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The Impact of Vitamin D Deficiency on Some Immunomodulatory Markers in Pregnant Women with Threatened Abortion

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ABSTRACT

The protective mechanism of vitamin D (VD) on the immune system played an important role behind many health problems including abortion. Our aim was to investigate the levels of VD and some immunomodulatory markers (IL-6, TNF-α and INF-γ) in pregnant women with threatened abortion (TA) and their effects on their obstetric health. This study including 132 pregnant participants with TA visiting maternity hospital in Babel province, Iraq. The demographic data including previous and current obstetrical history and body mass index were collected by well-trained researcher following structured questionnaire after obtaining their verbal consent. Laboratory Investigations were done with estimation of VD, IL-6, TNF-α and INF-γ. The mean level of VD was 19.83±43.59 ng/ml while the mean levels of IL-6: 389.45±70.88, TNF-α: 118.78±44.11 and INF-γ: 100.39±20.13 pg/ml with related percentage among participants for low VD was 84%, high IL-6 was 66%, high TNF-α was in 43% but only 23% had high INF-γ in relation with normal values. The frequency of abortion was significantly increased with low VD and high IL-6 ($p<0.05$) with high correlation between them ($r^2=0.89$). High incidence of TA associated with low level of VD accompanied with high serum levels of IL-6. This adverse pregnancy outcome may be associated with some form of autoimmune diseases with evidence that hypovitaminosis D was correlated with high proinflammatory markers (IL-6, TNF- α and IFN- γ) and worse form of TA and the role of VD is to correct these pro-inflammatory markers.

1. INTRODUCTION

Threatened abortion is a clinical pregnancy associated with an intrauterine viable fetus that commonly accompanied by painless vaginal bleeding and may normally continuation of the pregnancy or be accompanied by pain to become an inevitable abortion [1]. Identifying the associated factors can play an important role in preventing it and improving pregnancy outcomes. There is a crucial evidence for vitamin D in the body's immune system. Most of these researches have been done in cultured cells and in animals to prove the active control of vitamin D metabolism. Vitamin D receptor is found in significant concentrations in the T lymphocyte mostly the mature CD-8 T lymphocytes. This suggests that the action of T lymphocytes proved through calcitriol [2], [3]. The role of vitamin D is to block the effects of adaptive immune system leading to prevent the autoimmunity that proved by animal studies which showed with absent of calcitriol, the immune cells are attacked the healthy cells of the body leading to autoimmunity [2]. Moreover, vitamin D can be blocking the production of pro-inflammatory cytokines like interleukin-6 (IL-6), Tumor Necrotizing Factor-α (TNF- α) and Interferon-γ (IFN- γ) and that may also have associated with stimulating the production of anti-inflammatory cytokines and decreasing in killer T cells production [2], [4]. On other hand, the deficiency of vitamin D can inhibit the production of B lymphocytes leading to blocks the production of immunoglobulins [3- 5].

In normal immune response, T helper-1 cells produce INF-γ, IL-2, and TNF-α, and this response is strongly addressed to intracellular pathogens as viruses while T helper-2 lymphocytes produce mainly Tissue Growth Factor (TGF)-β1and IL-4 and IL-5, and these mediators are linked with extracellular pathogens as bacteria and parasites (6) that proved the same role of VD decreases the production of T helper-1 producers like INF-γ, IL-2, and TNF-α and increases the production of T helper-2 producers like IL-4 and IL-5 [6], [7].

Furthermore, the immunomodulating effects of VD and its analogs have been well-characterized in dendritic cells (DCs), which are as antigen presenting cells (APCs). In response to inflammatory signals, VD strongly impairs the migration and maturation of DCs, which reduces antigen presentation capacity and activation of T lymphocytes with marked reduction in pro-inflammatory ILs that contribute as an induction of tolerogenic state [3], [4].

The aim of study was to investigate the levels of VD and some immunomodulatory markers (IL-6, TNF-α and INF-γ) in pregnant women with TA and their effects on their obstetric health.

2. SUBJECTS AND METHODS

2.1 Patients

The number of patients was 132, study was carried out at maternity hospital in Babel province, Iraq during the period from June, 2019 until March, 2020. Sample size was taken by consecutive manner.

