

## ORIGINAL ARTICLE

**Relationship Between Serum 25-Hydroxy Vitamin D Level and Menstrual Cycle Irregularity: A Cross-Sectional Study**UZMA AFREEN<sup>1</sup>, MUBUSHRA SAMINA<sup>2</sup>, SHAIMA SULTANA MEMON<sup>3</sup>, HAFIZ SYED MUHAMMAD OSAMA JAFRI<sup>4</sup>, SUMAIRA RIFFAT<sup>5</sup>, SABA ABRAR<sup>6</sup><sup>1</sup>Assistant Professor, Department of Obstetrics and Gynaecology, Fazaia Ruth Pfau Medical College, Air Force Hospital, Faisal, Karachi, Pakistan.<sup>2</sup>Assistant Professor, Department of Obstetrics and Gynaecology, Karachi Institute of Medical Sciences Karachi, Pakistan.<sup>3</sup>Assistant Professor, Department of Pathology, Dow Medical College, Dow University of Health Sciences, Karachi, Pakistan.<sup>4</sup>Lecturer, Department of Biochemistry, Ziauddin Medical College, Karachi, Pakistan.<sup>5</sup>Lecturer, Department of Physiology, Sindh Medical College, Jinnah Sindh Medical University, Karachi, Pakistan.<sup>6</sup>Assistant Professor, Department of Physiology, Baqai Medical University, Karachi, Pakistan.Corresponding author: Uzma Afreen, Email: [uzmaafreen@yahoo.com](mailto:uzmaafreen@yahoo.com)**ABSTRACT****Background:** The significance of vitamin D deficiency in the progress of menstrual disorders in women is a topic of great interest in studies. Several studies have found that taking an increased vitamin D supplement can help restore the menstrual cycle.**Aim:** To investigate the relationship between vitamin D and menstrual cycle irregularity.**Material and Methods:** In this cross-sectional study, serum vitamin D levels were measured in 150 outpatient women with menstrual disorders (n=75), compared to women with comparable profiles (n=75) with symptoms other than menstrual disorders compared.**Results:** A decreased level of vitamin D was associated with a 13.3 times odds of an irregular cycle (95% CI):  $p < 0.001$ . 25-hydroxy vitamin D was not associated with age or body mass index. We found a significant difference ( $p < 0.001$ ) in mean vitamin D levels among the females with irregular cycles vs. regular cycles

Practical implication: There is scarcity of literature on vitamin D and menstrual periods so current study assessed the relationship between vitamin D and menstrual cycle irregularity in women.

**Conclusion:** Lower 25(OH)D levels are linked to irregular menstrual cycles. Our findings indicate that vitamin D has an effect on the reproductive axis, which has wider scope for reproductive potential.**Keywords:** Menstrual disturbances, Ovulation, Vitamin D deficiency, polycystic ovarian syndrome.**INTRODUCTION**

Vitamin D deficiency is regarded as a major public health issue.<sup>1</sup> Vitamin D is essential for bone health and might be necessary for reproduction.<sup>2</sup> It is known to play a variety of roles in affecting reproductive physiology via vitamin D receptors present in the female reproductive system, including the ovaries (especially in the granulosa cells), uterus, and placenta.<sup>3,4</sup>

Vitamin D deficiency is quite frequent in reproductive-age women, and it might impact menstrual cycle duration due to a prolonged follicular phase that causes prolonged ovulation.<sup>5,6</sup> Vitamin D controls ovarian function and thus the menstrual cycle most likely via anti-mullerian hormone (AMH) receptors, which further share a domain for the vitamin D response element.<sup>7,8</sup> A high dose of vitamin D supplementation helps address metabolic problems linked with PCOS.<sup>9,10</sup> Multiple research studies have linked Vitamin D deficiency to decrease in ovarian reserve and dysmenorrhea.<sup>11,12</sup> Moreover, gene mutations in vitamin D receptors have been associated to gonadotropins, sex hormone-binding globulin, testosterone, and insulin levels in the blood.<sup>13,14</sup> Furthermore, it has been proposed that higher serum vitamin D might enhance reproductive performance in PCOS women. A lack of vitamin D is a potential risk for polycystic ovarian syndrome.<sup>7</sup> Vitamin D supplementation can normalize menstrual periods and enhance ovarian folliculogenesis in women with this syndrome.<sup>15</sup> Even so, research on vitamin D and ovulatory function in healthy women is limited. Previous researchers have identified links between Vitamin D, a commonly used indicator of vitamin D status, and irregular cycles.<sup>9</sup> These studies used retrospectively remembered menstrual cycle data.

