The Relationship between Early Pregnancy Loss and Vitamin D3 Deficiency

Sahar Badea Haloob [1], Zainab Tareq Alyassin [2]

1. MBCHB, Basrah Health Directorate, Ministry of Health, Iraq.
2. Assistant Professor of Obstetrics and Gynaecology, College of Medicine / University of Basrah

Received:19.9.2024 **Accepted:20.11.2024**

Abstract

Background: Early pregnancy loss, occurring before 10-13 weeks, often involves an empty or nonviable gestational sac. Vitamin D deficiency is linked to higher miscarriage rates. Between 10% and 20% of diagnosed pregnancies end in loss, with true rates potentially higher.

Aims of the study: To assess if there is a relationship between serum vitamin D level and the spontaneous termination of pregnancy that occurs before reaching 10 to 13 weeks of gestational age.

Methods: A case-control study at Basrah Maternity and Child Hospital conducted from October 2023 to April 2024, involved 104 women divided into case (early pregnancy loss) and control (normal delivery) groups. Data collected included sociodemographic factors, medical history, and serum 25(OH)D levels, using the AFIAS™ Vitamin D assay.

Results: The study compared two groups of women (n = 52 each). Serum vitamin D3 levels were significantly lower in the case group (17.24 ng/ml) compared to the control group (27.74 ng/ml, p = 0.001). There was a significant statistical difference between both groups (P value = 0.05) regarding the time spent outdoors.

Conclusion: The study found a significant disparity in serum vitamin D levels in women with early pregnancy loss compared to those with normal pregnancies. Cases spent less time outdoors, reducing sun exposure and vitamin D synthesis. Despite higher average BMI in controls, they had better vitamin D levels, suggesting lifestyle and supplementation play key roles.

Keywords: Pregnancy Loss, Vitamin D3, Deficiency

Corresponding author: Sahar Badea Haloob MBCHB, Basrah Health Directorate, Ministry of Health, Iraq.

✉ **E-mail:** saharbadea532@gmail.com

Introduction

Early pregnancy loss is often defined as an involuntary termination of pregnancy that occurs before reaching 10 to 13 weeks of gestational age. It is characterised by the lack of a viable pregnancy, with the gestational sac being either empty or containing an embryo or fetus that lacks any fetal heart activity (1). Vitamin D deficiency is a significant problem that affects public health on a worldwide scale. Approximately 1 billion people worldwide suffer from a deficiency of vitamin D, with 50% of the population experiencing insufficient levels of vitamin D (2). Studies found that those with inadequate levels of vitamin D had a considerably higher incidence of miscarriage compared to those with adequate levels of vitamin D. The correlation persisted even when women with inadequate levels were included, and a clear biological gradient was seen (3).

Generally, between 10% and 20% of pregnancies that are identified and diagnosed by medical professionals may result in early pregnancy loss (4).

Vitamin D deficiency, characterised by insufficient levels of 25-hydroxyvitamin D (25(OH) D) in the

blood, is a significant worldwide health issue. Pregnant women and those intending to get pregnant are particularly vulnerable to this condition (5).

Vitamin D deficiency is more common in women who have significant reproductive and obstetric difficulties, such as preeclampsia, gestational diabetes, and premature delivery, despite its conventional association with bone disease in mothers and newborns (3, 6).

Multiple studies have shown that vitamin D has a protective effect on preventing miscarriage. Vitamin D is known to regulate decasualization by primarily controlling the HOXA10 gene, which plays a crucial role in regulating the growth of the endometrium and its ability to accept a fertilised egg during implantation. Vitamin D also controls the genes responsible for trophoblastic angiogenesis and invasion, which are crucial for the implantation of the embryo and placental function. This, in turn, promotes fetal growth and development. It acts as an immune modulator by exerting a strong anti-inflammatory effect at the interface between the mother and fetus, potentially influencing the immunological response of the mother and fetus. Vitamin D receptors are present on several immune cells that play a role in regulating antigen-receptor signalling pathways and the activation of T-cells. Activation of 1,25(OH)2 D has been suggested as a form of immunotherapy for spontaneous miscarriage. This is because it down-regulates the Th1 cytokines and inhibits the release of pro-inflammatory cytokines, such as interleukin-6, interferon-γ, and tumour necrosis factor-α, in the placenta (7,8).

