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The Correlation between Maternal Vitamin D and Interleukin-17 Levels and Fetal Biophysical Profile

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Abstract

This research aims to evaluate the serum levels of vitamin D and interleukin 17 (Il-17) in pregnant women, then finding the correlation between these maternal parameters and fetus biophysical profile. Healthy pregnant women (n=45) and non-pregnant control (n=45) were involved in the study, who attended Baghdad medical laboratory, Baghdad, Iraq, with an age range of 20 to 40 years. An analytical study was conducted from October 2019 until January 2020.

The results of the study show that the mean value of vitamin D level significantly increases ($P\leq0.05$) in pregnant women (11.07±0.93 ng/ml) in comparison with that in non-pregnant control (8.03±0.69 ng/ml). The level of IL-17 was significantly higher ($P\leq0.001$) in the pregnant women (468.38±50.62 Pg/ml) as compared to non-pregnant ones (144.39±3.98 Pg/ml). Also, the results show no significant correlation (0.162) between maternal vitamin D and (F.H.R.), which was measured by ultrasound sonography. Also, there is no significant inverse correlation between maternal Il-17 and fetal growth measurement.

It can be concluded from the current study that some of the signs of the physical appearance of the fetus that were studied in the second trimester (13-28 w) and third trimester (29-41 w) of pregnancy do not correlate with the level of vitamin D in the mother's blood. Also, there is a weak inverse relationship between this appearance and the mother's immune response, represented by measuring the level of IL-17, which needs more studies in the future.

Keywords: vitamin D, interleukin 17, fetus biophysical profile, pregnant women.

التحري عن العلاقة بين مستوى فيتامين (د) و الإنترلوكين-١٧ للأم و المظهر الفيزيائي الحيوي للجنين

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الخلاصة

تهدف هذه الدراسة إلى تقييم مستوى فيتامين د و الإنترلوكين 17 في النساء الحوامل، بعدها إيجاد العلاقة بين هذه المعلمات المادية والصورة الفيزيائية الحيوية للجنين. (العدد=45) من النساء الحوامل الأصحاء و (العدد=45) من النساء الغير حوامل شاركن في هذه الدراسة واللاتي ارتدن الى مختبر بغداد الطبي، في بغداد، واللاتي تتراوح أعمارهن من 20 إلى 40 سنة وقد أجريت هذه الدراسة التحليلية من أكتوبر 2019 حتى يناير 2020.

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النتائج: أظهرت نتائج الدراسة أن متوسط قيمة فيتامين (د) يزداد معنوياً (P <0.05) عند النساء الحوامل (11.07 ± 0.93 ng/ml) بالمقارنة مع غير الحوامل (8.03 ± 0.69 ng/ml) كما ان مستوى IL−17 أعلى بشكل ملحوظ (P <0.001) لدى النساء الحوامل (468.38 + 50.62 Pg /ml) مقارنة بغير الحوامل (144.39 ± 3.98 Pg / ml) كما أظهرت النتائج وجود ارتباط ضعيف جدا(0.162) بين فيتامين د للأم مع .F.H.R عن طريق التصوير فوق الصوتي. إضافة الى وجود ارتباط عكسي ضعيف وغير معنوي بين مستوى IL−17 للأم وقياس نمو الجنين.

الاستنتاج: يمكن الاستنتاج من الدراسة الحالية أن بعض دلائل المظهر الفيزيائي للجنين التي تم دراستها في الثلث الثاني(18−28w) والثالث (29−41w) من الحمل لا يرتبط مع مستوى فيتامين (د) في دم الأم وهناك علاقة عكسية ضعيفة بين هذا المظهر والمستوى المناعي للام المتمثل بقياس مستوى IL−17 ويحتاج هذا الاستنتاج الى دراسات أوسع مستقبلا.

Introduction

Vitamin D is considered as a pleiotropic seco-steroid hormone that is essential for the health of human and prevention of disease [1]. Pregnancy is a period of physiological and physical changes in both pregnant women and their fetuses [2,3]. Pregnancy was associated with a significant change in vitamin D metabolism, with an increase in the active form of maternal serum vitamin D (1,25dihydroxyvitamin) levels [4]. The deficiency of vitamin D during pregnancy is a worldwide problem [5]. Gestational diabetes mellitus, preeclampsia, [6], bacterial vaginosis, and an increased risk of C-section delivery, correlate with deficiency of vitamin D [1]. Also, evidence suggests that low in utero vitamin D levels might be associated with the risk of asthma in the offspring [7]. Nonclassical vitamin D has roles in pregnancy, where the placenta responds to vitamin D and produces it [1]. Vitamin D acts as a modulator of the production of cytokines, implantation, and responses of immunity to infection [1]. An emerging study supports the notion that vitamin D enhances immunity and provides protection against pathogens, while, at the same time, it confers immunosuppressive effects via the prevention of the detrimental impact of prolonged inflammatory responses in the host [8]. The immune cells (T cells, B cells, and antigen presenting cells) have vitamin D receptors and are capable of synthesizing the active metabolites of vitamin D. Vitamin D is capable of acting in a local immunologic milieu in an autocrine manner. It has the ability to moderate the innate and adaptive immune responses [9]. Deficiency in vitamin D was linked with increased autoimmunity and susceptibility to infection [9].

