, it is compared to coronary artery disease (Association 2022).

Age, gender, genetics, and a number of modifiable (hypertension, hyperlipidemia, and hyperglycemia) factors interact intricately to determine the overall cardiometabolic risk. An essential role is played by an entity known as atherogenic dyslipidemia, which

consists of qualitative and quantitative alterations in the lipoproteins LDL, HDL, and other Triglyceride Rich Lipoproteins (Eliasson *et al*. 2011).

2.7 Pathophysiology

Patients with diabetes types 1 and 2 experience dyslipidemia due to a variety of different reasons. Fasting plasma lipid and HDL levels in people with type 1 diabetes are frequently normal or even better than those of age- and gender-matched healthy individuals (Miettinen *et al*. 2004). This might have something to do with type 1 diabetic individuals' lower levels of hepatic cholesterol production (Forrest *et al*. 2000).

The dyslipidemia of the metabolic syndrome with hypertriglyceridemia and decreases in HDL cholesterol is the typical pattern in type 2 diabetes. Increases in the quantity of LDL particles, LDL, and apolipoprotein are additional lipoprotein modifications. Adipocytokines and hyperglycemia, along with insulin resistance/deficiency in type 2 diabetics, cause alterations in the normal lipid metabolism that are qualitative, quantitative, and kinetic in nature including: (1) High VLDL. (2) High LDL. (3) Low HDL (Chaudhury and Aggarwal 2018).

2.8 Vitamin B12

Cobalamin, often known as vitamin B12, is a water-soluble vitamin that is essential for neurological health, healthy hemopoiesis, and DNA synthesis. Thus, haematological and neurocognitive impairment are mostly seen in the clinical picture of vitamin B12 insufficiency. For proper cardiovascular, neurological, and hemopoetic function, vitamin B12 is a crucial micronutrient. Patients with type 1 and type 2 diabetes mellitus have been shown to have a significant prevalence of biochemical and clinical vitamin B12 insufficiency. It manifests clinically in a variety of ways, including memory loss, dementia, delirium, peripheral neuropathy, subacute combined spinal cord degeneration, megaloblastic anemia, and pancytopenia (Kibirige and Mwebaze 2013).

2.8.1 Physiological roles of vitamin B12

The two main biochemical mechanisms by which vitamin B12 exerts its physiological effects are the methylation of homocysteine to methionine and the conversion of methylmalonyl coenzyme A (CoA) to succinyl-CoA. Vitamin B12 makes it easier for homocysteine to be converted to methionine, which is then activated into S-adenosyl-methionine and gives its methyl group to methyl acceptors such myelin, neurotransmitters, and membrane phospholipids. Because of this, a metabolically significant vitamin B12 deficit will cause the methylation process to be disrupted and will cause an accumulation of intracellular and serum homocysteine (Malouf 2003).

Neurones and the vascular endothelium have been found to be potentially harmed by hyperhomocysteinemia. The transformation of dietary folate (methyl-tetrahydrofolate) into its active metabolic form, tetrahydrofolate, depends on this process as well. The co-factor vitamin B12 promotes the transformation of methylmalonyl coenzyme A (CoA) to succinyl-CoA in another crucial enzymatic pathway. This conversion route is impaired in vitamin B12 deficiency, which leads to an increase in serum methylmalonic acid (MMA). The neuronal membranes' faulty fatty acid production occurs next. The manufacture of monoamines, or neurotransmitters like serotonin and dopamine, requires vitamin B12. Vitamin B12 deficiency impairs this production (Malouf 2003).

3. MATERIALS AND METHODS

3.1 Study of Population

The subjects of this study were taken be from Thi-Qar province/ Iraq where the study were conducted. The study included 120 patients with Type – 2 diabetes that divided into three groups according to the levels of HbA1c as well as 40 healthy volunteers to obtain the normal values of the studied parameters.

Stedied groups

I. Group A: 40 patients with type 2 diabetes with poor control of glycemia.

II. Group B: 40 patients with type 2 diabetes with fair control of glycemia.

III. Group C: 40 patients with type 2 diabetes with good control of glycemia.

IV. Group C: 40 healthy subjects without DM (control group).

The instruments listed below will be using in accordance with the modus operandi specified by the manufacturing company and the mentioned in front of it the origin and the code: -

A. HbA1c Meter

B. Water bath

C. Visible Spectrometer

Tests:

The tests listed below will be carried out in accordance with the modus operandi (kit user manual) specified by the manufacturing company.

A. Serum FBG

B. Serum HbA1C

C. Serum Vit. B12

D. Serum TG

E. Serum Chol.

F. Serum HDL

G. Serum LDL

3.2 Samples Collection

After transferring the blood into a gel tube, the serum was extracted by centrifugation at room temperature. The results were obtained after being centrifuged at 2500 g for ten minutes. When it came time to analyze the serum, it was separated into three Eppendorf tubes and placed in the deep freezer (-20 C).

3.3 Data Collection

The kilos per square meter of body weight were used to determine BMI. Before getting their weight and height measured, patients were told to take off any bulky clothing or

shoes. The weight was measured using a medical balance. BMI of 18.5-24.9 kg/m, 25.0-29.9 kg/m, and 30.0 kg/m^2 were considered normal weight, overweight, and obesity, respectively.

3.4 Blood Sampling and Processing

TOS, glucose, and lipid profile in serum can be determined using spectrophotometric methods and ELISA kits, whereas asporsin is tested using ELISA kits. As a control group, the study includes 60 healthy individuals between the ages of 18 and 60 who are not overweight or obese.

3.5 General Information

Each person in this study had to be interviewed in person to get information about their age, body weight, matrial status

3.5.1 Inclusion criteria

Any budy buliding and normal persons between the ages of 20 and 45 who has been body buliding and agrees to take part in this study is considered a case.

3.5.2 Exclusion criteria

Persons with any of the following conditions were not included in this study.

- Thyroid disesase.
- Immune system disorders, such as lupus.
- Goodpasture's syndrome.

- Gout.
- Rhabdomyolysis.
- Muscle atrophy.
- Blood loss due to shock

3.6 Experimental Procedure

3.6.1 Human vitamin B12 (VB12) ELISA kit (competitive ELISA)

This VB12 ELISA kit is a 1.5 hour solid-phase ELISA designed for the quantitative determination of Human VB12. This ELISA kit for research use only, not for therapeutic or diagnostic applications, Catalog Number: MBS729208, Store all reagents at 2-8°C

Test prinicible:

The fundamental concept behind the examination For the purpose of carrying out a competitive enzyme immunoassay, the VB12 ELISA kit makes use of a polyclonal anti-VB12 antibody as well as a VB12-HRP conjugate. In a plate that has been coated beforehand, the assay sample and buffer are incubated with the VB12-HRP conjugate for one hour. After the allotted time for the incubation period has passed, the wells are drained and washed no less than five times. After that, the HRP enzyme substrate is incubated within the wells themselves. The interaction between the enzyme and the substrate results in the formation of a blue complex. The addition of a stop solution causes the color of the solution to change to yellow, which indicates that the reaction has been completed. The microplate reader is used to carry out the spectrophotometric measurement of the color intensity. The measurement is carried out at 450 nm. Because VB12 from samples and VB12-HRP conjugate fight for the anti-VB12 antibody binding

site, there is an inverse relationship between the concentration of VB12 and the intensity of the color. Because there are only a certain number of binding sites, the quantity of VB12 present in a sample has a direct bearing on how successfully the VB12-HRP conjugate will bind to it. On a standard curve, the optical density (O.D.) and the concentration are plotted against one another. Extrapolating from the standard curve allows for the determination of the VB12 concentration in each sample.

3.6.2 Human hemoglobin A1C%

It is possible to detect HbA1c in whole blood using the ZV-4001-0500-25 kit in conjunction with a manual HPLC approach. HbA1c is one of the most important markers that may be used for screening for and keeping an eye on diabetes. It is possible to determine a person's HbA1c level by combining their blood sugar with the hemoglobin protein they have in their blood. When there is an increase in blood sugar, there is also an increase in the generation of HbA1c. Levels of HbA1c that are very high are a strong indicator that a person has high blood sugar. There are four unique types of HbA1c that may be identified based on the kind of sugar that is bound to hemoglobin. These forms are referred to as A1a, A1b, s-A1c, and L-A1c respectively. The HbA1c test requires rigorous distinction of blood types in order to produce accurate results. Patients who have diabetes can have the disease diagnosed and its progression monitored with the use of the Zivak Hemoglobin A1c HPLC Analysis Kit. The analysis should take about one and a half minutes to complete. The duration of the experiment was increased by 0.5 minutes so that there would be sufficient time for column washing. EN ISO 13485 and Directive 98/79/EC serve as the foundation for the majority of the methodologies and procedures.

3.6.3 Determination of blood glucose

The guiding principles of the method Glucose oxidase (GOD) is a necessary enzyme for the production of gluconic acid from glucose. Hydrogen peroxide (H2O2) is detected in the presence of peroxidase using a chromogenic oxygen acceptor, specifically phenol, 4- aminophenazone (4-AP) (POD).

