FIRAT UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
TÜRKİYE

SERUM VITAMIN D AMONG PATIENTS WITH TYPE 2 DIABETES MELLITUS

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Master's Thesis
DEPARTMENT OF CHEMISTRY
Biochemistry

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GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
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Department of Chemistry
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THESIS APPROVAL

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DECLARATION

I hereby declare that I wrote this master's degree titled "Serum Vitamin D Among Patients with Type 2 Diabetes Mellitus" in consistent with the thesis writing guide of the Graduate School of Natural and Applied Sciences, Firat University. I also declare that all information in it is correct, that I acted according to scientific ethics in producing and presenting the findings, cited all the references I used, express all institutions or organizations or persons who supported the thesis financially. I have never used the data and information I provide here in order to get a degree in any way.

10 July 2020

Shwan Sulaiman AHMED

PREFACE

Our research is about serum vitamin D in T2DM patients, and the role of vitamin D in calcium and magnesium absorption. In our research we ran into several problems. In beginning many T2DM patients didn't believe us that we will do tests free and send results to him and they said (other researchers take samples from him and did not give him the results). Also we had problem in cobas 6000 analyzer controlling system and we solved the problem with the help of engineering staff in hospital. And I had to enter a course to learn to use HPLC D10 analyzer for measuring HbA1C level. Finally I had a problem in (SPSS) to analyze our results and we solved it with the help of my friend.

First of all, I thank God who gave me the strength and health and facilitated the ways for me to carry on my study.

It is my privilege to express my deepest gratitude and indebtedness to my supervisor Dr AYSEL SARI for her interest, close supervision, constant encouragement and generous devotion of her time and advice, which guided me throughout my study and enable me to complete it.

I would like to express my sincere thanks to the laboratory staff in Duhok Emergency teaching hospital and Amedi child friend hospital where I previously worked on their laboratories machines. And the staff of Duhok Diabetes center especially Dr Idress for their unlimited help and support.

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Shwan Sulaiman AHMED

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ABSTRACT

Serum Vitamin D Among Patients With Type 2 Diabetes Mellitus

Shwan Sulaiman AHMED

Master's Thesis

FIRAT UNIVERSITY
Graduate School of Natural and Applied Sciences

Department of Chemistry
Biochemistry

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Background: Vit D deficiency is a common health problem that increases the risk of Type 2 diabetic mellitus disease T2DM. Serum vitamin D care and the importance of Ca and Mg absorption were investigated. Serum vitamin D, Mg, Ca, cholesterol, triglyceride were evaluated. The role and importance of vitamin D in Ca and Mg absorption was evaluated by looking at vitamin D in patients with diabetic mellitus Type 2.

Materials and Methods: This study was done in the Azadi Education Hospital Endocrine and Diabetes Unit in Duhok, Iraq. This study was measured using 95 subjects, 70 patients with T2DM, HDL, LDL and FBS, auto analyzer Cobas 6000. Glycated hemoglobin (HPLC D10) was made with an auto analyzer device.

Results: Results: In patients with T2DM, serum vit D level was significantly lower than the control group. The relationship between fasting blood sugar and vitamin D and HbA1C was found statistically significant. There was also a significant difference between vit D and Ca and Mg.

Conclusion: As a result of high significant between Vit-D deficiency with increase in fasting blood glucose and HbA1C value of T2DM patient and the role of Vit-D in insulin resistance, T2DM patient should measure their Vit-D value periodically. Vit D is responsible for increasing intestinal absorption of Ca and Mg. It is very important to periodically measure Ca and Mg values in T2DM patients with Vit D deficiency.

Keywords: Vitamin D, Mg, Ca, Serum, Type 2 Diabetes Mellitus

ÖZET

Diyabet Mellitus Tip 2'li Hastalarda Serum Vitamin D'nin İncelenmesi

Shwan Sulaiman AHMED

Yüksek Lisans Tezi

FIRAT ÜNİVERSİTESİ

Fen Bilimleri Enstitüsü

Kimya Anabilim Dalı

Biyokimya

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Amaç: Vit D eksikliği, insulin direncinde etken ve Type 2 diyabetik mellitus hastalığı riskini artıran yaygın bir sağlık sorunudur. T2DM'l olan hastalarda serum D vitamin düzeylerinde bakılarak Ca ve Mg emilimindeki rolü ve önemi araştırtırıldı. Serum D vitamini, Mg, Ca, kolesterol, trigliserit değelendirildi.

Gereç ve Yöntem: Bu çalışma Irak'ın Duhok şehrinde bulunan Azadi Eğitim Hastanesi Endokrin ve Diyabet Ünitesinde yapıldı. Bu çalışma 95 denek, 70'i T2DM hastası, HDL, LDL ve FBS, oto analizör Cobas 6000 kullanılarak ölçüldü. Glikatlı hemoglobin (HPLC D10) oto analizör cihazı ile yapıldı.

Bulgular: T2DM'li hastalarda serum vit D düzeyi kontrol gubundan istatiksel anlamda önemli derecede düşüktü. Açlık kan şekeri ile D vitamini ve HbA1C arasındaki ilişki istatistiksel olarak anlamlı bulundu. Ayrıca vit D ile Ca ve Mg arasında da anlamlılık gözlendi.

Sonuç: Açlık kan şekerinde artışla vit D eksikliği T2DM hastalarında HbA1C değerindeki bu anlamlılık sonucunda T2DM hastalarında Vit D değerinin periyodik olarak ölçmesinin gerekliliği vurgulanmalıdır. Vit D, Ca ve Mg ‘un bağırsak emilimini arttırmaktan sorumludur. Vit D eksikliği olan T2DM hastalarında Ca ve Mg değerlerini periyodik olarak ölçülmesi oldukça önemlidir.

Anahtar Kelimeler: D Vitamini, Mg, Ca, Serum, Tip 2 Diabetes Mellitus.

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ABBREVIATIONS

ADA	American Diabetes Association
ANOVA	One Way Analysis Of Variance
BMI	Body Mass Index
Ca	Calcium
Dl	Deciliter
DM	Diabetes Mellitus
EDTA	Ethylene Diamine Tetra Acetic Acid
FBS	Fasting Blood Sugar
GDM	Gestational Diabetes Mellitus
HbA1c	Glycated Hemoglobin
HDL-C	High Density Lipoprotein Cholesterol
IDF	International Diabetes Federation
IDF	International Diabetes Federation
IR	Insulin Resistance
IS	Insulin Sensitivity
Kg	Kilogram
LDL-C	Low Density Lipoprotein Cholesterol
M	Meter
Mg	Magnesium
Mg	Milligram
N.S	No Significant
PPAR	Peroxisome Proliferative Activated Receptor
(r)	Pearson Correlation Coefficient
SNPs	Single Nucleotide Polymorphisms
SPSS	Statistical Package for the Social Sciences
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus
TG	Triglyceride
U.K	United Kingdom
U.S.A	United States
UV	Ultra Violet
VDR	Vitamin D Receptor
Vit-D	Vitamin D
Vit-D2 ($D_{2)}$	Ergocalciferol
Vit-D3 ($D_{3)}$	Cholecalciferol
WC	Waist Circumference
WHO	World Health Organization

1. INTRODUCTION

Diabetes mellitus is a heterogeneous state described by hyperglycemia. In general there are two main types of diabetes mellitus patients: type one diabetic (T1D), distinguished with other type of diabetes by an acute lack of insulin secretion, and type two diabetic (T2D) where the reason is a blend of insulin resistance (IS) and an insulin secretory imperfection. Diabetes difficulties are increasingly regular between (T2D) patients [1].

T2DM is a metabolic issue introduced by reduces the secretion of insulin from pancreatic beta cells, hyperglycemia, and insulin resistance. The International Diabetes Federation (IDF) estimated that in 2014 diabetes affected 387 million people around the world and that by 2035 it is expected that 592 million people will suffer from diabetes with an increase of 55%. The rise in prevalence in Asia and Africa by 2030 is primarily current. The increase in the prevalence of diabetes is mostly connected with bad lifestyles habits and obesity. Obesity is a main health issue mostly associated with T2D and results in increased mortality and morbidity [2].

Explaining levels of (T2DM) represent a worldwide health problem. Even as changes in diet, obesity levels and physical activity on the foundation of genetic hazard factors come out to be fuelling this epidemic, other environmental variables might be compelling in the improvement of T2DM [3].

The related yearly helpful and rehabilitative expenses of diabetes are onerous. A lot of the wellbeing related consumptions are committed to diabetes and it's complications [4].

Vit-D is a fat-soluble pro-hormone that acting an important character in Ca and Mg homeostasis and the protection of standard function in various tissues. People get Vit-D either legitimately from eating foods that contain Vit-D or from sun light ultraviolet beta radiation exposure. As well as its impact on skeletal health, Vit-D have a possible role in other illness and health conditions like cardiovascular disease, T2DM and another several diseases. A lot of studies have discovered that patients with D2TM are altogether bound to have lower Serum Vit-D level contrasted with those without DM [1].

The vital role of Vit-D has been reported in several non-skeletal diseases like T2DM, cardiovascular disease and another several diseases. Vit-D deficiency is a noteworthy risk factor for the improvement of (IR) insulin resistance and T2DM [5]. There are two main form of vit-D, Vitamin D2 and vitamin D3 and also called (Ergocalciferol and cholecalciferol) respectively. Ergocalciferol is taken from fungi and plant sources but cholecalciferol is obtained under the epidermis on exposure to UV from sunlight and also taken from dietary sources for example, supplement, fish and fortified foods [6].

In liver ergocalciferol and cholecalciferol hydroxylate and convert to 25-hydroxyvitamin D [25(OH)D2 and 25(OH)D3], a main circulating metabolite of Vit-D, It reflects both Vit-D intake

and endogenous product and is utilized to survey Vit-D status[5]. 25(OH)D hydrocylate to 1,25-dihydroxyvitamin D [1.25(OH)2D2 and 1,25(OH)2D3] (active form) in kidney and these active form bind to the Vit-D receptor and apply its biological activities [6]. The defensive role of Vit-D in T2DM is hypothesized because of its impact on Ca metabolism, stimulation of insulin receptor gene, raise the value of Ca in the cell and improving glucose take-up into the muscle [5]. 1,25(OH) 2D the active form of Vit-D increase insulin sensitivity (IS) of insulin-target tissues through-out of nuclear peroxisome proliferative activated receptor (PPAR) and upgrades the biosynthetic limit of β-cells and assists the transformation of pro insulin to insulin. The active form of Vit-D indirectly improves insulin sensitivity (IS) by decreasing adiposity and upgrading muscle mass. The relationship of 25(OH)D with β-cell function in type 2 diabetes mellitus patient and insulin resistance have been reported in several studies [5,7]. Notwithstanding, the fundamental mechanism through which 25(OH)D influences evolution and development of IR and T2DM isn't obviously comprehended in the moderately aged subjects [8].

1.1. Aim of study

The aim of this study is to

1. Evaluate the level of Serum vitamin D in patient with diabetic mellitus type2.
2. Evaluate the role of vitamin D in absorption of Ca.
3. Evaluate the role of vitamin D in absorption of Mg.

1.2. Diabetic mellitus (DM) (Definition)

Diabetes mellitus is a chronic illness that happens either if the pancreas doesn't create adequate amount of insulin or when the body can't adequately utilize the insulin it produces. The blood sugar is regulates by insulin hormone. Increased glucose or hyperglycemia is a typical impact of uncontrolled diabetes and over time leads to genuine harm to a many systems of human body particularly the nerves and veins [6].

8.5% of humans aged 18 years and older had diabetes in 2014. Because of diabetes and high blood sugar there is 2.2 million deaths in 2012 and 1.6 million deaths in 2016 [1].

