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Role Of Vitamin B_{12} Deficiency Among patients With Diabetes Mellitus type 2

Research project

Submitted to University of Salahaddin – Erbil, college of Education, Department of Biology in partial fulfillment of the requirements for Degree of bachelor in Biology

By

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CERTEFICATION

I attest that this thesis was completed under my direction at the University of Salahaddin – Erbil's Department of Biology, College of Education. I further suggest that it be approved in partial fulfilment of the requirements for the Bachelor of Science in Biology degree.

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Date: 4/2024

Chairman is Certification

In view of the available recommendation I forward this thesis for debate by the examining committee.

Signature:

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Date: /4/2024

Dedication:

I dedicate this work to :

My mother and father, who have always prayed for me and for everyone else.

My Supervisor prof. Dr.Kalthum Asaaf Maulood.

Sarah M. Abdullah

Acknowledgments

I would like to thank Allah, the most benevolent being who has guided me in all aspects of my life and career.

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Sarah M. Abdullah

Summary

Diabetes mellitus DM is group of metabolic disorders characterized by Hyperglycaemia resulting from defects in insulin secretion,insulin action or both. DM has many Subclassification: type 1, Type 2 and Gestational diabetes. Type 2 diabetes is high blood sugar level due to insulin it not be produced enough or insulin it makes not working Properly known as insulin Resistance. the aim of this study was to investigate various biochemical (vitamin B12, HbA1c) and Hematological parameters (RBC, Hb, WBC, PLT) in Patients with type 2 diabetes Mellitus (T2DM) and Compare those with non-diabetic subjects (Control group) this study was conducted on 50 subjects (ages ranging from 26-49 Years old; sex matched) who where classified into two groups :Diabetic group (n=35 Subjects) and non diabetic subject (n=15 Subjects) the study carried out during the period of December 2023 to January 2024 in the department of biology College of Education Salahaddin university .the following various parameter were assessed for all subjects: white blood cell count (WBC), Red blood cell count (RBC), Hemoglobin concentration (Hb),Packed cell volume (PCV), Platelets (PLT). The value of WBC count ,Hb and PCV level increase non significantly in male patients when compared with control group. Platelet count decrease non significantly in male patient with DM type 2 when compared to the control group, while the RBC count increase significantly in male patients as compared with control group. vitamin B12 increase non significantly and HbA1c level increase significantly in male patients when compared to the control group .The value of WBC count, PLT count increase non significantly in female patients when compared with control group, while RBC count, Hb and PCV level decrease non significantly in female patients with DM type 2 when compared with control group .Vitamin B12 and HbA1c level increase significantly in female patients with DM type2 when compared with control group.

Keyword: DMT2, anemia, vitamin B12,HbA1c.

List of content

List of tables

List of figures

1.Introduction:

the term diabetes mellitus (DM) describes several distinct metabolic disorders characterized by high blood glucose (hyperglycemia) (WHO,2019).Diabetes mellitus was previously tagged a disease associated with ‘sweet urine’ and high muscle loss (Karamanou et al.,2016) .There are three main types of diabetes mellitus: Type 1 DM results from the body's failure to produce enough insulin. Type 2 DM begins with insulin resistance, a condition in which cells fail to respond to insulin properly. As the disease progresses a lack of insulin may also develop. The primary cause is excessive body weight and not enough exercise. Gestational diabetes, is the third main form and occurs when pregnant women without a previous history of diabetes develop a high blood glucose level (kumar et al.,2020).Vitamin B12, also known as cobalamin, is a water-soluble vitamin involved in metabolism (ODS,2021).It is one of eight B vitamins. It is required by animals, which use it as a cofactor in DNA synthesis, and in both fatty acid and amino acid metabolism (Yamada.,2013). It is important in the normal functioning of the nervous system via its role in the synthesis of myelin, and in the circulatory system in the maturation of red blood cells in the bone marrow(ODS,2021).Vitamin B12 deficiency can cause a delay in the maturation of red blood cells (RBCs) and many changes in their shape, leading to megaloblastic anemia. Megaloblastic anemias are characterized by an imbalance between nuclear and cytoplasmic maturation and abnormal nuclear maturation in RBCs. Vitamin B12 deficiency and a lack of folates affect DNA synthesis, which slows nuclear replication and postpones all stages of development (Obeagu et al.,2021and Torrez et al.,2022).Metformin is considered one of the most important hypoglycemic drugs used to control the hyperglycemic state in patients with DM. It is mainly used in patients with Type 2 DM (T2DM) and both European and American recommendations recommend it as a first-line pharmacological treatment for T2DM (Herman et al.,2022).The aim of the present study is to investigate the role of serum vitamin B_{12} level in diabetes mellitus type 2 patients in kurdish population.