3.6.4 Determination of lipid profile

Determınation of cholesterol:

The number assigned to this item for purposes of identifying it is MAK043. The REACH Cholesterol Quantitation Kit was created by the company Sigma, which is the manufacturer. Intracellular trafficking, cell signaling, and cellular flexibility are all improved by the presence of cholesterol in the membranes of mammalian cells. Membrane lipids contain cholesterol, which is a fatty substance. There is a potential for the existence of free acid as well as cholesterol esters in the blood. One of the primary therapeutic goals in the treatment of atherosclerosis and in the prevention of cardiovascular disease is to bring down patients' cholesterol levels. Poor cholesterol homeostasis is connected to chronic inflammation, according to mounting data.

A Cholesterol Quantification Kit may be acquired from Princible, and with this kit, one can determine the levels of free cholesterol and cholesteryl esters that are present in their blood (total). An enzyme test that makes use of a colorimetric or fluorometric product reveals that the amount of cholesterol in a sample is directly proportional to the amount of enzyme that is present in that sample.

Determination of TG:

Method of Measurement Relying on Enzymes and Colorimetry for Triglycerides REF 1155005 Terminal Result Prınciple: Triglycerides are broken down by the enzyme lipoprotein lipase in the blood, which then results in the production of glycerol and free fatty acids (FFA) (LPL). ADP can be generated in the presence of glycerolkinase (GK) due to the fact that ATP is required for the production of glycerol-3-phosphate (G-3-P) when G-3-P is present (ADP). Glycerophosphate oxidase is responsible for the production of the byproducts hydrogen peroxide and dihydroxyacetone phosphate (DHAP) (GPO). The color of the chromogen changes when there is a greater amount of

triglycerides in the sample. This is due to the fact that 4-Aminoantipyrine (4-AA), phenol, and hydrogen peroxide can all be transformed into 4-AA by peroxidase (4-AA).

Determination of HDL:

Lipoproteins that contain apolipoprotein B can be isolated by precipitation with phosphotungstic acid/MgCl2, centrifugation, and enzymatic analysis of the clear supernatant as high-density lipoproteins (HDLs). The endpoint for this test is reference number 1133010 HDL-Cholesterol Enzymatic Colorimetric Test (HDL).

3.7 Statistical Analysis

We used a statistical program called SPSS-22 to do the analysis on the data (Statistical Packages for Social Sciences- version 22). The data were presented with only the most fundamental aspects of statistical analysis, such as frequency, percentage, mean, standard deviation, and range (minimum-maximum values). The Student's t-test, the Paired's t-test, and the ANOVA test were used to compare more than two independent means in order to discover whether or not there was a difference that might be considered statistically significant between them (quantitative data). To evaluate whether or not a given percentage difference was statistically significant, the Pearson's Chi-square test (two-test) with Yate's correction and, when appropriate, Fisher's Exact test were utilized (qualitative data). The significance of the Pearson correlation between two quantitative variables was analyzed with a t-test in order to get a handle on the relevant statistical information. The correlation coefficient (r) can be used to determine whether a connection is positive (direct) or negative (inverse), with r values of 0.3 indicating that there is no correlation, 0.3–0.5 indicating that there is a weak correlation, 0.5–0.7 indicating that there is a moderate correlation, and>0.7 indicating that there is a significant correlation (inverse correlation). Not only was the correlation calculated, but also the r2 (coefficient of determination), which demonstrates that knowing the values of either the y or the x variables may explain 34% of the variance in the y or x variables. A P value of less than 0.05 was required for the conclusion to be considered statistically significant.

4. RESULTS AND DISCUSSION

4.1 Characteristic Features for the Studied Parameters

This study included 160 participants, divided into four groups: The first group included healthy subjects as a control group. While the other three groups who suffer from diabetes with dyslipidemia, 40 participants of whom are patients with good diabetes control, 40 with fair control and 40 with poor control.

4.2 Genral Comparison

4.2.1 Comparsion of FBS in stedied groups

According to the results of this study presented in Table 4.1 and Figure 4.1, the concentration of FBS % was at its highest in the poorly controlled diabetic group compared to the other groups. The fair diabetic control group recorded similar results to the first group with a slight difference. While the good control group, their results were similar to the control group.

Table 4.1 Compartion of FBS in patients and control groups

Variables	Groups	No.	Mean	Std. Deviation	Std. Error
FBS mmol/L	Healthy Control	40	4.6640	0.45706	0.07227
	Poor control of glycaemia	40	10.0737	3.13494	0.49568
	Fair control of glycaemia	40	9.1888	2.69552	0.42620
	Good control of glycaemia	40	4.8665	0.39620	0.06264
	TOTAL	160	7.1983	3.21604	0.25425

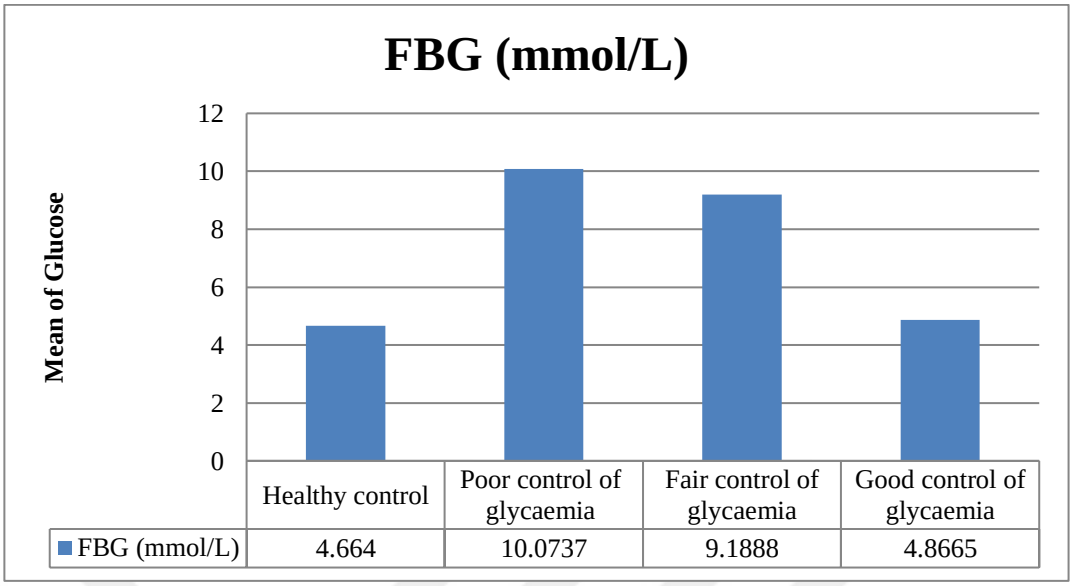

Figure 4.1 The level of serum FBS in control and patients groups

4.2.2 Comparison of HBA1C% in stedied groups

The HbA1C test, the average concentration of the poorly controlled group was higher than the other groups, and the fair group ranked second in terms of results, while the good group recorded results within the normal level as shown in Table 4.2 and Figure 4.2

Table 4.2 Compartion of HBA1C % in patients and control groups

Variables	Groups	No.	Mean	Std. Deviation	Std. Error
HBAIC%	Healthy Control	40	5.0650	0.48174	0.07617
	Poor control of glycaemia	40	8.0575	1.44007	0.22769
	Fair control of glycaemia	40	6.8325	0.79530	0.12575
	Good control of glycaemia	40	5.4600	0.78473	0.12408
	TOTAL	160	6.3538	1.50946	0.11933

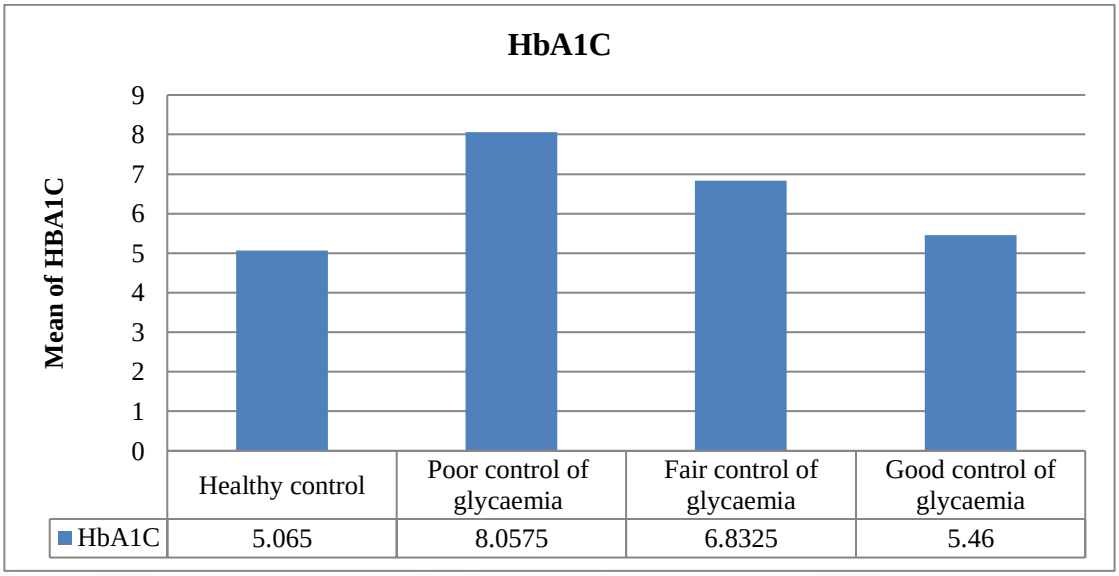

Figure 4.2 The level ofblood HBA1C in control and patients groups

4.2.3 Comparison of lipid profile in stedied groups

It was noted in the same table that the levels of triglycerides (TG) in the poor and fair group are close with a slight difference, higher in the poor group. While the good control group, their results were similar to the control group. The results showed that the cholesterol (CHOL) concentration in the poor control group was higher than the other groups. However, the fair control group was higher than the good and control group. While the good control group, cholesterol concentrations were slightly lower than the control group. When the concentrations of high-density lipoproteins (HDL) were observed, the lowest levels were recorded in the poor-control group, less than the other groups. He was at the highest level in the good control group as shown in Table 4.3, Figure 4.3, Figure 4.4 and Figure 4.5.