The presence of the vitamin D-activating enzyme CYP27B1 in the maternal decidua and fetal trophoblast during early pregnancy provides evidence for the potential role of vitamin D in pregnancy and miscarriage. Previous research has shown that the human placenta plays a crucial role in accumulating both 25(OH) D and active 1, 25-dihydroxyvitamin D (1,25(OH)2D) (9). This has the potential to significantly impact trophoblast invasion, placental spiral artery remodelling, and immune cell activity (10). The mechanisms involved in human miscarriage are disrupted, leading to abnormal endometrial receptivity and dysregulated placentation, which may be detected soon after conception (11, 12). Thus, it is plausible that a deficiency in blood 25(OH)D levels may contribute to the pathophysiology of miscarriage by causing a simultaneous reduction in placental 1,25(OH)2D levels and subsequent dysregulation of the placenta (3).

Aims of the study

To assess if there is a relationship between serum vitamin D level and the spontaneous termination of pregnancy that occurs before reaching 10 to 13 weeks of gestational age.

2. Patients and Methods

This is a case-control study, conducted at Basrah Maternity and Child Hospital to assess if there is a relationship between serum vitamin D levels and early pregnancy loss. Data was collected for the period from the 1st of October 2023 to the 30th of April 2024.

One hundred four women pregnant women were included in the study and divided into two groups.

- GROUP 1 (Case Group): fifty-two women presented with early pregnancy loss to the emergency department at Basrah Maternity and Child Hospital.
- GROUP 2 (Control Group): fifty- two women presented with normal vaginal delivery to the labour ward at Basrah Maternity and child hospital.

Direct interviews were used to collect data by the researcher. The questionnaire included the following aspects:

- The Socio-demographic and lifestyle characteristics such as age, residency, educational level, occupation, and family income.
- Pregnancy-related characteristics such as parity, inter-pregnancy intervals, any history of previous pregnancy loss and history of Antenatal care.

- Medical profile: any history of chronic disease, chronic use of drugs, usage of vitamin D supplement during pregnancy.

Participants enrolled in the study underwent an anthropometric measure which includes: The height was measured using a tape measure, and weight was measured using a weight scale, and then BMI was calculated (calculated as weight in kilograms divided by height in meters squared). Participants' BMI was then categorized into underweight (<18.5), normal weight (18.5–24.9), overweight (25–29.9) and obese (>30) (13).

Vital signs (blood pressure, heart rate, and temperature) were measured. Chest and abdominal examinations were also done. Routine investigations (CBC, RBS, and GUE) were done.

Ultrasonography was done for each woman included in the study, to determine the exact gestational age, and look for the risky signs of early pregnancy loss.

Fasting blood samples were collected from the antecubital vein of all enrolled pregnant women, for serum 25(OH)D concentrations assay.

We use AFIAS™ Vitamin D is a fluorescence immunoassay (FIA) that measures the total 25(OH)D2/D3 level in human serum or plasma quantitatively. The cut-off point of ≥ ٣٠ ng/ml was considered normal. Data was entered using computerized statistical software; Statistical Package for Social Sciences (SPSS) version 26 was used. The level of significance (p-value) is set at ≤ 0.05.

Results

The study includes two groups of women, 52 women in each group. Their mean age was 28.53 years for the case group and 27.02 years for the control. There is no significant statistical difference between the two groups p-value =0.807.

Regarding the residency, nearly 70% of women in both groups lived in urban areas. And there was no significant statistical difference p-value=0.830.

Table 2 shows the clinical characteristics of the participants, the women were asked if they had an

Of the women's educational level, 38.5% of women in the case group had a primary education in comparison to 34.6% of women in the control group. There are no significant statistical differences between women in both groups P-value= 0.157. More than 75% of women in both groups were housewives, there is no significant statistical difference between them p-value>0.05.

Regarding family income, nearly half of the participants had a middle outcome. All these data are presented in Table 1.

