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Comparative study of Vitamin D levels in diabetic and non- diabetic women and its correlation with age and seasonal variation

Abdulhadi Mohamed Jumaa

College of Medicine , Tikrit University , Tikrit , Iraq

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Corresponding Author:

Name: Abdulhadi Mohamed Jumaa

E-mail: RSY477@yahoo.com

Tel:

ABSTRACT

Background vitamin D has characteristics features of a hormone, and accordingly vitamin D is a pro-hormone, rather than a true vitamin. Active form of vitamin D is binding to vitamin D receptors (VDR) then started its main action inside the body.

This study is aimed to compare concentration of vit. D in a patients with diabetes mellitus, and healthy Iraqi women and its correlation with seasonal variation, and age.

Subjects and methods This study was conducted in the first of August 2017 till the first of April 2018, at the outpatient unit in especial private hospital at karbalaa city in the south of Iraq. 163 women have been enrolled in present study. The sample was divided into 3 groups in both diabetic and non-diabetic subjects.

Results the distribution of subjects in this study, 163 persons participated as a sample in a present research; 82 subjects are normal healthy women as controls, while 51 women suffering from non-insulin dependent diabetic mellitus, (T2DM), and 30 pregnant women suffering from gestational DM, (GDM). Stander deviation and mean of Vit. D levels in a non-insulin dependent diabetes mellitus and normal healthy women. There is significant reduction in the levels of vit.D that measured by using sera of women patients suffering from T2DM, (12.97 ± 8.9) as compared with control subjects, (18.95 ± 7.1).

Conclusions the present study conclude that, the measurement of vit.D are significantly reduced when measured in women patients with non-insulin dependent diabetes sera, as compare to healthy subjects and also reduced in the sera of women patients suffering from GDM, as compared with control subjects.

Introduction

Vit. D is a steroidal hormone synthesized at superior epidermal layer by conversion cholesterol to cholecalciferol by the effect of the sun. calcidiol and calcitriol also may come from dietary supplementations. The original type of vit. D (calcitriol) in addition to calcidiol are found in the some dietary sources[1].

Many components synthesized by many process from sterols belong to the vit.D family, and their functions not so different from vit.D. Vit. D (has another name cholecalciferol), which is the famoust one of them, and is carried out by the sequential action of the skin by irradiation of 7-dehydrocholesterol, a precursor used by epidermal keratinocytes and convert it, by warmth of sunlight,

as a result, regular daily sun bath prevents hypovitaminosis D [2].

The skin is a chemical factory, fueled in part by the sun's rays, and modified cholesterol molecules to vitamin D with sufficient quantity, vit. D liberated at cold months from fat poles, because vitamin cannot be modified from cholesterol in the skin . sun bath at summer months need a certain time and duration to produce sufficient amount of vitamin and can be prevented or altered by many factors like sun screens even with SPF 30% and more, and also by wearing a long clothes that cover the lower and upper extremity, degree of skin color also affect the formation of vit.D , so formation is more in those with dark than with light skin [3,4,5].

There is great association between sugar metabolism and vit.D , by increasing sensitivity of insulin receptors in addition to liberation of insulin from the pancreas and overcome or diminish the resistance to insulin hormone , at the level of peripheral tissue there will be increment in the response to glucose and enhancing it is entry to the cells [6,7].

Hyperglycemia may occur during pregnancy and start at the 24 weeks of gestation, but reach maximum at last stage of pregnancy , pregnancy is a diabetogenic and associated with glucose intolerance. At the second and third trimesters of pregnancy there will be increase in levels of hormones that secreted mainly by placenta like human placental lactogen, prolactin , progesterone and estrogen which in addition to thyroxin secreted by thyroid gland act as anti-insulin hormones so this will lead to increase the production of glucose by the liver and decrease glycogen storage in the tissues ,and decrease the sensitivity of peripheral tissues to insulin. All the previous events will lead to what's called pregnancy diabetes (Gestational diabetes mellitus GDM) [10,11].

Aim of the study:

It's to find out whether there is any correlation between vitamin D levels in female patients with diabetes and non- diabetics Iraqi females with seasonal variation and age.