2.2 Exclusion criteria

Eligible patients should not document with known chronic liver, kidney, thyroid or gastrointestinal diseases, hypertension, diabetes, or using any medications that affect vitamin D level (e.g. anticonvulsants, antituberculosis drugs, etc)

2.3 Criteria of threatened abortion

Persistent vaginal bleeding at first 20 weeks of gestation with viable fetus [1].

2.4 Ethics statement

The study was carried out in compliance with the Declaration of Helsinki principles and was reviewed and

approved by local research ethics committees of Faculty of Pharmacy, University of Babylon (Protocol No. 193/2019)). All-important study material was communicated to the patient group, and they were told that their rejection or withdrawal from the trial would have no impact on the medical care they received. After gaining their verbal consent, the data was collected by a well-trained researcher using a standardized questionnaire.

2.5 Demographic and clinical variables

Maternal age, residency, socioeconomic states, educational and occupation states, previous and current obstetrical history and body mass index (BMI) were collected by questionnaire to all participants.

The effects of maternal age were investigated using continuous and categorical approaches (coded as age < 25, 25-30, 30-35, and 35-40 and > 40 years).

Medical records were abstracted to a certain their anthropomorphic characteristics as well as their medical status throughout gestation. Pregnancy BMI was calculated using the admission weight and height measurements.

2.6 Laboratory Investigation

During admission period, 5ml venous blood sample was collected to measure vitamin D, IL-6, TNF-α, and IFN-γ.

2.7 Level of Serum vitamin D3 (25-hydroxyvitamin-D)

Estimation of vitamin D level from blood samples were determined by using a commercial enzyme-linked immunosorbent assay (ELISA) (Demediteq: DE1971, Germany). According to the manufacturer's protocol, each assay was run with known standards (provided with the kit) that were used to determine the quantity of vitamin D in each sample in ng/ml. The range of detection was 3–300 ng/mL. Patients' vitamin D status was evaluated according to vitamin D concentrations into deficient Levels below 25 ng/ml, insufficient between 25–75 ng/ml and normal level ≥75 ng/ml [8].

2.8 Immunomodulatory markers: IL-6, TNF-α, and IFN-γ levels

Inflammatory cytokines indices were measured by ELISA technique (elabscience company, USA). IL-6 is by E-EL-H0192 kit with normal values ranging from (104.35-238.90 pg/ml), TNF-α by E-EL-H 0109 kit with normal values ranging from (24.10-86.50 pg/ml), and IFN-γ by E-EL- H0108 kit with normal values ranging from (51.90-100.10 pg/ml). According to the manufacturer's protocol; each assay was run with known standards (provided with the kit) that were used to determine the quantity of cytokines in each sample.

2.9 Statistical analysis

To explore relationships and differences in Demographic, clinical factors, VD, and immunomodulatory markers, descriptive measures, one way and two-way ANOVA analysis were utilized. For all analyses, the significance level was fixed at a probability (P) of less than or equal to 0.05. GraphPad Prism 5.3 on Windows was used to conduct all analyses (GraphPad Software, San Diego, CA, USA).

3. RESULTS

Serum VD levels ranged from 2.8 to 68.5 ng/ml with mean level was 19.83±43.59 ng/ml. The distribution of VD among participant population was 84% (mean: 16.2±10.6 ng/ml) with deficient vitamin D (< 25 ng/ml), 11% (mean: 32.8±17.6) with insufficient vitamin D (25-75 ng/ml) and only 5% (mean: 76.5±3.6 ng/ml) with normal vitamin D level (>75 ng/ml) (Table 1). The rang of IL-6 levels was 133.94-438.90 pg/ml with mean

level of 389.45±70.88 and associated with 66% of participant higher than normal rang. The rang of TNF-α levels was 78.51-162.31 pg/ml with mean level of 118.78±44.11 and associated with 43% of participant higher than normal rang. The rang of INF-γ levels was 80.22-119.93 pg/ml with mean level of 100.39±20.13 and associated with only 23% of participant higher than normal rang (Table 1).