The effects of numerous demographic and biological factors on vitamin D have been investigated, such as ageing, body mass index (BMI), calcium intake, genetics, ethnicity, dietary fat installation, and some medications and diseases, in addition to the dose, type, and period of the supplementation of vitamin D [10]. Also, seasonal variation in vitamin D levels was reported, with an increase after summer and a decrease after winter, along with a habitat related differences in sunlight exposure [11].
The recommended dosage of vitamin D during pregnancy differs between societies. For a Middle Eastern pregnant woman, 3000 IU/day vitamin D doses were essential to reach an eligible maternal level of 25-hydroxy-vitamin D and to positively impact the infant's bone mineral content [12]. This would create a close relationship between the vitamin D status of the mother and her fetus. Maternal supply status could significantly affect the development and health of the fetus in utero and in later life [13]. The aim of this study is to estimate the mean values of vitamin D and IL-17 in the blood of healthy Iraqi pregnant and non-pregnant women and determine the possible correlation between these two components with fetal parameters.

Materials and Methods

Study design and population

This study was an analytical case study conducted from October 2019 until January 2020. The population in this study was a total number of 90 females, 45 are pregnant and 45 are non-pregnant control group, collected from Baghdad medical laboratory, Baghdad, Iraq, with an age range of 20-40 years. The inclusion criteria of pregnant women were that a woman should be healthy and without any chronic disease, gestational diabetes, or pregnancy hypertension. Also, the gestational age was selected to be in the second (18-28w) and third (29-41w) trimesters of pregnancy.

Vitamin D assay

Three milliliter of blood samples were taken using a venipuncture technique. Blood samples were added into labelled, vacuumed, gel & clot activator tube and left for 30 minutes at room temperature to clot before all samples were put in a centrifuge that was set at 3000 rounds per minute (rpm) for 10 minutes. About 1.5ml serum sample was used for vitamin D tests [14]. In this test, a competitive immunodetection method was used, using ichroma Vitamin D kit (boditech, Korea). The test was applied according to the kit's instructions.

Human Interleukin-17 (IL-17) Assay

About 1.5ml of serum sample was transferred by sterile micropipette into sterile Eppendorf tubes for the immunological test and kept frozen until time of analysis. The Pre-Coated ELISA (Enzyme-Linked Immunosorbent Assay) kit of Human IL-17 (BOSTER, USA) is based on a solid phase immunoassay. The test was applied according to the kit's instructions.

Fetal biophysical profile

All pregnant women in the second and third trimester (between 13 - 41 weeks of gestation) underwent doppler ultrasound scanning by a Radiologist, then a copy of the report was taken to estimate:

1. fetal weight (E.F.W)
2. biparietal diameter (B.P.D)
3. abdominal circumference (A.C)
4. head circumference (H.C)
5. femur length (F.L)
6. amniotic fluid (A.F.I)
7. fetal heart rate (F.H.R)

This assessment was compared with the other parameters of the mother. This is considered a widely accessible strategy of evaluating fetal parameters, which has been demonstrated to be a significant marker of fetal health [15].

Statistical analysis

The statistical analysis for the collected results was performed using SPSS program v.16, by applying Student's t-test and ONE WAY ANOVA (LSD) [16], as appropriate, at a significance probability level of 0.05. Pearson correlation coefficient was used to investigate the correlation between the studied parameters. The results were presented as mean + S. E and the Significant differences were considered at $p<0.05$.

Results and Discussion

The results of the current study showed that 69% of the pregnant and 80% of the non-pregnant women had vitamin D deficiency (<10ng/ml), whereas 26.6% of pregnant and 20% of non-pregnant women showed insufficient (10-30ng/ml) vitamin D level. Only 4.4 % of pregnant women had sufficient vitamin D level (30-100ng/ml), as shown in Table-1.

Table 1- Vitamin D levels as percentage in the serum of both groups.