Table 4.3 Compartion of lipid profile in patients and control groups

Varible	Level of Cholesterol mmol/L					
	Healthy Control	Poor control of glycaemia	Fair control of glycaemia	Good control of glycaemia	Total	Chi-sequre
Mean	4.5775	5.4700	5.1225	4.4150	4.89	
Std. Deviation	0.42936	0.73177	0.80654	0.53837	0.76598	
Std. Error	0.06789	0.11570	0.12753	0.08512	0.06056	
	Level of Triglyceride mmol/L					
	Healthy Control	Poor control of glycaemia	Fair control of glycaemia	Good control of glycaemia	Total	Chi-sequre
Mean	1.3130	1.8737	1.8295	1.3006	1.5792	
Std. Deviation	0.53264	0.65245	0.66572	0.45779	0.63957	
Std. Error	0.08422	0.10316	0.10526	0.07238	0.05056	
	Level of HDL mmol/L					
	Healthy Control	Poor control of glycaemia	Fair control of glycaemia	Good control of glycaemia	Total	Chi-sequre
Mean	1.3332	1.0382	1.0730	1.6268	1.2678	
Std. Deviation	0.16950	0.29522	0.31146	0.20579	0.34483	
Std. Error	0.02680	0.04668	0.04925	0.03254	0.02726	

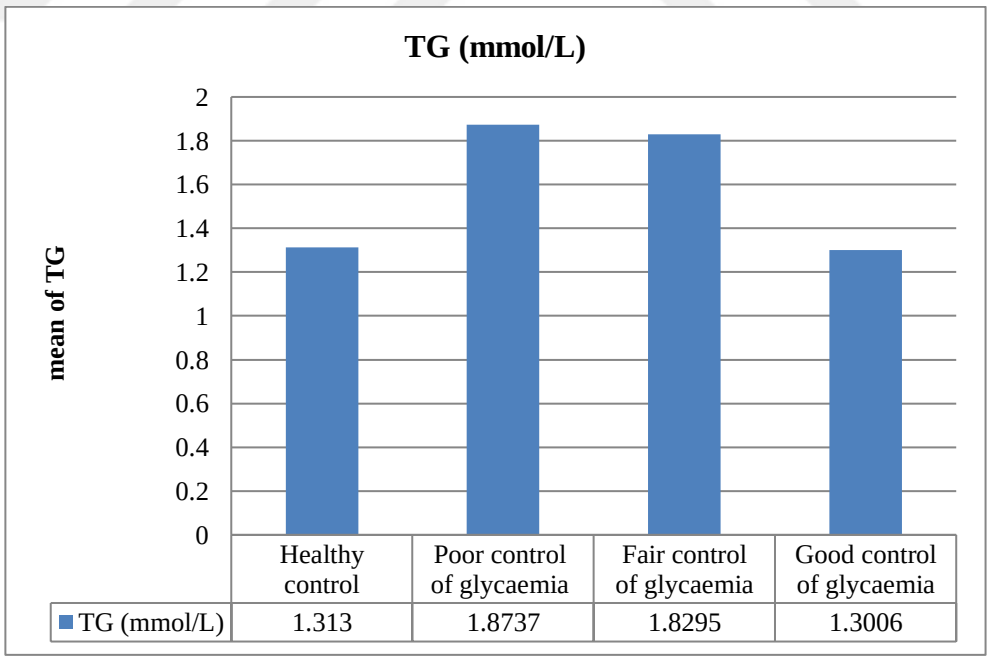

Figure 4.3 The level of serum TG in control and patients groups

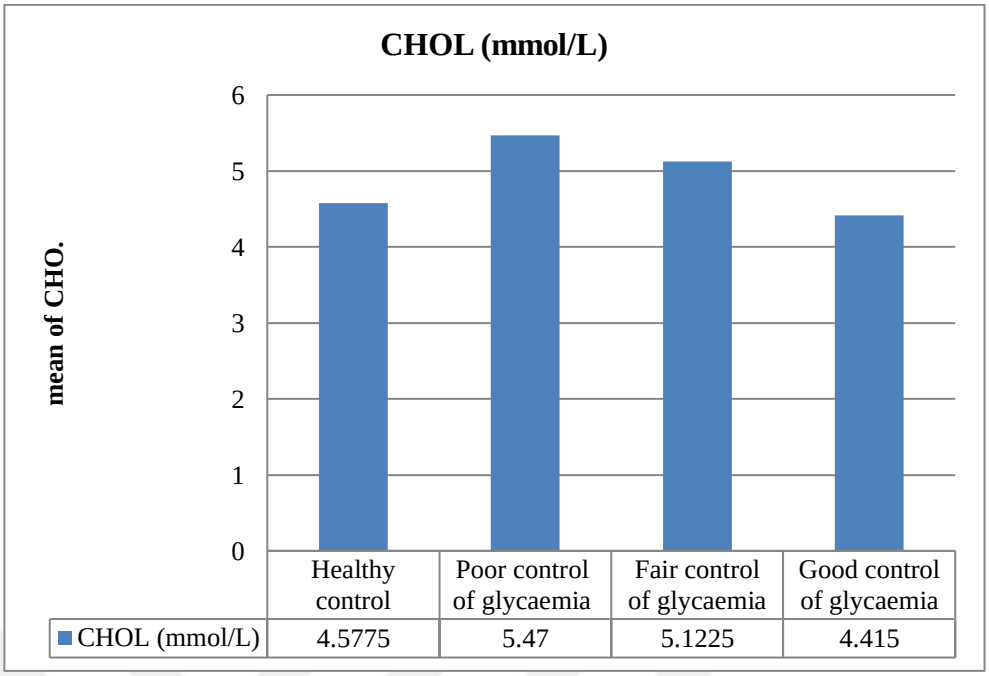

Figure 4.4 The level of serum cholesterol in control and patients groups

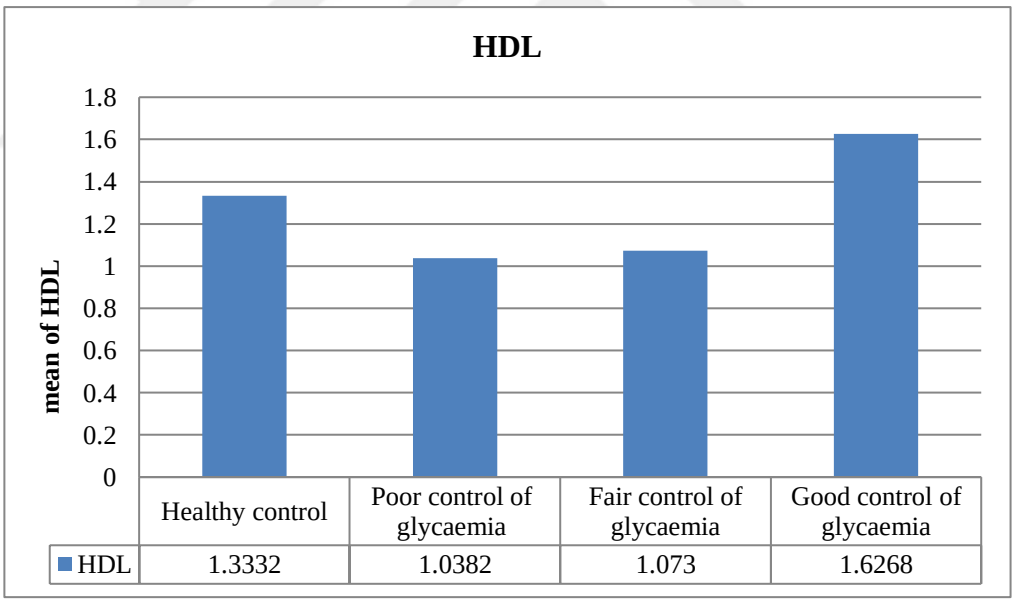

Figure 4.5 The level of serum HDL in control and patients groups

4.2.4 Comparison of Vitamin B12 in stedied groups

The findings of the current study achieved that the levels of vitamin B12 were lower in the poor-control group compared to the other groups, while the good-control group had

high levels, although the control group recorded higher results as shown in Table 4.4 and Figure 4.6.