2.LITERETURES REVIEW

2.1Diabetes mellitus (DM):

is the most common endocrine disorder characterized by increased fasting or post prandial plasma glucose levels more than upper limits during oral glucose tolerance test or random plasma measures as defined by the criteria for the diagnosis of DM (Thomas.,2017 and Ralston et al.,2018).Diabetes and it's complications is the fifth leading cause of death worldwide (Ralston et al.,2018).According to the pathogenic process that lead to hyperglycemia, diabetes mellitus is classified into; type 1 DM, type 2 DM that is the most common type of DM which account for about 85% of cases, gestational DM (Loscalzo et al.,2017).

3.Types of Diabetes:

3.1Type1 Diabetes:

Type 1 diabetes occurs when the immune system attacks and de- stroys insulin-producing β-cells in islets of Langerhans in the pancreas, leads to the inability to produce enough insulin to regulate blood glucose levels resulting in symptoms like excessive thirst, frequent urination, unexplained weight loss, fatigue, blurred vision, and increased hunger (Mayo Clinic, 2022). Due to complete insufficiency of endogenous insulin, T1D patients require daily insulin injections. Consequently, T1D is often referred to as insulin-dependent diabetes (Akil et al.,2021).Furthermore, diagnosis of T1D typically involves measuring blood glucose levels using a variety of tests, such as fasting blood glucose, oral glucose tolerance test, and random blood glucose test (Klonoff, 2015). The management of T1D involves insulin therapy to regulate blood glucose levels. Insulin can be administered through injections or insulin pumps, and their dosage and timing must be carefully monitored to prevent hypoglycaemia (low blood glucose levels) or hyperglycaemia (Mathieu et al., 2016). Insulin remains the primary medication for treating T1D (Danne et al., 2018). Moreover, newer medications, such as glucagon-like peptide-1 (GLP-1) receptor agonists and sodium-glucose co-transporter 2 (SGLT2) inhibitors, have been studied in individuals with T1D. GLP-1 receptor agonists work by stimulating the release of insulin from the pancreas and reducing appetite, thereby promoting weight

loss (Henry et al., 2015). Previously, combination therapy of GLP-1 receptor agonist with basal insulin resulted in a significant reduction in HbA1c and body weight (Carris et al., 2014). Moreover, SGLT2 inhibitors act by blocking the reabsorption of glucose in the kidneys, causing an increase in urinary excretion of glucose, and sub- sequently reducing blood sugar levels (Vallon and Verma, 2021).

3.2Type 2 diabetes

Type 2 diabetes previously referred to as "non-insulin-dependent diabetes" or "adult-onset diabetes," accounts for 90–95% of all diabetes. Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder characterized by persistent hyperglycemia, and it is a leading cause of morbidity and mortality worldwide (Khan et al.,2020).This form en- compasses individuals who have relative (rather than absolute) insulin deficiency and have peripheral insulin resistance. In T2DM, the response to insulin is diminished, and this is defined as insulin resistance. At least initially, and often throughout their lifetime, these individuals may not need insulin treatment to survive.There are various causes of type 2 diabetes. Although the specific etiologies are not known, autoimmune destruction of Beta-cells does not occur, and patients do not have any of the other known causes of diabetes.The risk of developing type 2 diabetes increases with age, obesity, and lack of physical activity (International Diabetes Federation,2021).Most, but not all, people with type 2 diabetes have overweight or obesity. Excess weight itself causes some degree of insulin resistance. Individuals who do not have obesity or overweight by traditional weight criteria may have an increased percentage of body fat distributed predominantly in the abdominal region. Insulin resistance may improve with weight reduction, physical activity, and/or pharmacologic treatment of hyperglycemia but is seldom restored to normal. Recent interventions with intensive diet and exercise or surgical weight loss have led to diabetes remission (Lean et al.,2018 and Cresci et al.,2020).Currently, the incidence of (T2DM) is becoming an epidemic, and the treatment of complications caused by chronic hyperglycemia is extremely economically burdensome. Chronic hyperglycemia exerts a direct toxic effect on different cell types, including pancreatic β-cells and vascular endothelial cells. Specifically, in the insulin resistance state preceding symptomatic T2DM, prolonged hyperglycemia contributes to oxidative stress that is highly dangerous to β-cells. Moreover, as a result of increased secretion

of insulin, β-cells become exhausted and die. Thus, secretion of insulin is disturbed. In turn, vascular endothelial cells are particularly sensitive to hyperglycemia since they transport glucose in an insulin-independent manner, and intracellular glucose concentration is proportional to its blood concentration. Thus, endothelial cells are directly exposed to the toxic effect of high glucose and related oxidative stress, which leads to micro- and macrovasculature dysfunction, initiating the development of diabetes complications in multiple organs that significantly affect the length and quality of life. (Yaribeygi et al.,2019).Currently, there is no complete treatment for T2DM, relying mainly on existing medications and a healthy lifestyle to control the development of the disease (Ismail et al .,2021).