Table 1. Socio-demographic characteristics of participants

Variables		Case (n=52)	Control (n=52)	P-value
Age	Mean ±SD	28.53± 5.87	27.02± 5.14	0.807
	<20	4 (7.7)	5 (9.6)	
	20-29	32(61.5)	28 (53.8)	
	≥ 30	16 (30.8)	19 (63.5)	
Residency	Rural	15(28.8)	16 (30.8)	0.830
	Urban	37(71.2)	36 (69.2)	
Educational level	Illiterate	2 (3.8)	5 (9.6)	0.157
	Primary	20 (38.5)	18 (34.6)	
	Secondary	11(21.2)	18 (34.6)	
	Higher education	19 (36.5)	11 (21.2)	
Employment status	Housewives	40 (76.9)	43 (82.7)	0.464
	Employed	12 (23.1)	9 (17.3)	
Family income	Good	12 (23.1)	10 (19.2)	0.632
	Middle	30 (57.7)	28(53.9)	
	Poor	10 (19.2)	14 (27.9)	

ANC, and around 30% of women had an ANC, there is no significant statistical difference between women in both the groups. P-value=0.680.

Regarding vitamin D supplementations, 55.8% of women in the case group and 53.8% in the control group received vitamin D and there was no significant statistical difference between them p-value=0.844.

Regarding the daily time they spend outdoors, women in the case group spent around 5.1 hours in comparison to 7.07 hours for women in the control group. There was a significant statistical difference between both groups P value=0.05.

Regarding their parity, there was no significant statistical difference between women in both groups p-value=0.973.

Table 2: the clinical characteristics of the participants

Variables		Case (n=52)	Control (n=52)	P-value
ANC	Yes	19 (36.5)	17 (32.7)	0.680
	No	33 (63.5)	35 (67.3)	
Vitamin D supplementation	Yes	29 (55.8)	28 (53.8)	0.844
	No	23 (44.2)	24 (46.2)	
Number of hours spent outdoor	Mean ±SD	5.1± 2.01	7.07± 2.31	0.05
Parity	0	10 (19.2)	10 (19.2)	0.973
	1-2	29 (55.8)	28 (53.8)	
	>3	13 (25.0)	14 (26.9)	

Table 3 shows the anthropometric measurement of the participants, women in the case group had a mean weight of 37.04 while women in the control group had a higher mean of 83.86 Kg. There is a significant statistical difference between women and both groups. P-value=0.001.

Regarding the height, there is no significant statistical difference between the women's height, P value=0.959.

The women's BMI has been measured, the mean BMI was 28.18 among women in the case group and it was higher at 32.36 among women in the control group. There is a significant statistical difference between both of them. P value=0.001.

Table 3: The anthropometric measurements of the participants

Variables		Case (n=52)	Control (n=52)	P-value
Weight	Mean ±SD	73.04 ±14.33	83.86 ±11.32	0.001
Height	Mean ±SD	161.02± 6.0	161.08± 5.48	0.959
BMI	Mean ±SD	28.18± 5.36	32.36± 4.45	0.001
	18.5-24.9	16 (20.8)	4 (7.7)	
	25-29.9	21(40.4)	10 (19.2)	
	≥ 30	15 (28.8)	38 (73.1)	

The serum vitamin D3 level among the case and control groups was presented in Table 4. The mean vitamin D level was 17.24 in the case group and 27.74 in the control group, this difference in the mean vitamin D level was statistically significant p-value=0.001.

Table 4: The serum Vitamin D3 level among the case and control group

Variables		Case	Control	P-value
Vitamin D level	Mean ±Sd	17.24 ± 1.7	27.74± 8.5	0.001
	Normal (≥30 ng/ml)	15 (28.8)	28 (53.8)	
	Deficient (< 30 ng/ml)	37 (71.2)	24 (46.2)	

Figure 1 shows the percentage of vitamin D3 deficiency among the case and control groups. 37 women in the case group and 24 women in the control group had vitamin D levels below 30 ng/ml.

