ISSN 2321 3361 © 2022 IJESC

Research Article Volume 12 Issue No.1

Statistical evaluation of the role of vitamin D supplementation on glycated hemoglobin levels in Libyan patients with diabetes mellitus type 2

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Background/ Objectives: Vitamin D deficiency has been found to be associated with glycated hemoglobin (HbA1c) levels amongst patients with diabetes mellitus type 2 (DM2). However, the beneficial effect of vitamin D supplementation on HbA1c levels in DM2 patients has been investigated and its role remains controversial. The purpose of this study was to examine the role of vitamin D supplementation on HbA1c levels amongst Libyan patients suffering from DM2.
Methods and design: Ninety-seven DM2 patients of both genders (M = 51) (F = 46), with an age range of 26-59 years, were recruited from the Al-Wafi Medical Laboratory in Tripoli, Libya. All patients were informed about the nature of the study and oral consent was obtained from all patients. Weight and height were measured, and dietary and lifestyle information was obtained. Blood samples were collected and HbA1c and vitamin D levels were measured pre-and post-the administration of vitamin D supplementation for three months. The participants were divided into three groups according to HbA1c level, and then into four groups, based on their vitamin D serum levels. The relationship between vitamin D and HbA1c was investigated.
Results: Vitamin D levels have increased after the administration of vitamin D supplementation amongst DM2 patients. We noticed that the level of HbA1c also decreased from 6.97% at the baseline to 5.65% after supplementation of vitamin D. The results showed that the difference in HbA1c levels was significant after administration of vitamin D as a supplement (P-value = 0.000). The level of vitamin D increased from 8.34±2.59 to 39.95±2.85 ng/ml after the administration of the supplements. In mixed ANOVA analysis, the present study confirmed that the relationship between HbA1c and the factors measured appear to be weak and these findings indicate that HbA1c was not influenced by all these factors studied.
Conclusions: The results of this study have shown that vitamin D supplementation significantly reduced the level of HbA1c amongst patients suffering from DM2. These results may be beneficial for the overall health and blood glucose control of those patients.

Keywords: Diabetes mellitus type 2, Vitamin D, HbA1c, Supplementation, Libya.

Introduction

Subjects with diabetes mellitus type 2 (DM2), formerly known as noninsulin-dependent diabetes, have relative insulin deficiency and peripheral insulin resistance [1, 2]. There are numerous causes of DM2 which are divided into genetic factors and environmental factors. Genetic factors are related to impaired insulin secretion and insulin resistance, while environmental factors include obesity, excess food consumption, physical inactivity, stress and aging [3]. Diabetes is usually diagnosed through the Fasting Plasma Glucose (FPG) test, Oral Glucose Tolerance Test (OGTT), and glycated hemoglobin (HbA1c) test. In the FPG test, plasma glucose is measured in a subject's sample of venous blood after a 12-hour overnight fast. In the case of the OGTT test, glucose is measured in a subject's sample of venous blood after a 12-hour overnight fast, and measured in another venous blood sample, collected two hours after the subject is administered a 75g oral dose of glucose [4]. As for the HbA1c test, glycated hemoglobin is measured to identify the average plasma glucose concentration over a long period of time. Glycated hemoglobin is formed non-enzymatically by binding hemoglobin to plasma glucose in an irreversible glycation reaction at one or both N-terminal valines of the beta chains, and when a hemoglobin molecule is glycated, it remains in the red blood cell throughout its four-month lifespan [5]. It has been reported that HbA1c laboratory tests are performed using many methods such as: ion-exchange chromatography; electrophoresis; immunological methods [6] and, as the HbA1c test reflects the mean blood glucose levels over the last 3 months [7], its value has been employed to evaluate the role of different supplementations in DM2 patients [8-10]. Some randomized controlled trials (RCTs) have reported evaluating the effect of vitamin D supplementation on HbA1c levels in DM2 patients and the results have been inconsistent [11-14]. These inconsistent findings have been pooled in some review studies to systematically review the effect of vitamin D supplementation on glycemic measures in DM2 patients [15-18], and even the results of those review studies have not been consistent. For instance, in one of the reviews studies the meta-analysis procedure has been used to review the effect of vitamin D supplementation on glycemic measures in DM2 patients in twenty-four RCTs, and it has been found that vitamin D supplementation was associated with reduction of HbA1c levels among DM2 patients with vitamin D deficiency [16]. In contrast, in another systematic review study of nineteen RCTs, in which the meta-analysis procedure has been applied, there has been no

