RELATIONSHIP BETWEEN SERUM 25-HYDROXYVITAMIN D LEVEL AND PREECLAMPSIA COMPONENTS AND METABOLIC PARAMETERS AMONG OVERWEIGHT OR OBESE PREGNANT WOMEN

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ABSTRACT

BACKGROUND: Studies have shown that vitamin D deficiency can be related to the occurrence of preeclampsia.

OBJECTIVE: The present study was conducted with the aim of determining the relationship between the serum level of 25-hydroxyvitamin D and preeclampsia and its related risk factors among obese or overweight pregnant women.

METHODS: This cross-sectional-analytical study was conducted on 83 pregnant obese or overweight women referred to comprehensive health service centers in Makoo city. Anthropometric indices and biochemical factors including vitamin D serum level, glycemic indices and lipid parameters were investigated in these women. In addition, the food intake of the participants was evaluated using an Food Frequency Questionnaire (FFQ).

RESULTS: The results showed that there is a direct correlation between vitamin D serum level and dietary fat level (r=0.269, p=0.007) and high-density lipoprotein (HDL-C) (r=0.478, p<0.0001). Also, there was an inverse correlation between serum vitamin D level and pre-pregnancy body mass index (r=-0.625, p<0.0001), systolic blood pressure (r=-0.592, p<0.0001), diastolic blood pressure (r=-0.592, p<0.0001), fasting blood sugar (FBS) (r=-0.511, p<0.0001), proteinuria (r=-0.422, p<0.0001), triglyceride (TG) (r=-0.36, p = 0.011), total cholesterol(TC) (r=-0.428, p<0.0001) and low-density lipoprotein (LDL-C) (r=-0.602, p<0.0001).

CONCLUSION: There is a direct relationship between dietary fat intake and serum vitamin D level. There is also a positive correlation between serum levels of this vitamin and serum HDL-C. On the other hand, there is an inverse relationship between the serum level of vitamin D and systolic blood pressure, degree of proteinuria, pre-pregnancy body mass index and fasting blood sugar total cholesterol and low-density lipoprotein .

KEYWORDS: Vitamin D, preeclampsia, pregnancy, Obesity, Glycemic Index, Body mass index

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INTRODUCTION

Preeclampsia is a specific syndrome during pregnancy that manifests itself as high blood pressure and proteinuria in pregnant women who were healthy before pregnancy and usually appears in the 20th or later week of pregnancy. Preeclampsia occurs in approximately 5 to 7% of all pregnancies[1]. Of course, according to the new guidelines of the American College of Obstetricians and Gynecologists (ACOG), the diagnosis of preeclampsia does not need to determine proteinuria; in fact, high blood pressure is sufficient to confirm preeclampsia[2]. This disease is the most common medical complication of pregnancy and one of the causes of pregnant women death. Preeclampsia causes 50,000 deaths every year[3]. For this reason, WHO considered one of the Millennium Development Goals be reducing maternal mortality by 75% between 1990 and 2015[4]. Vitamin D is one of the body's essential fat-soluble vitamins that is not produced in the body and is present in small amounts in food sources. The most important source of this vitamin is sunlight. Vitamin D plays an essential role in bone metabolism, absorption of calcium and phosphorus, and maintenance of muscle function; thus vitamin D may play a role in the prevention of preeclampsia[5]. Also, recently, some studies have shown that there is a relationship between vitamin D deficiency and preeclampsia. A study that investigated the relationship between vitamin D and preeclampsia has shown that women with preeclampsia have a lower level of vitamin D than healthy women. Also, the body mass index (BMI) of them was in the range of overweight, while healthy women had normal weight[6]. Due to the fact that vitamin D is a fat-soluble vitamin, this is unavailable to the body in overweight and obese people due to its deposition in the fat tissue, which causes a deficiency of it in the body and causes its complications[7]. Two systematic reviews and meta-analyses with clinical trials method did not show any beneficial effect of vitamin D supplementation in preventing of preeclampsia[8, 9]. This difference in the results of the studies may be due to different variables in the studied communities.

A pilot study in Iran showed that the concentration of 25 hydroxyvitamin D [25(OH)D] was less than 25 nmol/l in 80% of pregnant women[10]. Also, the prevalence of severe, moderate and mild deficiency of vitamin D was 9.5%, 57.6% and 14.2% respectively in a general Iranian population[11].