The women in the study ranged in age from 18 to 41 years old, with 70% of them being between the ages of 25 and 35. The majority of the participants were from a middle socioeconomic status (40%) with a secondary education (54%) and were housewives (77%) or multipara (1-3 para) (47%). Participants had a 41% chance of having a previous miscarriage, and a 12% chance of having a fetal death. BMI was 56% of the women were between 25-30 kg/m^2, while 31 % for participant more than 30 kg/m^2 and only 12 % was below 25 kg/m^2. Regarding the anemia, most of participant was non-anemic (58%) in spite of bad dietary history during pregnancy (59%) (Table 2).

Moreover, there was a significant relation ($P> 0.001$) between the numbers of previous mischarge (Table 3) and the levels of VD (Figure 1) that increase in the significances between the numbers of previous miscarriage in the deficient status of vitamin D ($P<0.001$). Moreover, there was also a significant relation between the numbers of previous mischarge and the levels of IL-6, TNF-α and INF-γ (Figure 2), these significances varied according the numbers of previous mischarge (significances ranging from $P<0.05$ to $P<0.001$). On other hand, the frequency of abortion was significantly increased with low VD and high IL-6 ($p<0.05$) with high correlation between them ($r^2=0.89$) (Figure 3).

4. DISCUSSION

There are several physiological alterations associated with pregnancy including changes in VD metabolism making only 16% of women had normal levels of VD [9]. Furthermore, the immune system is also involved in various pathological and physiological events during pregnancy [10].

The presence of different proteins, cytokines, and cells that can contribute to the development and maintenance of pregnancy can lead to the rejection or even termination of the fetus due to the disruption of its immunomodulation [11]. T helper cells are known to be very pathogenic and can trigger autoimmune and inflammatory diseases [12], [13].

Moreover, T helper cells can also contribute to the development and maintenance of pregnancy by regulating the expression of chemokines and proinflammatory cytokines [14]. These cells can also infiltrate and destroy tissue. The presence of cytokines in pregnancy can also alter the effects of different hormones [15].

Some studies revealed that the presence of high levels of proinflammatory cytokines in women who were threatened with an abortion [16], [17] was associated with an increased likelihood of having a negative outcome. These findings support the idea that low levels of these cytokines during early pregnancy are associated with a better chance of having a successful pregnancy [18], [19].

In vitro investigations also shown that participants with spontaneous abortion had higher IL-6 levels than those with normal pregnancy [20], [21], but that IL-6 levels were relatively high in in vitro fertilization produced pregnancies [22]. The release of cytokines into the surrounding fluid by activated T cells, and the rise of these cytokines (IL-6, TNF-, and INF-) in peripheral levels after they were released, suggests immune system activation and may reflect immunologic rejection of the fetus as a cause of spontaneous abortion [23].

Previous research has linked high amounts of IL-6 in pregnant women's peripheral blood and placenta to low

levels of VD [24]. VD has the ability to lessen the occurrence of spontaneous combustion [25].

In pregnancy IL-6 plays a crucial role in chorioamniotitis with involving of rupturing the fetal membranes at term [26]. However, TNF-α is believed to play a role in pre-implantation embryo transport by inducing local contractions [27]. No significant evidence that the changing in the level of INF-γ may affected the outcome of pregnancy in spite of antiviral and cytotoxic effects of it [28] but according to several research, VD status and considerable secretion of greater amounts of IFN- women with recurrent abortion have a high link with women who have at least one healthy life birth without spontaneous abortions or infertility [29].

Our findings point to a more immediate and subtle role for the immune system in threatening abortion than an eventual inflammatory pathogenesis, which has been identified as an immunomodulator in different mechanisms during pregnancy, including VD.

5. CONCLUSIONS

In summary, High incidence of Threatened Abortion associated with low level of Vitamin D accompanied with high serum levels of IL-6. This adverse pregnancy outcome may be associated with some form of autoimmune diseases. Hypovitaminosis D was correlated with high proinflammatory markers (IL-6, TNF- α and IFN- γ) and worse form of Threatened Abortion and the role of Vitamin D is to correct these pro-inflammatory markers.

6. CONFLICT OF INTEREST

No conflict of interest

7. ACKNOWLEDGEMENT

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8. AUTHOR CONTRIBUTION

All authors shared in conceptualization with direct and intellectual contribution to the work, writing, reference search, table and figures preparation and approved it for publication.

