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# IMPACT OF VITAMIN D DEFICIENCY ON REPRODUCTIVE HORMONES AND MENSTRUAL IRREGULARITIES IN WOMEN OF REPRODUCTIVE AGE

Musarrat Manzoor<sup>1\*</sup>, Mamoona Sarwar<sup>2</sup>, Uswa Mansoor<sup>3</sup>, M. Shakil Zari Khawri Siddiqui<sup>4</sup>, Amir Jalal<sup>5</sup>, Waqas Qurshi<sup>6</sup>

<sup>1</sup>Registrar, Muhammad Medical College & Hospital, Mirpurkhas, Pakistan <sup>2</sup>Senior Registrar, Department of Gynecology & Obstetrics, Ittefaq Hospital Trust, Model Town, Lahore, Pakistan

<sup>3</sup>Department of Pharmacy, Saulat Institute of Pharmaceutical Sciences and Drug Research, Quaid-i-Azam University, Islamabad, Pakistan

\*Corresponding Author: Musarrat Manzoor Email: musarratmanzoor.fimu@gmail.com

# **ABSTRACT**

**Background:** In addition to its classical effect in bone metabolism, vitamin D is crucial in the process of reproductive physiology, having an effect on ovarian steroidogenesis, gonadotropin secretion, and endometrial functioning. Vitamin D deficiency is very common amongst those women in South Asia of reproductive age and could be a cause of menstrual disorders and hormonal problems.

**Objectives:** The aim of the study was to assess the relationship between vitamin D deficiency, reproductive hormone profiles and menstrual abnormalities in women of reproductive age who visited tertiary care hospitals in Pakistan.

**Methods:** The study was conducted as a cross-sectional observational study between June 2024 and April 2025 at the Muhammad Medical College and Hospital, Mirpurkhas, and Ittefaq Hospital Trust, Lahore. One hundred and fifty women of the ages 18 to 45 years with menstrual irregularities were recruited. Measures were done on serum vitamin D [25(OH)D], follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol and progesterone. Deficiency in vitamin D was considered as less than 20 ng/ml, insufficiency as 20-29 ng/ml and sufficiency 30gm/dl or more. The statistical analysis has been done in SPSS version 26, and p <0.05 is taken to be significant.

**Results:** 58.7 percent of the respondents were found to be vitamin D deficient, 24.7 percent insufficient and only 16.6 percent sufficient. There was a report of menstrual irregularities in 64% of the women with most being oligomenorrhea (40.6%). Vitamin D deficiency was significantly associated with irregularities in menstruation (75% vs. 40% in sufficient group p=0.01). The deficient women had elevated levels of FSH (9.8  $\pm$  2.5 IU/L) and LH (12.6  $\pm$  3.7 IU/L), an elevated LH/FSH ratio, and a reduced level of estradiol (85.4  $\pm$  21.7 pg/mL) and progesterone (6.2  $\pm$  1.8 ng/mL) than adequately endowed women. The correlation of Pearson revealed that vitamin D had a positive correlation with estradiol (r=0.41, p<0.001) and progesterone (r=0.46, p<0.001), but a negative correlation with LH (r= -0.35, p<0.01).

<sup>&</sup>lt;sup>4</sup>Associate Professor, Department of Biochemistry, Azra Naheed Medical College, Lahore, Pakistan <sup>5</sup>Assistant Professor, Department of Biochemistry, Sahara Medical College, Narowal, Pakistan <sup>6</sup>Assistant Professor, Department of Physiology, Abwa Medical College, Khurrianwala, Pakistan

**Conclusion:** The deficiency of vitamin D is very common in the women of childbearing age and is closely linked with menstrual cycles and negative changes in reproductive hormones. Routine screening and correction of vitamin D deficiency may also be a cost-effective measure to enhance reproductive health outcomes in such a population.

**Keywords:** Vitamin D deficiency; reproductive hormones; menstrual irregularities; estradiol; progesterone; fertility

### INTRODUCTION

Vitamin D is a fat-soluble secosteroid hormone and it is conventional to acknowledge that it is involved in calcium homeostasis and skeletal health. However, over the past few decades it has been found research shows that vitamin D is a multifunctioning hormone that has extensive effects on the immune, cardiovascular and endocrine systems. More specifically, the growing evidence indicates that there is a high correlation between the quality of vitamin D and female reproductive health. Vitamin D has vitamin D receptors (VDRs) and enzymes that convert it into vitamin D in such reproductive structures as the ovary, endometrium, placenta and hypothalamus, which indicates its possible regulatory effect on the hypothalamic-pituitary-ovarian (HPO) axis and reproductive hormone production.