1.2.1. Type 1 Diabetes Mellitus (T1DM)

T1DM is also known as (insulin dependent or childhood onset) is characterized by lacking insulin creation and requires daily administration of insulin. The reason for type1 diabetes mellitus isn't known and it isn't preventable with current knowledge. Symptoms of (T1DM) include polydipsia (thirst), increase in excretion of urine (polyurea), weight reduction, hunger, fatigue and vision changes [9].

1.2.2. Type 2 diabetes mellitus (T2DM)

T2DM is also known as (adult onset, or non insulin independent) results from the body's inadequate utilization of insulin. T2DM comprises the majorly of population with diabetes in world, and is largely result from obesity and physical inactivity [6].

T2DM symptoms are similar to T1DM symptoms, but are regularly less noticeable. Thus, it may be diagnosed after several years onset, when complications have emerged. As of not long ago, this kind of diabetes was seen uniquely in adult however now it is additionally happening progressively frequently in children [10].

T2DM is a type of diabetes that is described by insulin resistance, high glucose level, and relatively absence of insulin. Regular symptoms include increased hunger, thirst, weight loss, urination, feeling tired and sores [6]. Often symptoms appear slowly. Long term complications from high glucose level incorporate coronary disease, kidney failure, strokes, and amputations. Because of poor blood flow in the limp and diabetic retinopathy which can result in blindness. The abrupt beginning of hyperosmolar hyperglycemic condition may happen; but ketoacidosis case is not common [3].

Symptoms

Improved thirst (polydipsia), urination (polyurea), weight loss, improved hunger, itchiness, recurrent vaginal infections, blurred vision, recurrent vaginal infections, peripheral neuropathy, fatigue, and loss of taste. During the first several years many people have no symptoms and they are diagnosed on routine testing. Hyperosmolar hyperglycemic state may occur only in a small number of patients with T2DM [11].

Causes

Lifestyle and genetic factors are main causes of type 2 diabetes mellitus. People can control some of factors such as obesity and diet, but another factors such as genetics, age and female gender are out of personal control [12].

Lifestyle

Lifestyle factors including obesity, being overweight (BMI more than25), poor diet, physical inactivity, stress, and urbanization are important factors in the development of type2 diabetes mellitus [13]. Dietary factors additionally impact the risk of creating T2DM. Utilization of sugar in overabundance is relate with an increase in risk [14, 15]. The sort of fat in the eating

routine are significant, with trans fatty acid or saturated fats increasing the risk, But monounsaturated and polyunsaturated fat diminishing risk [12]. White rise have a main role in increasing risk when it is consumed excessively [16]. Few minutes of activity as believed to cause 7% of cases [17].

Genetics

Nearly all cases of DM include numerous genes, by means of each being a little supporter of an increased likelihood of becoming T2DM. The extent of diabetes that is inherited is assessed at 72% [18] . More than 80 single nucleotide polymorphisms (SNPs) and 36 genes that contribute type 2 diabetes mellitus risk were discovered. These all genes collectively just record for only (10%) of the absolute heritable segment of the T2DM Disease [19].

Medical conditions

Various medical conditions and another health problem can incline to diabetes. A portion of mediations for example: thiazides, statins, beta blockers, glucocorticoids, and psychotics. The individuals that have gestational diabetes recently run a higher risk of creating T2DM [11]. Several health problems are related with T2DM such as: Cushing's syndrome, acromegaly, hyperthyroidism, some cancers for example; glucagonomas and pheochromocytoma. Cancer's patient risk of mortality might be higher in the event that they likewise have diabetes [20]. Type 2 diabetes mellitus also may cause testosterone deficiency [21, 22]. Dietary problems may likewise interact with T2DM. The risk increases with bulimia nervosa and the anorexia nervosa decreases [23].

Pathophysiology of T2DM

T2DM is because of lacking insulin creation from beta cell in the situation of insulin resistance,IR, which is cell's inability to respond appropriately to standard insulin levels, happens principally inside the liver, fat tissue, and inside the muscles.

Inside liver, normally insulin stifles glucose release, other than in the situation of insulin resistance; liver releases glucose in to blood incorrectly. The extent of insulin resistance (IR) versus beta cell dysfunction contrasts between peoples, with a little having fundamentally insulin resistance (IR) and just a small imperfection in insulin secretion, and other with minor insulin resistance (IR) and mainly an absence insulin secretion [13].

Another possibly significant mechanism related with T2DM and insulin resistance (IR) include: improved breakdown of lipid within fat cell, high value of blood glucagon, increased

maintenance of water and salt by kidneys, And inappropriate central nervous system metabolism regulation [10]. However, not every individuals who have insulin resistance create diabetes, as weakness of insulin secretion by pancreatic beta cells is additionally needed [13].

Diagnosis

Both types of diabetes mellitus was defined by (WHO) as a single raise glucose reading with symptoms; Plasma glucose in fasting equal or more than 126mg/dl. A blood glucose in random more than 200 mg/dl in involvement with representative symptoms, or a glycated hemoglobin (HbA1C) ≥6.5 % is another way of diagnosing diabetes [11].

The extent to which diabetes is diagnosed depends on the relation among glucose tolerance test results, fasting glucose or HbA1C and intricacy, for example retinal problem. A random or fasting blood glucose is favored more than the glucose tolerance test (GTT), for the reason that (FBS and RBS) are more available and easier to do for individuals. HbA1C had the focal points that fasting isn't necessary and the result is stable more than (FBS and RBS) however had the drawback that the test is more expensive than blood sugar measurement [24]. In United state it is assessed that 20% of individuals that had diabetes don't know that they have DM disease [10].

T2DM is described by high level of glucose in blood with regards to insulin resistance and proportional insulin deficiency. Both types of diabetes can commonly be recognized depending on the presenting conditions. In the event that the diagnosis is uncertain, the test of antibody might be valuable to affirm T1DM and the level of C-peptide might be helpful to affirm T2DM, in T2DM the level of C-peptide is normal or high, but in T1DM the level is low [25].

Preventation:

Beginning of T2DM be able to postponed or avoided via adequate diet and normal exercises [26, 27]. The risk of diabetes might be decrease over half by heavy lifestyle actions [28]. The advantage of physical activity occurs not considering of the individual's initial weight or ensuing weight loss [29]. The risk of DM can be decrease about 28% by physical activity in high levels [30]. Proof to serve dietary changes alone, be that as it may, is limited [31], With some proof for an eating routine contain high amount of green vegetables and some for constraining the admission of sweet drink [32, 33]. There really is a relationship between increased admission of sugar sweetened fruit juice and diabetes, yet no proof of a relationship with 100% fruit juice [34]. A review in 2019 discovered proof of advantage from nutritional fiber [35].

Management

Lifestyle

Diabetes care establishments are an appropriate healthy diet and exercise [11]. Better result is yielding with a greater amount of exercise [36]. Exercise improve glucose control, decrease blood lipid levels, decreases body fat content, and these impact are apparent even without weight loss [31]. Decreasing HbA1C and improving insulin resistance occurs when aerobic exercise is done. Resistance preparation is likewise helpful and the exercises might be more effective when human combine two types of it [37]. A diabetic eating routine which incorporates the calorie limitation to advance weight loss is commonly suggested [38]. Other suggestions incorporate affirming admission of vegetables, fruits, decreased saturated fat, dairy products that contain low-fat value, and with a macronutrient consumption followed to the person, carbohydrates and calories were convey during the day [38, 39].

Medication of T2DM

Just a few types of anti diabetic drugs are obtainable. Metformin is by and large suggested as a more common treatment worldwide, there is some proof that metformin diminishes death [40, 41]. In any case, the result is being challenged [42]. Metformin shouldn’t being utilized in those patients that have diseases in kidney or liver [11].

A second oral drug of another class or insulin might be added if metformin isn’t adequate after three months

Insulin or another types of drugs might be used if metformin isn’t adequate after four months of using [43]. Further classed of anti-diabetic drugs such as: Dipeptidyl peptidase-4 inhibitors, sulfonylureas, SGLT2 inhibitors, glucagon like peptide-1 analogs, and thiazolidiones [43].

Epidemiology

In 2015 statistical of diabetes 90% of diabetes cases had Type 2 diabetes mellitus and their number are more than 392 million of people. Identically this is about 6% of the world's peoples. Diabetes mellitus is popular in the developing as well as in the developed population. It stays unpopular ,notwithstanding, in the developed world [44].

1.3. Vitamin D

Vit-D is a gathering of fat-dissolvable secosteroids answerable for raising absorption of Mg, phosphate, and Ca, in intestine, and a number of additional biological effects [45].

1.3.1. Vitamin D definition

Vit-D3 and Vit-D2 also known as (cholecalciferol and ergocalciferol) respectively are most common and essential types of Vit-D in human body [46].

The significant source of Vit-D is production of cholecalciferol in the deeper epidermis layers of the skin by chemical process depend on UV of sunlight [47].

By the ingestion of ergocalciferol and cholecalciferol from the food and from supplement humans can increase Vit-D level [46, 48].

Just a several foods, such as the flesh fatty fish meat, and cod liver oil naturally contain large quantities of Vit-D. In many countries around the world, Vitamin D fortifies milk of cow and soy milk, as do other breakfast cereals. Ultra violet light exposed mushrooms contribute valuable amounts of Vit-D [49].

Dietary guidelines usually suggest that total amount of a human's Vit-D is better and safer to taken by mouse because sun exposure is unpredictable between inhabitant sand guidance for how much sun exposure is healthy remain unclear considering the risk of skin cancer [49].

Vitamin D from food sources, or from synthesis in the skin, is inactive biologically. To convert it to the active form a protein enzyme must hydrocylate it. This occurs in the kidney and the liver, since most mammals exposed to sufficient sunlight will synthesize Vit-D in adequate quantities, it isn't a critical dietary factor, even though it isn't exactly a vitamin. Rather Vit-D can be consider as a hormone, and actuation of the pro-hormone (Vit-D) bringing about active form, calcitirol, which at the point creates impacts by means of a nuclear receptor in multiple locations [48].

Inside liver calcifediol (25-hydroxycholecalciferol) produced from cholecalciferol, and (25-hydroxyergocalciferol) is produced from ergocalciferol. These structures known as (25-hydroxyvitamin D or 25(OH)D) is used to determine level of serumVit-D status of human's [50]. The biologically active form of Vit-D calcitriol (1,25-dihydroxyholecalciferol) is produced from hydroxylation of calcifediol in kidneys. Calcitriol circulates in the blood as a hormone, playing a significant function in controlling phosphate and Ca concentration, encouraging healthy growth and bone renewing. Even, calcitriol had another effects, such as those on cell formation, neuro muscular, reducing inflammation, and immune functions [51].

1.3.2. Types of Vitamin D:

Vit-D3 and Vit-D2 also known as (cholecalciferol and ergocalciferol) respectively are two main form of Vit-D in humans, but generally there are several form of Vit-D such as; Vit-D1(a mixture of compounds of lumisterol and ergocalciferol), Vit-D4 (22-dihydroxyergocalciferol), and Vit-D5 (sitocalciferol).

Vitamin D without a subscription is either both or each one of D2 and D3, collectively these two are known as calciferol. In 1931, Vit-D2 has been characterized chemically. In 1935, the chemical formation of Vit-D3 has been identified and demonstrated to be the product of UV radiation of (7-dehydrocholesterol). Secosteroids is a various chemical form of Vit-D, such as; steroids which break one of the steroid ring bond. Main difference in structure between Vit-D2 and Vit-D3 is the Vit-D2 have a double bond in side chain between carbon 22 and carbon 23, also contain methyl group in carbon24 [52].