Table 4.4 Compartion of Vitamin B12 in patients and control groups

Varible	Level of Vitamin B12					
	Healthy Control	Poor control of glycaemia	Fair control of glycaemia	Good control of glycaemia	Total	Chi-sequre
Mean	674.6005	175.0750	203.1500	584.4000	409.3064	
Std. Deviation	317.32020	28.72182	64.89461	211.35288	294.62351	
Std. Error	50.17273	4.54132	10.26074	33.41782	23.29203	

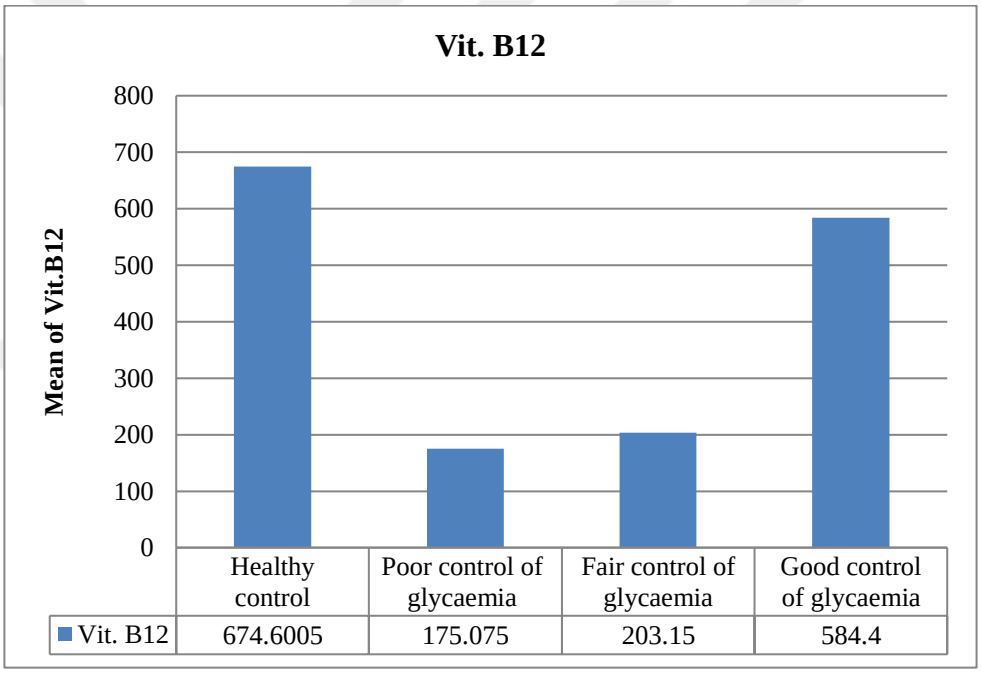

Figure 4.6 The level of serum B12 in control and patients groups

4.3 The Significance of the Studied Groups

4.3.1 Significance of glucose panale

It was noticed in Table 4.5 that there was a high significance (P-value < 0.001) among the four studied groups in FBS and HBA1C% parameters. Therefore our stedied shown a highly significant when we compared between groups in glucose panle.

Table 4.5 The significance between Glucose panle the studied groups

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
FBG (mmol/L)	Between Groups	963.603	3	321.201	73.587	0.001
	Within Groups	680.923	156	4.365	-	
	Total	1644.526	159	-	-	
HBAIC %	Between Groups	223.665	3	74.555	83.907	0.001
	Within Groups	138.612	156	0.889	-	
	Total	362.278	159	-	-	

4.3.2 Significance of lipid profile

It was noticed in Table 4.6 that there was a high significance (P-value < 0.001) among the four studied groups in lipid profile parameters.Therefore our stedied shown a highly significant when we compared between groups.

Table 4.6 The significance between the lipid profile in studied groups

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
CHOL (mmol/L)	Between Groups	28.543	3			
	Within Groups	64.747	156	9.514	22.924	0.001
	Total	93.291	159	0.415		
TG (mmol/L)	Between Groups	11.916	3	3.972	11.664	0.001
	Within Groups	53.123	156	0.341		
	Total	65.039	159			
HDL	Between Groups	8.952	3	2645380.510	70.357	0.001
	Within Groups	9.954	156			
	Total	18.907	159			

4.3.3 Significance of vitamin B12

It was noticed in Table 4.7 that there was a high significance (P-value < 0.001) among the four studied groups in B12 parameters. Therefore our stedied shown a highly significant when we compared between groups.

Table 4.7 The significance between the lipid profile in studied groups

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
Vit. B12.	Between Groups	7936141.531	3	2645380.510	70.357	0.001
	Within Groups	5865537.677	156	37599.600		
	Total	13801679.20	159			

4.4 Multiple Comparisons

It was noted in Table 4.8 that there was a significant difference between the control group and the poor and fair group (P value <0.01), but it did not record a significant difference between the good group and the control group (P value > 0.05) in FBG concentrations. The results of the current study did not record a significant difference between the fair group and the poor group when monitoring glucose levels (P value > 0.05). While there was a clear significance between the poor group and the good group (P value <0.01). Moreover, the results recorded a significant difference between the good group and the fair group (P value <0.01).

Table 4.8 Multiple comparison between the studied groups in FBG concentratio

Dependent Variable	(I) GROUP		Mean Difference (I-J)	Std. Error	Sig.
FBG (mmol/L)	CONTROL	Poor control of glycaemia	-5.40975-*	0.4671	0.001
		Fair control of glycaemia	-4.52475-*	0.4671	0.001
		Good control of glycaemia	-0.20250-	0.4671	0.665
	Poor control of glycaemia	Control	5.40975*	0.4671	0.001
		Fair control of glycaemia	0.88500	0.4671	0.060
		Good control of glycaemia	5.20725*	0.4671	0.001
	Fair control of glycaemia	Poor control of glycaemia	4.52475*	0.4671	0.001
		Good control of glycaemia	-0.88500-	0.4671	0.060
		Control	4.32225*	0.4677	0.001
	Good control of glycaemia	Control	0.20250	0.4671	0.665
		Poor control of glycaemia	-5.20725-*	0.4671	0.001
		Fair control of glycaemia	-4.32225-*	0.4671	0.001

As shown in Table 4.9, this study indicated that there was a significant difference in HbA1C levels between the control group and the other groups (P value < 0.01), except for the good group, where it did not give a significant difference (P value > 0.05). When comparing the poor group with the fair and good groups, a high significant difference (P value < 0.01) was observed.

Table 4.9 Multiple comparison between the studied groups in HbA1C level

Dependent Variable	(I) GROUP		Mean Difference (I-J)	Std. Error	Sig.
HbA1C	CONTROL	Poor control of glycaemia	-2.99250-*	0.2107	0.001
		Fair control of glycaemia	-1.76750-*	0.2107	0.063
		Good control of glycaemia	-0.39500-	0.2107	0.001
	Poor control of glycaemia	Control	2.99250*	0.2107	0.001
		Fair control of glycaemia	1.22500*	0.2107	0.001
		Good control of glycaemia	2.59750*	0.2107	0.001
	Fair control of glycaemia	Poor control of glycaemia	1.76750*	0.2107	0.001
		Good control of glycaemia	-1.22500-*	0.2107	0.001
		Control	1.37250*	0.2107	0.063

As in the other parameters, the data in Table 4.10 showed that there was no significant difference between the control group and the good control group in the concentration of triglycerides (P value > 0.05), but there was a clear indication between the control group and the fair and poor control groups (P value < 0.01). However, the data did not record a significant difference between the poor and fair control group (P value > 0.05). It created a difference between the poor and good control group and a difference between the good and fair group (P value < 0.01).

Table 4.10 Multiple comparison between the studied groups in TG concentration

Dependent Variable	(I) GROUP		Mean Difference (I-J)	Std. Error	Sig.
TG	CONTROL	Poor control of glycaemia	-0.56075-*	0.1304	0.001
		Fair control of glycaemia	-0.51650-*	0.1304	0.001
		Good control of glycaemia	0.01240	0.1304	0.924
	Poor control of glycaemia	Control	0.56075*	0.1304	0.001
		Fair control of glycaemia	0.04425	0.1304	0.735
		Good control of glycaemia	0.57315*	0.1304	0.001
	Fair control of glycaemia	Poor control of glycaemia	0.51650	0.1304	0.001
		Good control of glycaemia	-0.04425-	0.1304	0.735
		Control	0.52890*	0.1304	0.001
	Good control of glycaemia	Control	-0.01240-	0.1304	0.924
		Poor control of glycaemia	-0.57315-*	0.1304	0.001
		Fair control of glycaemia	-0.52890-*	0.1304	0.001

Table 4.11 shows that cholesterol concentration did not register a significant difference between the control group and the good-controlled group (P value > 0.05). While the fair control group and the poor control group recorded a significant difference when compared with the control group (P value < 0.05). When searching for the least significant difference, it was found that all disease groups recorded significant differences between them (P value < 0.05).