significant effect on HbA1c levels among DM2 patients after vitamin D intervention [17].

Libya, where the present study was conducted, is an Arab country located in North Africa and bordered by the Mediterranean Sea to the north. There is a limited number of studies in Arab countries in which the effect of vitamin D supplementation on HbA1c levels in DM2 patients has been investigated. In a study in Saudi Arabia, one of the Arab Gulf countries, 41 DM2 patients (27 males and 14 females) were supplemented once weekly for 8 weeks with 45,000 units of cholecalciferol, followed by 22,500 units of cholecalciferol once weekly for 16 weeks. The mentioned study has revealed that a significant reduction of HbA1c (7.9% to 7.4%, $p < 0.001$) was linked to Vitamin D3 replacement [19]. In another study in Egypt, an Arab country located to the east of Libya, 100 DM2 patients (68 males and 32 females) were supplemented with 4500 IU/day of vitamin D for two months. The results have pointed out that vitamin D supplementation significantly decreased HbA1c (7.7% to 7.2%, $p < 0.05$) [20].

As it is still uncertain whether vitamin D supplementation has a favorable effect on DM2 patients by significantly reducing HbA1c levels, the aim of this study was to investigate the role of vitamin D supplementation on HbA1c levels between Libyan patients suffering from DM2, and to the best of our knowledge, this is the first study to address this research theme in Libyan DM2 patients. This was achieved by statistically analyzing HbA1c levels for a group of patients suffering from DM2, pre- and post-administration of vitamin D as a supplement.

Materials and methods

Subjects and supplementation:

This study was conducted at Al-Wafi Medical Laboratory in Tripoli, Libya. It involved a group of 100 DM2 patients of both genders (M = 47) (F = 53), who were residents in Tripoli, Libya, with an age range from 26-59 years old. Each patient was informed about the nature of the study and oral consent was obtained from all patients. Before the study commenced, the weight and height of each patient were measured, and all patients were asked specific questions to obtain information about their diet and lifestyle. This study was reviewed and granted permission by the Bioethics Committee at the Libyan Center for Biotechnology Research in Tripoli, Libya, under the Ethics reference number (BEC-BTRC 21-2021).

To investigate the role of vitamin D on HbA1c levels, each DM2 patient was supplemented monthly by intramuscular injection of 200000 ng/ml of vitamin D for three months. Serum HbA1c levels and vitamin D levels for all patients were measured (triplicate for each sample) pre-and post-administration of the vitamin D supplementation.

Blood Sample Collection:

Two blood samples were collected from each DM2 patient pre- and post-administration of vitamin D supplementation for three months. Each sample was collected and treated as follows: 5 ml of blood were collected, by venipuncture, from each patient after 8 hours of fasting, then transferred into a tube containing no additives and centrifuged for 15 minutes at 8000 rpm, and finally, the obtained serum was transferred to a clean specimen tube and stored at -20°C.

Measurement of HbA1c and vitamin D level:

Measurement was performed using ichroma™ II (Boditech Med Incorporated, Gangwon-do, Korea). The applied methods for measuring HbA1c and vitamin D levels were carried out according to the manufacturer's protocol and were based on fluorescence immunoassay technology**.**

Statistical analysis:

Collected data were reviewed and all statistical analyses were performed using R package version 4.0.2 (R Foundation for Statistical Computing, Vienna, Austria). Categorical data were described in numbers and percentages, while continuous data were presented as Mean±Standard Deviation and Range (Max and Min).