In another study, the level of 25-hydroxyvitamin D in 552 pregnant mothers who had referred to the teaching hospitals of Tehran University of medical sciences was measured during delivery. The results showed that the prevalence of vitamin D deficiency in the maternal blood samples were 66.8% and there was a significant relationship between the concentration of vitamin D in the mothers' blood serums and umbilical cord[12].

Makoo is a city in the West Azerbaijan province of northwest Iran, situated at a high latitude. It is one of the colder regions of Iran, receiving limited sunlight. Consequently, this study has been designed and conducted to explore the relationship between serum levels of 25-hydroxyvitamin D and preeclampsia, along with its associated risk factors, among overweight or obese pregnant women who visited the comprehensive health service centers in Makoo city.

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Method

The participants of this study were selected from those who were referred to health centers in Makoo city in 2019. This is an analytical cross sectional study. This project has been approved by the Ethics Committee of Tabriz University of Medical Sciences with tracking code "IR.TBZMED.REC.1399.1087". Sampling was done by the accessible random method. The records of pregnant women who were obese or overweighed before pregnancy were extracted from the SIB (system information health) system of three comprehensive urban health service centers. Based on the available information, if the pregnant women were in the 20th week of pregnancy or later during sampling, they were invited to participate in the study, with considering the inclusion and exclusion criteria.

The final confirmation was done the by the centers' physicians, based on the diagnostic criteria of preeclampsia.

The sample size was calculated based on the mean and standard deviation of the serum concentration of 25-hydroxyvitamin D in pregnant women suffering from preeclampsia. Data related to the mean and standard deviation of the serum concentration of 25-hydroxyvitamin D were determined using Cochran's formula[13]. In this research, considering 95% confidence and d= 0.03, p=0.7, the sample size was 75. By including 10% non-response, the sample size was 83. It was considered[14]. The following formula was used for calculating the sample size:

$$N = \frac{Z^2 P(1-P)}{d^2}$$

The sampling process of mothers is shown in the figure 1.

In order to check the anthropometric indicators, height and weight of participants were measured and their body mass indexes were calculated. Their Systolic and diastolic blood pressure were measured twice, using a mercury sphygmomanometer from their right arms in sitting position with 15 minutes interval. The average of these two amounts was used to determine the participants' blood pressure.

Data on food intake:

In order to determine the participants' food intake, a semi-quantitative Food Frequency Questionnaire (FFQ) with 80 items was used. The capability and reliability of this questionnaire for the Iranian adult community had already been examined and confirmed[15]. The questionnaire evaluated the consumption pattern of the participants in the past year and was completed face to face. The participants reported the frequency of each questionnaire item on a daily, weekly, monthly and yearly basis. The nutritional data were converted into standard values

