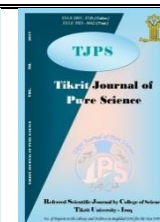




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# Estimation of vitamin D status and calcium level among pregnant women in Tikrit city: case control study

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## ABSTRACT

The demand for calcium and vitamin D rises during pregnancy, increasing the chance of hypocalcemia and vitamin D deficiency. Hypocalcemia may be associated with pregnancy-related complications. This study thus sought to ascertain the frequency of hypocalcemia and vitamin D insufficiency in pregnant women seeking secondary care, as well as to explore the possible associations between these disorders and pregnancy outcomes.

**Subjects, Materials and method:** This study was conducted without the use of vitamin D supplements in the first, second, and third trimesters at gestational ages more than 40 weeks, even before the convenience sample number 36 became pregnant. Control groups for Case number 10 non pregnant women. The age of cases and control women in range from 20-35years. Using the Roche Cobas e411 immunoassay device, we calculated the serum concentrations of calcium and vitamin D. The study carried out between December 2023 and February 2024 in Tikrit Teaching Hospital.

**Results:** A total of 36 pregnant women were enrolled in the study Mean (SD) serum Vitamin D level was  $17.9 \pm 0.12$  mg/dl pregnant women with First trimester,  $11.8 \pm 0.18$  mg/dl pregnant women with second trimester and  $10.5 \pm 0.20$  mg/dl pregnant women with third trimester compared with control groups  $44.4 \pm 0.40$  (p-value =  $<0.001$ ). As well as Mean (SD) serum calcium level was  $(7.9 \pm 0.10, 8.2 \pm 0.18, 7.5 \pm 0.20$  mg/dl) pregnant women with First, second and third trimester respectively, compared with control groups  $8.4 \pm 0.40$  (p-value = 0.06).

## تقدير حالة فيتامين د ومستوى الكالسيوم بين النساء الحوامل في مدينة تكريت

تفاؤل جابر حميد

كلية الصيدلة ، جامعة تكريت ، تكريت ، العراق

## الملخص

خلال فترة الحمل يرتفع الطلب على الكالسيوم وفيتامين د ، مما يزيد من فرصة الإصابة بنقص كالسيوم الدم ونقص فيتامين د. قد يترافق نقص كالسيوم الدم مع المضاعفات المرتبطة بالحمل. وبالتالي سعت هذه الدراسة للتأكد من وتيرة نقص كالسيوم الدم ونقص فيتامين (د) لدى بعض النساء الحوامل ، وكذلك لاستكشاف الارتباطات الأخرى المحتملة خلال فترة الحمل.

**المواضيع والمواد والطريقة:** أجريت هذه الدراسة على مجموعة من الحوامل دون استخدام مكملات فيتامين د في الثلث الأول والثاني والثالث من عمر الحمل لمدة أكثر من 40 أسبوعاً، شملت الدراسة 36 عينة من النساء الحوامل وشملت 10 عينات تمثل المجموعات المراقبة لنساء غير حوامل . قمنا بقياس تراكيز الكالسيوم وفيتامين د باستخدام جهاز Roche Cobas e411 .

**النتائج:** تم استخدام 36 امرأة حامل في الدراسة، وكان متوسط مستوى فيتامين د في مصل الدم  $0.12 \pm 17.9$  ملجم / ديسيلتر للنساء الحوامل في الثلث الأول من الحمل، و  $0.18 \pm 11.8$  ملجم / ديسيلتر للنساء الحوامل في الثلث الثاني من الحمل و  $10.5 \pm 0.20$  ملجم/ ديسيلتر للنساء الحوامل في الثلث الثالث من الحمل مقارنة بمجموعات المراقبة  $44.4 \pm 0.40$  (قيمة  $> 0.001$ ). وكذلك كان متوسط مستوى الكالسيوم في مصل الدم لدى النساء الحوامل  $(7.9 \pm 0.10)$  ( $0.18 \pm 8.2$ ) ( $0.20 \pm 7.5$ ) في الثلث الأول والثاني والثالث على التوالي، مقارنة بمجموعات السيطرة  $8.4 \pm 0.40$  قيمة  $p=0.06$ .