Table 4.11 Multiple comparison between the studied groups in CHOL concentration

Dependent Variable	(I) GROUP		Mean Difference (I-J)	Std. Error	Sig.
CHOL	CONTROL	Poor control of glycaemia	-.89250-*	.14406	0.001
		Fair control of glycaemia	-.54500-*	.14406	0.001
		Good control of glycaemia	.16250	.14406	0.001
	Poor control of glycaemia	Control	.89250*	.14406	.261
		Fair control of glycaemia	.34750*	.14406	0.001
		Good control of glycaemia	1.05500*	.14406	0.001
	Fair control of glycaemia	Poor control of glycaemia	.54500*	.14406	0.001
		Good control of glycaemia	-.34750-*	.14406	.017
		Control	.70750*	.14406	0.001
	Good control of glycaemia	Control	-.16250-	.14406	.261
		Poor control of glycaemia	-1.05500-*	.14406	0.001
		Fair control of glycaemia	-.70750-*	.14406	0.001

It was noted in Table 4.12 that there is a clear significance between all the studied groups when compared with the control group when measuring HDL (P value < 0.05), and this sign is present when comparing the poor-control group against the good-control group, as well as when comparing the fair-control group against the good control, but it did not notice a significant difference between Poor control group and fair group control (P value > 0.05).

Table 4.12 Multiple comparison between the studied groups in HDL concentration

Dependent Variable	(I) GROUP		Mean Difference (I-J)	Std. Error	Sig.
HDL	CONTROL	Poor control of glycaemia	.29500*	.05648	0.001
		Fair control of glycaemia	.26025*	.05648	0.001
		Good control of glycaemia	-.29355-*	.05648	0.001
	Poor control of glycaemia	Control	-.29500-*	.05648	0.001
		Fair control of glycaemia	-.03475-	.05648	.539
		Good control of glycaemia	-.58855-*	.05648	0.001
	Fair control of glycaemia	Poor control of glycaemia	-.26025-*	.05648	0.001
		Good control of glycaemia	.03475	.05648	.539
		Control	-.55380-*	.05648	0.001
	Good control of glycaemia	Control	.29355*	.05648	0.001
		Poor control of glycaemia	.58855*	.05648	0.001
		Fair control of glycaemia	.55380*	.05648	0.001

4.5 Pearsonal Correlation

The results in Table 4.13 show that there is a high positive correlation between FBG and HbA1C (r = 0.520), TG (r =0.333), CHOL (r =0.387), while the correlation was also high but negative between FBG and HDL (r = -0.404), Vit. B12 (r = -0.579) with a significant P value < 0.01. The results of the study achieved a clear positive correlation between HbA1C and TG (r= 0.503), CHOL (r = 0.459), while there was a high negative correlation between HbA1C and HDL (r = -0.617), Vit. B12 (r = -0.595) with a significant difference of P value < 0.01. In the same table, when comparing the correlation of triglycerides with other parameters, a clear positive correlation was found between TG and CHOL (r = 0.540), while a negative correlation was observed between TG and HDL (r = -0.612), Vit. B12 (r = -0.391), the significant difference was clear among all studied parameters compared with triglycerides, P value < 0.01. There was a positive correlation between HDL and Vit. B12 (r = 0.471) with clear significance (P value < 0.01).

Table 4.13 Correlation between all studied parameter

		B12	Cholesterol_ mmol_l_	Glucose_ mmol_l	HbA1 c_%	HD L	TG_mm ol_l_
B12	Correlation coefficient Significance Level P n		0.016 0.8477 160	0.392 <0.0001 160	-0.513 <0.00 01 160	- 0.23 6 0.00 51 160	0.100 0.2406 160
Cholesterol _mmol_l_	Correlation coefficient Significance Level P n	0.016 0.847 7 160		0.399 <0.0001 160	0.142 0.094 4 160	- 0.56 9 <0.0 001 160	0.517 <0.0001 160
Glucose_ mmol_l	Correlation coefficient Significance Level P n	0.392 <0.00 01 160	0.399 <0.0001 160		0.024 0.777 4 160	- 0.78 5 <0.0 001 160	0.574 <0.0001 160
HbA1c_%	Correlation coefficient Significance Level P n	- 0.513 <0.00 01 160	0.142 0.0944 160	0.024 0.7774 160		- 0.11 1 0.19 36 160	0.125 0.1424 160
HDL	Correlation coefficient Significance Level P n	- 0.236 0.005 1 160	-0.569 <0.0001 139	-0.785 <0.0001 139	-0.111 0.193 6 139		-0.581 <0.0001 160
TG_mmol_l _	Correlation coefficient Significance Level P n	0.100 0.240 6 160	0.517 <0.0001 160	0.574 <0.0001 160	0.125 0.142 4 160	- 0.58 1 <0.0 001 160	

4.6 Discussion

For healthy blood, nerves, and a heart, vitamin B12 is an absolute necessity. It has been proven that both biochemical and clinical vitamin B12 deficiency are common in patients who have diabetes mellitus type 1 and type 2. A small subset of the clinical symptoms include amnesia, dementia, delirium, peripheral neuropathy, subacute mixed spinal cord degeneration, megaloblastic anemia, and pancytopenia. In this review article, up-to-date information on the physiological roles of vitamin B12, suggested

pathophysiological processes of deficiency, screening for deficiency, and supplementation in diabetic patients is offered (Selhub *et al*. 2009).

In various stedied refers that are two primary metabolic pathways by which vitamin B12 exerts its physiological effects; one is the conversion of homocysteine to methionine, and the other is the oxidation of methylmalonyl coenzyme A (CoA) to succinyl-CoA. Both of these pathways are important. Because vitamin B12 functions as a co-factor in the conversion of homocysteine to methionine, myelin, neurotransmitters, and membrane phospholipids are all able to accept methyl groups (Selhub *et al.* 2009).

Because of this, a diet that is deficient in vitamin B12 to a significant degree can disrupt the methylation process and lead to an increase in the concentration of homocysteine in both the blood and the cells themselves. According to a number of studies, having high amounts of homocysteine in the blood can cause harm to the vascular endothelium as well as the neurons that are found in the brain. This process must also be carried out in order for dietary folate, which is in the form of methyltetrahydrofolate, to be transformed into the active metabolic form, which is tetrahydrofolate. In addition, vitamin B12 is necessary for the conversion of methylmalonyl coenzyme A (CoA) to succinyl-CoA, which is an essential step in another enzymatic pathway. This conversion takes place because vitamin B12 acts as a co-factor. In vitamin B12 insufficiency, this conversion mechanism does not work as well, which results in increased amounts of MMA in the serum. As a consequence of this, the synthesis of fatty acids in neuronal membranes becomes hindered Without vitamin B12, the monoamines serotonin and dopamine cannot be created . Monoamines are also known as neurotransmitters. This production is hindered when vitamin B12 is lacking in the body (Selhub *et al*. 2009).

When considered as a whole, the neuro-cognitive or mental symptoms that accompany a deficiency in vitamin B12 can be explained by the components that have been discussed so far. Vitamin B12 deficiency can cause neuronal damage, which can manifest as severe peripheral or autonomic neuropathy, subacute combined degeneration of the spinal cord, delirium, and dementia Axonal demyelination, degeneration, and eventual death are the hallmarks of this type of neuronal damage. There is a correlation between

hyperhomocysteinemia and an increased risk of cardiovascular events This is likely owing to the effects of hyperhomocysteinemia on both cells and blood vessels (Selhub *et al*. 2009).

Vitamin B12, along with other micronutrients such as folate and iron, is required for the synthesis of DNA, the repair of damaged cells, and the production of hemopoietin in an adequate amount. Macrocytic red blood cells (MCV > 100 fl) with or without anemia, ovalocytes, excessively segmented white blood cells (i.e. >5% of neutrophils with 5 lobes), and pancytopenia are the fundamental haematological signs of vitamin B12 deficiency. Ineffective cell repair mechanisms frequently manifest themselves clinically as atrophic glossitis, stomatitis, malabsorption resulting from atrophied villi, and mucositis (Reinstatler *et al*. 2012).

According to a study that compares patients with type 2 diabetes mellitus and the general population, patients with type 2 diabetes mellitus are more likely to have vitamin B12 insufficiency than the general population. According to the findings of a number of cross-sectional studies and case reports vitamin B12 deficiency is more prevalent among individuals who have type 2 diabetes (T2DM). There is a significant correlation between the use of metformin and vitamin B12 deficiency in patients who have type 2 diabetes Studies indicated that patients with type 2 diabetes who took metformin had a prevalence of vitamin B12 insufficiency ranging from 5.8% to 33%. This information was gleaned from the patients' blood samples. Differences in the way that vitamin B12 deficiency was characterized across studies is one potential factor that may explain the substantial variance in the prevalence that has been reported (Reinstatler *et al*. 2012).