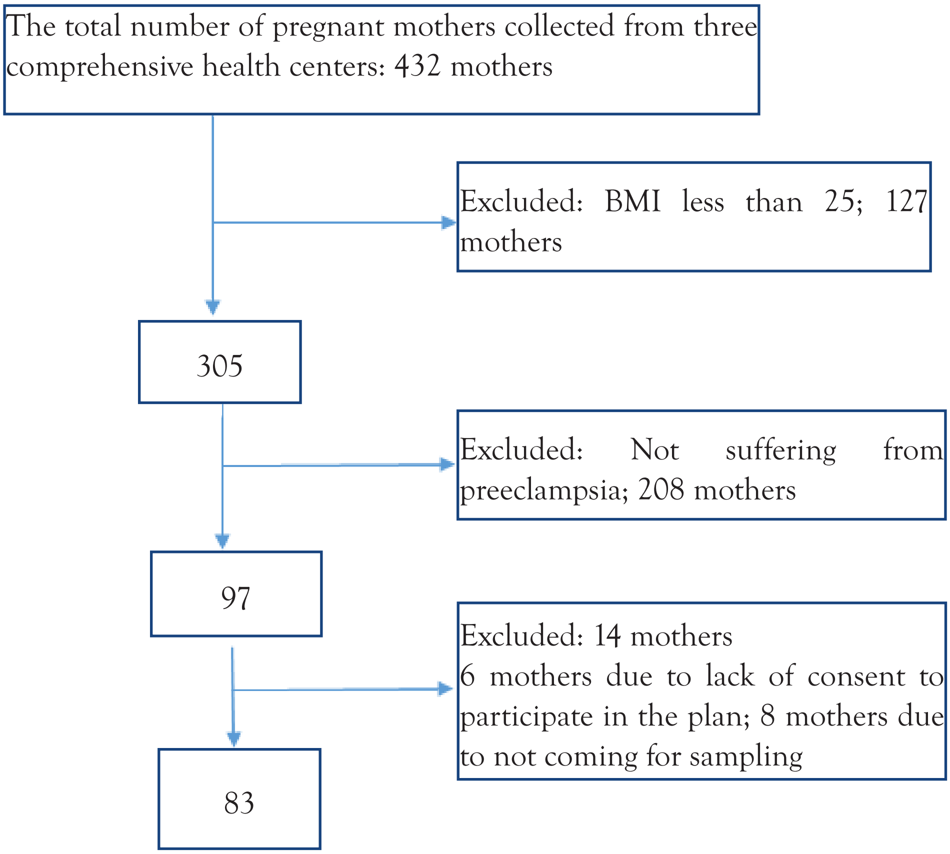


Figure 1: Sampling of mothers from 3 comprehensive health service centers

and then converted into grams using a home scale book. Due to the incompleteness of the table of Iranian food ingredients, some food items included in USDA food ingredients table to determine their energy and nutrients amounts. Also, the Iranian food ingredients table was used for Iranian foods that were not in the USDA food composition table, including food items such as curd.

Examination of biochemical factors:

To measure the biochemical factors, 5 cc of the venous blood samples were taken from the participants after 8 hours of fasting and their serum levels of 25-hydroxyvitamin D, fasting serum glucose, total cholesterol, triglyceride and HDL-C were measured. ECLA commercial kits were used to evaluate the serum levels of 25-hydroxyvitamin D. Serum concentration of fasting glucose, total cholesterol, triglyceride and HDL-C were measured by enzymatic colorimetric method by Pars Azmoon kits. In order to calculate serum LDL-C concentration, the Fried Ewald formula [TC (mg/dL) - HDL-C (mg/dL) - (TG(mg/dL) /5) = LDL-C] was used (16). To measure proteinuria to diagnose preeclampsia, Med-test combi strip tests were used in the urine samples. In case of color change in favor of the presence of protein, sulfosalicic acid solution was used to trace protein in urine. According to the degree of turbidity, it was scored from positive one to positive four.

Data analysis:

The obtained data was analyzed by "SPSS" software version 20. Kolmogrov smirnov test was used to determine the data distribution. Quantitative data were presented as mean and standard deviation and qualitative data were presented as frequency and percentage. If the data were normal, Pearson correlation coefficient was used; if they were not normal, Spearman correlation coefficient was used. Also, taking into account the confounding factors of the relationship evaluation, they were re-examined by Partial correlation. Multi linear regression tests were used to model and better understand the relationship between dependent and independent factors. P-value>0.05 was considered significant.

Results

83 pregnant women with average age of 28.19 ± 6.13 years and gestational age of 29.1 ± 5.6 weeks were included in the study. 68.7% of the samples were overweight and 31.3% were obese. Proteinuria 1+, 2+ and 3+ in the studied samples was 65.1, 32.5 and 2.4%, respectively. Average pre-pregnancy body mass index, serum level of 25-hydroxyvitamin D, systolic and diastolic blood pressure, as well as the average metabolic indexes is shown in Table 1.