1.3.3. Source of vitamin D

Vit-D2 was generally found in the Fungi, and Vit-D3 in animal sources [53].

1.3.3.1. Natural sources

Vitamin D2 is created by ergosterol UV irradiation, Main source of ergosterol is in numerous fungi (Table 1.1) [54].

Table 1.1. Natural source of Vit-D

Foods	Content (IU/100gm)
Breastfeeding woman milk	2.5-3
Standard milk formulae	40-60
Egg yolk	125
Mushroom	75
Chicken liver	50
Sea fish	200-650
Shells	320
Cod liver oil	8400

Food fortification

Produced foods fortified with Vit-D include: meal replacement energy bars, some juices and juice drinks, certain cheese and cheese products, many breakfast cereals, soy protein-based beverages, flour products, milk and infant formulas [55, 56].

Supplements

Vitamin D supplementation is a safe approach used to avoid or treat rickets. Vit-D supplementation impacts on non-skeletal health are unsure [57]. A 2013 review found no effect on non-skeletal illness levels from supplementation, other than a slight reduction in mortality among older people. Supplementations with Vit-D don't actually change the outcomes of stroke or vascular disease, myocardial infarction, cancer, and bone fractures [58].

1.3.4. Daily requirement

There is deference in daily requirement between humans, pregnant woman and elderly require more amount of Vit-D than others (Table 1.2) [54].

Table 1.2. Daily requirements of Vit-D

Age	RDA/day
0-12months	400IU….10mcg
1-13years	600IU….15mcg
14-18years	600IU….15mcg
19-50years	600IU….15mcg
51-70years	600IU….15mcg
>71years	800IU….20mcg

1.3.5. Vitamin D biology

Calcitiriol the active metabolite form of Vit-D mediate its biological impacts by binding to the Vit-D receptor (VDR), Vit-D receptor is located mainly in the nuclei of target cells. Calcitriol binding to (VDR) allows (VDR) to act as a transcription factor that tweaks the gene expression of transportation proteins (for example calbindin), associated with intestinal absorption of Ca. The (VDR) corresponds to steroid/thyroid hormone receptor super family of nuclear receptors and

VDRs are represented by very many organs of the cell, such as the heart, brain, gonads, skin, breast, and prostate [59].

Activation of Vit-D receptor in bone cells, intestine, kidneys and P-thyroid gland lead to the construction of phosphorus and Ca value in human's blood (with the help of calcitonin and P-thyroid hormone) and also have a role in the protection of bone contents [45].

1.3.6. Action mechanism

Vit-D has been transported through bloodstream to inside liver, where even it is transformed to pro-hormone (calcifediol).

Metabolic activation

In the kidneys, circulating calcifediol can after that been transformed in to the biological active structure of Vit-D (calcitriol) [60]. Either it is produced in the skin or taken by ingestion, at location 25 (upper right of the molecule), Inside liver Vit-D is hydroxylated to the structure 25-hydrocycholecalciferol(25(OH)D or calcifedion) (Fig 1.1) [61]. The microsomal enzyme vitamin D 25-hydrocylase catalyze this reaction, to produce (CYP2R1) human gene, and with liver cells articulated [62]. On one occasion produced, product was created in to the plasma, in which it has been bound to α-globulin transported protein called Vit-D-binding protein [61].

After transporting to the kidney proximal tubules, calcifidiol, hydroxylatedby 25-hydroxyvitaminD31-alpha-hydroxylase enzyme as catalyzes at the 1- α position to produce calcitriol (1,25-dihedroxycholecalciferol, 1,25(OH)2D). 25-hydroxyvitaminD31-alpha-hydroxylase enzyme is produced by human gene CYP27B1.CYP27B1 and it will be more active by parathyroid hormone, as well as by decrease in value of phosphate and Ca. Calcitriol is released into circulation in the final conversion stage in renal. Calcitriol has been transmitted across human body by a bound with Vit-D-binding protein, Such as for the classic main organ of intestine, bone, and kidney **(**Figure 1.2**)**. The vitamin D receptor's (Calcitriol) most potent natural ligand which mediated most of vitamin D physiological actions [48, 60].

Besides the renal, calcitriol is however produced by other a lot of cells in the immune system including monocyte macrophages. Once mono cytemacrophages are synthesized, calcitriol functions on the place as a cytokine and modulates organs responses against microbial pathogens by activating the innate immune system [61].

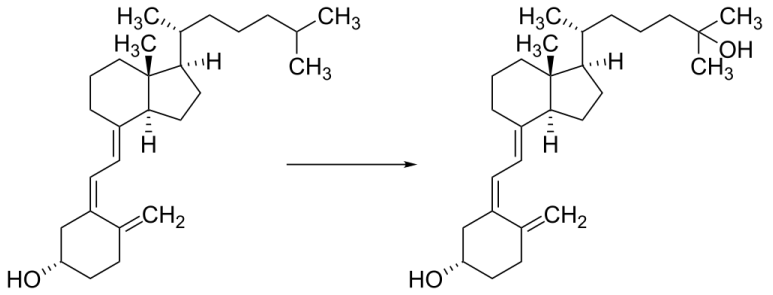

Figure 1.1. Hydroxylation of cholecalciferol to calcifediol in liver

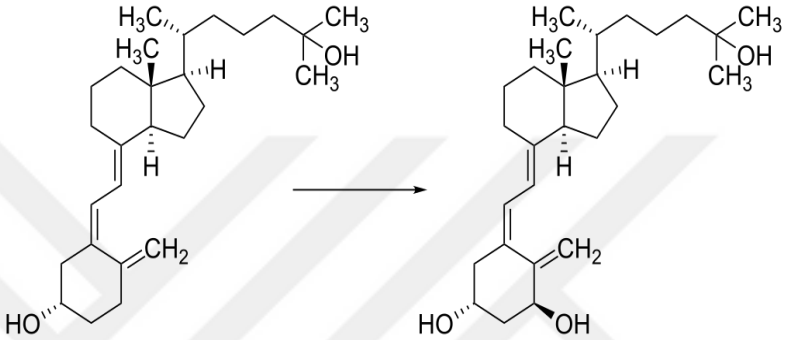

Figure 1.2. Hydroxylation of calcifediol to calcitriol in kidney

Inactivation

Hydroxylation at position 24 can reduce the activity of calcifediol and calcitriol by Vit-D3 24-hydrocylase, producing inactive form of secalciferol and calcitetrol, in that order [61].

1.3.7. Diagnoses

Usually, The 25(OH)D serum concentration has been used to assess the Vit-D form. Nearly every Vit-D in the serum was transformed to 25(OH)D, which gives an accurate image of vitamin D state. Serum level 1,25(OH)D is characteristically not use to assess the form of Vit-D as it's also controlled by certain hormones in human body for example; the parathyroid hormone.
The levels of 1,25(OH)D may stay normal yet if the human might be deficient in Vit-D.25(OH)D level in human serum is the laboratorical test ordained to decide if a human has a insufficiency or deficiency of vitamin D [63].

1.3.8. Vitamin D deficiency

Vit-D deficiency is also known as (hypo vitaminosis D), is mainly caused because of in sufficient sunlight exposure [54]. Vit-D deficiency be able to cause by insufficient dietary eating

of Vit-D, vitamin D decreases disorders, and factors that hinder Vit-D's transformation into active metabolites include the kidney, liver, and hereditary disorders [64]. Vitamin D insufficiency or deficiency affects bone mineralizing, resulting in bones disease including children rickets. Vit-D deficiency also be able to contribute to increased risk of osteomalacia and osteoporosis in adults[54, 64]. Weakness of muscles also has been a popular condition of deficiency of Vit-D and additionally raises the hazard of adult falling as well as bones fracture [54].

There is one billion human around the world are either vitamin D insufficient or deficient [45].

Ranges of Vit-D in Human body:

- Deficiency <20 ng/ml
- Insufficient between 20 to 29 ng/ml
- Normal 30 to100ng/ml
- Toxic>100 ng/ml. [45]

1.3.9. Risk factor

Age:

Older human have higher hazards of Vit-D deficiency because of combined some risk factors include: reduced dietary eating of Vit-D, reduced exposure to sunlight, and decreased skin thickness resulting in further decreased sunlight UV absorption [65].

Fat percentage

Because Vit-D3 and Vit-D2 are soluble in fat, some fat needs to be stored by human and other animals with a skeleton. In the cases of lack of fat, it is difficult to humans to absorb Vit-D2 and Vit-D3 and the relatively low ratio of fat, they increase the hazard of vit-D deficiency, and that would be valid in athletes that are trying hard to become as lean as probable [66].

Malnutrition

Osteomalacia cases in several immigrant communities involved women with apparently sufficient sunlight exposure outside wear traditional western cloths [66]. Humans that have darker skin and less access to sunlight didn't cause rickets and when the nutrition deviate from a western omnivorous lifestyle described by high intakes of fish, meat, and eggs and low intakes of high-extraction cereals [67]. Vit-D deficiency was due to reduced intake of Ca in the eating routine in sunny countries where wickets occur among older infants and children. That is characterization of

diets depend on cereals with inadequate access to diary product [67]. Decreases in the cases of rickets in USA is because of increase in the amount of animal protein in nutrition and increase in utilization of Vit-D fortified products such as milk and juices [45] A study of kids in Uganda anyway indicated that there is no critical differentiation in Vit-D level of malnourished kids contrasted with non-malnourished kids. Since two gatherings have been at risk because of dark skin pigmentation, the two gatherings have been Vit-D deficiency [68].

Obesity

Human who regarded the classification as overweight or obese depended on certain body mass index determining are at increased risk for Vit-D deficiency [69]. The relation among those cases is incomprehensible. There have been various agents that may participate to this relation such as exposure to sunlight and particular diet [69].

Sun exposure

Covering the skin by using sunscreen will decrease the development of Vit-D in human skin to more than 95% [45, 70]. Rather, wearing clothes has been more protective at decreasing the portion of skin exposed to UVB and in increasing the production of natural Vit-D. When worn consistently and regularly, clothing that hides a substantial part of human's skin has been connected with lower value of Vit-D and a raised popularity of hypo vitaminosis D [71].

Countries that far from equator have been higher seasonal variation in the amount and intensity of sunlight. In UK, lower Vit-D occurrence is found to be higher in children and adolescents in winter rather than in summer. Another factor play a significant role in Vit-D deficiency like indoor versus, outdoor work, and outdoor recreational time [72].

Darker color in skin:

The diminished pigment of light-skinned population may cause increase in Vit-D levels and since melanin works such as a sun-block, particularly dark-skinned individuals might be need more Vit-D to prevent deficiency at higher attitudes [64].

Malabsorption:

Levels of vitamin D deficiency are higher among people with untreated celiac disease [73, 74]. Inflammatory intestinal disease, exocrine cystic fibrosis pancreatic insufficiency, and short bowel syndrome [74]. Which can all cause malabsorption problems. Vit-D deficiency is also

much more popular after surgical procedures that decrease absorption from the intestine, such as losing weight procedures [75].

Critical illness:

Deficiency of Vit-D is released to increased mortality in critical disease. Those who take Vit-D supplement before they are admitted for intensive care will die less than human who don't take Vit-D supplement [76] . In addition, Vit-D levels decrease during intensive care stays. Vitamin D3 (cholecalciferol) or calcitriol orally administered may reduce mortality without major harmful effects [77].

1.3.10. Effect of vitamin D deficiency

Rickets, a childhood illness, is described by obstructed growth and soft, weak, disfigured long bones that twist and bow under their weight as kids begin walking.