In a cross-sectional study of 203 outpatient type 2 diabetic patients conducted at a large military primary care clinic in the United States by (Pflipsen *et al.* 2009), serum vitamin B12 concentrations of 100 pg/ml or elevated serum methylmalonic acid of >243 nmol/L or homocysteine concentrations of >11.9 nmol/L were used to define definite vitamin B12 deficiency. In the United States National Health and Nutrition Examination Survey of 1999-2006, identified serum vitamin B12 values of 148 pmol/l and >148-221 pmol/l,

respectively, as definite and borderline biochemical vitamin B12 deficiency . (Reinstatler *et al*. 2012).

These values were based on the serum concentration of vitamin B12. In a cross-sectional study that found a significant prevalence of vitamin B12 insufficiency among adult patients with type 2 diabetes, vitamin B12 deficiency was defined as blood vitamin B12 values of 150 pg/ml . This study found that 33% of adult patients with type 2 diabetes had vitamin B12 insufficiency. Patients who were taking metformin for long periods of time (more than four years) and high doses (more than two grams per day) were included in this analysis. These are two clinical criteria that have been connected to vitamin B12 deficiency (Qureshi *et al*. 2011).

The comparison of the prevalence of vitamin B12 deficiency in people with type 2 diabetes and healthy general populations is made more difficult by the fact that different studies use different definitions of vitamin B12 deficiency, as well as the fact that cultural and religious perspectives can vary greatly from one region of the world to another. According to the findings of a study that was conducted on an entire population, 12.1% of adults in Finland who were 65 and older were vitamin B12 deficient . 2.6% of the respondents who participated in the study reported that they had been given a diagnosis of vitamin B12 deficiency at some point in the past. Only 2.6% of participants were found to have taken vitamin B12 pills, according to the survey. In this study, vitamin B12 deficiency was defined as either a total serum vitamin B12 concentration of less than 150 pmol/l or a total serum vitamin B12 concentration of between 150 and 250 pmol/l and a holotranscobalamin concentration of less than 37 pmol/l and a homocysteine concentration of less than 15 mol/l. Another definition for vitamin B12 deficiency was a total serum vitamin B12 concentration of between 150 and 250 (Qureshi *et al*. 2011).

Insufficiency in vitamin B12 is particularly prevalent among the general population in India, a country in which a significant number of individuals abstain from eating meat due to cultural or religious beliefs. In a study that looked at the prevalence of vitamin B12 insufficiency and hyperhomocysteinemia among 441 healthy middle-aged Indian

men, (Yajnik 2006) found that vitamin B12 deficiency, which was defined as vitamin B12 values of 150 pmol/L, was recorded in 67% of study participants. The study was looking at the prevalence of vitamin B12 insufficiency and hyperhomocysteinemia. According to the findings of a multivariate analysis, adhering to a vegetarian diet was the only significant predictor associated with having low levels of vitamin B12 (OR 4.4, 95% CI 2.1-9.3) (Shobha *et al*. 2011).

According to the findings of a recent cross-sectional study , among 175 healthy senior Indian individuals aged >60 years, the prevalence of vitamin B12 insufficiency was found to be 16%. Vitamin B12 levels that were measured to be below 150 pmol/L were considered to be deficient. Five hundred and fifty percent of the study participants showed increased levels of MMA in their serum, which is a more sensitive indicator of a lack of vitamin B12 (Shobha *et al*. 2011).

Diabetes type 1, also known as T1DM, is an autoimmune illness that results from the immune system of the body attacking and destroying the pancreatic beta cells that are responsible for the production of the hormone insulin. Autoimmune polyglandular syndromes are characterized by the simultaneous appearance of a wide range of endocrine and immune system disorders, both systemic and localized. Autoimmune polyglandular syndromes are characterized by the simultaneous appearance of a wide range of endocrine and immune system disorders (Koshy *et al*. 2012).

People who have type 1 diabetes frequently suffer with pernicious anemia, which is brought on by autoimmune gastritis. Pernicious anemia affects approximately one percent of the general population, while chronic autoimmune gastritis affects two percent. Patients who were diagnosed with Type 1 Diabetes Mellitus (T1DM) had a prevalence that was three to five times higher (Koshy *et al*. 2012).

Pernicious anemia, which is brought on by a deficiency in vitamin B12, is a disorder that is commonly seen in people who have type 1 diabetes. 45.5% and 54.0% of the people with T1DM had low vitamin B12 levels, respectively, when the makers' cutoff threshold of 180 pg/ml and the published cutoff point of 200 pg/ml were used by the

researchers in a cross-sectional study that was carried out in South India [32]. This was discovered by the researchers who discovered that 45.5% of the people with T1DM had low vitamin B12 levels. There was no correlation seen between low levels of vitamin B12 and gender, age, length of diabetes, or glycemic control (Koshy *et al*. 2012).

Patients with type 1 diabetes typically have autoantibodies to intrinsic factor (AIF) types 1 and 2 and parietal cell antibodies (PCA) . This is especially true for patients who also have antibodies against glutamate decarboxylase-65 (GAD-65) and the HLA-DQA1*0501-B1*0301 haplotype (Joffe B *et al*. 2010).

People who have type 1 diabetes have a tenfold increased risk of developing pernicious anemia when compared to the general population. This is because the PCA inhibits the production of intrinsic factor. Because it prevents vitamin B12 from binding to IF, type 1 AIF is responsible for the development of vitamin B12 insufficiency. Because of this, it is unable to get to the spot in the terminal ileum that is responsible for absorption. The research that has been done indicates that 70 percent of people who have pernicious anemia have these autoantibodies (Joffe B *et al*. 2010).

Both primary autoimmune hypothyroidism and celiac disease are common comorbidities associated with type 1 diabetes . It has been established that both of these conditions have a direct impact on the metabolism of vitamin B12. The prevalence of concomitant auto immune hypothyroidism was found to be 20.2% overall in a cross-sectional study of 504 ambulatory T1DM patients in South Africa. It was shown to be considerably higher in female patients (30.9% vs. 10.1%, p0.0002) than in male patients. The study was conducted in South Africa. Celiac disease was found in three (0.6%) of the people who participated in the study (Joffe B *et al*. 2010).

Patients who have autoimmune hypothyroidism have an increased risk of developing vitamin B12 deficiency due to a number of factors, including: decreased oral intake; dyserythropoesis related to thyroid hormone shortage; and faulty absorption due to decreased intestinal motility, gut wall oedema, and bacterial overgrowth . Patients with

autoimmune hypothyroidism are also more likely to have an increased risk of developing anemia related to thyroid hormone shortage (Selimoglu *et al*. 2010).

Celiac disease, a prevalent autoimmune-mediated gastrointestinal condition, was reported to be present in 1-16% of type 1 diabetes patients compared to 0.3-1% of the normal population This finding contrasts with the normal population, which has a prevalence of 0.3-1%. It has been found that those who are genetically predisposed to developing this condition are more likely to develop it if they consume wheat gluten and other proteins that are comparable to it. Failure to thrive, recurrent diarrhea, and anemia are clinical manifestations of micronutrient malabsorption with folate and vitamin B12 being the most common nutrients affected (Rewers *et al*. 2004).

There are currently no guidelines that have been agreed upon by the diabetes community as a whole for screening type 1 diabetics for vitamin B12 insufficiency. The high prevalence of pernicious anemia and subsequent vitamin B12 deficiency among T1DM patients was reported in most cross sectional studies . Because vitamin B12 deficiency can occur at any time, screening patients at the time of diagnosis, then annually for the following three years, then every five years after that, or in the presence of any clinical indication would be practical. Those with type 1 diabetes who also have thyroid peroxidase antibodies and the indicators of stomach autoimmunity PCA and AIF should have their vitamin B12 levels measured as part of the screening process. The presence of these autoantibodies is associated with an increased likelihood of developing a deficiency in vitamin B12 (De Block *et al*. 2008).

Patients who have non-insulin dependent diabetic mellitus (NIDDM) and who do not get enough vitamin B12 may have an increased risk of developing the serious condition of peripheral neuropathy if they continue to take metformin for an extended period of time. Around thirty percent of diabetics over the age of forty who suffer a lessened sensory sensitivity in their feet are affected by neuropathy, an approaching health concern caused by a shortage of vitamin B12. This condition is brought on by a lack of vitamin B12. There is a correlation between a deficiency in vitamin B12 and a loss of proprioception, commonly known as the vibration sense , as well as an increased

sensitivity to vibrations. These are symptoms that are also experienced by those who suffer from paresthesia and diabetic neuropathy. Recent research that attempted to explain the putative connection between long-term metformin use and its vitamin B12 deficiency-related peripheral neuropathy produced contradicting data. These investigations were conducted in an effort to shed light on this connection (Pflipsen *et al.* 2009).