According to HbA1c levels, the participants were divided into three categories: Diabetes (6.5% or higher), Pre-diabetes (6.4% - 5.7%) and Normal (4.0% - 5.6%) [21]. The participants were divided into two groups based on BMI: Normal weight (18.5 kg/m^2 -24.9 kg/m^2) and non-normal weight which includes the overweight and obese categories (≥25.0 kg/m^2) [22]. Patients were also grouped according to base vitamin D levels: Severe Deficiency (<10 ng/ml) [23], Deficiency (10-20 ng/ml), Insufficiency (20-30 ng/ml) and Sufficient (>30 ng/ml) [24].

Univariate analyses of serum levels of HbA1c at the baseline and after three months of vitamin D administration were accomplished using the unpaired *t-test* and paired *t-test* where appropriate. A mixed ANOVA was conducted to evaluate the vitamin D effect on HbA1c levels in serum of DM2 patients across the groups of the study factors and to study interactions in order to find out if any factor supports vitamin D in reducing HbA1c levels in patients. This analysis was performed to test for the between-subject factors (vitamin D and study factors), and the within-subject factor of Time (two levels, pre-and post-administration of vitamin D). According to Cohen [25], values of the effect size of 0.1, 0.3 and 0.5 were classified as small, medium, and large respectively. A P-value of <0.05 was considered significant.

Results

In the present study, 100 diabetic patients were recruited as participants; three of them were considered as outliers and were excluded. The whole analysis was based on 97 patients: 53% were female and 47% were male. The mean age and BMI of participants were 42.76 years and 26.63 kg/m^2 respectively. Tables 1 and 2 present demographic and laboratory data of selected participants.

Table 1: Frequency distribution of variables in all selected participants

Variables		n	Percentage
Gender	Male	51	53%
	Female	46	47%
BMI (kg/m^2)	Normal weight	18	19%
	Overweight & Obese	79	81%
Age (y)	<35	22	23%
	35-50	56	57%
	≥50	19	20%
Diabetic	Yes	43	44%
	No	54	56%
Sport	Yes	15	15%
	No	82	85%
Food	Normal	46	47%
	Fat	51	53%
Smoking	Yes	36	37%
	No	61	63%

As shown in Table 1, more than half of the patients were nonsmokers (63%) and 85% did not practice sport. About half of the participants eat fatty food (53%), and 44% of patients have a family history of diabetes. As illustrated in Table 2, the average vitamin D level was 8.34 ng/ml before supplementing the patients with vitamin D, and the full range was between 4.09-19.01 ng/ml. However, the average vitamin D level increased to 39.95 ng/ml after administration of vitamin D and its highest value reached 68.76 ng/ml.

Table 2: Mean and standard deviation of quantitative variable in selected participants

Variables	Mean $\pm SD$	Min	Max
Age (years)	42.76±8.76	26	36
Height (m)	1.71±0.07	1.55	1.88
Weight (kg)	78.10±7.61	64	92
BMI (kg/m^2)	26.63±2.11	21.70	31.90
Vitamin D^1 (ng/ml)	8.34±2.59	4.09	19.01
Vitamin D^2 (ng/ml)	39.95±12.85	20.66	68.76

SD: Standard deviation, [1] [2]Measured before and after administration of vitamin D respectively

Before vitamin D administration, all DM2 patients were suffering from variable degrees of vitamin D deficiency; however, this number decreased to 23 patients, while 74 patients' vitamin D status improved to sufficient levels (Table 3). According to the HbA1c level in serum of DM2 patients, the results have shown that 91 out of 97 participants had a high level of HbA1c before administration of vitamin D (Table 3); of these 66 patients had severe vitamin D deficiency, 25 patients had a deficiency in vitamin D and only two participants had pre-diabetes (1 with severe deficiency and 1 with deficiency). Therefore, all diabetic patients in this study had a low level of vitamin D.