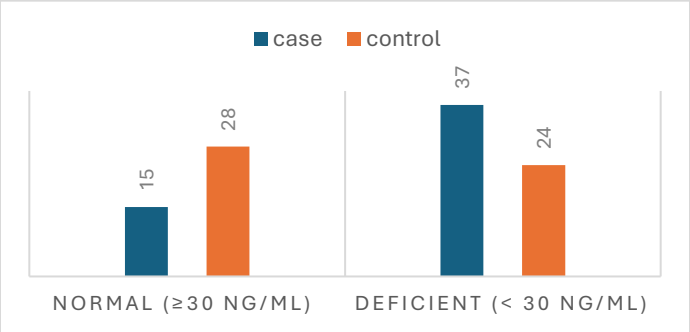

Figure 1: the comparison between vitamin D3 among case and control groups

Discussion

Recently, there has been increasing attention towards the possible association between vitamin D insufficiency and undesirable pregnancy outcomes, such as early pregnancy loss (14). This research aimed to find a relationship between serum vitamin D levels in women who had early pregnancy losses and those with normal pregnancies. By doing so, it aims to enhance our awareness of the possible influence of vitamin D on reproductive health.

The research maintained thorough matching between the case and control groups for several socio-demographic factors, such as age, residency, educational level, work status, and family income. No significant changes were seen between ANC attendance, vitamin D supplementation, and parity. By using this matching technique, the impact of socio-demographic variables is reduced, resulting in a more precise evaluation of the relationship between vitamin D levels and pregnancy outcomes. The strict matching process enhances the validity of our results and minimises the potential effect of socio-demographic factors, parity, ANC, and vitamin D supplementation on the observed disparities in clinical outcomes.

The notable disparity in blood vitamin D levels between the case and control groups (17.24 ng/ml vs. 27.74 ng/ml, p = 0.001) highlights the potential impact of vitamin D insufficiency on early pregnancy loss. This finding aligns with multiple studies that have shown a relationship between inadequate levels of vitamin D and adverse outcomes during pregnancy, such as miscarriages. For instance, research conducted by Al-Rawaf et al. (2022) established a statistically significant association between deficient levels of vitamin D and the occurrence of missed abortions. Furthermore, it was shown that the concentration of serum 25-hydroxyvitamin D was significantly lower in women who had a missed abortion compared to those who had a normal pregnancy ($P \leq 0.05$) (15). Furthermore, this prevalence aligns with, but slightly exceeds, our findings (71.2%). Furthermore, research conducted by Al-Mogbel et al. (2019) said that despite the abundance of sunshine accessible in Saudi Arabia, the prevalence of hypovitaminosis D among young, healthy Saudi ladies is 100%, which they deemed a matter of public concern (16). Iraq-based research conducted by Sofihussein et al. (2023) revealed a significant prevalence of hypovitaminosis D (71.3%) in pregnant women. The author also said that there is a strong correlation between advanced gestational age and elevated levels of vitamin D (17). A separate team of researchers from Basrah discovered a high prevalence of vitamin D insufficiency among pregnant women, accounting for 45.1% of the population (18).

The research showed a notable disparity in the amount of time spent outside daily between the case and control groups (5.1 hours vs. 7.07 hours, p = 0.05). This finding emphasises the need for sun exposure to maintain sufficient amounts of vitamin D. Insufficient exposure to sunlight may result in an insufficient amount of vitamin D since sunlight is the main source of this vitamin. The findings of Kareem et al. (2023) and Raymond-Lezman et al. (2023) research provide support for this finding, demonstrating that insufficient exposure to sunlight leads to a significant decrease in serum vitamin D levels, hence elevating the likelihood of insufficiency and related health complications (18,19).

Many variables contribute to the high incidence of this insufficiency. One of these causes is the restricted availability of vitamin D in several foods, such as egg yolk, salmon, mushrooms, and cod liver oil (20). Furthermore, the body needs sunshine to synthesise vitamin D effectively (19). Several variables influence

the production and absorption of vitamin D in the body, such as the season, use of sunscreen, indoor workplace, and type of clothes, obesity, pollution, ageing, and higher levels of melanin in those with dark skin (21). Another potential factor in this research is the prevalence of dark skin tones among the majority of Basrah people.