## Introduction

Vitamin D comes in two forms. Vitamin D<sub>2</sub> is the name of the initial form of vitamin D. Ergocalciferol gets its name from being extracted from UV-exposed yeast ergosterol. The second is termed vitamin D<sub>3</sub> (cholecalciferol), which is the naturally occurring form of vitamin D that is produced in the skin and derived from animal sources [1]. Vitamin D is converted by enzymes in the liver and kidneys into two forms in the human body: active (1,25-dihydroxy vitamin D) and 25-hydroxy vitamin D (25(OH)D), which is a circulating and storage form of vitamin D [2]. Traditionally, vitamin D has been involved in maintaining normal blood levels of phosphorus and calcium by enhancing the absorption of calcium from the digestive system. Enzyme activity, blood coagulation, and muscle contraction are among the physiological activities that depend on these levels for maintenance [3]. Vitamin D regulates several physiological systems fundamentally and has a wide range of impacts. According to recent studies, vitamin D has anti-inflammatory properties because it controls the expression of immune system-related genes. The production and expression of vitamin D receptor (VDR) by immune cells, including dendritic cells, macrophages, B and T cells [4]. Over the past few decades, a large number of

research have been published worldwide that demonstrate the health benefits of vitamin D. The development of robust fetal bone throughout pregnancy depends on vitamin D, even if study on this topic is still underway [5]. Insufficient vitamin D intake during pregnancy increases the risk of small for gestational age, neonatal rickets, tetany, and gestational diabetes mellitus in the fetus [6,7]. Numerous studies have documented vitamin D insufficiency in nations like Saudi Arabia and India that receive lots of sunshine for most of the year [8]. The skeletal, cardiovascular, neurological, muscular, hormonal, and enzymatic systems of the human body all depend on calcium, an essential mineral. The skeletal system, which gives bones their rigidity and structure, contains 99% of the calcium in the body, the remaining 1%, which is split between intra- and extracellular fluids; most metabolic processes, muscular contraction, nervous system transmission, enzyme activation, and hormonal activity all need calcium in this environment [9,10]. Inadequate dietary calcium consumption can cause a decrease in blood calcium levels. Apart from the quantity of calcium obtained from diet, the human body's blood calcium level is also influenced by several other factors, such as vitamin D, exposure to sunlight, and parathyroid

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hormone levels [11]. The World Health Organization (WHO) and the Food and Agriculture Organization (FAO) of the United Nations recommend a dietary intake of 1200 mg/day of calcium for pregnant women and 1000 mg/day for non-pregnant adults (19–50 years old) [12]. During pregnancy, there is a significant increase in intestinal  $\text{Ca}^{2+}$  absorption, which is mediated by the active metabolite of vitamin  $\text{D}_3$  1,25-dihydroxycholecalciferol (1,25-(OH) $_2\text{D}_3$ , calcitriol), parathyroid hormone (PTH), and calcitonin [13]. Reduced bone density in both the mother and the unborn child is the result of inadequate calcium consumption during pregnancy. Pregnancy-induced hypertension, low birth weight, premature delivery, and maternal and prenatal mortality are other possible outcomes [14]. Thus, we attempted to ascertain the frequency of hypocalcaemia among pregnant mothers who attend the prenatal clinic at a secondary level hospital. The secondary purpose was examining the association between blood calcium levels and pre-eclampsia, infant birth weight, early delivery, and neonatal mortality.

#### **Aim of study**

The current study set out to ascertain the incidence of vitamin D deficiency and hypocalcemia in a sample of expectant mothers. The link between calcium levels and vitamin D in control groups and pregnant women in different trimesters will also be examined in this study.

### **Materials and methods**

#### **Study Design and Population**

The design of the present work is a case-control study carried out between December 2023 and February 2024. The number of pregnant women (36), their gestational ages in the first, second, and third trimesters, and their non-use of vitamin D and calcium supplements even before conception are among the predetermined inclusion criteria. Control groups no.10.

The research samples were obtained by a systematic convenience sample sampling process from all patients who visited the prenatal care unit of the Salahaddin Teaching Hospital in Tikrit city and met the inclusion criteria. This case control study successfully recruited 36 expecting moms to participate.

#### **Data Collection Trained**

Individuals conducted direct interviews in order to collect data. The first of the questionnaire's three main sections was on gestational age. Sun exposure was defined as exposure to direct

sunlight that made body parts visible, as opposed to via windows. This study does not include pregnant women in order to account for any confounding factors that might alter the level of vitamin D. Food history, with particular attention to the amount of milk and fish regularly consumed. Outdoor recreation and exercise were taken into account.