There is a scarcity of literature on this topic vitamin D and menstrual periods and also some researchers challenge the relevance of vitamin D (in conjunction with calcium) in any type of female menstrual issues. To the best of author's knowledge no such study has been conducted in Pakistani population so far. So we conducted this study to determine whether there is a link between low vitamin D levels and menstrual irregularity.

**MATERIAL AND METHODS**

It was cross-sectional based observational study conducted at a tertiary care hospital of Karachi between May to July 2018. The survey included women of the reproductive age who presented to the gynaecology outpatient department with menstrual irregularities. With 80% power and a 5% level of significance, the sample size was calculated using a previous study's<sup>7</sup> standard deviation and minimum difference detected for mean vitamin D levels between irregular and regular cycles. The sample size calculated is 150. Each group contain 75 individuals.

Women of reproductive age who presented to the gynaecology outpatient clinic complaining of menstrual irregularity (defined as an 8-10 day cycle variation) were included. Regular (intervals of 21 to 35 days) and irregular participants were separated into two groups (intervals of more than 35 days). The following were the exclusion criteria: 1) Women with a known cause of irregular menstruation, such as thyroid problems, hyperprolactinemia, uterine pathogenesis, or ovarian carcinoma; 2) Patients taking hormone treatment, insulin therapy, vitamin D, calcium, or glucocorticoids. Women with similar demographics who attended the gynaecology outpatient clinics with complaints other than menstrual irregularities were recruited as controls.

Each respondent underwent a thorough general investigation, including a BMI calculation. Serum vitamin D levels were assessed after 5 ml of venous blood was withdrawn from respondents' antecubital veins in a plain red vacutainer for analysis by an automated kit method utilizing Electrochemiluminescence enzyme immunoassay. For this assay, blood serum was obtained utilizing basic type of sampling tubes or tubes comprising separating gel. At 20-25°C, 25-hydroxyvitamin D is secure for 8 hours. Precipitated samples were centrifuged for 2 hours before measurement.

SPSS version 21 was used to analyze the data. The categorical variable was represented as frequencies and percentages. The Chi Square statistics was used for testing relationships between categorical variables. The mean vitamin D levels of women with irregular vs. regular cycles were compared using the Student's t test. A p-value of  $< 0.05$  was considered statistically significant.

**RESULTS**

Table 1: shows the demographic features of the women in the both study groups. There was no significant difference between the two groups in terms of age, BMI, or marital status.

Table 1: Demographic Data of Study Participants

Variables	Regular Cycle	Irregular Cycle	p-value
Age (years)	n (%)	n (%)	
18-20	20 (26.6)	14 (18.6)	0.503
21-30	31 (41.3)	34 (45.3)	
31-40	24 (32)	27 (36)	
BMI (kg/m <sup>2</sup> )			
Underweight	13	11	0.515
< 18.5	28	23	
Normal	16	24	
18.5-22.9	18	17	
Overweight	45 (60)	50 (66.6)	0.532
23-24.9	30 (40)	25 (33.3)	
Obese			
>25			
Marital Status			
Married			
Unmarried			
Data is presented as n (%)			
Chi square test was used			
p-value <0.05 was considered significant			

Table 2: shows that there was a substantial difference in mean vitamin D levels (p < 0.001) between those who had irregular cycles and those who had regular cycles. Women with regular menstrual cycles had considerably higher vitamin D levels than those with irregular menstrual cycles.