3.3Gestational diabetes:

Gestational Diabetes Mellitus (GDM) is a collection of symptoms in pregnant women caused by an increase in blood glucose levels due to a progressive decrease in insulin secretion, this diabetes is characterized by an increase in blood sugar during pregnancy in 24th week of pregnancy and blood sugar levels will return to normal after pregnancy(Zhang.,2021 and Retnakaran.,2021). Gestation diabetes mellitus (GDM) traditionally refers to the first occurrence or discovery of abnormal glucose metabolism during pregnancy. Due to substantial increase in the levels of growth hormone ,Adrinocorticotropic hormone, human placental prolactin, pituitary prolactin, estrogen and progesterone in the systemic regulation, insulin resistance gradually increase and typically manifests during the second and third trimesters of pregnancy (Lain and Catalano,2007).

4.Pancreas:

The human pancreas is a retroperitoneal organ in the upper abdomen weighing between 100-150 g and measuring between 15-25 cm in length. It is connected to other abdominal organs such as the spleen, stomach, duodenum and colon (Leung.,2010). This organ is surrounded by a fibrous capsule that divides its parenchyma into distinct lobes and lobules (Dolenśek et al.,2015). separated by connective tissue that divides the pancreas into two structurally distinct components: The exocrine pancreas, which consists mainly of acinar cells and duct cells; and the endocrine pancreas, which is the site of islet cells (Low.,2004). The endocrine portion is composed of groups of cells known as islets of Langerhans, which are attributed with the secretion of several pancreatic peptide hormones for glucose homeostasis, including insulin. There are five major cell types that constitute the islet: α cells; β cells; δ cells; PP cells; and ε cells. They are responsible for producing glucagon, insulin, somatostatin, pancreatic polypeptide and ghrelin, respectively (Leung.,2010 and A-Kader et al.,2012). The most numerous are the β cells that synthesize and secrete insulin.

5.Insulin hormone:

Insulin from Latin insula, 'island') is a peptide hormone that plays an important role in glucose homeostasis, cell growth and metabolism It is considered to be the main anabolic hormone of the body.It regulates the metabolism of carbohydrates, fats and protein by promoting the absorption of glucose from the blood into liver, fat and skeletal muscle cells. In these tissues the absorbed glucose is converted into either glycogen via glycogenesis or fats (Triglyceride) via lipogenesis or, in the case of the liver, into both (Rahman et al.,2021). This hormone is synthesized in the β cells of the pancreatic islets; its transcription and translation is regulated in part by nutrients, specifically in response to glucose concentrations(Rahman et al.,2021 and Fu .,2013). The active structure of this hormone is formed by two chains named “chain A” with 21 amino acid residues and “chain B” with 30 amino acid residues linked by three disulfide bonds between both chains (Fu et al.,2013). Proinsulin, a single-chain 86 amino acid peptide, is cleaved into insulin and C-peptide (a connecting peptide); both are secreted in equimolar portions from the beta cell upon stimulation from glucose and other insulin secretagogues.While C-peptide has no known physiologic function, it can be measured to provide an estimate of endogenous insulin secretion.The insulin is stored in vesicles to be released into the

bloodstream when β cells take up glucose from the extracellular medium (Rahman et al.,2021). through the bloodstream it will reach all peripheral organs and the brain (Park et al.,2021).Insulin is a peptide hormone that was discovered in 1922 by surgeon Frederick Grant Banting and physician Charles Herbert Best and purified by biochemist James Bertam Collip (Karamitsos.,2011).

6. Vitamins

Vitamins are important and necessary elements in small quantities, and these are not produced by our body and therefore we need to use external sources to suppress our need , being the source of animal or Vegetable origin (Rubert et al .,2017).