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Table 1. Serum Levels of Vitamin D, Interleukin-6 (IL-6), Tumor Necrotizing Factor-α (TNF- α) and Interferon-γ (IFN- γ) in Threatened Abortion Participants (n=132).

Immunomodulatory Markers	Means	Std. Deviation	Std. Error	Lower 95% CI of mean	Upper 95% CI of mean
Vitamin D	19.83 ng/ml	43.59	3.794	12.32	27.34
IL-6	389.5 pg/ml	70.88	6.169	377.2	401.7
TNF-α	118.8 pg/ml	44.11	3.839	111.2	126.4
INF-γ	100.4 pg/ml	20.13	1.752	96.92	103.9

Table 2. sociodemographic data, previous obstetrical and medical information in the studied population in relation to the serum level of vitamin D.

Parameters	Sub-parameters	Normal vitamin D N = 12	Insufficient vitamin D N = 22	Deficient vitamin D N = 98	*P value*
Age	< 25 yrs	3 (2%)	4 (3%)	20 (15%)	*P<0.001*
	25-30 yrs	3 (2%)	8 (6%)	60 (45%)	
	31-35 yrs	1 (1%)	3 (2%)	17 (13%)	
	36-40 yrs	0 (0%)	4 (3%)	8 (6%)	
	> 40 yrs	0 (0%)	0 (0%)	1 (1%)	
Socioeconomic state	Low	0 (0%)	4 (3%)	37 (34%)	*P<0.001*
	Medium	2 (2%)	5 (4%)	42 (39%)	
	High	3 (3%)	4 (4%)	14 (11%)	
Educational Level	Elementary	0 (0%)	4 (3%)	29 (22%)	*P<0.001*
	Secondary	3 (2%)	8 (6%)	63 (48%)	
	Bachelor or >	4 (3%)	3 (2%)	18 (14%)	
Occupation	Housewife	4 (3%)	11 (8%)	89 (68%)	*P<0.001*
	Employee	3 (2%)	4 (3%)	21 (16%)	
BMI (kg/m^2)	<25	0 (0%)	3 (2%)	13 (10%)	*P<0.001*
	25-30	3 (2%)	3 (2%)	68 (52%)	
	> 30	4 (3%)	10 (8%)	28 (22%)	
Number of Parity	Primiparous	6 (4%)	6 (4%)	42 (32%)	*P<0.001*
	1-3 Para	1 (1%)	8 (6%)	55 (42%)	
	> 3 Para	0 (0%)	1 (1%)	13 (10%)	
Status of Pregnancy	Singleton	4 (3%)	10 (7%)	87 (66%)	*P<0.001*
	Multiple	3 (2%)	5 (4%)	23 (17%)	

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History of abortion	Yes	1 (1%)	6 (5%)	34 (26%)	*P<0.001*
	No	5 (4%)	8 (6%)	77 (58%)	
History of Fetal Death	Yes	0 (0%)	3 (2%)	14 (10%)	*P<0.001*
	No	6 (5%)	12 (9%)	98 (74%)	
Dietary History	Bad	1 (1%)	6 (5%)	73 (55%)	*P<0.001*
	Good	5 (4%)	8 (6%)	39 (29%)	
Sun exposure	> One hr	3 (2%)	3 (2%)	16 (12%)	*P<0.001*
	< One hr	4 (3%)	12 (9%)	94 (72%)	

BMI: body mass index

Table 3. The numbers of previous abortions among participant women (n=132).