Subjects and methods:

This study was conducted in the first of August 2017 till the first of April 2018 , at the outpatient unit in especial private hospital at karbalaa city in the south of Iraq. 162 women have been enrolled in present study. The sample was divided into 3 groups in both diabetic and non-diabetic subjects.

group 1= female age less than 18 years

group 2= female age 18-45 years

group 3= female age 46-60

Full history was taken from all participants and all of them undergo through physical examination. All blood samples had been collected at afternoon after 5 pm from the vein at the level of antecubital fossa, subjects were a mixture of urban and rural residents.

Renal function test, serum alkaline phosphatase, blood sugar, HbA1c were done for all of them, serum vitamin D was measured by immune assay methods by Abbot equipment and by minividas equipment.

All females who participate in present study were wearing Hijab (is a veil that covers head , arms , and chest) and wearing Abayah also which is a black cloth worn by Muslim women in some parts of middle east especially in Arabic countries over usual clothes when leaving the house .

Including criteria:

1. diabetic patients.
2. non-diabetic healthy subjects.
3. 18-60 years.
4. Normal healthy normotensive pregnant women.

Excluding criteria:

1. Chronic renal and liver disease .

2. Patients who was treated with vitamin D supplements in the last 6 months.

3. Malignancy.

4. Chronic anti-epileptic and glucocorticoid use.

Statistical analysis:

All data were presented as a mean and standard deviation, (SD). Unpaired student T- test was used to compare between means and stander deviations measured variables. SPSS version 21 has been used for analysis of data. P value less than 0.05 , ($P \leq 0.05$) was used as significant value.

Result:

One hundred and sixty three women participate in the present study, distributed as follows in (Table 1);

-82 subjects non diabetic subjects as control.

-51 patients gave diabetic diseases, (type 2 diabetes mellitus).

-Thirty pregnant women with gestational diabetes mellitus.

Table (1) shows that the distribution of subjects in the present study, 163 subjects participated in the present study; 82 subjects are normal healthy women as controls, while 51 women suffering from type 2 diabetic mellitus, (T2DM), and 30 pregnant women suffering from gestational DM, (GDM).

Table (1) the distribution of patients and controls

Subjects	Number
Control	82
T2DM	51
Gestational DM	30
Total	163

However, Table(2) shows that the standard deviation and mean of vit.D levels in patients with type two diabetes, the concentration of vitamin D is significantly decline in samples were taken from serum of female patients suffering from T2DM, (12.97 ± 8.9) as compared with control subjects, (18.95 ± 7.1).

Table(2) Standard deviation and mean of D3 vit. concentration inT2DM and normal healthy women

	Mean+SD	P value
T2DM	12.97 ± 8.9	0.05
Controls	18.95 ± 7.1	

Also, in Table(3) Vit. D measures are significantly decline in young age of T2DM patients. In T2DM patients less than 18 years old have significant reduction in vitamin D measures, (13.78 ± 7.1), and in T2DM patients aged 18-40 years, as compared with the older age 42-50 years, (17.78 ± 6.4).

Table (3) the mean and standard deviation of vitamin D concentration in T2DM patients distributed according to age groups

Age groups	Number of patients	Mean +SD	P value
≤ 18 years	10	13.78 ± 7.1	
18-40	26	12.79 ± 8.8	
41-50	15	17.78 ± 6.4	0.05
Total	51		

In the present study, there is no significant differences regarding vitamin D concentration in 5-10 years and 11-20 years, as compare with normal healthy subjects of same age and gender.

However, vit. D measures are significantly decline T2DM patients there is significant reduction in the concentration of vitamin D in patients with T2DM in the following age divisions (21-30, 31-40 and 41-60 years), as compare to control subjects, (Table 4).

Table (4) The mean and standard deviation (SD) of Vitamin D concentration in T2DM and normal healthy subjects according to age groups.

Age groups (years)	T2DM patients (51)	Controls (82)	P value
5-10	8.31 ± 1.23	11.23 ± 2.1	NS
11-20	14.38 ± 2.38	16.8 ± 3.2	NS
21-30	23.7 ± 2.1	27.2 ± 3.5	0.05
31-40	31.5 ± 5.8	35.8 ± 6.4	0.05
41-60	29.4 ± 6.7	37.3 ± 8.7	0.05

Moreover, Table (5) shows that the mean and standard deviation of vit. D. concentration GDM and normal healthy females . There is significant reduction of vit.D measurements in a samples of serum that had been taken from female patients suffering from GDM, (10.3 ± 5.4) as compared with control subjects, (18.95 ± 7.1).