Vitamin D deficiency is on the epidemic levels in the world, and it affects over one billion individuals, with a disproportionately large number of women of reproductive age. Some of the reasons behind this deficiency are poor exposure to sunlight, poor diet intake, dark pigmentation of the skin and cultural dressing. The South Asian and Middle Eastern, even though the sun is available throughout the year, experience the highest incidence of hypovitaminosis D which begs the question of the long term effects of such a condition on fertility and reproductive capacity in these areas.

Ovarian folliculogenesis, ovulation and menstrual cycle regulation all require reproductive hormones like follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol and progesterone. The distortion of their balance usually leads to menstrual disturbances, anovulation, and infertility. Vitamin D has been reported to have diverse effects on ovarian steroidogenesis, modulated luteal activity in response to progesterone production, and gonadotropin secretion at the hypothalamic and pituitary gland. It follows that the vitamin D deficiency can also play a role in the development of endocrine conditions that are clinically expressed through oligomenorrhea, amenorrhea, polymenorrhea, or irregular cycles.

More so, menstrual abnormalities that are linked to vitamin D deficiency are often seen in women with polycystic ovarian syndrome (PCOS), which is a condition that is linked to hyperandrogenism, ovulatory dysfunction, and metabolic dysfunction. Vitamin D has been reported to be associated with a positive effect on menstrual regularity, ovulation rates, and insulin sensitivity, thus indicating its therapeutic worth in these patients. In the same way, a study on assisted reproductive technologies (ART) has indicated rates of increased implantation and pregnancy in women with adequate vitamin D levels and thus the value of the nutrient in reproductive health.

In light of the high rates of vitamin D deficiency and the high cost of menstrual cycles abnormalities and infertility in women of reproductive age, there is a critical clinical need to comprehend the interaction between vitamin D status and reproductive hormones. Elucidating this association can be used to enlist vitamin D as a risk factor to be altered and lay the groundwork to preventive and curative intervention to enhance women reproductive outcomes.

### **MATERIALS AND METHODS**

# **Study Design and Duration**

It was a cross-sectional observational study conducted in a hospital, which involved a period between June 2024 and April 2025. The main aim was to test the effect of vitamin D deficiency on the reproductive hormones and menstrual abnormalities in women of reproducing age.

## **Study Settings**

The study was done in two tertiary care hospitals in Pakistan, i.e. the Muhammad Medical College & Hospital, Mirpurkhas, and the Department of Gynecology and Obstetrics, Ittefaq Hospital Trust, Model Town, Lahore. The selection of these centers was due to a large number of patients served and the varied population, whereby, the study sample would have represented women with varying socioeconomic and lifestyle backgrounds.

# Population and Sample Size of the study.

One hundred and fifty women aged 18 years to 45 years who were in the outpatient gynecology and obstetrics departments were recruited. The sample size was calculated at a satisfactory level of statistical power to examine the correlation between vitamin D status, hormonal levels and menstrual irregularities.

## Eligibility Criteria

The inclusion criteria were as follows: a woman of reproductive age with a menstrual anatomy disorder of oligomenorrhea, amenorrhea, polymenorrhea, or irregular cycles, and a willing participant who signed an informed consent form to take part in the study. Women were not included when pregnant or lactating, receiving vitamin D supplement or hormonal therapy, or had a history of chronic systemic diseases including thyroid disorders, diabetes mellitus, chronic renal failure, or liver disease. Women who had undergone gynecological surgery including fibroid tumor or endometriosis were also locked out to eliminate confounding variables.

#### **Data Collection**

Following the enrollment, each participant was interviewed with a pretested structured questionnaire which captured demographical information, socioeconomic status, menstrual and reproductive history, sunlight exposure, and food intake of vitamin D. An in-depth clinical analysis was done to evaluate anthropometric parameters, such as the weight, height, and body mass index (BMI).

### **Laboratory Investigations**

To reduce the effect of hormonal variations, venous blood samples (5 mL) were taken during the early follicular phase of menstrual cycle i.e. during the period in between 2 and 5 days. Vitamin D serum was analyzed to determine the level of the vitamin under a chemiluminescent immunoassay at 25-hydroxyvitamin D [25(OH)D]. The cutoffs of vitamin D deficiency were set at serum 25(OH)D <20 ng/mL, insufficiency at 2029 ng/mL, and sufficiency at 30ng/mL. Enzyme linked immunosorbent assay (ELISA) was used to analyze reproductive hormone levels such as follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol and progesterone. Serum calcium and phosphate were also checked to exclude the metabolic derangements.