Rickets

Rickets occur usually between the ages of 3 to 18 months. In North American and other Western countries, cases continue to be reported and are seen primarily in breastfed infants and those with darker skin complexions [71]. This disease is described by bow legs, which could be caused by deficiencies of Ca and phosphorus, as a lack of Vit-D [78].

In most countries, Vit-D deficiency remains the main cause of rickets among young children as breast milk is low in Vit-D and social customs and environmental effects can inhibit sufficient sun exposure [79].

Vit-D fortified milk, infant Vit-D supplement, and Vit-D supplements in Canada and United States have assisted with annihilating most of instances of rickets for youngsters with fat malabsorption conditions [78].

Osteoporosis and Osteomalacia

Osteomalacia is a condition caused by a Vit-D deficiency in adults. Characteristics of this condition are bone softening, leading to spine folding, leg bowing, bone fragility, proximal muscle weakening and high risk of fractures. Osteomalacia decreases the absorption of Ca and increases bone Ca loss which raises the risk of bone fractures. Osteomalacia typically occurs when levels of 25-hydroxyvitamin D are below about 10 ng/ml. Though it's believed that the symptoms of osteomalacia lead to chronic musculoskeletal pains [80].

Skin pigmentation:

It has been shown that dark-skin humans residing in temperature climates have low levels of Vit-D, but the meaning of this is not clear [81,82]. Dark-skinned human are much less effective at producing Vit-D, because melanin impedes the production of Vit-D [83]. Lack in Vit-D levels is more popular in the USA among Hispanics as well as African-Americans, with rates falling dramatically in the winter. This is because of the amounts of melanin in their bodies, since it serves as a natural protection against sun exposure [84].

Diabetes and insulin resistance

Vit-D tends to specifically stimulate the production of insulin in b-pancreatic cells via the nuclear Vit-D receptor (VDR), as well as Vit-D helps to minimize inflammation. Which is a major process in inducting insulin resistance [1,10].

1.3.11. Treatment of vitamin D deficiency

Treatment of deficiency of Vit-D depends on the extent of the deficit [85]. Treating require an initial high-dosage treatment process before achieving the appropriate serum levels, following by sustaining the acquired levels. The lower concentration of the serum 25(OH)D before treating, the higher is the dose needed to achieve an appropriate serum level of Vit-D quickly [85]. The initial high-dose treatment can be taken daily or weekly or in one or more single doses [86].

The treatments for therapy differ, and there is still no agreement about how best to reach an optimal serum level of Vit-D. Whilst there is proof that vitamin D3 raised blood value of 25(OH) D greater than vitamin D2 [80]. Another research indicates that D2 and D3 are equal for The 25(OH)D status [85].

1.4. Ca in Human body

Ca is vital-needed mineral. Ca helps human blood to clot, human muscles to contract, and human heart to beat, in addition to building bones and keeping them in good health. Around 99% of human body's Ca is in bones and teeth [87].

Humans lose Ca every day by human skin, hair, nails, urine, feces and sweat. Therefore, human body can't generate their own Ca, it's vital that humans get enough calcium from their food. If humans don't get the Ca that their body needs it will be taken from their bones. This is fine every now and then, but if it occurs too often, the bones become weaker and easier to break [87].

By Vit-D dependent and Vit-D independent mechanisms Ca is absorbed in the intestine [87].

Ca normal range in human body is between (8.5 to 10.5) mg/dl. The daily requirement of body for Ca is between 1000 to 1200 mg/day [88].

1.5. Mg in human body

For more than 300 biochemical reactions within the body is required to Mg. It help keep up normal functioning of the nerve and muscle, keeps the heart beat steady, help bones stay strong and support a healthy immune system. It's also adjusting blood glucose levels. It helps in energy and protein production [88].

Mg's role in the prevention and management of disorders like heart disease, high blood pressure and diabetes is being investigated. But it's not actually recommended to take Mg supplements. Diets high in protein, Ca or Vit-D will make Mg more needed. Mg normal range in human body is between (1.7 to 2.5) mg/dl [89].

2. MATERIALS AND METHOD

This cross segment research was carried out at Endocrine and Diabetes unit at Azadi Teaching Hospital, and at Emergency Teaching Hospital in the Duhok city in Iraq. The study spanned the period from September 2019 to September 2020 using convenient sampling techniques.

2.1. Subjects and study design

A total number of 70 of diabetic outpatients visiting the endocrine and diabetic unit were selected. Based on the diabetes record for each diabetic patient, the following conditions were excluded: Chronic liver diseases patients, Chronic kidney Diseases patients, Patients with thyroidism diseases, Patients who are taking folic acid and multivitamin (A – Z) or vitamin B12 and iron, patients who are taking Ca and Mg supplements, patients who are taking insulin, Pregnant women, Inflammatory conditions, and T1DM patients. In this study we take 150 subjects, 100 subjects are patients in diabetes mellitus type 2 (case group). And another 25 individual healthy (control group) were recruited by personal request from the staff of Azadi Teaching Hospital and Emergency Teaching Hospital in Duhok city, the blood samples will be collected after an overnight fast for the test.

The study group ages were between 35 and 75 years. Informed consent was obtained from each participant commenced before they study and the recruited one were permitted by the Ethics Committee of the Directorate of Health, of Duhok city Governate.

A meeting interview was conducted for filling a questionnaire form that was designed to match the study needs. The questionnaire form was similar to the diabetic clinic questions at the Endocrine and Diabetic Unit with some modifications.

The questionnaire form includes questions such as : name, age, sex, smoking habit, type of drugs taken for diabetes mellitus (only for patients), patient family history in diabetes, physical activity, duration of diabetes, and minerals taking (Ca, Mg), with measurement waist circumference and calculation of body mass index.

2.2. Assessment of studied variables

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2.2.1. Ages groups

The patients were classified according to their age into two groups:

1. Less than 40 years.

2. Equal to, or more than 40 years.

2.2.2. Family history of diabetes mellitus

Patients were classified according to the family history of the disease into two groups:

1. Negative family history of diabetes mellitus.
2. Positive family history of diabetes mellitus.

2.2.3. Duration of diabetes mellitus

Patients were classified depending on the duration of diabetes into two groups:

1. Equal to or less than 5 years.
2. More than 5 years.

2.2.4. Physical activity

According to physical activity patients were classified into two groups:

1. Inert physical activity when the patient did not exercise or for less than 15 minutes daily.
2. Active physical activity when the patient did exercise for 15 minutes or more daily.

2.2.5. Body mass index (BMI)

BMI was determined for each participant by calculating the weight in kilograms (Kg) divided by the height square in meter [90]

1. BMI from 18.5 to 24.9 kg/m^2 was considered normal weight.
2. BMI from 25 to 29.9 kg/m^2 was considered overweight.
3. BMI equal or more than 30 kg/m^2were considered obese.

BMI uses heights and weights to assess an adult fall within in the underweight, healthy weight category, Overweight, or obese. BMI= weight (kg)/$Height^2$ m^2

2.2.6. Smoking habit

Depended on the smoking habits, the patients were classified into two groups:

1. Non-smoker, when no cigarette was smoked daily.
2. Smoker (either light or heavy smoker) when more than ten cigarette were smoked daily.

2.2.7. Diabetes status (Glycemic control and fasting blood sugar)

For glycemic control, Glycated Hemoglobin was measured for all diabetic patient:

1. HbA1c less than 6.5% was considered good control.
2. HbA1c equal to, or more than 6.5% was considered fair control.

For fasting blood sugar,

1. FBS equal to or less than 110mg/dl was considered as normal.
2. FBS more than 110 mg/dl was considered as high level.

2.2.8. Lipid profile status

1. Total serum cholesterol

Cut points of lipids were done based on national cholesterol education program guidelines below:

1. Total serum cholesterol less than 200 mg/dl was considered as normal.
2. Total serum Cholesterol equal to or more than 200 mg/dl was considered as hypercholesterolemia.

2. Serum triglyceride (S.TG)

1. S.TG less than 150 mg/dl was considered as normal.
2. S.TG equal, and more150 mg/dl was considered as high.

3. Serum high density lipoprotein-cholesterol (S.HDL-C).

1. S.HDL-C less than 40 mg/dl was considered as low level.
2. S.HDL-C from 40 to 60 mg/dl was considered as normal level.
3. S.HDL-C more than 60 mg/dl was considered as high level.

4. Serum low density lipoprotein-cholesterol (S.LDL.C)

1. S.LDL-C less than 130 mg/dl was considered as optimal.
2. S.LDL-C equal, and more than 130 mg/dl was considered as high risk factor.

2.2.9. Serum Mg level

1. Serum Mg levels less or w\equal to 1.7 mg/dl was considered as low level.
2. Serum Mg level between 1.7-2.5 mg/dl was considered as normal level.
3. Serum Mg level > 2.5 mg/dl was considered as high level.

2.2.10. Serum vitamin D level

1. Serum Vitamin D<10ng/ml was considered as deficient.

2. Serum Vitamin D between10and 30ng/dl was considered as insufficient.

3. Serum vitamin D >30ng/ml was considered as normal range.

2.2.11. Serum Ca Level:

1. Serum Ca level <8.6 mg/dl considered as low level.
2. Serum Ca level between 8.6 and 10.2 mg/dl was considered as normal level.
3. Serum Ca level >10.2 was considered as high level.

2.3. Methods

70 blood samples were collected from T2DM patients diagnosed according to the WHO protocol.25 blood sample were also collected from apparently healthy individuals (control group).

2.3.1. Collection and processing of blood sample

Participants who attended at the endocrine and diabetic unit in the morning were fasting overnight for 12 to 14 hours. Vinous blood samples (6ml) were collected between 8:30-11:30 AM, withdrawn by picture using vacutainer from the antecubital vein. Two ml were collected immediately into a vacuum tube containing K3 EDTA as anticoagulant for estimation of HbA1c .the remainder 4 ml was collected in vacutainer system gel separator tubes.

The serum was separated from the whole blood after clotting using centrifugation process (HITACHI centrifuge, model O5P-21) at 6000 rpm for 8 minutes, the serum was processed immediately for measuring glucose, total cholesterol, triglycerides, HDL, LDL, Mg, Ca and vitamin D.

2.3.2. Biochemical analysis

Most of the tests were performed by the Cobas c 311 and cobas6000 devices for laboratory analyze, and here we review all these tests:

a. Fasting blood sugar

The sugar level is detected by using Glucose HK Gen.3 300 tests Roche (Hitachi) cobas c311, cobas c501/502 with reference no. 04404483 190.

b. Cholesterol

The cholesterol level is detected by using Cholesterol Gen.2 400 tests Roche (Hitachi) cobas c311, cobas c501/502 with reference no. 03039773 190.

c. Triglyceride

The Triglyceride level is detected by using Triglyceride. 250 tests Roche (Hitachi) cobas c311, cobas c 501/502 with reference no. 20767107 322.

d. HDL

The HDL level is detected by using tests Roche (Hitachi) cobas c311, cobas c 501/502 with reference no. 07528566 190.

e. LDL

The LDL level is detected by using tests Roche (Hitachi) cobas c311, cobas c 501/502 with reference no.07005717 190.

f. Mg

The Mg level is detected by using Mg Gen.2 250 tests Roche (Hitachi) cobas c311, cobas c 501/502 with reference no. 06481647 190.

g. Ca

The Ca level is detected by using Ca Gen.2 300 tests Roche (Hitachi) cobas c311, cobas c 501/502 with reference no. 05061482 190.

(www.roche.com 2013-10, V 3.0 English)

h. Vitamin D

The detection of serum vitamin D3 is performed by using Vitamin D3 total 25-Hydroxy vitamin D3 kit (Roche) with reference number 05894913 190 and modular analytics cobas e 601, cobas e 602 and E170 cobas e 411.

i. HbA1c

Determination of HbA1c level was done by (HPLC D10) auto analyzer device.