Table 2: Comparison and association of vitamin D levels based on regularity

Variables	Mean ± SD	p-value
Regular menstrual cycle	26.8 ± 8.6	<0.001
Irregular menstrual cycle	19.6 ± 9.0	
Data is presented as Mean ± SD		
Independent t test was used		
p-value <0.05 was considered significant		

The relationships between vitamin D levels, age, BMI and menstrual irregularity are shown in Table 3. There was no significant difference in age and BMI. Only 20% of patients with normal menstruation were vitamin D deficient, whereas 53.3% of patients with menstrual irregularities were vitamin D deficient, with a vitamin D level of 20 ng/ml.

Table 3: Evaluation of vitamin D levels with age, and BMI

Vitamin D (mg/dl)			
Variable	< 20	>20	p-value
Age (years)			
18-20	11	23	0.831
21-30	25	40	
31-40	19	32	
BMI (kg/m <sup>2</sup> )			
Underweight < 18.5	10	14	0.869
Normal 18.5-22.9	17	34	
Overweight 23-24.9	14	26	
Obese >25	14	21	
Menstrual Cycle			
Regular cycle	15 (20)	60 (80)	<0.001
Irregular Cycle	40 (53.3)	35 (46.6)	
Data is presented as n (%)			
Chi square test was used			
p-value <0.05 was considered significant			

**DISCUSSION**

The findings of our study revealed a significant difference (p 0.001) in mean vitamin D levels between women with irregular cycles and those with regular cycles. Women who had regular periods had considerably higher vitamin D levels. A low vitamin D level was

linked to an 18.4 fold increase in the likelihood of having an irregular cycle.

Multiple research studies have observed that patients with PCOS are deficient in vitamin D.<sup>5,6,7</sup> Also, Previous research has found a link between low levels of 25(OH)D and a higher likelihood of irregular menstruation.<sup>11</sup> Nonetheless, the current study supports previous findings that lower levels of 25(OH)D may cause irregular menstrual cycle.

Decreased levels of 25(OH)D were linked to both long menstrual periods and long follicular phases, as well as a proclivity for brief luteal phases. The greatest links were found for vitamin D deficiency (20 ng/ml). Both the likelihood of a long follicular phase and the likelihood of a brief luteal phase risen as 25(OH)D decreased.<sup>18</sup>

There was no discrepancy in deficiency of vitamin D in between PCOS and control groups, with 80% vs. 70%, respectively (p = 0.14).<sup>19</sup> A cross-sectional research conducted on African American women of child bearing age, it was discovered that low 25(OH)D levels were linked with a sustained follicular phase, resulting in late ovulation and thus long menstrual periods (but not short or irregular cycles).<sup>11</sup> Other research has concentrated on combining vitamin D and calcium supplements with other drugs such as metformin to achieve better ovulation and menstrual disturbances in PCOS patients.<sup>20,21</sup> Although our study found no link between age or BMI and vitamin D levels, there have previously been research in which participants with Polycystic ovary syndrome benefited in their complaints of infertility, BMI, and biochemical and metabolic abnormalities after getting calcium And vitamin d supplements.<sup>16,22</sup> A few other research findings, including one randomized, single-blind, placebo-controlled intervention trial, discovered proof of an increase in menstrual regularity after vitamin D and calcium supplementation in addition to Metformin.<sup>5,23</sup> Vitamin D deficiency is very prevalent in child bearing women, and it can affect menstruation length due to a pronged follicular phase, which causes slow ovulation. As this is a new area of study, bigger sample sizes and longer study durations are required to investigate the function of vitamin D in reproduction particular to the menstruation and fertility of child bearing women, the fundamental mechanisms, efficient cutoff values, and accurate dose of supplementation required.<sup>24</sup>

**CONCLUSION**

Menstrual irregularities in child bearing women are a very prevalent issue in our society with vitamin D deficiency. Vitamin D is cost-effective and readily accessible, with few adverse effects, therefore it should be regarded for diets supplemented in women who have irregular menstrual cycles. More research is needed to reach more conclusive conclusions about the effectiveness of vitamin D supplementation for irregular periods, fertility issues, and other advantages.

**Conflict of Interest:** There is no conflict of interest in the present study.

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