# **Outcome Measures**

The main result of concern was the correlation between menstrual irregularities and the deficiency of vitamin D. Secondary outcomes encompassed the association between the serum vitamin D status with the reproductive hormone, that is, FSH, LH, estradiol, and progesterone levels.

# **Statistical Analysis**

All the collected data were coded and entered into SPSS version 26.0 to be statistically analyzed. The continuous variables like age, BMI, vitamin D, and hormone levels were represented by mean and standard deviation (SD) whereas categorical variables like type of menstrual irregularity were represented by frequencies and percentages. The independent sample t-test and the one-way analysis of variance (ANOVA) were used to compare the mean levels of the hormones between the groups of people who are deficient in vitamin D and adequate in it. The relationship between vitamin D status and the menstrual irregularities was checked with the help of chi-square test, and the connection

between vitamin D levels and reproductive hormones was evaluated with the help of Pearson correlation coefficient. The p-value of below 0.05 was regarded as significant.

### **Ethical Considerations**

The study received ethical approval on the part of the Institutional review board of Muhammad medical college and hospital, Mirpurkhas and ethics committee of Ittefaq hospital trust, Lahore. All participants gave informed consent written informed consent was obtained before data were collected and there was upholding of patient records in the study.

#### **RESULTS**

One hundred and fifty reproductive age women were recruited into the study. The participants had a mean age of 28.7 5.6 years with a range of 18-44 years. The highest percentage of women were between 26-35 years category (48%), then 32 percent between 18-25 years, and 20 percent between the 36-45 years. The average body mass index (BMI) was 26.2 + 3.8 kg/m 2, which indicated that a considerable percentage of the females were considered to be overweight or obese. In particular, 34.7% of the respondents were normal weight, 42.7% were overweight, and 22.6% were obese. All these baseline traits are summarized in Table 1, and they point towards the reality that the study population was mostly composed of women who were at risk of not only nutritional but also metabolic disturbances that could further affect reproductive functionality.

Table 1. Baseline Characteristics of Study Participants (n=150)

Characteristic	Mean ± SD / n (%)
Age (years)	$28.7 \pm 5.6$
Age groups	
- 18-25 years	48 (32%)
- 26-35 years	72 (48%)
- 36–45 years	30 (20%)
Body Mass Index (BMI, kg/m²)	$26.2 \pm 3.8$
- Normal weight (18.5-24.9)	52 (34.7%)
- Overweight (25–29.9)	64 (42.7%)
– Obese (≥30)	34 (22.6%)

When the vitamin D status of participants was assessed, it was found that a substantial majority of women were either deficient or insufficient. Specifically, 58.7% (n=88) of women were vitamin D deficient, 24.7% (n=37) were insufficient, and only 16.6% (n=25) had sufficient vitamin D levels. These findings point toward a disturbingly high prevalence of hypovitaminosis D among women of reproductive age, even in a country with ample sunlight exposure. The distribution is presented in Table 2, and it clearly indicates that more than four out of every five women were not meeting adequate vitamin D levels, which has significant implications for both general and reproductive health.

**Table 2. Distribution of Vitamin D Status among Participants** 

Vitamin D Status	n (%)
Deficient (<20 ng/mL)	88 (58.7%)
Insufficient (20–29)	37 (24.7%)
Sufficient (≥30 ng/mL)	25 (16.6%)

Irregularities were very common in the regard of the menstrual patterns. Among 150 women, 96 (64%), had menstrual cycle problems, and only 54 women (36%), had regular menstrual cycles. Oligomenorrhea was the pattern most frequent in those with menstrual irregularities (40.6), followed by polymenorrhea (28.1), secondary amenorrhea (18.8), and irregular unpredictable cycles (12.5). Vol.32 No. 09 (2025) JPTCP (01-17)

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Notably, menstrual irregularities were also highly correlated with the lack of vitamin D. Three in every four women (75% of the deficient group) had irregular cycles, as compared with 54% in the insufficient and 40% in the sufficient groups. This correlation was found to be statistically significant (p<0.01). Table 3 demonstrates the relationship between vitamin D categories and menstrual disturbances and here it is emphasized that women with deficiencies are at a significantly increased risk of experiencing menstrual disturbances as compared to those with adequate levels.