6.1Vitamin B complex

B vitamins, also known as B-complex vitamins, play essential roles in catabolic and anabolic metabolism. These 8 water-soluble vitamins are excreted in urine and require repletion daily. The B vitamins are identified as follows: thiamine (B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), pyridoxine (B6), biotin (B7), folate (B9), and cobalamin (B12) B vitamins act as coenzymes in several enzymatic processes that support every aspect of cellular physiological functioning, including major functions within the brain and nervous system. Any B vitamin deficiency can negatively affect mitochondrial metabolism of amino acids, glucose, and fatty acids through the citric acid cycle and electron transport chain (Kennedy.,2016).

6.2Function of Vitamin B

Independently, each B vitamin is essential for the body's need to start the chemical processes that regulate numerous activities of the body. As an illustration, cells use B vitamins to convert fatty acids, carbohydrates, and other nutrients into energy. So, if the body is deficient in B-complex vitamins, it may not function properly at all. Hence, it is crucial to routinely absorb B vitamins through food or supplements to prevent deficiency because the body cannot readily retain them for extended periods because B vitamins are water-soluble so these may be radially eliminated from the body through urination (Alachram et al., 2021). B vitamins are also required for the production of red blood cells that transport oxygen throughout the body, the adrenal glands' ability to produce hormones associated with sex and stress, the operation of the liver and nervous system, the health of the gastrointestinal tract, and the growth of healthy skin, hair, and eyes (Hanna et al., 2022; Sarwar et al., 2022).Each vitamin of B complex protein is different structurally and plays a particular function in the body. A role in wound healing has been demonstrated for the B vitamins i.e. thiamine, pantothenic acid, and others. It has been suggested that taking supplements of vitamins B_1, B_2, and B_6 will help treat canker sores.(Mikkelsen and Apostolopoulos, 2019).Group B vitamins are required for various cortical processes involved in metabolism, such as the methylation of homocysteine to methionine (specifically B6, folic acid and B12), which is essential for the synthesis, repair and synthesis of DNA. other methylation reactions in the central nervous system (Saiki.,2018).

7. Vitamin B_{12}

Vitamin B_{12} (Cobalamin or Cynocobalamin) Vitamin B_{12} is often called cobalamin or cyanocobalamin. It can be found in some foods naturally as well as in dietary supplements and prescription medications. Cobalt is a compo- nent of vitamin B12, which wherefore substances that operate like vitamin B12 are commonly referred to as "cobalamin." Methylcobalamin and 5- deoxy adenosylcobalamin are the forms of vitamin B_{12} that are metabolically active. However, two further forms, hydroxocobalamin and cyanocobalamin, become physiologically active after being converted into methylcobal- amin or 5- deoxy adenosylcobalamin (Niklewicz et al., 2022).For the synthesis of DNA, the growth, myelination, and proper operation of the central nervous system, and

the production of healthy red blood cells, vitamin B_{12} is crucial. Vitamin B12 functions as a cofactor for the enzymes methionine synthase and L-methyl malonyl-CoA. The enzyme methionine synthase converts homocysteine into the essential amino acid methionine. Vitamin B_{12} is necessary for both DNA and red blood cells. Vitamin B_{12} supplementation reduces hyper-homocysteinemia, a complication of chronic kidney disease (CKD) that has harmful cardiovascular implications.(Theobald & Lim, 2019).

7.1Source of vitamin B_{12}

Vitamin B12 (cobalamin) is a water-soluble vitamin that is derived from animal products such as red meat, dairy, and eggs. Main sources in Nordic and Baltic diets are meat, liver, dairy products, fish, and shellfish. Intrinsic factor is a glycoprotein that is produced by parietal cells in the stomach and necessary for the absorption of B12 in the terminal ileum. Once absorbed, B12 is used as a cofactor for enzymes that are involved in the synthesis of DNA, fatty acids, and myelin. As a result, B12 deficiency can lead to hematologic and neurologic symptoms. B12 is stored in excess in the liver; however, in cases in which B12 cannot be absorbed for a prolonged period (e.g., dietary insufficiency, malabsorption, lack of intrinsic factor), hepatic stores are depleted, and deficiency occurs (Fritz et al.,2019). Among humans, the primary source of vitamin B12 is considered to be food of animal origin. Some bacteria that make up the human intestinal flora have the ability to synthesize vitamin B12, but its bioavailability in this case is limited. The availability of vitamin B12 produced by intestinal bacteria depends, among other things, on the location of the bacteria in the appropriate section of the intestine, with vitamin B12 being absorbed in the small intestine; the ratio of the amount of cobalamin-producing bacteria to the amount of cobalamin-consuming bacteria; and the presence of diseases caused by bacteria (Wan et al.,2022).