Table 3: Distribution of HbA1c level in all study participants before and after administration of vitamin D

	Grouping by vitamin D level	Grouping by HbA1c level		
		Diabetes	Pre-diabetes	Normal
Before administration of vitamin D	Severe Deficiency	66	1	3
	Deficiency	25	1	1
	Total	91	2	4
After administration of vitamin D	Insufficient	1	-	22
	Sufficient	38	-	36
	Total	39	-	58

After administration of vitamin D, the findings of this study also showed that 39 participants had a high level of HbA1c. Consequently, the level of HbA1c of 54 patients reduced after taking the supplementation.

The average level of HbA1c was decreased from 6.97% at baseline to 5.65% after supplementation of vitamin D. The result of the paired t-test in Table 4 showed that the mean difference of HbA1c level pre- and post-administration of vitamin D was significant (P-value =0.000).

Table 4: Results of Descriptive Statistics and paired t-test for HbA1c level

	Before administration of vitamin D		After administration of vitamin D		
Outcome	Mean	SD	Mean	SD	p-value
	6.97%	0.68%	5.65%	1.01%	0.000

According to the different factors, the mean and standard deviation of the HbA1c level in patients was shown in Table 5. In each group of study factors, the result showed that the difference was significant in HbA1c levels before and after administration of vitamin D as a supplement. (P-value = 0.000).

Table 5: Mean and standard deviation of HbA1c level according to different factors before and after administration of vitamin D.

Factors		**Baseline**	**After 3months**	**P**
Gender	Male	6.92±0.67	5.59±0.99	0.000
	Female	7.02±0.70	5.71±1.04	0.000
BMI	Normal weight	7.09±0.78	5.84±1.12	0.000
	Overweight & Obese	6.95±0.66	5.61±0.99	0.000
Age	<35	6.88±0.87	5.53±1.04	0.000
	35-50	7.02±0.60	5.79±1.09	0.000
	≥50	6.95±0.69	5.65±0.99	0.000
Family history of diabetes	Yes	7.07±0.65	5.82±1.05	0.000
	No	6.86±0.71	5.44±0.94	0.000
Sport	Yes	6.54±0.70	5.42±0.94	0.000
	No	7.05±0.65	5.70±1.03	0.000
Food	Normal	6.93±0.77	5.70±1.07	0.000
	Fat	7.01±0.60	5.61±0.97	0.000
Smoking	Yes	6.82±0.63	5.49±0.99	0.000
	No	7.06±0.70	5.75±1.02	0.000

The findings showed that the HbA1c level of males reduced more than females after administration of vitamin D. The patients whose age was lower than 35 years old had a lower level of HbA1c compared to others, while the participants whose age was higher than 50 years old had the highest level of HbA1c before and after administration of vitamin D. The patients whose weight was abnormal, had a lower level of HbA1c than other patients. Furthermore, the participants who have a family history of diabetes and the participants who did not practice sport had a higher level of HbA1c.

The 3-way mixed ANOVA was conducted to evaluate the effect of the study factors and the effect of their interaction with vitamin D on interest in HbA1c levels. The main effect of time (before and after administration of vitamin D) was significant with a large effect size (P-value = 0.000), indicating that across all the participants, HbA1c levels were not similar before and after taking vitamin D and reduced after taking the supplementation. This result confirmed the result of the paired t-test.

There was a significant interaction between the time at which the level of HbA1c was measured and the level of vitamin D (sufficient and insufficient) with a small effect size (P-value = 0.000). Pairwise, comparisons in Table 6 revealed that the average level of HbA1c was significantly different between the participants with sufficient and insufficient levels of vitamin D after taking the supplementation. Participants were more likely to achieve a lower level of HbA1c if their level of vitamin D reached between 20 - 30 ng/ml.