Table (5) The mean and standard deviation of Vitamin D concentration in gestational DM and normal healthy women

	Mean±SD	P value
Gestational DM	10.3±5.4	0.01
Controls	18.95±7.1	

Table (6) shows that the concentration of vitamin D during summer months and compare to winter months. There is high significant elevation in the concentration of vitamin D in summer, (25.34 ± 8.2) , as compare with winter status, (14.5 ± 7.2).

Table (6) The mean and standard deviation of Vitamin D concentration in normal healthy subjects according to season.

Variables	Mean±SD	P value
January	15.76±7.2	
February	12.94±6.3	
March	10.67±7.8	
August	14.7±7.1	
September	15.8±8.4	
October	19.5±9.5	
Winter	14.5±7.2	0.01
Summer	25.34±8.2	

Discussion

In the present study, there is significant reduction of vit. D measurements of female T2DM, (12.97±8.9) as compared with control subjects, (18.95 ±7.1).

Vitamin D reduction could be due to long periods of indoor especially at summer months and decrease exposure to sun lights and low consumption of vitamin D rich foods in addition to absence of foods fortified with vitamin D (e.g cereals), and also could be due to wearing Hijabs and Abayah that cover large

parts of the body and frequent use of sun screens also considered as an important cause [33].

The mechanisms for increased insulin resistance in vitamin D insufficiency have not been fully elucidated. Many tissues and cells including the b cells of the pancreas express 1-OHase and can produce 1,25-dihydroxy vitamin D. The b cells have a vitamin D receptor, which may improve insulin secretion and production and an increase in serum 25(OH)D3 levels leads to reduction in b-cell glucose insensitivity and increases Phase 1 and 2 of insulin secretion after a glucose challenge [9,22]. Vitamin D can also affect insulin secretion by increasing the intracellular calcium concentration via the nonselective voltage-dependent calcium channels [23].

In clinical studies, vitamin D deficiency has been shown to cause impairment of insulin secretion and an increase in insulin resistance among patients with T2DM [3].

Previous studies revealed that the number of cases having vitamin D deficiency and insufficiency were significantly higher than that of controls. Hypovitaminosis D and sub-normal were found to be more prevalent among type 2 diabetic patients, [12,13,14].

Also, Previous study was done in Gaza strip on T2DM done by Abed El-Raouf ,(2014) they found the mean level of vit.D was significantly reduced in T2DM patients cases in comparison to control healthy persons of same age and gender, (25.9 ±11.0 vs. 34.6±13.8 ng/dl, P=0.01), [15].

The present result showed that significant reduce in the mean level of vit.D in patients sera. This means vitamin D levels deficiency are linked to type 2 diabetes. Such finding is in agreement with that demonstrated by , [14,15].

Vitamin D play apart in the type 2 diabetes pathophysiology is an important issue in a network of interacting scientist. Specific interactions are suggested to describe how T2DM is encouraged by hypovitaminosis D. The vitamin D receptors (VDRs) are highly expressed in adipose, pancreatic tissues, and perhaps in a muscle cells [17,18].

Insulin production by beta-cell of langerhance in the endocrine part of pancreas is directly modulated by effect on the nuclear VDR, which also regulates genes affecting insulin synthesis [19].

Vitamin D can also act indirectly on the control of diabetes by acting on osteoblast to stimulate the formation of osteocalcin hormone. The high increase in osteocalcin formation appears to maintain glucose levels within certain range triggering insulin impulses from cells of langerhance [20,21].

Shanthi et al. (2012) investigated that the clinically meaningful associations which implicated low serum levels of vitamin D with impaired diabetic control in type 2 diabetes. The serum vitamin D and the HbA1c levels were determined in 50 patients with type 2 diabetes, along with their (FBS), 2 hours after meal

blood sugar (RBS) and other parameters which were required to assess the diabetic control were also checked. The results revealed a trend towards an inverse vitamin D- FBS ($r=-0.090$) and inverse vitamin D- PPBS ($r=-0.095$) association. The lower serum vitamin D levels were associated with the higher HbA1c levels [22,23].