Limitations

It is important to recognise certain limitations of this research. Initially, the sample size was slightly small, potentially restricting the applicability of the results. In addition, the research used self-reported data for some factors, such as hours spent outdoors and nutritional consumption, which may add recall bias. The study's cross-sectional design limits the capacity to make causal assessments. Longitudinal studies are necessary to demonstrate the causal association between vitamin D insufficiency and early pregnancy loss.

Conclusion

the study revealed a significant disparity in serum vitamin D levels between women who had early pregnancy loss and those who had normal pregnancies. The cases had a considerably reduced amount of time spent outdoors daily compared to the control group. This difference in outdoor time affects sun exposure and, as a result, the synthesis of vitamin D.

References

1. Lees C, Bourne T. Dewhurst’s textbook of obstetrics & gynaecology. John Wiley & Sons; 2018.

2.Sizar O, Khare S, Goyal A, et al. Vitamin D Deficiency. [Updated 2023 Jul 17]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024. Available from: https://www.ncbi.nlm.nih.gov/books/NBK532266/

3.Tamblyn JA, Pilarski NSP, Markland AD, Marson EJ, Devall A, Hewison M, et al. Vitamin D and miscarriage: a systematic review and meta-analysis. Fertil Steril. 2022;118(1):111–22.

4. American College of Obstetricians and Gynecologists' Committee on Practice Bulletins—Gynecology. ACOG Practice Bulletin No. 200: Early Pregnancy Loss. Obstet Gynecol. 2018;132(5): e197-e207.

5. Kramer CK, Ye C, Swaminathan B, Hanley AJ, Connelly PW, Sermer M, Zinman B, Retnakaran R. The persistence of maternal vitamin D deficiency and insufficiency during pregnancy and lactation irrespective of season and supplementation. Clin Endocrinol (Oxf). 2016;84(5):680-6.

6. Chu J, Gallos I, Tobias A, Tan B, Eapen A, Coomarasamy A. Vitamin D and assisted reproductive treatment outcome: a systematic review and meta-analysis. Hum Reprod. 2018;33(1):65-80.

7. RCOG. Vitamin D in Pregnancy: Scientific Impact Paper No. 43; 2014. Available from: https://www.rcog.org.uk/en/guidelines-research-services/guidelines/sip43/

8. Ganguly A, Tamblyn JA, Finn-Sell S, Chan SY, Westwood M, Gupta J, Kilby MD, Gross SR, Hewison M. Vitamin D, the placenta and early pregnancy: effects on trophoblast function. J Endocrinol. 2018;236(2): R93-R103.

9.Tamblyn JA, Susarla R, Jenkinson C, Jeffery LE, Ohizua O, Chun RF, Chan SY, Kilby MD, Hewison M. Dysregulation of maternal and placental vitamin D metabolism in preeclampsia. Placenta. 2017; 50:70-77.

10. Zhang JY, Wu P, Chen D, Ning F, Lu Q, Qiu X, Hewison M, Tamblyn JA, Kilby MD, Lash GE. Vitamin D Promotes Trophoblast Cell Induced Separation of Vascular Smooth Muscle Cells in Vascular Remodeling via Induction of G-CSF. Front Cell Dev Biol. 2020; 8:601043.

11. Dimitriadis E, Menkhorst E, Saito S, Kutteh WH, Brosens JJ. Recurrent pregnancy loss. Nat Rev Dis Primers. 2020;6(1):98.

12. Lucas ES, Vrljicak P, Muter J, Diniz-da-Costa MM, Brighton PJ, Kong CS, Lipecki J, Fishwick KJ, Odendaal J, Ewington LJ, Quenby S, Ott S, Brosens JJ. Recurrent pregnancy loss is associated with a pro-

senescent decidual response during the peri-implantation window. Commun Biol. 2020;3(1):37.

13. Nuttall FQ. Body Mass Index: Obesity, BMI, and Health: A Critical Review. Nutr Today. 2015;50(3):117-128.

14.Zhang H, Wang S, Tuo L, Zhai Q, Cui J, Chen D, Xu D. Relationship between Maternal Vitamin D Levels and Adverse Outcomes. Nutrients. 2022; 14(20):4230.