Table 6: Mean and standard deviation of HbA1c level according to vitamin D level after administration of vitamin D

	Insufficient		**Sufficient**		
Outcome	Mean	SD	Mean	SD	p-value
Baseline	6.75	0.90	7.04	0.58	0.075
After 3 months	4.92	0.53	5.88	1.02	0.000
p-value	0.000		0.000		

The main effect of all the study factors and their interaction with vitamin D was not significant, indicating that the level of HbA1c was almost similar between the groups of the study factors before and after taking vitamin D as a supplement for three months. Consequently, the change in HbA1c level did not

depend on these factors. More importantly, no factor assisted vitamin D to reduce HbA1c levels in serum of DM2 patients.

Discussion

Sources of vitamin D for humans include food, fortified food, supplements, and exposure to sunlight [26, 27]. Vitamin plays a significant role in regulating calcium-phosphorus homeostasis and control of bone metabolism [28] and has some effects on cell proliferation and the immune system [29, 30], while its deficiency is associated with rickets [27], the incidence of diabetes and cardiovascular diseases [30, 31].

The results of the present study agree with previous studies [19] where the level of vitamin D in the serum increased from 8.34±2.59 ng/ml before to 39.95±2.85 ng/ml after administration of vitamin D, respectively. Together, the present findings showed that the average level of HbA1c has significantly decreased after the administration of vitamin D from 6.97% to 5.65%. Overall, these results agree with the results reported by [16, 19 and 20]. In accordance with a previous study [17], a significant decrease was found in the HbA1c level after vitamin D was taken particularly between the insufficient group (20 – 30 ng/ml) and sufficient group (>30ng/ml), where a 1.17% and 1.16% decrease can be seen in the HbA1c level compared to the baseline after 3 months, respectively. This result ties well with a previous study [19], which indicated that the higher the level of HbA1c at the baseline among DM2 patients, the greater the decrease in HbA1c concentration after vitamin D supplementation is taken for 3 months [19,21].

The findings of this study have also shown an association between vitamin D and HbA1c level, where the level of HbA1c reduced significantly between subjects after the administration of vitamin D. Nevertheless, the main effect of all the study factors and their interaction with vitamin D was not significant, indicating that the levels of HbA1c were quite similar between the study factors before and after the administration of vitamin D for three months. In addition, a relationship between HbA1c and BMI was also reported, and, in this respect, overweight subjects had lower HbA1c levels and higher 25(OH) D levels compared with subjects with a BMI classified as a healthy weight. This could be related to the absorption and metabolism of vitamin D which might be influenced by body composition and insulin resistance in overweight subjects [32]. There is another explanation for these differences, where race is referred to as causing individual variance due to the different vitamin D receptor polymorphisms and vitamin D-binding protein polymorphisms. [17].

In our study, the main conclusion that can be drawn is that there was a significant relationship between serum vitamin D level and HbA1C levels. The results showed that the level of HbA1C was significantly reduced after the administration of vitamin D to DM2 patients. The effect of risk factors (gender, BMI, age, food, smoking, and family history of diabetes) on HbA1C levels was studied and the main effect of time in HbA1c level was indicated after administration of vitamin D. It is worth mentioning that there was no significant difference in the glucose level at the beginning; however, the effect was clear after 3 months of vitamin D administration and HbA1C concentration decreased. The present findings underline that the relationship between the HbA1c level and the factors studied after vitamin D supplementation, that the HbA1c level educed but that reduction in HbA1c was not dependent on the factors studied in this study. The observed decrease in HbA1c could be attributed to other factors such as time and dosage of the vitamin D administered.

There are several limitations of this study. Firstly, the study was conducted at Al Wafi Medical Laboratory Center which is a single center and the samples collected might be small and not representative of every patient with DM2 in the city. Secondly, the sample that was recruited were those patients who came to the center for a routine blood check, which could lead to bias to some extent in the results achieved. Moreover, some factors affecting vitamin D metabolism were not studied and this might have limited our method. Factors such as dietary intake, sun exposure, baseline medication and exercise might be very important in the process of vitamin D synthesis, and they could have made it very difficult to interpret the results and confirm the effect of vitamin D supplementation on HbA1c levels.