A cross-sectional study was conducted in Pakistan with the purpose of describing the incidence of vitamin B12 deficiency among patients taking metformin for the treatment of Type II Diabetes Mellitus (T2DM). This was done in order to analyze the factors that are related to vitamin B12 insufficiency that occurs in patients who are taking metformin. Patients older than 45 years old who were diagnosed with type II diabetes were aggressively recruited between the months of January and December of 2016 at the Endocrinology Unit, Medical Complex, and Diabetic Center Hayatabad in Peshawar, Pakistan (Jager *et al.* 2016).

Patients who fulfilled the requirements for the study were asked to complete out a questionnaire that requested information about their demographics, the medications they were currently taking, and whether or not they took any supplementary multivitamin supplements. This study was given informed consent (Mahajan *et al*. 2010).

Patients who had undergone previous weight-loss surgery, gastrectomy, previous gut resection, or had a history of inflammatory bowel disease (especially Crohn's disease), amongst other conditions, were not permitted to participate in the study. This included patients who had undergone previous weight-loss surgery in the past. A creatinine level that was greater than 3.0 served as an accurate indicator of chronic renal disease. Metformin, insulin, additional diabetes drugs, acid blockers (H2 blockers and/or proton pump inhibitors), herbal supplements, and B complex vitamins were some of the pharmaceuticals and supplementary multivitamins that were put to the test. Age, gender, and the timeframes of diabetes development and diagnosis are some examples of the types of biodata that are required (Mahajan *et al*. 2010).

In the cross-sectional study that we conducted, we discovered that 29.66 percent of persons who had diabetes type 2 (T2DM) also had an insufficient amount of vitamin B12. It is not easy to compare the prevalence that we have now with that which was found in past study because there are a variety of factors that come into play. Despite the fact that the particular causes of this impairment are not yet completely understood, a number of researchers have arrived at the same results (Lindenbaum *et al*. 1988).

In this particular sector, there is a dearth of data. In a similar vein, there has been no research conducted to determine the extent to which diabetics in Pakistan who take metformin are at risk for vitamin B12 insufficiency. Previous research conducted by (Jager *et al.* 2016) found that using metformin was associated with a 19% (95% CI -24% to -14%) drop in vitamin B12 levels when compared to taking a placebo. Participants in the study were required to have type II diabetes and to have taken metformin at a dosage of 2,550 milligrams each day for a minimum of 4.3 years (Jager *et al*. 2016).

The usage of metformin in conjunction with H2-blockers or proton pump inhibitors has been demonstrated to be one of the conditions that has a strong correlation with a lack of vitamin B12. The correlation between vitamin B12 deficiency and the use of proton pump inhibitors or H2-blockers provides support for the hypothesis that lower stomach acidity contributes significantly to vitamin B12 malabsorption. This hypothesis states that lower stomach acidity contributes significantly to vitamin B12 malabsorption. Both of these drugs suppress the generation of stomach acid, which is necessary for the breakdown of dietary vitamin B12 . The breakdown of vitamin B12 requires stomach acid. The existence of this relationship is one of the rarest occurrences imaginable , there is no connection between the use of omeprazole and the amount of vitamin B12 present in the blood. However, due to the potential cumulative effect of metformin, proton pump inhibitors (PPI), and/or H2-blockers on vitamin B12 uptake, caution is urged while utilizing so many different medications at the same time (Pflipsen *et al*. 2009).

Metformin users who take the drug on a regular basis have a significantly higher danger of becoming vitamin B12 deficient. This study recommended screening for vitamin B12 deficiency and recommended that patients with Type II diabetes using metformin take multivitamins on a consistent basis to reduce the risk of developing vitamin B12 insufficiency. It was found that diabetics who took multivitamins had a lower risk of developing vitamin B12 deficiency than those who did not. The usage of multivitamins has been associated in the research that has been done with higher serum B12 levels. It was discovered that the levels of vitamin B12 in the blood of individuals who routinely consumed between six and nine micrograms were much higher than those of adults who did not take the supplement (Pflipsen *et al* . 2009).

When it comes to placebos, it can be difficult to discover points of comparison between the findings of our study and those of other investigations . Additionally, it has not been confirmed whether or not taking a multivitamin every day helps prevent vitamin B12 deficiency. This is something that has to be investigated. Because vitamin B12 insufficiency is often treated with significantly higher dosages of supplements, either orally consumed or given parenterally, the significance of our findings is especially noteworthy considering this fact. However, taking a vitamin B12 supplement with six to twenty-five micrograms of the nutrient is typically enough to prevent vitamin B12 deficiency in foods and supplements such as multivitamins. To determine whether or not multivitamins are useful in preventing vitamin B12 deficiency, additional research needs to be conducted (Pflipsen *et al*. 2009).

In addition, age and the use of acid blockers, both of which are known to increase the likelihood of B12 insufficiency, were not discovered to be statistically significant predictors of B12 deficiency (Miller *et al*. 2009).

This is an alarmingly high rate of vitamin B12 deficiency, and it should serve as a warning to doctors that this nutrient is often depleted in people who have diabetes, particularly if they have been taking metformin for an extended period of time or at a high enough dose. This should serve as a warning to doctors because it is often depleted in people who have diabetes who have been taking metformin for an extended period of

time or at a high enough dose. Although it has been hypothesized by some that insufficiency is a major risk factor for the onset and/or worsening of neuropathies and anemias in a population that is already predisposed to these complications due to the presence of underlying co-morbid diabetes, the precise medical significance and impact of insufficiency are unknown at this time. According to the findings of previous studies , taking vitamin B12 through a doctor's prescription can help lower the likelihood of getting neuropathy (Vasconcelos *et al*. 2006).

The research has a few flaws that need to be addressed. Due to the fact that the research was carried out on a limited number of participants at a specific location, it is probable that the findings won't be applicable to the normal diabetic living in the community. Because of this, the first question regarding the study's validity to the outside world arose. Second, the researchers did not measure the amounts of methylmalonic acid in the participants' blood, which would have boosted the study's sensitivity by allowing for the diagnosis of vitamin B12 deficiency at an earlier, asymptomatic stage. In addition, the results of vitamin B12 replenishment were not evaluated at any point. As a result, it would have been beneficial to check in with these individuals on a regular basis to see how well the dosage was working for them and for how much longer it is likely that they will need to continue taking a vitamin B12 supplement (Vasconcelos *et al*. 2006).

5. CONCLUSIONS AND RECOMMENDATION

5.1 Conclusions

- FBG, HbA1C, TG, CHOL, HDL and vit B12 levels in type2 diabetic patients with dyslipidemia presented significant compare with healthy persons.
- The level of the studied parameters in the group with good diabetes control was close to that of the control group
- The level of vitamin B12 decreased in the poor control group, and its levels were also low in the fair group, but were similar to the healthy people in the good-controlled group.
- A clear negative correlation between vitamin B12 and the other studied parameters, except for HDL, the correlation was positive.

5.2 Recommendations

- Further study of vitamin B12 with type 1 diabetes.
- Further study on vitamin B12 with those taking metformin and comparing it with those not taking it.
- Further study on comparing vitamin B12 levels between type 1 and type 2 diabetes.

REFERENCES

Acara, A. Ç., Bolatkale, M., Kızıloğlu, İ., İbişoğlu, E. and Can, Ç. 2018. A novel biochemical marker for predicting the severity of ACS with unstable angina pectoris: asprosin. American Journal of Emergency Medicine, 2: 13-15.

Alhaji, J. H. 2022. Vitamin B12 Deficiency in Patients with Diabetes on Metformin: Arab Countries. Nutrients, 14(10): 2046.

Alhameed, A. 2022 . Biochemical Comparative Study for the Role of Selenium and Arab Countries. Nutrients, 1: 4-10.

Association, A. D. 2022 . Classification and Diagnosis of Diabetes : Standards of Medical Care in Diabetes 2022. Diabetes Care, 45(l): 17–38.

Block, C. E., De Leeuw, I. H. and Van Gaal, L. F. 2008. Autoimmune gastritis in type 1 diabetes: a clinically oriented review. The Journal of Clinical Endocrinology and Metabolism, 93(2): 363-371.

Chaudhury, D. and Aggarwal, A. 2018 . Diabetic dyslipidemia: Current concepts in pathophysiology and management. Journal of Clinical and Diagnostic Research, 12(1): OE06–OE09.

Cho, N. H., Shaw, J. E., Karuranga, S., Huang, Y., da Rocha Fernandes, J. D., Ohlrogge, A. W. and Malanda, B. 2018. IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Research and Clinical Practice, 138: 271–28.

Dixit, A. K., Dey, R., Suresh, A., Chaudhuri, S., Panda, A. K., Mitra, A. and Hazra, J. 2014. The prevalence of dyslipidemia in patients with diabetes mellitus of ayurveda Hospital. Journal of Diabetes and Metabolic Disorders, 13(1): 2–7.

Durruty, P. and Sanhueza, M. S. 2019. Pathogenesis of Type 2 Diabetes Mellitus. Seikagaku, 71(11): 1281–1298.

Eliasson, B., Cederholm, J., Eeg-Olofsson, K., Svensson, A. M., Zethelius, B. and Gudbjörnsdottir, S. 2011. Clinical usefulness of different lipid measures for prediction of coronary heart disease in type 2 diabetes: A report from the Swedish National Diabetes Register. Diabetes Care, 34(9): 2095–2100.