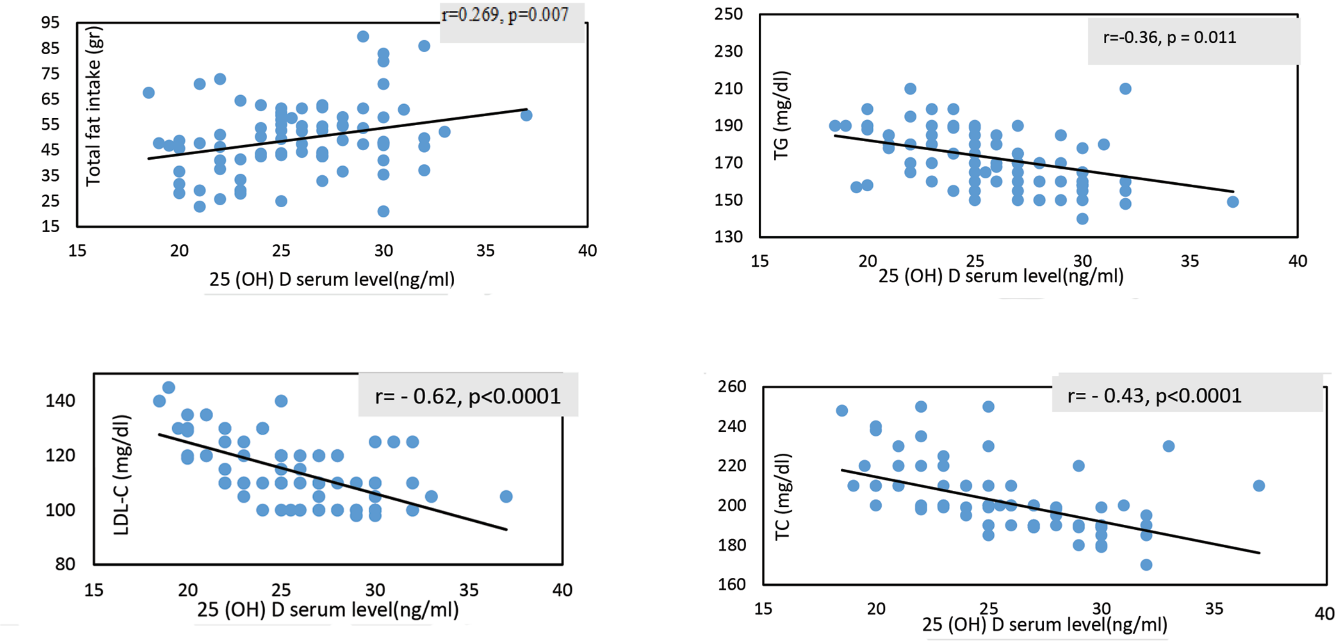
Table 1: Anthropometry and blood pressure in pregnant mothers participating in the study

Variable	Mean ± SD
Body mass index before pregnancy (kg/m2)	29.59±3.56
25 (OH) Vitamin D (ng/ml)	3.80 ±25.88
Systolic Blood pressure (mmHg)	7.74 ±149.94
Diastolic Blood pressure (mmHg)	5.17±94.54
FBS (mg/dl)	17.79 ±91.00
TG (mg/dl)	19.87±173.23
TC (mg/dl)	20.11±201.16
LDL-C (mg/dl)	11.89 ±13.78
HDL-C (mg/dl)	4.40 ±45.05

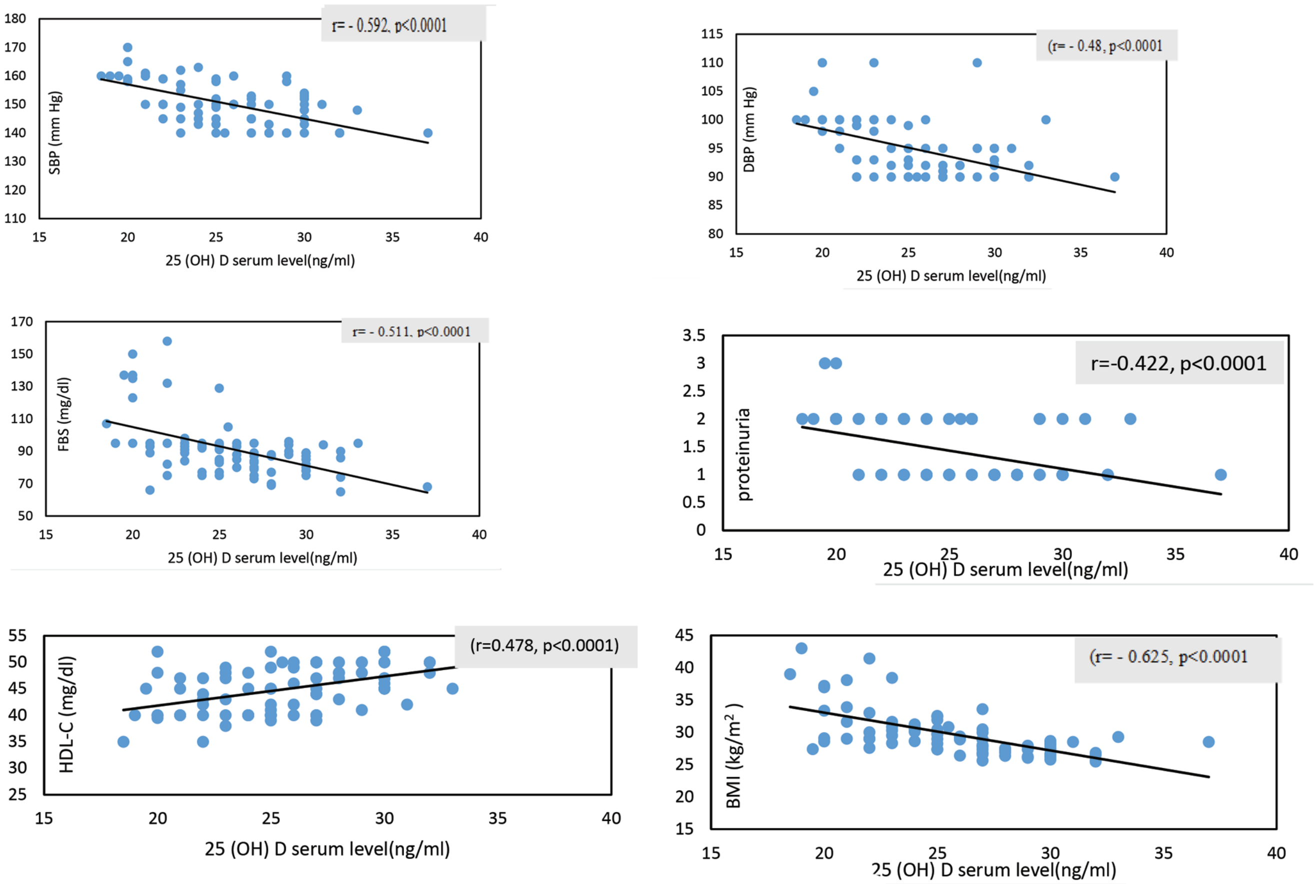
25 (OH) D serum level was normal in 21.7% of the samples (≥30ng/ml) and insufficient in 74.7% of them (20-29 ng/ml). 3.6% of the samples were also suffering from vitamin D deficiency (<20 ng/ml).