Future studies are needed to confirm the physiological and biological association between vitamin D supplementation and HbA1c level in DM2 patients. Moreover, a large-scale randomized intervention study is needed to further investigate the relation between vitamin D and DM2. This may answer some questions about whether the administration or the dietary intake of vitamin D has positive or negative effects on HbA1c levels between patients with DM2. The main conclusion that can be drawn is similar to previous studies, where vitamin D supplementation significantly reduced the level of HbA1c amongst patients suffering from DM2.

Acknowledgment: The authors thank the study participants for their participation in this study.

Funding: The author(s) received no financial support for the research, authorship, and/or publication of this paper.

Conflict of interest: The authors declare that they have no conflict of interest.

References

1- American Diabetes Association. 2. Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes-2020. Diabetes Care. 2020 Jan;43(Suppl 1):S14.
2- Olokoba AB, Obateru OA, Olokoba LB. Type 2 diabetes mellitus: a review of current trends. Oman medical journal. 2012 Jul;27(4):269.
3- Kohei KA. Pathophysiology of type 2 diabetes and its treatment policy. JMAJ. 2010 Feb;53(1):41-6.
4- Olga SC, James HN. The importance of testing for prediabetes—using the right tool. US Endocrinology. 2009;5(1):22-5.
5- Gupta S, Jain U, Chauhan N. Laboratory diagnosis of HbA1c: a review. J Nanomed Res. 2017;5(4):00120.
6- Kobold U, Jeppsson JO, Dulffer T, Finke A, Hoelzel W, Miedema K. Candidate reference methods for hemoglobin A1c based on peptide mapping. Clinical chemistry. 1997 Oct 1;43(10):1944-51.
7- Zhao Y, Xu G, Wu W, Yi X. Type 2 diabetes mellitus-disease, diagnosis and treatment. J Diabetes Metab. 2015 May 1;6(533):2.
8- Daily JW, Yang M, Kim DS, Park S. Efficacy of ginger for treating Type 2 diabetes: A systematic