Fargion, S., Dongiovanni, P., Guzzo, A., Colombo, S., Valenti, L. and Fracanzani, A. L. 2005. Iron and insulin resistance. Alimentary Pharmacology and Therapeutics, Supplement, 22(2): 61–63.

Federation, I. D. 2021. IDF Diabetes Atlas. In Diabetes Research and Clinical Practice., 10: 123-125.

Forrest, K. Y. Z., Becker, D. J., Kuller, L. H., Wolfson, S. K. and Orchard, T. J. 2000. Are predictors of coronary heart disease and lower-extremity arterial disease in type 1 diabetes the same: A prospective study. Atherosclerosis., 148(1): 159–169.

Guariguata, L., Whiting, D. R., Hambleton, I., Beagley, J., Linnenkamp, U. and Shaw, J. E. 2014. Global estimates of diabetes prevalence for 2013 and projections for 2035. Diabetes Research and Clinical Practice., 103(2): 137–149.

Gupta, K. K. D. S. 2021. Biochemistry, Ketogenesis. Health Measures Survey. American Journal of Clinical Nutrition, 94(4): 1079– 1087.

Ho, M., Halim, J. H., Gow, M. L., El-Haddad, N., Baur, L. A., Cowell, C. T., Garnett, S. P. and Marzulli, T. 2014. Vitamin B12 in obese adolescents with clinical features of insulin resistance. Nutrients, 6(12): 5611–5618.

Jager, J., Kooy, A., Lehert, P., Wulffelé, M. G., Van der Kolk, J., Bets, D. and Stehouwer, C. D. 2016. Long term treatment with metformin in patients with type 2 diabetes and risk of vitamin B-12 deficiency: randomised placebo controlled trial. Bmj., 3: 40.

Joffe, B., Distiller, L., Landau, S., Blacking, L., and Klisiewicz, A. 2010. Spectrum of Autoimmune Disorders in Type 1 Diabetes-A Cross-Sectional Clinical Audit. J Diabetes Metab., 1(11): 22.

Jones, A. G., McDonald, T. J., Shields, B. M., Hagopian, W. and Hattersley, A. T. 2021. Latent Autoimmune Diabetes of Adults (LADA) Is Likely to Represent a Mixed Population of Autoimmune (Type 1) and Nonautoimmune (Type 2) Diabetes. Diabetes Care, 44(6): 1243–1251.

Katsarou, A., Gudbjörnsdottir, S., Rawshani, A., Dabelea, D., Bonifacio, E., Anderson, B. J., Jacobsen, L. M., Schatz, D. A. and Lernmark, A. 2017. Type 1 diabetes mellitus.Nature Reviews Disease Primers, 3: 1–18.

Kibirige, D. and Mwebaze, R. 2013. Vitamin B12 deficiency among patients with diabetes mellitus: Is routine screening and supplementation justified? Journal of Diabetes and Metabolic Disorders, 1: 21.

Knight, J. and Nigam, Y. 2017. Diabetes management 1: disease types, symptoms and diagnosis. Nursing Times, 4(11): 34.

Koshy, A. S., Kumari, S. J., Ayyar, V. and Kumar, P. 2012. Evaluation of serum vitamin B12 levels in type 1 diabetics attending a tertiary care hospital: A preliminary cross–sectional study. Indian Journal of Endocrinology and Metabolism, 16(1): S79.

Lindenbaum, J., Healton, E. B., Savage, D. G., Brust, J. C., Garrett, T. J., Podell, E. R. and Allen, R. H. 1988. Neuropsychiatric disorders caused by cobalamin deficiency in the absence of anemia or macrocytosis. New England journal of medicine, 318(26): 1720-1728.

MacFarlane, A. J., Greene-Finestone, L. S. and Shi, Y. 2011. Vitamin B-12 and homocysteine status in a folate-replete population: results from the Canadian Health Measures Survey. The American journal of clinical nutrition, 94(4): 1079-1087.

Mahajan, R. and Gupta, K. 2010. Revisiting metformin: annual vitamin B12 supplementation may become mandatory with long-term metformin use. Journal of Young Pharmacists, 2(4): 428-429.

Malouf, A. A. S. 2003. Vitamin B12 for cognition. Sciences., 3: 45-46.

Miettinen, T. A., Gylling, H., Tuominen, J., Simonen, P. and Koivisto, V. 2004. Low Synthesis and High Absorption of Cholesterol Characterize Type 1 Diabetes. Diabetes Care, 27(1): 53–58.

Miller, J. W., Garrod, M. G., Allen, L. H., Haan, M. N. and Green, R. 2009. Metabolic evidence of vitamin B-12 deficiency, including high homocysteine and methylmalonic acid and low holotranscobalamin, is more pronounced in older adults with elevated plasma folate. The American Journal of Clinical Nutrition, 90(6): 1586-1592.

National Diabetes Data Group. 1979. Guide to diagnosis and classification of diabetes mellitus and other categories of glucose intolerance. Diabetes Care, 28: 1039–1057.

Pflipsen, M. C., Oh, R. C., Saguil, A., Seehusen, D. A., Seaquist, D. and Topolski, R. 2009. The prevalence of vitamin B12 deficiency in patients with type 2 diabetes: a cross-sectional study. The Journal of the American Board of Family Medicine, 22(5): 528-534.

Qureshi, S. A., Ainsworth, A., and Winocour, P. H. 2011. Metformin therapy and assessment for vitamin B12 deficiency: is it necessary. Practical Diabetes, 28(7): 302-304.

Reinstatler, L., Qi, Y. P., Williamson, R. S., Garn, J. V. and Oakley Jr, G. P. 2012. Association of biochemical B12 deficiency with metformin therapy and vitamin B12 supplements: the National Health and Nutrition Examination Survey, 1999–2006. Diabetes Care, 35(2): 327-333.

Rewers, M., Liu, E., Simmons, J., Redondo, M. J. and Hoffenberg, E. J. 2004. Celiac disease associated with type 1 diabetes mellitus. Endocrinology and Metabolism Clinics, 33(1): 197-214.

Rivellese, A. A., Bozzetto, L. and Massaro, P. 2007. Advances in Diabetes Diagnostics. European Endocrinology, 00(1): 20.

Saeedi, P., Petersohn, I., Salpea, P., Malanda, B., Karuranga, S., Unwin, N., Colagiuri, S., Guariguata, L., Motala, A. A., Ogurtsova, K., Shaw, J. E., Bright, D. and Williams, R. 2019. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. Diabetes Research and Clinical Practice, 157: 107843.

Selhub, J., Morris, M. S., Jacques, P. F. and Rosenberg, I. H. 2009. Folate–vitamin B-12 interaction in relation to cognitive impairment, anemia, and biochemical indicators of vitamin B-12 deficiency. The American Journal of Clinical Nutrition, 89(2): 702S-706S.

Selimoglu, M. A. and Karabiber, H. 2010. Celiac disease: prevention and treatment. Journal of Clinical Gastroenterology, 44(1): 4-8.

Shahwan, M. J., Jairoun, A. A., Farajallah, A. and Shanabli, S. 2019. Prevalence of dyslipidemia and factors affecting lipid profile in patients with type 2 diabetes. Diabetes and Metabolic Syndrome: Clinical Research and Reviews, 13(4): 2387–2392.

Shobha, V., Tarey, S. D., Singh, R. G., Shetty, P., Unni, U. S., Srinivasan, K. and Kurpad, A. V. 2011. Vitamin B12 deficiency & levels of metabolites in an apparently normal urban south Indian elderly population. The Indian Journal of Medical Research, 134(4): 432.

The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. 1997. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care, 20(7): 1–15.

Tracy L. Setji, M., Ann J. Brown, M. and Mark N. C. 2016. Gestational diabetes mellitus. Gynecologie Obstetrique Et Fertilite, 44(4): 244– 247.

Tshivhase, A., Matsha, T. and Raghubeer, S. 2021. Diagnosis and treatment of MODY: An updated mini review. Applied Sciences (Switzerland), 1: 18-20.

Vasconcelos, O. M., Poehm, E. H., McCarter, R. J., Campbell, W. W. and Quezado, Z. 2006. Potential outcome factors in subacute combined degeneration. Journal of General Internal Medicine, 21(10): 1063-1068.

WHO. 1965. Diabetes Mellitus. Report of a WHO Expert Committee, pp. 1–44.

WHO. 1980. WHO Expert Committee on Diabetes Mellitus second report, pp. 1–80.

WHO. 2019. Classification of diabetes mellitus. In Clinics in Laboratory Medicine

Yajnik, V. 2006. B12 deficiency and hyperhomocysteinemia in rural and urban Indians. J Assoc Physicians India., (54): 775.

Yuan, Y., Chen, W., Luo, L. and Xu, C. 2021. Dyslipidemia: Causes , Symptoms and Treatment International Journal of Trend in Scientific Research and Development (IJTSRD) Dyslipidemia: Causes, Symptoms and Treatment, 5: 72-73.

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