A positive and significant correlation was observed between serum level of vitamin D with the level of fat intake and HDL-C, in contrast to a negative and significant correlation between serum vitamin D or preeclampsia components (systolic and diastolic blood pressure and proteinuria), FBS, TG, TC and LDL-C(Graph 1 and 2).

Graf1: Correlation of serum 25 hydroxy vitamin D with fat intake and lipid profil



Graf 2: Correlation of serum hydroxyvitamin D with fasting blood sugar, body mass index and systolic and diastolic blood pressure



The results of multiple linear regression analysis showed a positive and significant relationship between fat intake and serum vitamin D level and no correlation between calories, protein and carbohydrate intake (Table 2).

Table 2: Linear regression results of daily food intake and serum vitamin D level

Variable	model	β estimate	P value
2energy	0	-0.15	0.17
	1	-0.12	0.24
	2	-0.10	0.23
Carbohydrate	0	-0.21	0.08
	1	-0.21	0.09
	2	-0.18	0.13
Protein	0	0.06	0.16
	1	0.06	0.60
	2	0.01	0.90
Fat	0	0.27	0.005
	1	0.27	0.005
	2	0.19	0.010
Pre-pregnancy BMI	0	-0.62	0.000
	1	-0.62	0.000
	2	-0.59	0.000

25-hydroxy vitamin D as dependent variable
Model 0 = crude model
Model 1 = after adjustment for age
Model 2= after adjustment for age, Use of sunscreen, exposure to sunlight, type of Clothing and physical activity

After removing the effect of confounding factors of age, body mass index and daily intake of energy and macronutrients, multiple linear regression analysis showed an inverse and significant relationship between serum level of vitamin D and systolic and diastolic blood pressure levels, proteinuria, FBS, TC and LDL-C. There was a direct and significant relationship between serum level of vitamin D and HDL-C level (Table 3).