Vitamin D levels	Pregnant	Non-pregnant
Deficiency <10ng/ml	69%	80 %
Insufficiency 10-30ng/ml	26.6 %	20 %
Sufficiency 30-100ng/ml	4.4 %	____

The mean value of vitamin D level significantly increased ($P\leq0.05$) in pregnant women compared with non-pregnant ones. The mean value of vitamin D in pregnancy was 11.07±0.93 ng/ml, whereas it was 8.03±0.69 ng/ml in the control group, as shown in Table-2 and Figure-1.

Table 2- Statistical results of parameters between pregnant and non-pregnant women

Parameters	Mean ± S.E.		P-value
	Pregnant	Non-pregnant	
Vitamin D	11.07±0.93	8.03±0.69	0.01 *
IL-17	468.38±50.62	144.39±3.98	0.001 **
** ($P\leq0.001$), * ($P\leq0.05$)			

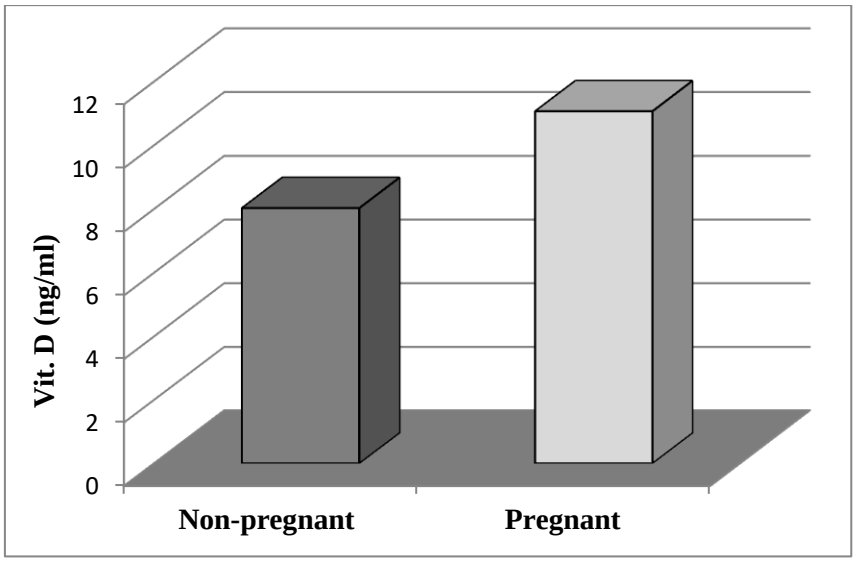

Figure 1- Comparison between the different groups in terms of serum vitamin D level (ng/mL)

A previous study revealed that the serum level of vitamin D was higher among pregnant females compared with non-pregnant ones [17].

Another study explained that, during pregnancy, women have a relatively persistent vitamin D supply, that arises from the synthesis in the cutaneous tissue, and a good vitamin D status. The concentration of vitamin D3 increases across pregnancy (11 nmol/L) in women who do not receive supplemental vitamin D. However, in pregnant women receiving supplement vitamin D within a micronutrient supplement, the concentration of vitamin D3 increases across pregnancy. Additionally, in pregnant women who do not receive vitamin D as supplementation, 3- epi-25 (OH) D3 continually increases in concentration across pregnancy, which suggests an independent impact of pregnancy on 3-epi-25(OH)D3 production [18]. Also, a high dose of vitamin D support was necessary during pregnancy. Limited exposure to the sun, use of sunscreens regularly, dark skin colour, poor nutritional status, use of medications, and women s indoor work or preferred covered dressing style have been reported as risk factors for vitamin D deficiency [19].

The results of the measurement of serum interleukin-17 levels showed significantly higher levels ($P \leq 0.001$) in pregnant women (468.38±50.62 Pg/ml) as compares to non-pregnant ones (144.39±3.98 Pg/ml), as shown in Table-2 and Figure-2.