#### **Sample collections and treatment**

Blood samples from the pregnant women who satisfied the eligibility conditions were taken, and the samples were centrifuged for five minutes at 5000 rpm. When it came time to test the blood calcium level and serum 25 dehydroxyl vitamin D, the serum was isolated and stored in a deep freezer at  $-80^\circ\text{C}$ . The Roche Cobas e411 immunoassay analyzer was used to assess the blood levels of calcium using biolabo kit, France and vitamin D using the Roche Elecsys Vitamin D3 test (Roche Diagnosis, Mannheim, Germany). Vitamin D deficiency was identified by serum levels less than 20 ng/mL, as insufficiency, and acceptable level recognition by serum levels between 20 ng/mL and 30 ng/mL. The content validity was evaluated using a panel, and the reliability of the data was evaluated using the statistically adequate correlation coefficient ( $1 = 0.884 = 0.88.4$ ). In a preliminary study, thirty six pregnant women from Tikrit Teaching Hospital took part.

#### **Statistical analysis**

Utilizing SPSS (Statistical Package for Science Services), computerized statistical analysis was carried out. Probability (P value) were used to compare between groups, where P values of 0.05 or less regarded as statistically significant (S), those of 0.01 or less as highly significant (H.S.), and those of 0.05 or more as non-significant (N.S.).

### **Results and discussion**

Table1. Illustrates the Vitamin D levels mean $\pm$  SD (range) in the pregnant women and case control groups. Results displayed that pregnant women with First trimester recorded that mean $\pm$  SD range (17.9 $\pm$ 0.12), while pregnant women with Second trimester recorded that mean $\pm$  SD range (11.8 $\pm$  0.18), pregnant women with Third trimester recorded that mean $\pm$  SD range (10.5  $\pm$  0.20), compared to the control recorded that mean $\pm$  SD range (76.4  $\pm$  0.40) with highly significant difference p-values were  $<0.01$ .

**Table 1: The Average values of Vitamin D level among pregnant women**

Variable Vitamin D level	No.	Mean ± SD (range)
pregnant women with First trimester	12	17.9±0.12
pregnant women with Second trimester	12	11.8± 0.18
pregnant women with Third trimester	12	10.5 ± 0.20
Case control	10	76.4 ± 0.40
Total	46	
P-Value	<0.001	

**Table 2: The Average values of Calcium level among pregnant women**

Variable Calcium level	No.	Mean ± SD (range)mg/dl
pregnant women with First trimester	12	7.9±0.10
pregnant women with Second trimester	12	8.2± 0.18
pregnant women with Third trimester	12	7.5 ± 0.20
Case control	10	8.4 ± 0.40
Total	46	
P-Value	0.06	

Table 2. Illustrates the Calcium level levels mean± SD range in the pregnant women and control group. Results displayed that pregnant women with First trimester recorded that mean± SD range (7.9±0.10), while pregnant women with Second trimester recorded that mean± SD range (8.2± 0.18), pregnant women with Third trimester recorded that mean± SD range (7.5 ± 0.20), compared to the control recorded that mean± SD range (8.4 ±0.40) with non- significant difference p-values were >0.06.

Table 3. Illustrates the Vitamin D percentage in the pregnant women and control group. Results displayed that pregnant women with First trimester recorded that 6(50%), as well as pregnant women with Second trimester recorded 7(58%) was Insufficient25-50nmol/l, while pregnant women with Third trimester recorded that 7(58%) was Deficient > 25 nmol/l, compared to the control recorded that 10 (100%) was Optimum<75nmol/l with highly significant difference p-values were <0.001.

**Table 3: The percentage of Vitamin D level among pregnant women according to stages of trimester**

Vitamin D level	Pregnant women			
	First trimester	Second trimester	Third trimester	Case control
Deficient < 12 ng/ml	5(42%)	5(42%)	7(58%)	0 (0%)
Insufficient12-20 ng/ml	6(50%)	7(58%)	5(42%)	0 (0%)
Adequate20-50ng/ml	1 (8%)	0(0%)	0(0%)	0 (0%)
Optimum>50 ng/mL	0(0%)	0(0%)	0(0%)	10 (100%)
Total	12	12	12	10 (100%)
P-Value		0.001		

Table 4. Illustrates the Calcium percentage in the pregnant women and control group. Results displayed that pregnant women with First trimester recorded that 10(83%), as well as pregnant women with Second trimester and Third

trimester were recorded that 11(90%) and 12(100%) were Low Calcium>8.5mg/dl, with highly significant difference p-values were <0.001.