7.2Vitamin B12 deficiency

It is worth noting that although vitamin B12 belongs to the group of water-soluble vitamins, it is excreted in both urine and feces (together with bile) (Shibata et al.,2014).Vitamin B12 deficiency can also be caused by diseases of the gastrointestinal tract: gastritis, intestinal malabsorption, Crohn's disease, Helicobacter pylori infection, parasite infection and chronic pancreatitis. Other causes include old age, difficulty swallowing and the long-term use of proton pump inhibitor drugs and metformin (Obeid et al.,2019).Vitamin B12 deficiency, due to insufficient intake, is seen in low-income, malnourished populations and among vegans, vegetarians and the elderly(Rizzo et al.,2016)

7.3Absorption of vitamin B_{12}

In healthy patients, dietary vitamin B12 binds to a protein called R-factor, which is secreted from salivary glands. Once the complex arrives at the small intestine, B12 is cleaved from R-factor by pancreatic enzymes, allowing it to bind to a glycoprotein called intrinsic factor, which is secreted by gastric parietal cells. The newly formed complex of B12 and intrinsic factor can then bind to receptors on the ileum, which allows for absorption of B12. Once absorbed, B12 is involved in metabolic pathways important in both neurologic and hematologic functions. If B12 cannot be absorbed, regardless of the etiology, many impairments may occur.Vitamin B12 is a cofactor for the enzyme methionine synthase, which is used in the conversion of homocysteine to methionine. As a byproduct of this reaction, methyl-THF is converted to THF, which is converted to intermediates used in the synthesis of pyrimidine bases of DNA. In B12 deficiency, homocysteine cannot be converted to methionine, and thus, methyl THF cannot be converted to THF. As a result, homocysteine levels accumulate, and pyrimidine bases cannot be formed, slowing down DNA synthesis and causing megaloblastic anemia. The anemia then leads to symptoms such as fatigue and pallor that are commonly seen in patients with B12 deficiency. The impaired DNA synthesis causes problems for other rapidly proliferating cell lines, such as polymorphonuclear leukocytes (PMNs). Thus, B12 deficiency characteristically results in the formation of hyper segmented neutrophils. Vitamin B12 is also used as a cofactor for the enzyme methyl malonyl-CoA mutase, which converts methyl malonyl-CoA to succinyl-CoA. In patients with B12 deficiency, methylmalonic acid (MMA)

levels will accumulate, as it cannot be converted to succinyl-CoA. It is hypothesized that elevated levels of MMA, along with elevated levels of homocysteine, contribute to myelin damage, accounting for the neurologic deficits, such as neuropathy and ataxia, seen in these patients. The damage to the myelin results in a condition known as subacute combined degeneration of the spinal cord (SCDSC). This condition affects various parts of the spinal cord, including the dorsal columns, the lateral corticospinal tracts, and the spinocerebellar tracts, resulting in a loss of proprioception, ataxia, the development of peripheral neuropathy, and dementia (Covalcoli et al.,2017).

8.Metformin

Metformin (N, N-dimethyl biguanide) belongs to the biguanide class of anti-diabetic drugs. These drugs contain 2 linked guanidine rings. The main target tissue of metformin is the liver, and its main effect is the reduction of glucose secretion in the liver due to the inhibition of gluconeogenesis, which leads to a reduction in blood glucose levels (Maruthur et al.,2016).Metformin slows intestinal glucose absorption(Rena et al.,2017).In addition, metformin indirectly increases insulin sensitivity by increasing peripheral glucose utilization (Maruthur et al.,2016).Metformin rarely causes side effects such as hypoglycaemia , hyperinsulinaemia ,Vitamin B12 deficiency ,peripheral neuropathy, or Acidosis, comparing to other antidiabetic drugs (Schulten.,2018).other common side effects of metformin includes loss of appetite, epigastric pain, nausea,and diarrhea (American Diabetes Association.,2018). The US Food and Drug Administration (FDA) approved metformin in 1994 for the treatment of type 2 Diabetes (Schulten.,2018). Currently, metformin is recommended as the first-line treatment for type 2 diabetes (American Diabetes Association,2018).Metformin is mainly absorbed from the small intestine but is excreted unchanged in the urine. The elimination half-life of metformin during multiple dosages in patients with good renal function is approximately 5 hours (Graham et al.,2011).

8.2How can metformin cause malabsorption of vitamin B12?