Previous abortion	**0**	**1**	**2**	**3**	**4 or more**

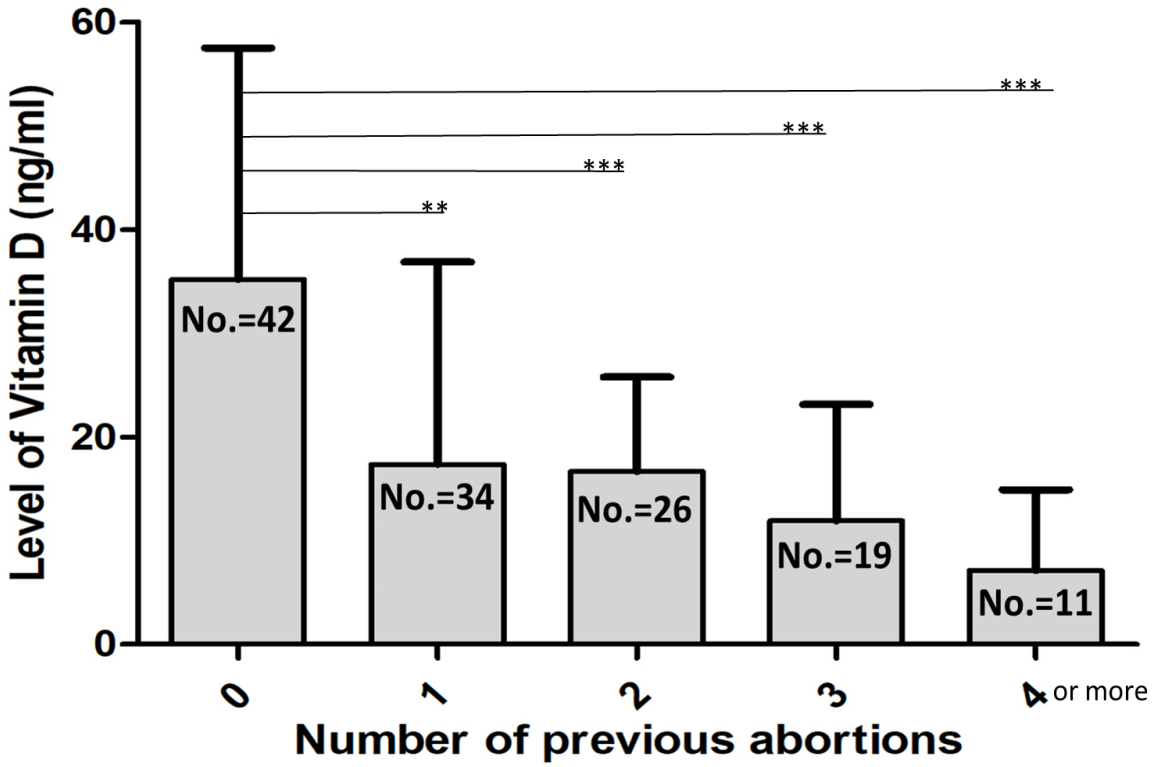

Figure 1. Relation between the levels of vitamin D and the numbers of previous abortion in study participant (n=132). The number of asterisks (*) corresponds to the level of the statistical significance (**P<0.01, ***P<0.001).