2.4. Statistical analysis

Statistical package for social science program (SPSS) version (23) was used to analyzing our data. In comparison between proportions we were used T-test. A P-value of ≤ 0.05 was considered statistically significant, while P-value was 0.01 is considered statistically as a highly significant. In comparison among more than two groups one way ANOVA was used. The correlation coefficient between study parameters was determined by using the pearson correlation.

3. RESULTS AND DISCUSSION

In this study we compare 70 T2DM patients with 25 healthy control persons. The study participants were 46 males and 49 females.

3.1. General characteristic of studied participants for frequency distribution

The study includes 79 subjects 83.2% of whom non-smokers, and out of 70 subjects, 73.7% did not do exercise in comparison with 16 smokers 16.8% and 25 physically active subjects 26.3%. The age of 17 persons 17.9% are equal or less than 40 years old and 78 persons 82.1% are more than 40 years old. 32 patients 33.7% have duration of diabetes equal or less than 5 years, 38 patients 40% have duration of diabetes more than 5 years. 25 persons 26.3% have no duration (controls). BMI of 16 persons 16.8% are less than 25 kg/m^2, 31 persons 32.6% are between 25 to 29.9 kg/m^2 and 48 persons 50.6% are more than 30 kg/m^2 (Table 3.1).

Table 3.1. General characteristic of studied participants for frequency distribution

Subject characteristics (n=95)		Frequency Distribution	
		Frequency (n)	Percentage (%)
Subject categorizes	Control	25	26.3
	Diabetic	70	73.7
Age (years)			
	≤40 years	17	17.9
	>40 years	78	82.1
Gender	Male	46	48.4
	Female	49	51.6
BMI			
	<25kg/m^2	16	16.8
	25-29.9 kg/m^2	31	32.6
	>30 kg/m^2	48	50.6
Duration of diabetes (years)			
	≤5 years	32	33.7
	>5 years	38	40.0
No Duration(control)		25	26.3
Family history of diabetes	Positive	61	64.2
	Negative	34	35.8
Exercise	Yes	25	26.3
	No	70	73.7
Smoking	Yes	16	16.8
	No	79	83.2

3.2. General characteristics of biochemical indicators for frequency distribution

The biochemical indicators for this study are: 36 persons 37.9% have equal or less than 110 mg/dl glucose and 59 persons 62.1% have more than 110 mg/dl glucose. HbA1c of 33 persons 34.7% are less than 6.5% and 62 persons 65.3% has equal or more than 6.5%. vitamin D of 33 persons 34.7% are less than 10 ng/ml, 51persons 53.7% are between 10 to 29.9 and 11 persons 11.6% are equal or more than 30ng/ml. Mg of 26 persons 27.4% are equal or less than 1.7 mg/dl and 69 persons 72.5% are more than 1.7 mg/dl. Ca of 21 persons 22.1% are less than 9 mg/dl and 74 persons 77.9% are equal or more than 9 mg/dl. The cholesterol value of 65 persons 68.4% are less than 200 mg/dl and 30 persons 31.6% are equal or more than 200mg/dl.The triglyceride value of 34 persons 35.8% are equal or less than 150 mg/dl and 61 persons 64.2% are more than 150 mg/dl. HDL value of 32 persons 33.7% are less than 40mg/dl and 63 persons 66.3% are equal and more than 40 mg/dl. LDL value of 71 persons 74.7% are less than 130 mg/dl and 41 persons 25.3% are equal or more than 130 mg/dl (Table 3.2).

Table 3.2. General characteristics of biochemical indicators for frequency distribution

biochemical indicators		Frequency Distribution	
		Frequency (n)	Percentage (%)
Glucose	≤110 mg/dl	36	37.9
	>110 mg/dl	59	62.1
HbA1c (%)			
<6.5 %		33	34.7
>=6.5 %		62	65.3
Vitamin D	<10 ng/ml	33	34.7
	10-29.9 ng/ml	51	53.7
	>=30 ng/ml	11	11.6
Mg (mg/dl)	<=1.7	26	27.4
	>1.7	69	72.5
Ca	<9 mg/dl	21	22.1
	>=9 mg/dl	74	77.9
Cholesterol (mg/dl)	<200	65	68.4
	>=200	30	31.6
Triglyceride	≤150 mg/dl	34	35.8
	>150 mg/dl	61	64.2
HDL	<40 mg/dl	32	33.7
	≥40 mg/dl	63	66.3
LDL	<130 mg/dl	71	74.7
	>=130 mg/dl	41	25.3

3.3. Patients and subjects characteristics according to control, and diabetic patients

In this table we compare all characteristics according to control and diabetic patients. The Mean±SD of control age is (42.48 ±8.11) and for diabetic patients is (52.12 ±9.09) with p-value (<0.001). 11 persons (44.0%) of controls are male and 14 persons (56.0%) are female, 35 persons (50.0%) of diabetic patients are male and 35 persons (50.0%) are female with No significant in p-value. The Mean±SD of control BMI are (26.90±4.09) and (30.52±4.56) for diabetic patients with P-value (<0.01). 20 (80.0%) of controls have positive family history of diabetes and 5 (20.0%) have negative family history of diabetes, 41 (58.6%) of patients have positive family history of diabetes and 29 (41.4%) have negative family history of diabetes with p-value(<0.05). 32 persons (45.7%) of patients have equal or less than five years duration of diabetes and 38 persons (54.3%) of patients have more than five years duration of diabetes. 9 persons (36.0%) of controls are physically active and 16 persons (64.0%) of controls are physically inactive, 16(22.9%) persons of patients are physically active and 54 persons (77.1%) of patients are physically inactive with no signification in p-value. 5 persons (20.0%) of controls are smokers and 20 persons (80.0%) are non-smokers, 11 persons (15.7%) of diabetic patients are smokers and 59 persons (84.3%) are non-smokers with no significant in p-value. The Mean±SD of glucose is (93.48±8.48) for controls and (176.64±59.90) for diabetic patients with (p-value <0.001). The Mean±SD of HbA1c is (5.28±0.19) for controls and (8.81±1.71) for diabetic patients with p-value (<0.001). The Mean±SD of Vit-D is (21.09±8.54) for controls and (13.48±6.77) for diabetic patients with p-value (<0.001). The Mean±SD of Mg is (2.13±0.23) for controls and (1.98±0.29) for diabetic patients with p-value (<0.05). The Mean±SD of Ca is (9.57±0.44) for controls and (9.53±0.43) for diabetic patients with no significant in p-value. The Mean±SD of cholesterol is (176.16±27.63) for controls and (178.44±41.61) for diabetic patients with no significant in p-value. The Mean±SD of triglyceride is (133.84±57.70) for controls and (203.24±80.88) for diabetic patients with p-value (<0.001). The Mean±SD of HDL is (47.00±8.10) for controls and (42.3±8.59) for diabetic patients with p-value (<0.01). The Mean±SD of LDL is (109.16±25.10) for controls and (100.65±39.40) for diabetic patients with no significant in p-value (Table 3.3).

Table 3.3. Patients and subjects characteristics according to control, and diabetic patients

Subjects, characteristics (n=95)		Frequency Distribution or Mean±SD		p-value
		Controls (n=25)	Diabetic (n=70)	
Age (years)		42.48 ±8.11	52.12 ±9.09	<0.001
Gender	Male	11(44.0%)	35(50.0%)	NS
	Female	14(56.0%)	35(50.0%)	
BMI ($kg/m^{2)}$		26.90±4.09	30.52±4.56	<0.01
Family history of diabetes				
	Positive	20(80.0%)	41(58.6%)	<0.05
	Negative	5(20.0%)	29(41.4%)	
Duration of diabetes (years)				
	≤5	No Diabetic	32(45.7%)	
	>5		38(54.3%)	
Exercise	Yes	9(36.0%)	16(22.9%)	NS
	No	16(64.0%)	54(77.1%)	
Smoking	Yes	5(20.0%)	11(15.7%)	NS
	No	20(80.0%)	59(84.3%)	
Glucose (mg/dl)		93.48±8.48	176.64±59.90	<0.001
HbA1c (%)		5.28±0.19	8.81±1.71	<0.001
Vit.D (ng/ml)		21.09±8.54	13.48±6.77	<0.001
Mg (mg/dl)		2.13±0.23	1.98±0.29	<0.05
Ca (mg/dl)		9.57±0.44	9.53±0.43	NS
Cholesterol (mg/dl)		176.16±27.63	178.44±41.61	NS
Triglyceride (mg/dl)		133.84±57.70	203.24±80.88	<0.001
HDL (mg/dl)		47.00±8.10	42.3±8.59	<0.01
LDL (mg/dl)		109.16±25.10	100.65±39.40	NS

NS: Statistically No significant. P-value<0.05 is considered significant, P-value> 0.05 is considered No significant. Independent t-test and Chi-square was performed for statistical analysis.

3.4. Vitamin D level in T2DM patients, and healthy subjects in relations with age group

The Mean±SD of Vit-D of T2DM patients that its age equal or less than 40 years is (14.39±3.20) and the Mean±SD of Vit-D of T2DM patients that its age more than 40 years is (13.43±6.94) with no significant in p-value. The Mean ±SD of Vit-D of healthy subjects that its age equal or less than 40 years is (17.99±8.65) and the Mean ±SD of Vit-D of healthy subjects that its age more than 40 years is 24.46±7.34 with no significant in p-value (Table 3.4).

Table 3.4. Vitamin D level in T2DM patients, and healthy subjects in relations with age group

Parameter	Diabetic (n=70) Mean ±SD			Controls (n=25) Mean ±SD		
	<=40 year (n=4)	>40 year (n=66)	p-value	<=40 year (n=13)	40 year (n=12)	p-value
Vit.D	14.39±3.20	13.43±6.94	NS	17.99±8.65	24.46±7.34	NS

NS : Statistically No Significant

3.5. Vitamin D level in T2DM patients, and healthy subjects in relations with BMI group.

In comparison between Vit-D of T2DM patient and healthy subject with BMI group we found that: The Mean ±SD of Vit-D for T2DM patients which its BMI is less than 25 kg/m^2 is (19.03±10.98), the Mean ±SD of Vit-D for T2DM patients which its BMI is between (25-29.9) kg/m^2 is (14.41±6.66) and the Mean ±SD of Vit-D for T2DM patients which its BMI is equal and more than 30 kg/m^2 is (11.84±5.51) with p-value (<0.05). The Mean ±SD of Vit-D for healthy subjects which its BMI is less than 25 kg/m^2 is (26.79±7.86), the Mean ±SD of Vit-D for healthy subjects which its BMI is between (25-29.9) kg/m^2 is (18.86±2.83) and the Mean ±SD of Vit-D for healthy subjects which its BMI is equal and more than 30 kg/m^2 is (16.90±7.58) with p-value (<0.05) (Table 3.5).

Table 3.5. Vitamin D level in T2DM patients, and healthy subjects in relations with BMI group

Parameter	Diabetic (n=70) Mean ±SD			
	<25 kg/m^2 (n=6)	25-29.9 kg/m^2 (n=28)	>=30 kg/m^2 (n=36)	p-value
Vit.D	19.03±10.98	14.41±6.66	11.84±5.51	<0.05
Parameter	Controls (n=25) Mean ±SD			
	<25 kg/m^2 (n=10)	25-29.9 kg/m^2 (n=3)	>=30 kg/m^2 (n=12)	p-value
Vit.D	26.79±7.86	18.86±2.83	16.90±7.58	<0.05

NS : Statistically No Significant

3.6. Vitamin D level in T2DM patients, and healthy subjects in relations with gender group

In comparison between Vit-D of T2DM patients and healthy subjects with gender group we found that: The mean ±SD of Vit-D for T2DM patients for male is (14.90±5.71) and for female is (12.07±7.51) with no significant in p-value. The Mean ±SD of Vit-D for healthy subjects for male is (22.72±10.37) and for female is (19.81±6.93) with no significant in p-value (Table 3.6).