Metformin can reduce the absorption of vitamin B12 through a mechanism that has not been established clearly(Infante et al.,2021).Until now, several theories describe how metformin prevents the absorption of vitamin B12. These include compromised enterohepatic B12 circulation, increased vitamin B12 hepatic storage, decreased IF production, and decreased intestinal motility with bacterial overgrowth,the most accepted theory is that metformin antagonizes the calcium cation and prevents the calcium-dependent IF-vitamin B12 complex from binding to the ileal cubilin receptor and consequently will reduce the endocytosis process of vitamin B12(Bell.,2022).It is proposed that metformin could give a positive charge to the membrane's surface of cubilin receptor(Di magno et al.,2022).The positively charged receptor will push the divalent calcium cations by repulsion forces. This will lead to vitamin B12 malabsorption because the calcium-dependent binding of the IF-vitamin B12 complex to the ileal cubilin receptor is compromised (Bell.,2022).

9.Megaloblastic anemia

Megaloblastic anemia (MA) encompasses a heterogeneous group of macrocytic anemias characterized by the presence of large red blood cell precursors called megaloblasts in the bone marrow (Wickramasinghe.,2006).This condition is due to impaired DNA synthesis, which inhibits nuclear division. Cytoplasmic maturation, mainly dependent on RNA and protein synthesis, is less impaired. This leads to an asynchronous maturation between the nucleus and cytoplasm of erythroblasts, explaining the large size of the megaloblasts (Green and Datta,2017).The process affects hematopoiesis as well as rapidly renewing tissues such as gastrointestinal cells. Megaloblastic anemia is most often due to hypovitaminosis, specifically vitamin B12 (cobalamin) and folate deficiencies, which are necessary for the synthesis of DNA (Sayar et al.,2020).

Materials and methods

this study was conducted at Layla qasm center for diabetes mellitus diseases in Erbil, Iraq. 35 Patients with 15 Person participate as control group in both male and female with age between (26-49)Years. diabetes mellitus type2 & were selected in both male and female with age between (26-49)Years. After an overnight fast Five ml of whole blood samples were draw in patients and control group, then separated into two parts, the first part 2ml was collected in an EDTA tube and processed for hematological asessment. The second part was transferred to gel tubes and centrifuged at 3000 rpm=round per minute for 15 minute to separate the Serum, then the Serum used for determination of vitamin B12 and glycated hemoglobin HbAlc Serum level.To determine the RBC count, RBC count, Hemoglobin concentration, Packed cell volume level and platelet count by using Coulter counter (Medonic.Sweden). Determination of serum vitamin B12 and HbAlc Level, was done by (Cobas e411.Germany) was used while serum HbAlc level was determined by (BIOLIS30i).

Statistical analysis

Graph pad prism Software vision 9, San Diego CA, USA was used for data analysis, Mean±SEM Calculated P-values 0.05 was considered significant.

Results

Table 1 demonstrated the Mean±SE of White blood cell count, Red blood cell count, hemoglobin concentration, Packed Cell Volume level and platelet Level in male patients with DM type 2. The Value of WBC count, Hb and PCV Level increased non significantly (P>0.05) in patients (8.4755 0.811, 15-725±0727 and 45.633+1981) when compared with control group as value (7.160±0.888, 14.240±0.630 and 41-220±2.116). respectively. Platelet count decreased non significantly in Patients with DM type2 (255.5831-94.59) compared with Control group as value (5.718±0.200), while RBC count increased significantly (P<0.05) in patients as value 5.71840200 compared with control group as value (4.808±0.256).

Table 1: Mean±SE of some hematological parameters in male Patients with DM type2 & and control group.

Group/Parameters	Control	Patient	P.value
WBC 10^9/L	7.160±0.888	8.475±0.811	0.3600 (ns)
RBC 10^{12}/L	4.808±0.256	5.718±0.200	0.0212 (*)
Hb g/dl	14.240±0.630	15.725±0.727	0.2393 (ns)
PCV %	41.220±2.116	45.633±1.981	0.2123 (ns)
PLT 10^9/L	334.000±5.348	255.583±24.59	0.0624 (ns)

Table 2 and figure 1 and 2 showed non significant increase in serum vitamin B_{12} in Patients as value (575-316±106.00) compared with control group as value (334.520±45.011).Serum HbAlc Level increased significantly in patients value (7-448±0.442) compared with control group as value (5-340±0.074).

Table 2: Serum vit. B12 and HbAlc in male patients with DM type 2 and control group.