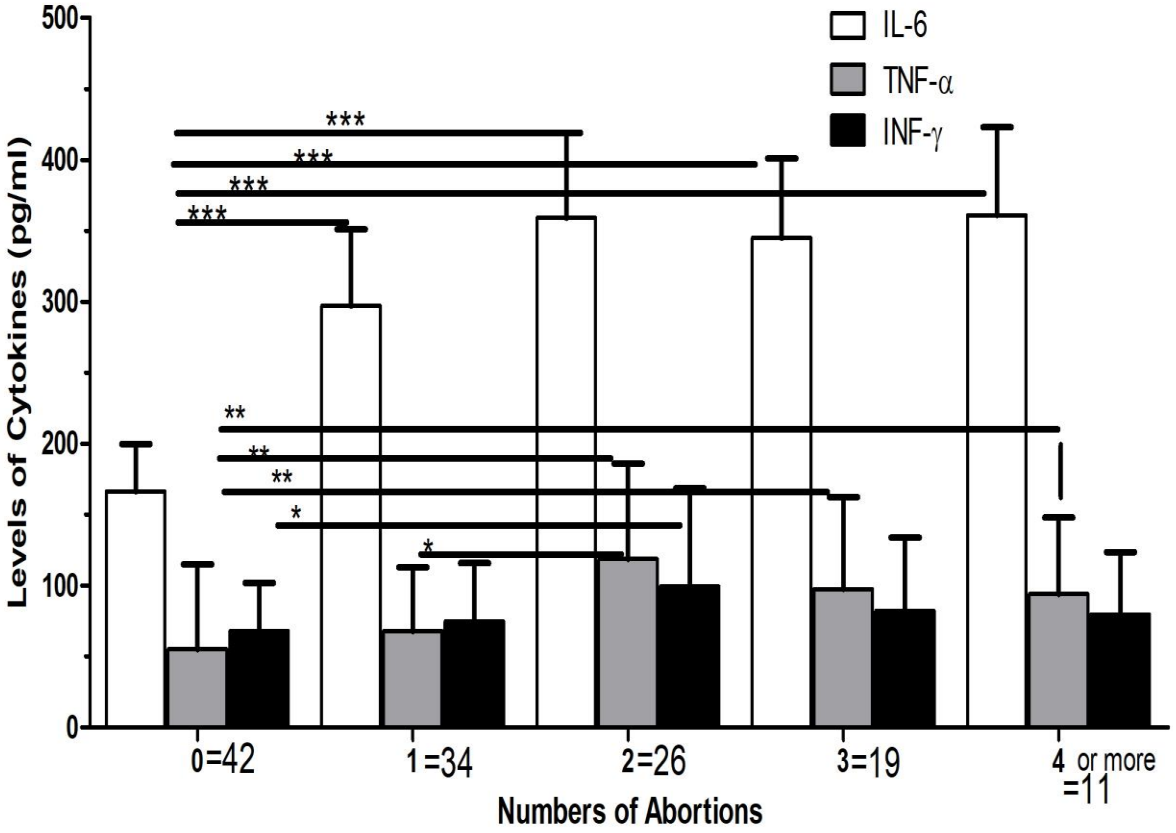

Figure 2. Relation between the levels of Interleukin-6 (IL-6), Tumor Necrotizing Factor-α (TNF- α) and Interferon-γ (IFN- γ) and the numbers of previous abortion in study participant (n=132). The number of asterisks (*) corresponds to the level of the statistical significance (*P<0.05, **P<0.01, ***P<0.001).

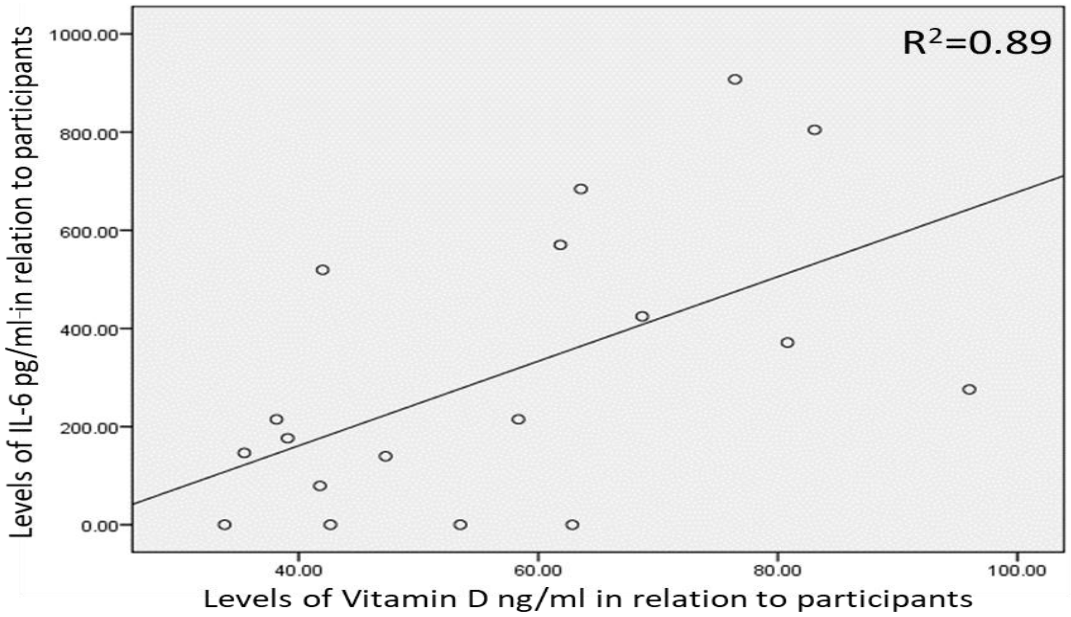

Figure 3. Correlation between the levels of vitamin D levels and Interleukin-6 (IL-6) in relation to participant (n=132)