Table 3: The results of linear regression of the relationship between serum level of vitamin D and preeclampsia components and the studied metabolic indicators

Variables	model	β estimate	P value
Systolic blood pressure	0	-0.59	0.000
	1	-0.41	0.000
	2	-0.38	0.004
Diastolic blood pressure	0	-0.48	0.000
	1	-0.35	0.006
	2	-0.26	0.067
Proteinuria	0	-0.42	0.000
	1	-0.37	0.002
	2	-0.35	0.013
FBS	0	-0.51	0.000
	1	-0.46	0.000
	2	-0.56	0.000
TG	0	-0.36	0.011
	1	-0.04	0.75
	2	-0.10	0.54
TC	0	-0.43	0.000
	1	-0.25	0.046
	2	-0.24	0.08
LDL	0	-0.61	0.000
	1	-0.47	0.000
	2	-0.53	0.000
HDL	0	0.48	0.000
	1	0.29	0.020
	2	0.39	0.004

25-hydroxy vitamin D as independent variable
Model 0 = crude model
Model 1 = after adjustment for before pregnancy BMI
Model 2= after adjustment for age, before pregnancy BMI, energy and macronutrients Consumption and physical activity

DISCUSSION

In the current study, 97% of pregnant women had mild preeclampsia, with 83% reporting insufficient vitamin D status and 9.6% indicating a deficiency in vitamin D, and none were taking vitamin D supplements. Additionally, analyses conducted on the energy intake and macronutrient data of participants in this study showed that there was only a correlation between vitamin D levels and dietary fat intake, with no significant relationship to other macronutrients. Regression analyses also indicated that only serum vitamin D levels had a relationship with fat intake. Vitamin D is a fat-soluble vitamin that is found in limited food sources including eggs, liver, and fish in small amounts, and the consumption of these foods can play a crucial role in achieving adequate vitamin D levels in the body[17].

One suitable strategy for increasing vitamin D intake and improving vitamin D status in the community is through educating about proper nutrition, utilizing food sources rich in vitamin D, and the fortification of food products. Considering that vitamin D is a fat-soluble vitamin and oils are essential food items consumed daily for cooking, this food category could be an appropriate choice for fortification, as in many countries around the world, oils are fortified with vitamin D[18]. Statistical analyses of the data regarding blood pressure and proteinuria from this study indicated that there is an inverse correlation between systolic and diastolic blood pressure and proteinuria with vitamin D status in overweight and obese pregnant women suffering from preeclampsia.

Razavand and colleagues conducted a study in 2018 aimed at examining the relationship between vitamin D status and blood pressure, preeclampsia, and body mass index in pregnant women. The results of this study indicated that serum vitamin D levels in pregnant women with preeclampsia were lower than those in healthy pregnant women; furthermore, lower vitamin D levels were associated with higher blood pressure in pregnant women suffering from preeclampsia [19].Singla and colleagues investigated vitamin D status and its relationship with preeclampsia in 74 pregnant women, and statistical analyses in this study showed that serum vitamin D levels in the control group were lower than in the study group (p=0.0001). Additionally, systolic and diastolic blood pressure levels in the study group were found to be higher compared to the control group[20]. Moreover, Al-shaikh and colleagues conducted a descriptive study in 2016 to investigate the relationship between vitamin D status and pregnancy outcomes in mothers and newborns. A total of 1,000 pregnant women participated in this descriptive study. The results revealed that the prevalence of vitamin D deficiency in the first trimester of pregnancy was 82.8%. Pregnancy-related hypertension and preeclampsia were reported only in women who had vitamin D deficiency[21].

Several mechanisms have been mentioned regarding the role of vitamin D in preventing the onset of preeclampsia; a reduction in vitamin D levels leads to inflammatory responses and an increase in oxidative stress in the body, which contributes to endothelial dysfunction of the blood vessels, resulting in elevated blood pressure and preeclampsia[22, 23]. Additionally, this vitamin affects the expression of genes involved in angiogenesis and trophoblast invasion, which may play a role in the pathophysiology of preeclampsia[24]. Deficiencies in the body's immune and vascular systems can lead to poor placental invasion, which results in the release of vasoconstrictor factors derived from the placenta and, consequently, leads to maternal hypertension and proteinuria[25].Vitamin D receptors on the heart and blood vessels indicate that vitamin D has a protective effect on the heart and can influence the function of endothelial smooth muscle cells as well as control inflammation and regulate blood pressure through its impact on the renin-angiotensin-aldosterone system. Vitamin D is one of the strongest hormones that suppresses the renin-angiotensin system, thereby regulating blood pressure. Additionally, vitamin D can reduce the risk of preeclampsia through its influence on angiogenesis[26].The present study reports an average body mass index of 29.59 ± 3.56 kg/m2 in the women studied, and an average serum vitamin D level of 49.63 ± 15.06 ng/ml. Statistical analyses also indicate a significant and inverse relationship, which remains significant even after adjusting for confounders of serum vitamin D levels. Bodnar and colleagues conducted a study in 2007 aimed at examining the relationship between the body mass index of pregnant mothers and the vitamin D status of mothers and infants. This study found an independent relationship between vitamin D status and body mass index after adjusting for potential confounders such as race, season of blood sampling, gestational age, physical activity, and multivitamin use. Furthermore, a comparison between underweight and obese women revealed that underweight pregnant women had lower serum levels of vitamin D during weeks 4 to 22 of pregnancy (56.5 nmol/L versus 62.7 nmol/L), and vitamin D deficiency was more prevalent (61% versus 36%)[27].