**Table 4: The percentage of Calcium level among pregnant women according to stages of trimester**

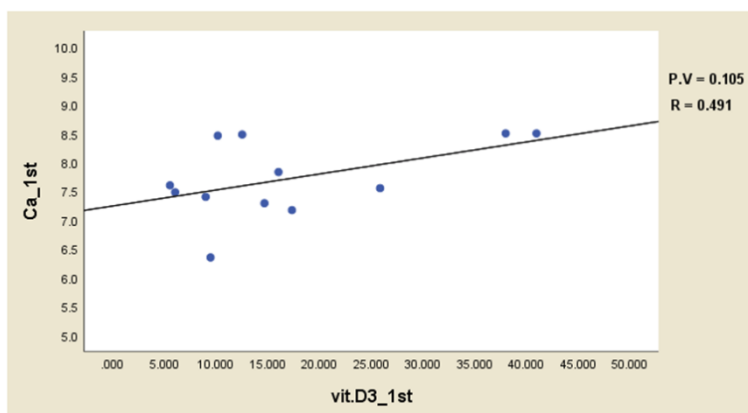
Calcium level	Pregnant women			
	First trimester	Second trimester	Third trimester	Case control
LowCalcium>8.5mg/dl	10(83%)	11(90%)	12(100%)	0 (0%)
NormalCalcium8.5-10.2 mg/dl	2(17%)	1(10%)	0(0%)	10 (100%)
High Calcium <10.2 mg/dl	0(0%)	0(0%)	0(0%)	0 (0%)
Total	12	12	12	10 (100%)
P-Value		0.001		

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**1. Correlation between Ca and Vit.D3 in 1<sup>ST</sup> trimesters pregnant women**

This study found that there was a negative correlation between Ca and Vit.D3 in 1<sup>ST</sup>

trimesters pregnant women ( $r = - 0.154$ ), Figure (1).

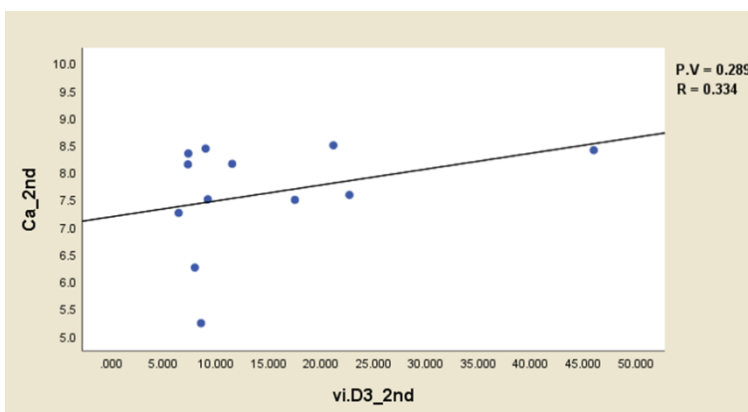


**Fig. 1: Correlation between Ca and Vit.D3 in 1<sup>ST</sup> trimester's pregnant women**

**2. Correlation between Ca and Vit.D3 in 2<sup>nd</sup> trimesters pregnant women**

This study found that there was a positive correlation between Ca and Vit.D3 in 2<sup>nd</sup>

trimesters pregnant women ( $r = + 0.169$ ), Figure (2).

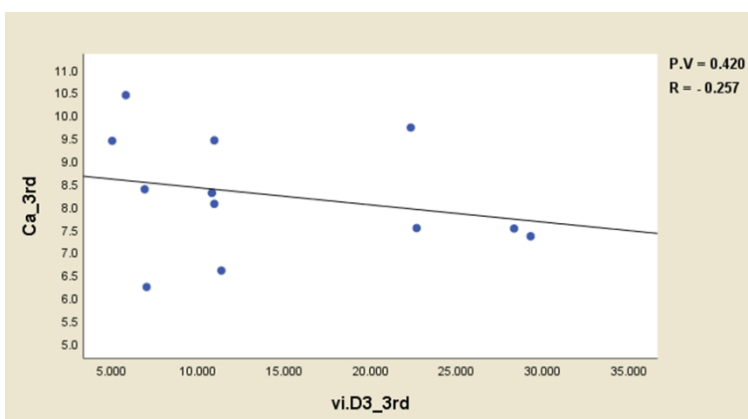


**Fig. 2: Correlation between Ca and Vit.D3 in 2<sup>nd</sup> trimester's pregnant women**

**3. Correlation between Ca and Vit.D3 in 3<sup>rd</sup> trimesters pregnant women**

This study found that there was a positive correlation between Ca and Vit.D3 in 3<sup>rd</sup>

trimesters pregnant women ( $r = + 0.177$ ), Figure (3).