Table 3.6. Vitamin D level in T2DM patients, and healthy subjects in relations with gender group

Parameter	Diabetic (n=70) Mean ±SD			Controls (n=25) Mean ±SD		
	Male (n=35)	Female (n=35)	p-value	Male (n=11)	Female (n=14)	p-value
Vitamin D	14.90±5.71	12.07±7.51	NS	22.72±10.37	19.81±6.93	NS
NS : Statistically No Significant						

3.7. Vitamin D level in T2DM patients, and healthy subjects in relations with physical activity group.

In comparison between Vit-D of T2DM patients and healthy subjects with physical activity group we found that: The Mean±SD of Vit-D for T2DM patients for active persons is (14.87±6.32) and for inactive person is (13.08±6.91) with no significant in p-value. The Mean±SD of Vit-D for healthy subjects for active persons is (21.92±9.33) and for inactive persons is (20.63±8.35) with no significant in p-value.(Table 3.7)

Table 3.7. Vitamin D level in T2DM patients, and healthy subjects in relations with physical activity group

Parameter	Diabetic (n=70) Mean ±SD			Controls (n=25) Mean ±SD		
	Yes(n=16)	No(n=54)	p-value	Yes(n=9)	No(n=16)	p-value
Vit.D	14.87±6.32	13.08±6.91	NS	21.92±9.33	20.63±8.35	NS
NS : Statistically No Significant						

3.8. Vitamin D level in T2DM patients, and healthy subjects in relations with smoking group:

In comparison between Vit-D of T2DM patients and healthy subjects with smoking group we found that: The Mean±SD of Vit-D for T2DM patients for smokers is (13.79±5.10) and for non-smokers is (13.43±7.08) with no significant in p-value. The Mean ±SD of Vit-D for healthy

subjects for smokers is (25.37±8.96) and for non-smokers is (20.02±8.32) with no significant in p-value (Table 3.8).

Table 3.8. Vitamin D level in T2DM patients, and healthy subjects in relations with smoking group

Parameter	Diabetic (n=70) Mean ±SD			Controls (n=25) Mean ±SD		
	Yes(n=11)	No (n=59)	p-value	Yes (n=4)	No(n=18)	p-value
Vit.D	13.79±5.10	13.43±7.08	NS	25.37±8.96	20.02±8.32	NS

NS : Statistically No Significant

3.9. Vitamin D level in T2DM patients, and healthy subjects in relations with family history group.

In comparison between Vit-D of T2DM patients and healthy subjects with family history group we found that: The Mean ±SD of Vit-D for T2DM patients for negative family history is (14.50±7.36) and for positive family history is (12.05±5.66) with no significant in p-value. The Mean ±SD of Vit-D for healthy subjects for negative family history is (21.00±7.95) and for positive family history is (21.46±11.70) with no significant in p-value (Table 3.9).

Table 3.9. Vitamin D level in T2DM patients, and healthy subjects in relations with Family history group

Parameter	Diabetic (n=70) Mean ±SD			Controls (n=25) Mean ±SD		
	Negative (n=41)	Positive (n=29)	p-value	Negative (n=20)	Positive (n=5)	p-value
Vit.D	14.50±7.36	12.05±5.66	NS	21.00±7.95	21.46±11.70	NS

NS : Statistically No Significant

3.10. Vitamin D level in T2DM patients in relations with Duration group.

In comparison between Vit-D of T2DM patients with Duration group we found that: The mean ±SD of Vit-D for T2DM patients for equal and less than five years duration is (13.81±7.54) and for more than five years duration is (13.21±6.15) with no significant in p-value (Table 3.10).

Table 3.10. Vitamin D level in T2DM patients in relations with duration group

Parameter	Diabetic (n=70) Mean ±SD		
	<=5 year (n=32)	>5 year (n=38)	p-value
Vit.D	13.81±7.54	13.21±6.15	NS

NS : Statistically No Significant

3.11. Vitamin D level in T2DM patients, and healthy subjects in relations with cholesterol

In comparison between Vit-D of T2DM patients and healthy subjects with cholesterol level we found that: The mean ±SD of Vit-D for T2DM patients which its cholesterol value is less than 200 mg/dl is (14.49±7.22) and for patients which its cholesterol value is equal or more than 200 mg/dl is (11.68±5.57) with no significant in p-value. The Mean ±SD of Vit-D for healthy subjects which its cholesterol value is less than 200 mg/dl is (19.61±8.44) and for persons which its cholesterol value is equal or more than 200 mg/dl is (27.02±6.65) with no significant in p-value (Table 3.11).

Table 3.11. Vitamin D level in T2DM patients, and healthy subjects in relations with cholesterol

Parameter	Diabetic (n=70) Mean ±SD			Controls (n=25) Mean ±SD		
	Serum total cholesterol level<200 (n=45)	Serum total cholesterol level>=200 (n=25)	p-value	Serum total cholesterol level<200 (n=20)	Serum total cholesterol level>=200 (n=5)	p-value
Vit.D	14.49±7.22	11.68±5.57	NS	19.61±8.44	27.02±6.65	NS

NS : Statistically No Significant

3.12. Vitamin D level in T2DM patients, and healthy subjects in relations with triglyceride group

In comparison between Vit-D of T2DM patients and healthy subjects with triglyceride level we found that: The Mean±SD of Vit-D for T2DM patients which its triglyceride value is less than 150 mg/dl is (14.84±7.67) and for patients which its triglyceride value is equal or more than 150 mg/dl is (13.02±6.45) with no significant in p-value. The Mean ±SD of Vit-D for healthy subjects which its triglyceride value is less than 150 mg/dl is (18.82±7.57) and for

persons which its triglyceride value is equal or more than 150 mg/dl is (25.13±9.11) with no significant in p-value (Table 3.12).

Table 3.12. Vitamin D level in T2DM patients, and healthy subjects in relations with triglyceride group

Parameter	Diabetic (n=70) Mean ±SD			Controls (n=25) Mean ±SD		
	Serum total Triglyceride level<150 (n=18)	Serum total Triglyceride level>=150 (n=52)	p-value	Serum total Triglyceride level<150 (n=16)	Serum total Triglyceride level>=150 (n=9)	p-value
Vit.D	14.84±7.67	13.02±6.45	NS	18.82±7.57	25.13±9.11	NS
NS : Statistically No Significant						

3.13. Vitamin D level in T2DM patients, and healthy subjects in relations with HDL group

In comparison between Vit-D of T2DM patients and healthy subjects with HDL level we found that: The Mean ±SD of Vit-D for T2DM patients which its HDL value is less than 40mg/dl is (13.18±6.81)and for patients which its HDL value is equal or more than 40mg/dl is (13.68±6.83) with no significant in p-value. The Mean±SD of Vit-D for healthy subjects which it's HDL value is less than 40mg/dl is (21.50±8.78) and for persons which its HDL value is equal or more than 40mg/dl is (21.02±8.71) with no significant in p-value (Table 3.13).

Table 3.13. Vitamin D level in T2DM patients, and healthy subjects in relations with HDL group

Parameter	Diabetic (n=70) Mean ±SD			Controls (n=25) Mean ±SD		
	Serum HDL Level<40 (n=28)	Serum HDL Level>=40 (n=42)	p-value	Serum HDL Level<40 (n=4)	Serum HDL Level>=40 (n=21)	p-value
Vit.D	13.18±6.81	13.68±6.83	NS	21.50±8.78	21.02±8.71	NS
NS : Statistically No Significant						

3.14. Vitamin D level in T2DM patients, and healthy subjects in relations with LDL group

In comparison between Vit-D of T2DM patients and healthy subjects with LDL level we found that: The Mean ±SD of Vit-D for T2DM patients which its LDL value is less than 130 mg/dl is (13.89±7.05) and for patients which its LDL value is equal or more than 130mg/dl is (12.39±6.00) with no significant in p-value. The Mean ±SD of Vit-D for healthy subjects which

its LDL value is less than 130 mg/dl is (21.51±7.90) and for persons which its LDL value is equal or more than 130 mg/dl is (19.42±11.71) with no significant in p-value (Table 3.14).

Table 3.14. Vitamin D level in T2DM patients, and healthy subjects in relations with LDL group

Parameter	Diabetic (n=70) Mean ±SD			Controls (n=25) Mean ±SD		
	Serum LDL Level<130 (n=51)	Serum LDL Level>=130 (n=19)	p-value	Serum LDL Level<130 (n=20)	Serum LDL Level>=130 (n=5)	p-value
Vit.D	13.89±7.05	12.39±6.00	NS	21.51±7.90	19.42±11.71	NS
NS : Statistically No Significant						

3.15. Vitamin D level in whole study population in relations with fasting blood glucose level group:

In comparison between Vitamin D level in whole study population with fasting blood glucose level group we found that: The Mean±SD of Vit-D for whole population which its fasting blood glucose level is equal or less than 110 mg/dl is (19.22±9.23) and for persons which its fasting blood glucose level is more than 130 mg/dl is (12.21±6.15) with p-value (<0.001) (Table 3.15).

Table 3.15. Vitamin D level in whole study population in relations with fasting blood glucose level group

Parameter	Whole study population (n=92) Mean ±SD		
	Fasting blood glucose level <=110 mg/dl (n=36)	Fasting blood glucose level >110 mg/dl (n=59)	p-value
Vit.D	19.22±9.23	12.21±6.15	<0.001
NS : Statistically No Significant			

3.16. Vitamin D level in whole study population in relations with HbA1C level group:

In comparison between Vitamin D level in whole study population with HbA1C level group we found that: The Mean±SD of Vit-D for whole population which its HbA1C level is equal or less than 6.5% is (20.46±8.56) and for persons which its fasting blood glucose level is more than 6.5% is (12.84±6.26) with p-value (<0.001) (Table 3.16).

Table 3.16. Vitamin D level in whole study population in relations with HbA1C level group

Parameter	Whole study population (n=92) Mean ±SD		
	HbA1C level<6.5 (n=33)	HbA1C level>=6.5 (n=62)	p-value
Vit.D	20.46±8.56	12.84±6.26	<0.001
NS : Statistically No Significant			

3.17. Vitamin D level in T2DM patients, and healthy subjects in relations with Mg group.

In comparison between Vit-D of T2DM patients and healthy subjects with Mg level we found that: The mean ±SD of Vit-D for T2DM patients which its Mg value is equal or less than 1.7mg/dl is (10.22±5.51) and for patients which it's Mg value is more than 1.7mg/dl is (14.88±6.83) with p-value (<0.05). The Mean±SD of Vit-D for healthy subjects which its Mg value is equal or less than 1.7mg/dl is (10.84±84) and for persons which its Mg value is more than 1.7 mg/dl is (23.66±7.50) with p-value (<0.05) (Table 3.17).