In the present study, in Table(3), vitamin D concentration is significantly reduced in young age of T2DM patients. In T2DM patients less than 18 years old have significant reduction in vitamin D concentration, (13.78 ± 7.1), and in T2DM patients between 18-40 years, in comparison with the older age 42-50 years, (17.78 ± 6.4).

Also, in the present study, vit. D measurements are significantly declined in patients with T2DM at age the following, 21-30, 31-40 and 41-60 years, as compare to control subjects, (Table 4).

Effects of vitamin D supplementation on glucose homeostasis have been shown in numerous studies. Study done by Talei et al (2012) suggested that the insulin resistance appears to be decreased in T2DM patients who had received vitamin D. Von Hurst (2009) showed that vitamin D supplementation significantly improved insulin sensitivity and insulin resistance [25]. There are some mechanisms for the effects of vitamin D presence of vitamin D receptors on pancreatic β cells, Vitamin D activating 1α hydroxylase is expressed in pancreatic β cells, presence of vitamin D response element in the insulin gene, presence of vitamin D receptor in skeletal muscle and the fact that $1,25$ (OH)D increases transcription of insulin receptor genes, and also suppresses the renin gene reducing hyperglycemic-induced increases in renin levels in pancreatic β cells and blockade of renin-angiotensin has been proposed as a novel target for diabetes treatment[26].

Protective effects of vitamin D on diabetes, may be due to well-known effects of vitamin D such as its anti-inflammatory properties, its effects on calcium and phosphorus metabolism and regulation of the insulin receptor gene. It seems that vitamin D increases in calcium content of the cells, which in turn leads to increased transport of glucose into the muscle. Vitamin D also regulates nuclear PPAR important role in the insulin sensitivity. Vitamin D deficiency is associated with increases in inflammation the expression of pro-inflammatory cytokines involved in insulin resistance such as interleukins, IL-1, IL-6, TNF- α also down regulates NF-K β (Nuclear factor) activity [19,26].

Previous study found that Vitamin D status is significantly reduced in young females patients in comparison to younger, (16). Also, the prevalence of vit. D deficiency (53.25) is higher in young pregnant (age 20-34 years) had significantly (p -value =0.029) with older women (35-49 years), 38.2%, [21,27,28]. In the present study, table (5) shows that the mean and standard deviation of vit. D concentration in a

patients with GDM and normal healthy female. There is significant reduction in the concentration of vitamin D in the sera of women patients suffering from GDM, (10.3 ± 5.4) as compared with control subjects, (18.95 ± 7.1).

The pathophysiology behind the development of GDM is not fully understood, but the maternal changes in metabolism are substantial during pregnancy. The glucose metabolism changes to meet the nutritional demands of the mother and fetus [24,25,26,27].

As the prevalence of GDM which ranges from 2% to 20% depending on the populations is increasing worldwide, and risk for developing DM in the postpartum 10 to 20 years is substantial (35-60%), the interest in GDM is growing rapidly. There are several evidences supporting a role for vitamin D in developing glucose intolerance and type 2 DM. Potential mechanisms of effects of vitamin D on glucose metabolism are as follows; the binding of active form of vitamin D to vitamin D receptors (VDR) on pancreatic beta-cells, the expression of 1α -hydroxylase in pancreatic β -cells, insulin secretion and sensitivity by regulating extracellular calcium and calcium flux through the pancreatic β -cell, the presence of vitamin response element in the human insulin gene promoter, the effects on stimulating the expression of insulin receptor and the effects on systemic inflammation by modulating the effects of cytokines for beta cell function, since insulin resistance (IR) and β -cell apoptosis could be induced by systemic inflammation[28,29,30].

In present study, there is high significant elevation in the concentration of vitamin D in summer, (25.34 ± 8.2), as compare with winter status, (14.5 ± 7.2). table (6) shows Difference between the two groups with seasonal variation due to high amount of sun exposure in summer in our country and the sun approximately over the head, and there are more UV-B radiation which is important in photosynthesis of vitamin D from fat under the skin or from 7-dehydrocholesterol. The decrement of vitamin D level in winter is no doubt attributable to the low sun ray in this season [31].