review and meta-analysis of randomized clinical trials. Journal of Ethnic Foods. 2015 Mar 1;2(1):36-43.
9- Dakhale GN, Chaudhari HV, Shrivastava M. Supplementation of vitamin C reduces blood glucose and improves glycosylated hemoglobin in type 2 diabetes mellitus: a randomized, double-blind study. Advances in pharmacological sciences. 2011 Jan 1;2011.
10- Yin RV, Phung OJ. Effect of chromium supplementation on glycated hemoglobin and fasting plasma glucose in patients with diabetes mellitus. Nutrition journal. 2015 Dec 1;14(1):14.
11- Jafari T, Faghihimani E, Feizi A, Iraj B, Javanmard SH, Esmaillzadeh A, Fallah AA, Askari G. Effects of vitamin D-fortified low fat yogurt on glycemic status, anthropometric indexes, inflammation, and bone turnover in diabetic postmenopausal women: A randomised controlled clinical trial. Clinical nutrition. 2016 Feb 1;35(1):67-76.
12- Krul-Poel YH, Westra S, ten Boekel E, ter Wee MM, van Schoor NM, van Wijland H, Stam F, Lips PT, Simsek S. Effect of vitamin D supplementation on glycemic control in patients with type 2 diabetes (SUNNY trial): a randomized placebo-controlled trial. Diabetes Care. 2015 Aug 1;38(8):1420-6.
13- Rad EY, Djalali M, Koohdani F, Saboor-Yaraghi AA, Eshraghian MR, Javanbakht MH, Saboori S, Zarei M, Hosseinzadeh-Attar MJ. The effects of vitamin D supplementation on glucose control and insulin resistance in patients with diabetes type 2: a randomized clinical trial study. Iranian journal of public health. 2014 Dec;43(12):1651.
14- Jehle S, Lardi A, Felix B, Hulter HN, Stettler C, Krapf R. Effect of large doses of parenteral vitamin D on glycaemic control and calcium/phosphate metabolism in patients with stable type 2 diabetes mellitus: a randomised, placebo-controlled, prospective pilot study. Swiss medical weekly. 2014;144(w13942): w13942.
15- Li X, Liu Y, Zheng Y, Wang P, Zhang Y. The effect of vitamin D supplementation on glycemic control in type 2 diabetes patients: a systematic review and meta-analysis. Nutrients. 2018 Mar;10(3):375.
16- Wu C, Qiu S, Zhu X, Li L. Vitamin D supplementation and glycemic control in type 2 diabetes patients: a systematic review and meta-analysis. Metabolism. 2017 Aug 1; 73:67-76.
17- Krul-Poel YH, Ter Wee MM, Lips P, Simsek S. The effect of vitamin D supplementation on glycaemic control in patients with type 2 diabetes mellitus: a systematic review and meta-analysis. Eur J Endocrinol. 2017 Jan 1;176(1): R1-14.
18- George PS, Pearson ER, Witham MD. Effect of vitamin D supplementation on glycaemic control and insulin resistance: a systematic review and meta-analysis. Diabetic Medicine. 2012 Aug;29(8): e142-50.
19- Nada AM, Shaheen DA. Cholecalciferol improves glycemic control in type 2 diabetic patients: a 6-month prospective interventional study. Therapeutics and clinical risk management. 2017; 13:813.
20- Mohamad MI, El-Sherbeny EE, Bekhet MM. The effect of vitamin D supplementation on glycemic control and lipid profile in patients with type 2 diabetes mellitus. Journal of the American college of nutrition. 2016 Jul 3;35(5):399-404.
21- Sherwani SI, Khan HA, Ekhzaimy A, Masood A, Sakharkar MK. Significance of HbA1c test in diagnosis and prognosis of diabetic patients. Biomarker insights. 2016 Jan;11:BMI-S38440.
22- Shiraishi M, Haruna M, MATSUZAkI M, Murayama R. Demographic and lifestyle factors associated with vitamin D status in pregnant Japanese women. Journal of nutritional science and vitaminology. 2014;60(6):420-8.
23- Kennel KA, Drake MT, Hurley DL. Vitamin D deficiency in adults: when to test and how to treat. InMayo Clinic Proceedings 2010 Aug 1 (Vol. 85, No. 8, pp. 752-758). Elsevier.
24- Ringe JD, Kipshoven C. Vitamin D-insufficiency: An estimate of the situation in Germany. Dermato-endocrinology. 2012 Jan 1;4(1):72-80.
25- Cohen J. A power primer. Psychological bulletin. 1992 Jul;112(1):155.
26- Holick MF. Vitamin D deficiency. New England Journal of Medicine. 2007 Jul 19;357(3):266-81..
27- Holick MF. Resurrection of vitamin D deficiency and rickets. The Journal of clinical investigation. 2006 Aug 1;116(8):2062-72.
28- Sassi F, Tamone C, D'Amelio P. Vitamin D: nutrient, hormone, and immunomodulator. Nutrients. 2018 Nov;10(11):1656.
29- Gil A, Plaza-Diaz J, Mesa MD. Vitamin D: classic and novel actions. Annals of Nutrition and Metabolism. 2018;72(2):87-95.
30- Wang H, Chen W, Li D, Yin X, Zhang X, Olsen N, Zheng SG. Vitamin D and chronic diseases. Aging and disease. 2017 May;8(3):346.
31- Kulie T, Groff A, Redmer J, Hounshell J, Schrager S. Vitamin D: an evidence-based review. The Journal of the American Board of Family Medicine. 2009 Nov 1;22(6):698-706.
32- Lee P, Greenfield JR, Seibel MJ, Eisman JA, Center JR. Adequacy of vitamin D replacement in severe deficiency is dependent on body mass index. The American journal of medicine. 2009 Nov 1;122(11):1056-60.