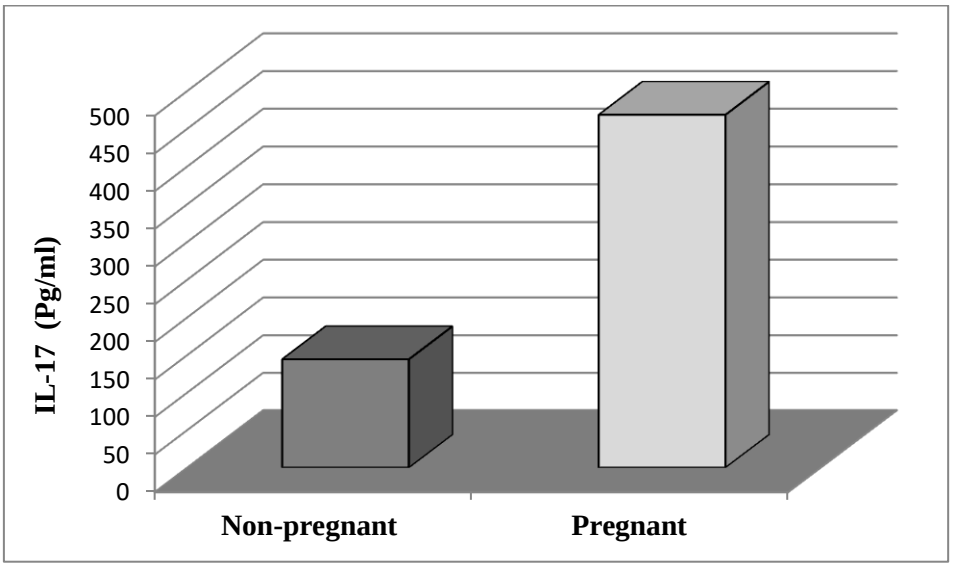

Figure 2- Comparison between different groups in terms of serum interleukin-17 level (Pg/ml).

Studies previously explained that serum IL-17 levels increase significantly in pregnant women at the third trimester in comparison with the beginning of the first trimester. The mean level of 17 increased up with the period of pregnancy to the last trimester [20, 21, 22]. Another study revealed no significant differences in the proportion of IL-17 cells among pregnant and non-pregnant women [23]. This finding may propose the potential function of interleukin -17 in angiogenesis and implantation. Also, it might propose the local synthesis of this cytokine in the placental tissue (maternal fetal interface). Interleukin -17 may be engaged in a more complex network that induces its production throughout the whole pregnancy period [20].

The results of Pearson correlation shown in Table-3 demonstrate non-significant inverse correlations between vitamin D level and some fetal growth parameters (E.F.W., H.C., A.C., F.L.). Also, a weak correlation was found between vitamin D and other fetal parameters (B.P.D., A.F.I., Heart Rate). These results indicate that each parameter is independent from the others, as shown in Table-3.

Table 3- The results of Pearson correlation between vitamin D levels in pregnant women and fetal growth parameters.

Parameters	Vitamin D	
	Pearson Correlation	**Sig. (2-tailed)**
E.F.W.	-0.062	0.685
B.P.D.	0.013	0.934
H.C.	-0.027	0.863
A.C.	-0.082	0.592
F.L.	-0.083	0.587
A.F.I.	0.021	0.889
F.H.R.	0.126	0.409

In 2015, a previous study reported similar results and found that the maternal and fetal serum concentrations of vitamin D positively correlate ; however, it found that this these levels do not impact the fetal growth measurements of head circumference and neonatal weight [24, 25]. Also, the study revealed no correlation between maternal vitamin D concentrations in the first trimester and neonatal birth weight [26].

Some previous studies reported that vitamin D status of the mother is associated with the growth of the fetus during pregnancy [27]. Hence, the current study does not corroborate such a relationship, which is possibly attributed to genetic differences [25].

Also, an earlier research found that the maternal vitamin D deficiency leads to reduced fetal circulating levels of vitamin D (25-(OH) D) and the active form of vitamin D (1,25(OH)2 D) concentrations that pass to the fetus by the placenta, which results in decreased fetal bone generation. Osteoblasts, which are cells that secrete the matrix for bone formation, have receptors for the active form of vitamin D and numerous osteoblast-specific genes are 1,25-(OH)-2D responsive [28]. Thus, low vitamin D concentrations in pregnancy and, consequently, low vitamin D and active vitamin D in the fetus, might reduce the activity of osteoblasts and leads to negative effects on bone growth [29].

The results in Table-4 show a weak inverse correlation between the maternal serum IL-17 levels and fetal growth measurements.

Table 4- The results of Pearson correlation between maternal serum IL-17 levels and fetal growth parameters

Parameters	IL-17	
	Pearson Correlation	**Sig. (2-tailed)**
E.F.W.	-0.23	0.129
B.P.D.	-0.146	0.339

H.C.	-0.161	0.292
A.C.	-0.172	0.258
F.L.	-0.151	0.323
AFI	-0.009	0.955
Heart Rate	-0.277	0.066

A previous study investigated the association between maternal pro-inflammatory profile and the adverse outcomes of pregnancy. It provided an evidence that supports the inverse association between the concentrations of the circulating cytokines and the methylation of the offspring [30]. The aberrant methylation leads to altered gene expression [31] and it has been involved in the growth and development of the fetus [32].

In conclusion, this study indicated that the maternal parameters (serum vitamin D and interleukin-17) do not correlate with the fetal biophysical profile.

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