Various reasons have been cited for the inverse relationship between body mass index and serum vitamin D levels. For instance, obese individuals, due to reduced social acceptance, may spend less time outdoors, leading to decreased sun exposure and subsequent vitamin D synthesis[28]. Additionally, fat tissue in the human body retains vitamin D metabolites produced through sunlight exposure or obtained from dietary sources, preventing their transport to the liver for hydroxylation[29]

Furthermore, a significant amount of the activity of the enzyme alpha-1-hydroxylase in the fat cells of obese individuals is directed towards the local utilization of vitamin D[28, 29]

For this reason, changes in the stores of 25-hydroxy vitamin D may be directly correlated with the amount of subcutaneous fat. Statistical analyses of the findings from the present study showed that serum vitamin D levels had an inverse correlation with fasting blood sugar, triglycerides, total cholesterol, and LDL-C in overweight and obese pregnant women, while showing a direct correlation with HDL-C levels. However, there was no significant relationship with triglycerides. Lithy and colleagues conducted a study aimed at investigating the relationship between serum vitamin D levels and glycemic control in pregnant women. The results of this study indicated an inverse relationship between serum vitamin D levels with fasting blood sugar (r=-0.386) and HbA1c (r=-0.492)[30].

Mansouri and colleagues conducted a study in 2018 to examine the relationship between vitamin D status and metabolic syndrome in the Iranian adult population. The findings of this study revealed an inverse relationship between serum vitamin D levels and blood sugar (OR=0.40), abdominal obesity (OR=0.41), and blood pressure (OR=0.37), but no relationship was observed between vitamin D and lipid parameters[31].The results of the study by Shakri et al., conducted to examine the relationship between vitamin D and biochemical parameters in pregnant mothers, showed that the average level of 25-hydroxyvitamin D in the mothers was 22.52 nanomoles per liter. 7.33% of the mothers had a vitamin D deficiency, 76.6% had insufficient vitamin D levels, and 15.9% were within the normal range. Additionally, there was an inverse relationship between the serum vitamin D level in mothers and fasting blood glucose; however, no correlation was observed between serum vitamin D levels and lipid parameters in these individuals[29].

The mechanism by which vitamin D affects glycemic control is not only through the regulation of plasma calcium levels in the synthesis and secretion of insulin but also by improving the sensitivity of target cells (liver, skeletal muscle, and fat) to insulin. Moreover, vitamin D directly enhances and improves the function of β-cells while protecting them against harmful immune attacks, and it indirectly affects various immune cells, including inflammatory macrophages and different types of T-lymphocytes, all of which play a role in regulating local immune responses[32].

Limitations of the study: One of the weaknesses of this study is that due to financial constraints, it was not possible to conduct the study with a larger sample size, and it was carried out as a cross-sectional analytical study. The absence of a control group made it impossible to achieve a better and more comprehensive comparison. It is suggested that the levels of many substances present in the blood decrease during pregnancy, which can occur for various reasons, including dilution, and that normal values are generally not standardized. Therefore, there is a need to establish a standard normal range for vitamin D during pregnancy.

Conclusion

There was an inverse relationship between serum 25-hydroxyvitamin D levels and systolic and diastolic blood pressure, proteinuriaBased on the findings of this study, it is recommended that maintaining a balanced level of vitamin D plays a crucial role in preventing hypereclampsia in pregnant women. Therefore, it is essential to obtain this vitamin through dietary intake, particularly from fat-containing foods due to the fat-soluble nature of this vitamin. It is important to note that global guidelines suggest a daily supplement of 1000 IU of vitamin D for pregnant mothers, which can be safer and help prevent excessive weight gain during pregnancy that may result from overconsumption. It is advisable to limit foods, especially those high in fats.

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