Group/Parameters	Control	Patient	P.Value
Vitamin B_{12} pg/ml	334.520±45.011	575.316±106.000	0.1764 (ns)
HbA1c %	5.340±0.074	7.448±0.442	0.0088 (**)

According to age character the patients differ with control group and there is a significant difference in figure 3. The value in patients was (41.909±1.048), in control group was (32.400±3.326).

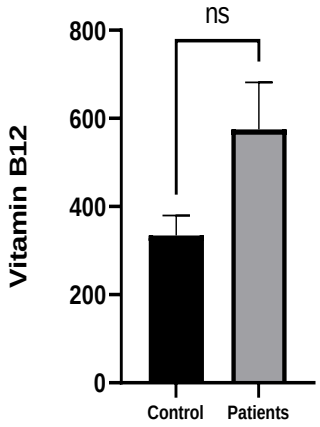

Figure 1: Serum vitamin B12 in male patients with DM type 2 and control group.

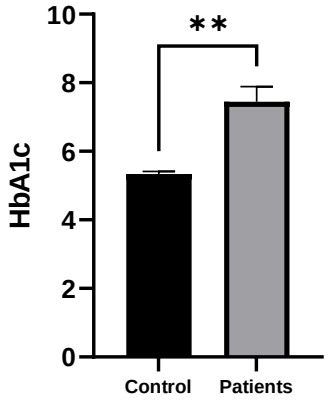

Figure 2: Serum HbA1c in male patients with DM type 2 and control group.

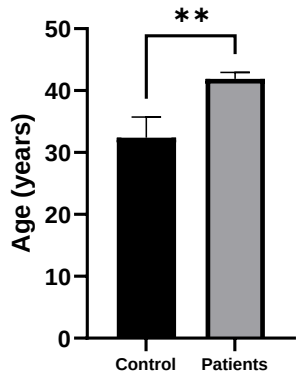

Figure 3: Ages character in male patients with DM type 2 and control group.

Table 3 demonstrated the Mean±SE of White blood cell count, Red blood cell count, hemoglobin concentration, Packed Cell Volume level and platelet Level in female patients with DM type 2.The value of WBC count,Platelet count increase non significantly in patient (8.343±0.434, 293.782±10.570) when compared with control group as value (7.160±0.344,265.800±17.230). respectively RBC count, Hb and PCV level decreased non significantly in patient with DM type 2 (4.656±0.079,13.104±0.414 and 37.739±1.039) compared with control group as value (4.718±0.273,13.110±0.255 and 37.940±0.584).

Table 3: Mean±SE of some hematological parameters in Female Patients with DM type2 & and control group.

Group/Parameters	Control	Patients	P.value
WBC 109/L	7.160±0.344	8.343±0.434	0.1018 (ns)
RBC 1012/L	4.718±0.273	4.656±0.079	0.7779 (ns)
Hb g/dl	13.110±0.255	13.104±0.414	0.9932 (ns)
PCV %	37.940±0.584	37.739±1.039	0.9030 (ns)
PLT 109/L	265.800±17.230	293.782±10.570	0.1640 (ns)

Table 4 and figure 4 and 5 showed significant increase in serum vitamin B12 and HbA1c level in Patients with DM type 2 as value (476.926±41.590, 7.241±0.349).compared with control group as value (307.530±36.740, 5.320±0.055).

Table 4: Serum vit. B12 and HbAlc in female potients with DM type 2 and control group.

Group/Parameters	Control	Patient	P.value
Vitamin B_{12} pg/ml	307.530±36.740	476.926±41.590	0.0182 (*)
HbA1c %	5.320±0.055	7.241±0.349	0.0011 (**)

Figure 6 showed non significant difference between female patients and control group as value (40.700±1.427) in patients and (38.800±1.960) in control group.

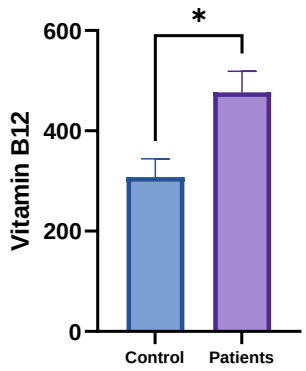

Figure 4: Serum vitamin B12 in female patients with DM type 2 and control group.

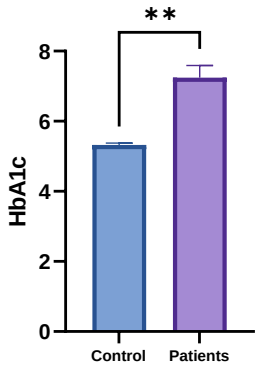

Figure 5: Serum HbA1c in female patients with DM type 2 and control group.