Table 3.17. Vitamin D level in T2DM patients, and healthy subjects in relations with Mg group

Parameter	Diabetic (n=70) Mean ±SD			Controls (n=25) Mean ±SD		
	Serum Mg level <=1.7 (n=21)	Serum Mg level >1.7 (n=49)	p-value	Serum Mg level <=1.7 (n=5)	Serum Mg level >1.7 (n=20)	p-value
Vit.D	10.22±5.51	14.88±6.83	<0.05	10.84±84	23.66±7.50	<0.05
NS : Statistically No Significant						

3.18. Vitamin D level in T2DM patients, and healthy subjects in relations with Ca group.

In comparison between Vit-D of T2DM patients and healthy subjects with Ca level we found that: The Mean ±SD of Vit-D for T2DM patients which its calcium value is less than 9mg/dl is (8.50±2.98) and for patients which its calcium value is equal or more than 9 mg/dl is (14.85±6.90) with p-value (<0.05). The Mean ±SD of Vit-D for healthy subjects which its calcium value is less than 9mg/dl is (13.13±5.48) and for persons which its calcium value is equal or more than 9 mg/dl is (21.61±7.82) with p-value (<0.05) (Table 3.18).

Table 3.18. Vitamin D level in T2DM patients, and healthy subjects in relations with Ca group

Parameter	Diabetic (n=70) Mean ±SD			Controls (n=25) Mean ±SD		
	Serum Ca level <9.0 (n=15)	Serum Ca level >=9.0 (n=55)	p-value	Serum Ca level <9.0 (n=6)	Serum Ca level >=9.0 (n=19)	p-value
Vit.D	8.50±2.98	14.85±6.90	<0.05	13.13±5.48	21.61±7.82	<0.05
NS : Statistically No Significant						

3.19. Ca and Mg level in T2DM patients, and healthy subjects in relations with Vitamin D group

In comparison between Ca and Mg level in T2DM patients, and healthy subjects in with vitamin D group we found that: The Mean±SD of Mg level for T2DM patients that it's Vit-D level less than 10 ng/ml is (1.88±0.34), the Mean±SD of Mg level for T2DM patients that it's Vit-D level between (10-29.9) ng/ml is (2.06±0.22) and the Mean±SD of Mg level for T2DM patients that it's Vit-D level equal or more than 30 ng/ml is (2.00±0.20) with p-value (<0.05).

The Mean±SD of Ca level for T2DM patients that it's Vit-D level less than 10 ng/ml is (9.37±0.48), the mean ±SD of Ca level for T2DM patients that it's Vit-D level between (10-29.9) ng/ml is (9.64±0.38) and the Mean±SD of Ca level for T2DM patients that it's Vit-D level equal or more than 30ng/ml is (9.68±0.17) with p-value (<0.05).

The Mean±SD of Mg for healthy subjects that it's Vit-D level less than 10ng/ml is (1.69±0.01), the Mean±SD of Mg level for healthy subjects that it's Vit-D level between (10-29.9) ng/ml is (2.18±0.17) and the Mean±SD of Mg level for healthy subjects that it's Vit-D level equal or more than 30ng/ml is (2.27±0.09) with p-value (<0.001).

The Mean±SD of Ca level for healthy subjects that it's Vit-D level less than 10ng/ml is (8.88±0.05), the Mean±SD of Ca level for healthy subjects that it's Vit-D level between (10-29.9) ng/ml is (9.70±0.37) and the Mean±SD of Ca level for healthy subjects that it's Vit-D level equal or more than 30ng/ml is (9.72±0.30) with p-value (<0.01) (Table 3.19).

Table 3.19. Ca and Mg level in T2DM patients, and healthy subjects in relations with vitamin D group

Parameter	Diabetic (n=70) Mean ±SD			
	<10 ng/ml (n=29)	10-29.9 ng/ml (n=37)	>=30 ng/ml (n=4)	p-value
Mg	1.88±0.34	2.06±0.22	2.00±0.20	<0.05
Ca	9.37±0.48	9.64±0.38	9.68±0.17	<0.05
Parameter	Controls (n=25) Mean ±SD			
	<10 ng/ml (n=4)	10-29.9 ng/ml (n=14)	>=30 ng/ml (n=7)	p-value
Mg	1.69±0.01	2.18±0.17	2.27±0.09	<0.001
Ca	8.88±0.05	9.70±0.37	9.72±0.30	<0.01

NS: Statistically No Significant

3.20. Correlation between vitamin D and other parameters in the study population

According to Pearson correlation coefficient (r), our results in the whole study population, showed that Vit-D level had negatively no significant correlation with each of [Age group, Cholesterol level and LDL level (r=-0.105, p=0.313),(r=-0.109, p=0.292),(r=-0.091,p=0.383) respectively], Vit-D level had negatively low significant correlation with Triglyceride(r=-0.209, p<0.05) , Vit-D level had negatively high significant correlation with each of [BMI, Fasting blood sugar and HbA1C (r=-0.308, p<0.01),(r=-0.334, p<0.01),(r=-0.380, p<0.001) respectively], Vit-D level had positively no significant correlation with HDL(r=0.100, p=0.336) and Vit-D level had positively high significant correlation with each of [Mg level, Ca level (r=0.402, p<0.001),(r=0.289, p<0.01) respectively] (Table 3.20 **).**

Table 3.20. Correlation between vitamin D and other parameters in the study population

Parameter	(r)	P-value
Age (year)	-0.105	0.313
BMI(Kg/m^2)	-0.308**	<0.01
Cholesterol	-0.109	0.292
Triglycerides	-0.209*	<0.05
HDL	0.100	0.336
LDL	-0.091	0.383
FBS	-0.334**	<0.01
HbA1c	-0.380**	<0.001
Mg	0.402**	<0.001
Ca	0.289**	<0.01

*represent low significant correlation, **represent High significant correlation
Person's correlation test was performed for statistical analysis.

T2DM is a metabolic issue introduced by reduces the secretion of insulin from pancreatic beta cells, hyperglycemia, and insulin resistance. The increase in the prevalence of diabetes is mostly connected with bad lifestyles habits and obesity. Obesity is a main health issue mostly associated with T2D and results in increased mortality and morbidity [2].

Explaining levels of (T2DM) represent a worldwide health problem. Even as changes in diet, obesity levels and physical activity on the foundation of genetic hazard factors come out to be fuelling this epidemic, other environmental variables might be compelling in the improvement of T2DM [3].

Vitamin D is a fat-soluble pro-hormone that acting an important character in Mg and Ca homeostasis and the protection of standard function in various tissues [1]. Due to it is exogenous source, Vit-D is called (vitamin), predominantly from oily fish in the form of vitamin D3 and D2. It is just a hormone, produced by the skin and metabolized to its active form (calcitriol) in the kidney, calcitriol, then work all over the body [88].

In comparison to it is well recognized role on skeletal health, Vit-D has been indicated as having a possible effect in many disease processes and health conditions including cardiovascular disease, type 2 Diabetes, cancers, and autoimmune disorders [1].

In our research the data confirmed that there is no significant in the relation between Vit-D patient and control and each of (Age, Gender, Activity, Smoking, family history, Duration) group. Similar observations were reported by Abdulhalem (2019), Maysaa et al (2019) Majed Yassin (2019) [1, 10, 91].

Body mass index (BMI) is known as the body mass divided by the body height square and is expressed uniformly in kg/m^2units, in this study there is negative Pearson correlation coefficient (r) between (BMI) group with Vit-D of patient and control therefore decreasing in Vit-D value was shown with increase in BMI value. Similar observations were reported by Abdulhalem (2019), Maysaa et al (2019) [10, 91].

In our research the data confirmed that statistically there is no significant in the relation between Vit-D of patient and control for each of (Cholesterol, triglyceride, LDL, HDL) level, there is positive pearson correlation coefficient (r) between HDL level with Vit-D of patient and control but there is negative Pearson correlation coefficient (r) between (Cholesterol, triglyceride, LDL) level with Vit-D of patient and control. Similar observations were reported by yassin (2019) [1].

The Mean±SD of Vit-D level for whole population which it's fasting blood glucose level is equal or less than 110 mg/dl is (19.22±9.23) and for persons which its fasting blood glucose level is more than 130 mg/dl is (12.21±6.15) with p-value (<0.001).The relation between vit-D level and fasting blood glucose statistically is significant with negative pearson correlation coefficient. Similar observations were reported by Ramuadela et al (2016) and Abdulhalem (2019)[6], [10].

The Mean ±SD of Vit-D for whole population which it's HbA1C level is equal or less than 6.5% is (20.46±8.56) and for persons which it's fasting blood glucose level is more than 6.5% is (12.84±6.26) with p-value (<0.001).The relation between Vit-D level and fasting blood glucose statistically is significant with negative Pearson correlation coefficient. Similar observations were reported by Ramuadela et al(2016), Abdulhalem (2019) and Maysaa et al (2019)[6], [10], [91].

The strong relation between Vit-D and fasting blood glucose and HbA1C is because of Vit-D tends to specifically stimulate the production of insulin in beta pancreatic cells via the nuclear Vit-D receptor (VDR), as well as Vit-D helps to minimize inflammation, which is a major process in inducting insulin resistance [1,10].

Ca is vital-needed mineral. Ca helps human heart to beat, human blood to clot, and human muscles to contract, in addition to strengthening bones and keeping them healthy. About 99% of human body's Ca is in human teeth and bones. Humans lose Ca every day during finger nails, human skin, hair, urine, feces, and sweat. By vitamin D-dependent and vitamin D independent mechanisms the intestine was absorbs Ca [87]. The Mean±SD of Vit-D for T2DM patients which it's Ca value is less than 9 mg/dl is (8.50±2.98) and for patients which it's Ca value is equal or more than 9 mg/dl is (14.85±6.90) with statistically significant and p-value (<0.05). The Mean±SD of Vit-D for healthy subjects which it's Ca value is less than 9 mg/dl is (13.13±5.48) and for persons which it's Ca value is equal or more than 9 mg/dl is (21.61±7.82) with statistically significant and p-value (<0.05). The relation between Vit-D level and Ca value statistically is significant with positive Pearson correlation coefficient. Similar observations were reported by Michael f.(2018), Yassin (2019) and M S Sheikh (1988) [1, 54, 87].

Mg is fourth mineral that is most common in the body of human. For over 300 biochemical reactions inside the body Mg is required. This helps maintain regular functioning of the nerve and muscles, keeps heart beat steady, helps bones remain strong and supports a healthy immune system. This also have a role in controlling blood glucose level. It helps in energy and protein synthesis [88]. Because of the role of Vit-D in stimulation of intestinal Mg absorption, there is positive Pearson correlation coefficient between Vit-D and Mg in our results. The Mean ±SD of Vit-D for T2DM patients which it's Mg value is equal or less than 1.7 mg/dl is (10.22±5.51) and for patients which its Mg value is more than 1.7 mg/dl is (14.88±6.83) with statistically significant and p-value(<0.05). The Mean±SD of Vit-D for healthy subjects which its Mg value is equal or less than 1.7mg/dl is (10.84±84) and for persons which it's Mg value is more than 1.7mg/dl is (23.66±7.50) with statistically significant and p-value (<0.05). Similar observations were reported by T. Vos et al (2015)and A. M. Uwitonze et al (2018) [44, 89]

4. CONCLUSION

Type 2 Diabetes mellitus is a complicated metabolic disease which has developed into a major health problem. Vitamin D deficiency is a main health problem around the worlds that is currently associated with chronic disease, such as diabetes mellitus, and cardiovascular disease. Because of high significant between Vit-D deficiency with increase in fasting blood glucose and HbA1C value of T2DM patient and the role of Vit-D in insulin resistance, T2DM patient should measure their Vit-D value periodically. Vit-D is responsible for increasing intestinal absorption of Mg and Ca; therefore T2DM patient who has Vit-D deficiency should measure their Ca and Mg value periodically.