**Fig. 3: Correlation between Ca and Vit.D3 in 3<sup>rd</sup> trimester's pregnant women**

Many researches have explained vitamin D's participation in many body functions, which has piqued the curiosity of many academics nowadays. Serum 25(OH)D is a reliable measure of vitamin D status because it represents both endogenous and exogenous synthesis of vitamin D. This study, conducted in Tikrit City, Iraq, may be the first to examine the prevalence of vitamin D deficiency in expectant mothers. Among individuals who were included in the current study, the percentage of pregnant women with third trimester deficits who also had vitamin D deficiency was rather high. Compared to the control groups, 12 of the 36 pregnant women had optimum vitamin D levels (25(OH)D 30 ng/ml). Similar outcomes were found in other research investigations including study in Sulaimaneyah City in Iraq on pregnant women showed prevalence of Vitamin D deficiency among pregnant women [15]. Other studies in Saudi Arabia confirm our results and showed prevalence of vitamin deficiency among pregnant women in Saudi Arabia [16,17]. The main way to raise serum vitamin D levels, which is responsible for many health advantages, is through UV exposure. Increased vitamin D levels are linked to defense against the development of cancer, particularly melanoma. Vitamin D production and UV absorption are dependent on latitude, season, skin color, and sun protection. Guidelines for sun protection from the public health department reduce the risk of skin cancer, but too much sun exposure can result in vitamin D deficiency [18]. A cultural element that could partially explain the higher degree of vitamin D insufficiency among females could be the dress practices of South Asian women, in addition to their longer indoor hours. Direct sunlight exposure is prevented by wearing traditional clothing such as burqas (a full body covering), hijabs (a Muslim head covering), and other non-religious body coverings [19]. Saudi Arabia produced data indicating a rather high prevalence of vitamin D deficiency in the general population and among women, both pregnant and non-pregnant, despite the dearth of study in the field [20]. The close closeness of the culture, location, or beliefs might support our conclusion and assumptions of vitamin D inadequacy in Tikrit City. This demonstrates that individuals living in hot regions do not always get the appropriate dosage of vitamin D. The study's control groups and pregnant women both had significantly higher blood vitamin D levels [21].

This study looked at the mean blood calcium level, the quantity of calcium in the diet, and the frequency of hypocalcaemia among pregnant patients who visited the prenatal clinic at a secondary level hospital. Just 18% of pregnant women consumed the recommended daily amount of calcium through food. The prevalence of hypocalcaemia was 23.9%. This study also looked at the connection between blood calcium levels, dietary calcium intake, pre-eclampsia, and other pregnancy outcomes. The mean blood calcium levels of pregnant women who gave birth to LBW kids were significantly lower than those of pregnant women who gave birth to newborns weighing two thousand grams or more. Calcium intake from food in the study participants the average daily consumption of calcium (SD) derived from diet was 3600.4 (796.4) mg. It was less than the recommended daily allowance (RDA) of 1,200 mg for expecting moms. Studies carried performed outside of India have also reported similar results [22]. A comparable study that we conducted a while back in the same vicinity revealed that the average daily intake of calcium from food was 858.4 mg. These findings concur with the earlier research that was released. [23]. Pregnant women's blood calcium levels were determined to be 9.56 (0.94) mg/dl on average (SD), which is within the normal range for pregnant women (9-11 mg/dl). According to Kumar et al. of New Delhi, the average blood calcium level in pregnant women was found to be 8.1 mg/dl, which is below the normal limit. Kumar et al. carried out this investigation in a tertiary care hospital in Central Delhi [24]. It's likely that most of the patients were urban residents. We also included women from rural locations in our study. Rural dwellers frequently spend more time in the sun [25], which might encourage the body to produce more vitamin D. Increased vitamin D levels help to maintain elevated blood calcium levels. Therefore, the variance in mean serum calcium levels may be influenced by one's place of residence. Still, additional research is required on this claim [23]. Patients who are pregnant and have other co-morbidities that might impact blood calcium levels may be drawn to tertiary care centers and referral hubs. Consequently, there is yet another reason for the lower mean blood calcium level. Variations in the morbidity status of the subjects in Kumar's study might exist [24].

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## Conclusions

This study draws attention to the high rates of calcium and vitamin D insufficiency among expectant mothers in Tikrit City. In order to lessen the negative health effects linked to

vitamin D insufficiency during pregnancy, methods to increase vitamin D status, such as encouraging sunshine exposure and dietary treatments, are justified.

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