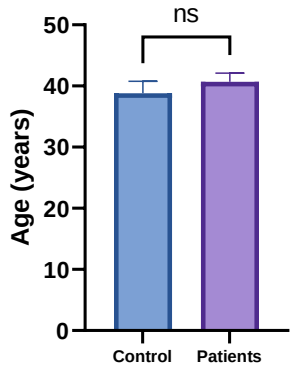

Figure 6: Ages character in female patients with DM type 2 and control group.

13.Discussion

The current study found a significant effect of T2DM on CBC parameters, including RBC count, Hb concentration, Serum Vitamin B12 and HbA1c level compared to healthy controls.The mean RBC count was lower in T2DM patients as compared to the control group, but the difference was not statistically significant. This finding is in coherence with the (Salhen at al.,2017).The possible explanation for decreased RBCs count might be that persistent hyperglycemia causes increased production of reactive oxygen species and nonenzymatic glycosylation of Hb and RBC membrane proteins leading to reduced deformability, increased aggregation, and accelerated aging of RBCs (Abdel and Hamed,2016).These changes in RBCs are also shown to markedly increase blood viscosity that adversely affects the microcirculation in diabetes, leading to microangiopathy (Cho et al .,2008).In contrary to our findings, studies carried out in kurdistan reported higher RBC count and Hb concentration in T2DM of male patients than male control and lower RBC count in female patient with DM than female control.This might be explained by the effect of insulin resistance, which is associated with the stimulation of erythroid progenitors increasing RBC count, and increased levels of Hb Chronic inflammation and increased level of oxidative stress are common in diabetes and they are known to reduce RBCs' survival that results in variation in RBCs size and decreased RBCs count (Sherif et al.,2013).other study showed that patients with T2DM had lower hemoglobin concentrations. Anemia is relatively common in patients with DM, and low hemoglobin concentration may contribute to many clinical aspects of diabetes mellitus or its progression. Low hemoglobin concentration is associated with a more rapid decline in glomerular filtration rate than that of other kidney diseases(Rossing et al.,2004).Hemoglobin concentration is closely associated with diabetic profiles.On logistic regression analysis, the duration of DM was significantly associated with anemia similar to the studies from Nigeria (Awofisoye et al.,2019). Patients who had DM for more than 7 years were 3 times more likely to have anemia compared to those who had DM for 7 years or less. Evidence suggested that the longer the duration of the disease the higher the inflammatory process, resulting in increased IL-6 with anti-erythropoietic effect, causing a decrease in the number of circulating RBCs and consequently causing a reduction of circulating hemoglobin (Sahay et al.,2017).meta-analysis showed that increased WBC corresponds to higher risk of T2DM(7). Nada et al found that higher WBC counts in patients with uncontrolled glycemia (HbA1c >7%)

than those with good glycemic control (HbA1c ≤7) (Nada.,2015).In present study showed that higher RBC count in both male and female patient with DM type 2 compared to the control group.In diabetes, hyperglycemia, dyslipidemia, insulin resistance, and oxidative stress could stimulate the production of pro-inflammatory cytokines, activation of inflammatory signaling pathways, and recruitment of immune cells that can contribute to the elevated level of white blood cells and its sub-population (Joanna et al.m2018).Moreover, Hyperglycemia, insulin resistance, and insulin deficiency contribute to increased platelet reactivity through direct effects via promoting glycation of platelet proteins leading to morphological and functional alteration of platelet indices (Tripura et al.,2016).The high prevalence of vitamin B12 deficiency in patients with T2DM who receive metformin treatment may be attributable to metformin-induced inhibition of calcium-dependent absorption of the intrinsic factor–vitamin B12 complex in the ileum (Khalaf et al.,2019).Therefore, vitamin B12 supplementation is considered an integral component of the routine treatment regimen for T2DM patients, particularly for those who receive prolonged metformin treatment (Parry-Strong.,2016). In contrast our study, showed that serum vitamin B12 higher in patient both male and female compared to the cotrol group, this differences may be due to the sample size of these patients which includes 35 sample, the life style of females and males,geographic situation for each patients, types of treatment, also the chronic disease for each patients and interacting between the activity of type of treatment all of these factors may affect on the level of vitamin B12.

14.Conclusion

This present study concluded that the WBC count, RBC count, Hb concentration, Packed cell volume and Platelet count changed in male and female patients with DM type 2 when compared with control group, furthermore the serum level of vitamin B12 and HbA1c increase in patients in our population.

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