Sunlight is an important to skin production of vitamin D. Environmental conditions where sunlight exposure is limited may reduce this source of vitamin D. The amount of vitamin D produced in the skin varies depending on the time of the day, season, and latitude. Hormones defined as the chemical substances that are made by one body organ to influence another. Vitamin D was originally thought to be a hormone rather than vitamin. This because vitamin D works just like hormone. Some vitamin D [31,32].

precursor compounds in the skin when exposed to the ultraviolet rays of the sun, they change their chemical structures and go back into the blood stream, so that sunlight exposure is the factor that influence the synthesis of vitamin D. The longer exposure to

sunlight, in early morning (sun rise to 11:00 a.m.) or late afternoon (3:00 p.m.) is believed to be reasonably safe. Geographic location and seasons of year affect vitamin D Production, In winter people have less sun exposure and the sun is at an angle that limits the amount of ultraviolet light UV-B) radiation that hits the earth[32,33].

Many people leave for work early in the morning, return home after dark, and drive to and from work, so that, during winter, they have limited sunlight exposure for five out of every seven days. In general, cutaneous synthesis provides most of vitamin D needs to the body (80%-100%), and with adequate sunlight exposure dietary vitamin D may be unnecessary. Vitamin D stored in the adipose tissue is available during the winter, when sunlight exposure is minimal. Most of people, who don't allow more UV-

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B to reach their skin, have to take vitamin D by diet [34].

The present study concludes the followings;

1- 1-There is significant reduction in vitamin D measurements in female patients with T2DM serum samples .

2- 2-Vitamin D measurements are significantly reduced in serum of women with GDM, as compare with control subjects.

3- 3-summer samples showed higher measurements of vitamin D than in winter.

Also , this study recommend the followings;

A-To do more studies on the vitamin D levels in pediatric patients with diabetes mellitus.

B-To give vitamin D. supplementations for the diabetic patients including gestational diabetes.

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دراسة مقارنة لمستويات فيتامين D لدى النساء المصابات بالسكري والنساء غير المصابات وعلاقتها مع الاختلافات الموسمية والعمر

عبدالهادي محمد جمعة

فرع الفلسفة ، كلية الطب ، جامعة تكريت ، تكريت ، العراق

الملخص

المقدمة: فيتامين D هو هورمون ستيرويدي ينتج في الجلد من خلال تحويل الكولستيرول تحت تأثير الأشعة فوق البنفسجية، يوجد فيتامين D ايضا في بعض الأطعمة ومنتجات الالبان. فيتامين D2 و فيتامين D3 يتواجدان في بعض الأغذية. تهدف هذه الدراسة الى المقارنة بين مستويات فيتامين D في مرضى السكري والمرضى الغير مصابين بالسكري من النساء العراقيات ودراسة العلاقة بين مستويات فيتامين D وفصول السنة وكذلك تغيرها مع تغير عمر المشاركين في الدراسة. هذه الدراسة تم تصميمها والبداية بها في بداية شهر آب من عام 2017 واستمرت حتى الاول من نيسان عام 2018 في وحدة الاستقبال للمرضى الخارجيين في احد المستشفيات الخاصة في مدينة كربلاء جنوب العراق. شارك 163 من النساء في هذه الدراسة وقد تم تقسيمهم الى ثلاث مجاميع سواء في مرضى السكري وغير المصابين بالسكري. تم توزيع المشاركين في هذه الدراسة وعددهم 163 من النساء الى 82 امرأة سليمة بينما 51 امرأة كانت مصابة بداء السكري من النوع الثاني وثلاثون امرأة حامل مصابات بسكري الحمل. المعدل والانحراف المعياري لتركيز فيتامين دي يبين وجود نقصان مهم في مصل المرضى الذين يعانون من داء السكري الثاني بالمقارنة مع مجموعة السيطرة. نستنتج من هذه الدراسة بأن مستويات فيتامين D قلت بشكل كبير في النساء المصابات بداء السكري الثاني وكذلك النساء الحوامل بالمقارنة مع مجموعة السيطرة.