15. Al-Rawaf SA, Mousa ET, Kamal Abdulhussein F. Correlation between Serum Vitamin D and Calcium Levels in Missed Miscarriage. Arch Razi Inst. 2022; 77(4):1349-1353.

16. Al-Mogbel ES. Vitamin D status among Adult Saudi Females visiting Primary Health Care Clinics. Int J Health Sci (Qassim). 2012; 6(2):116-26.

17. Sofihussein HQ. Prevalence of Vitamin D Deficiency among Pregnant Women in Sulaimaneyah City-Iraq. UHD J SCI TECH. 2023;7(1):1-7.

18.Kareem, H., Alsalami, K., Al-Yasin, Z. Prevalence of Vitamin D Deficiency among Pregnant Women In Basrah City. The Medical Journal of Basrah University, 2023; 41(2): 120-127.

19.Raymond-Lezman JR, Riskin SI. Benefits and Risks of Sun Exposure to Maintain Adequate Vitamin D Levels. Cureus. 2023;15(5): e38578.

20. Dominguez LJ, Farruggia M, Veronese N, Barbagallo M. Vitamin D Sources, Metabolism, and Deficiency: Available Compounds and Guidelines for Its Treatment. Metabolites. 2021; 11(4):255.

21.Palacios C, Gil K, Pérez CM, Joshipura K. Determinants of vitamin D status among overweight and obese Puerto Rican adults. Ann Nutr Metab. 2012;60(1):35-43.

العلاقة بين الإجهاض المبكر ونقص فيتامين D3

الخلفية:يُعد الإجهاض المبكر، الذي يحدث قبل ١٠-١٣ أسبوعًا من الحمل، غالبًا نتيجة لكيس حمل فارغ أو غير قابل للحياة. يرتبط نقص فيتامين D بارتفاع معدلات الإجهاض. تشير الإحصائيات إلى أن ١٠٪-٢٠٪ من حالات الحمل المكتشفة تنتهي بالإجهاض، مع احتمالية أن تكون المعدلات الحقيقية أعلى.

أهداف الدراسة:تقييم العلاقة بين مستوى فيتامين D في مصل الدم والإجهاض التلقائي الذي يحدث قبل الوصول إلى عمر حمل يتراوح بين ١٠ و١٣ أسبوعًا.

المنهجية:تم إجراء دراسة حالة-شاهد في مستشفى البصرة للولادة والأطفال خلال الفترة من أكتوبر ٢٠٢٣ إلى أبريل ٢٠٢٤، شملت ١٠٤ امرأة تم تقسيمهن إلى مجموعتين: مجموعة الحالات (إجهاض مبكر) ومجموعة الشواهد (ولادة طبيعية). تم جمع البيانات عن العوامل الاجتماعية والديموغرافية، والتاريخ الطبي، ومستويات فيتامين D3 في المصل باستخدام فحص AFIAS™ Vitamin D.

النتائج:قارنت الدراسة بين مجموعتين من النساء (n = 52 لكل مجموعة). كانت مستويات فيتامين D3 في مصل الدم أقل بشكل ملحوظ في مجموعة الحالات (١٧,٢٤ نانوغرام/مل) مقارنة بمجموعة الشواهد (٢٧,٧٤ نانوغرام/مل، p = 0.001). وُجد اختلاف إحصائي كبير بين المجموعتين فيما يتعلق بالوقت الذي يتم قضاؤه في الهواء الطلق (P = 0.05).

الاستنتاج:وجدت الدراسة اختلافًا كبيرًا في مستويات فيتامين D في مصل الدم بين النساء اللاتي تعرضن للإجهاض المبكر مقارنة باللاتي أتممن حملهن بنجاح. أمضت مجموعة الحالات وقتًا أقل في الهواء الطلق، مما قلل من التعرض للشمس وإنتاج فيتامين D. على الرغم من ارتفاع متوسط مؤشر كتلة الجسم في مجموعة الشواهد، كانت مستويات فيتامين D لديهن أفضل، مما يشير إلى أهمية عوامل نمط الحياة والمكملات الغذائية.

الكلمات المفتاحية:الإجهاض، فيتامين D3، نقص الفيتامينات.