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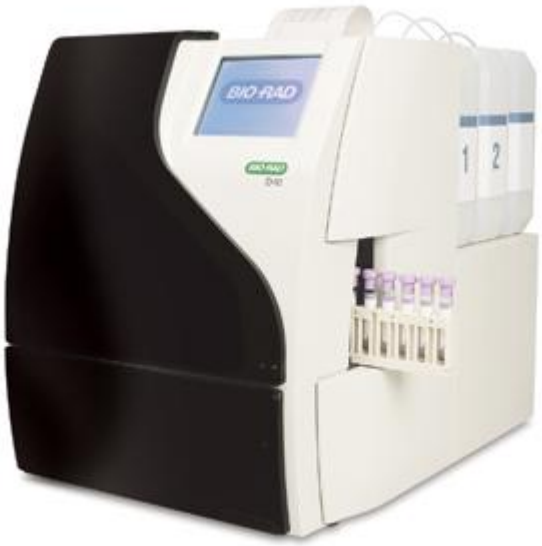
APENDICES

Appendix (1) Cobas C 311 Autoanalyzer

Appendix (2) Cobas6000 autoanalyzer

Appendix (3) HPLC Instrument Bio-Rad D10



Appendix (4) Research Questionnaire

1. Patient's Name: ……
2. Patient's ID……
3. Age: ……
4. Job: ……..
5. Address: ……..
6. Tel.: …….

Diseases History

- Chronic kidney diseases: Yes No
- Chronic liver diseases: Yes No
- General inflammation: Yes No
- Smoker Yes No
- Types of diabetes therapy
- Duration of daisies ≤5 Years >5 Years
- Physical activity Yes No
- Taking medications including:

-Folic acid Yes No

-Multi-Vitamins Yes No

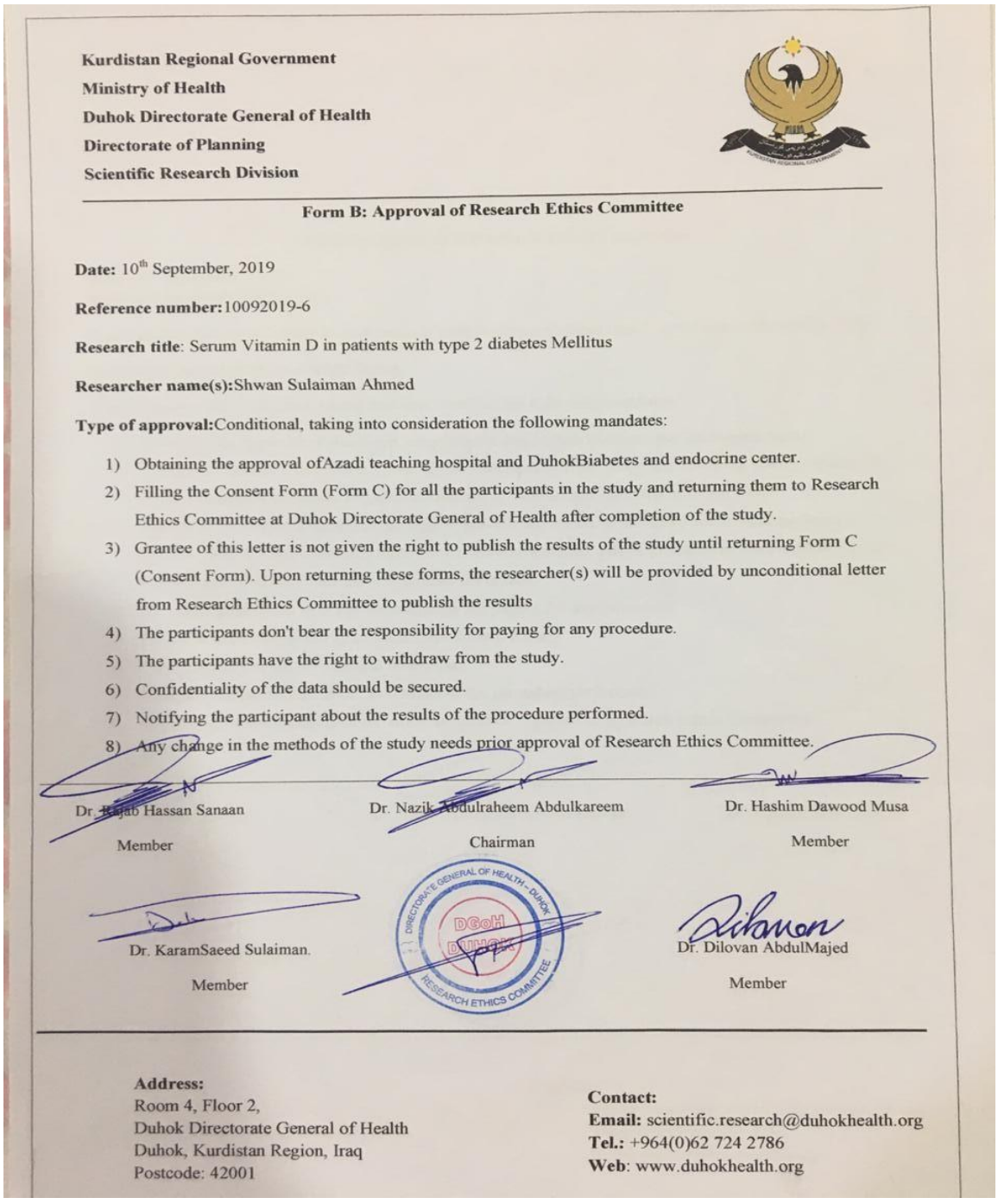
-Insulin Yes No

Physical examinations

1. Width circumferences (WC)…… ( )
2. Body mass index (BMI)……... (kg/m^2)

Weight: …….. (Kg) Height: …….. (cm)

Appendix (5): Form (B) Approval of Research Ethics Committee



Kurdistan Regional Government
Ministry of Health
Duhok Directorate General of Health
Directorate of Planning
Scientific Research Division

Form B: Approval of Research Ethics Committee

Date: 10th September, 2019

Reference number: 10092019-6

Research title: Serum Vitamin D in patients with type 2 diabetes Mellitus

Researcher name(s): Shwan Sulaiman Ahmed

Type of approval: Conditional, taking into consideration the following mandates:

1) Obtaining the approval ofAzadi teaching hospital and DuhokBiabetes and endocrine center.
2) Filling the Consent Form (Form C) for all the participants in the study and returning them to Research Ethics Committee at Duhok Directorate General of Health after completion of the study.
3) Grantee of this letter is not given the right to publish the results of the study until returning Form C (Consent Form). Upon returning these forms, the researcher(s) will be provided by unconditional letter from Research Ethics Committee to publish the results
4) The participants don't bear the responsibility for paying for any procedure.
5) The participants have the right to withdraw from the study.
6) Confidentiality of the data should be secured.
7) Notifying the participant about the results of the procedure performed.
8) Any change in the methods of the study needs prior approval of Research Ethics Committee.

Dr. Rajab Hassan Sanaan — Member

Dr. Nazik Abdulraheem Abdulkareem — Chairman

Dr. Hashim Dawood Musa — Member

Dr. KaramSaeed Sulaiman. — Member

Dr. Dilovan AbdulMajed — Member

DIRECTORATE GENERAL OF HEALTH - DUHOK — DGoH DUHOK — RESEARCH ETHICS COMMITTEE

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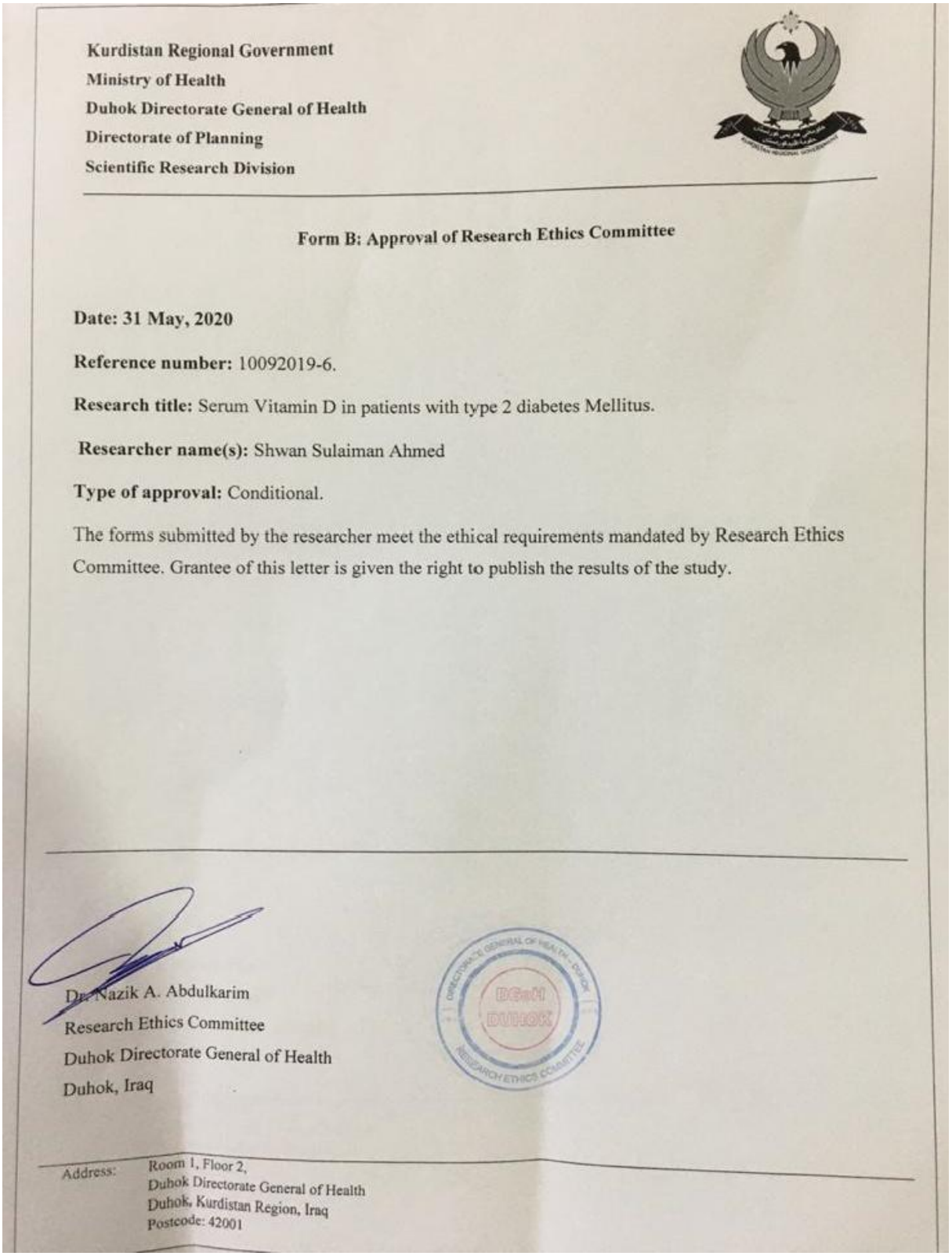
Appendix (6): Form (B) Approval of Research Ethics Committee



Kurdistan Regional Government
Ministry of Health
Duhok Directorate General of Health
Directorate of Planning
Scientific Research Division

Form B: Approval of Research Ethics Committee

Date: 31 May, 2020

Reference number: 10092019-6.

Research title: Serum Vitamin D in patients with type 2 diabetes Mellitus.

Researcher name(s): Shwan Sulaiman Ahmed

Type of approval: Conditional.

The forms submitted by the researcher meet the ethical requirements mandated by Research Ethics Committee. Grantee of this letter is given the right to publish the results of the study.

Dr. Nazik A. Abdulkarim
Research Ethics Committee
Duhok Directorate General of Health
Duhok, Iraq

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Duhok Directorate General of Health
Duhok, Kurdistan Region, Iraq
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CURRICULUM VITAE

Shwan Sulaiman AHMED

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