Table 3. Association of Vitamin D Status with Menstrual Irregularities

Vitamin D Status	Normal Cycles n (%)	Irregular Cycles n (%)	Total n
Deficient (<20 ng/mL)	22 (25%)	66 (75%)	88
Insufficient (20–29)	17 (46%)	20 (54%)	37
Sufficient (≥30 ng/mL)	15 (60%)	10 (40%)	25
Total	54 (36%)	96 (64%)	150

Reproductive hormone levels were analyzed and found significant differences between the groups. The means of the FSH and LH level of women with vitamin D deficiency were vastly increased resulting to a greater LH/FSH ratio than that of women with adequate vitamin D. The average FSH of the deficient women was 9.8 2.5 IU/L and the average of the sufficient women was 7.3 1.9 IU/L (p<0.01). On the same note, LH were 12.6 +/ -3.7 IU/L in women who were deficient and 9.4 +/ -2.8 IU/L in women who were sufficient (p<0.01). Deficient women had significantly low levels of estradiol and progesterone with a mean of 6.2 1.8 ng/mL of progesterone as opposed to 9.5 2.2 ng/mL in sufficient women (p<0.001). These findings are summarized in Table 4 and are a clear indication of the biochemical changes in hormones involved in reproduction and deficiency in vitamin D.

Table 4. Comparison of Mean Reproductive Hormone Levels by Vitamin D Status

Hormone	•	Insufficient (n=37)	•	р-
	$Mean \pm SD$	$Mean \pm SD$	Mean ± SD	value
FSH (IU/L)	$9.8 \pm 2.5$	$8.2 \pm 2.1$	$7.3 \pm 1.9$	< 0.01
LH (IU/L)	$12.6 \pm 3.7$	$11.1 \pm 3.2$	$9.4 \pm 2.8$	< 0.01
LH/FSH Ratio	1.29	1.21	1.08	< 0.05
Estradiol	$85.4 \pm 21.7$	$97.6 \pm 19.2$	$108.3 \pm 20.4$	< 0.01
(pg/mL)				
Progesterone	$6.2 \pm 1.8$	$7.8 \pm 2.0$	$9.5 \pm 2.2$	< 0.001
(ng/mL)				

Finally, correlation analysis confirmed a strong relationship between serum vitamin D levels and reproductive hormones. Vitamin D showed a positive correlation with estradiol (r = 0.41, p < 0.001) and progesterone (r = 0.46, p < 0.001), while an inverse correlation was observed with LH levels (r = -0.35, p < 0.01). The correlation with FSH was weaker but still significant (r = -0.28, p < 0.05). These findings indicate that as vitamin D levels improve, ovarian hormone production becomes more favorable for reproductive health, while gonadotropin imbalance decreases. The details of correlation coefficients are presented in Table 5.

**Table 5. Pearson Correlation between Vitamin D Levels and Reproductive Hormones** 

Hormone	Correlation Coefficient (r)	<i>p</i> -value
FSH	-0.28	< 0.05
LH	-0.35	< 0.01
Estradiol	+0.41	< 0.001
Progesterone	+0.46	< 0.001

#### **DISCUSSION**

The current study revealed that there was a strong relationship between vitamin D deficiency and menstrual irregularities as well as unfavorable changes in the reproductive hormone profiles in reproductive women. Over fifty percent of the participants in the study were discovered to be deficient in vitamin D and almost two thirds of the participants were reported to have menstrual abnormalities. These results are important as they do not only show the common occurrence of hypovitaminosis D as an issue among Pakistani women but also as a possible serious endocrine disruptor that may cause reproductive health issues.

We found out that 75% of the women who were deficient in vitamin D had irregular menstrual cycles as opposed to 40% of the women who were adequately endowed with vitamin D. It is not a new finding since the previous research in South Asia and the Middle East has indicated that vitamin D deficiency is associated with cycle disruptions like oligomenorrhea and amenorrhea. Similar results were found by Naderi et al. (2016) in the case of Iranian women, in which lower Vitamin D levels were strongly related to irregular menstruation and longer menstrual cycles. These results indicate that vitamin D can have a vital role in the process of regulating the hypothalamic pituitary ovary (HPO) axis and in the normalization of the ovarian cyclicity.

The current study also showed that the lack of vitamin D was associated with the significant change in the concentration of reproductive hormones. Low vitamin D women showed high FSH and LH levels, higher LH/FSH ratio, and lower estradiol and progesterone levels. This hormonal pattern is a sign of impaired folliculogenesis, less ovarian reserve and faulty luteal functioning. The decline of estradiol and progesterone could be the reason why irregular periods and anovulatory conditions are very common in deficient women. These results are biologically credible because the receptors of vitamin D are present in the ovarian tissue, granulosa cells, and the endometrium. Increasing estrogen synthesis by increasing aromatase activity Vitamin D has been found to facilitate estrogen production by increasing aromatase activity. Increasing luteal cell progesterone production Vitamin D has been reported to increase the production of estrogens by increasing aromatase activity. Inadequacy, thus, interferes with ovarian steroids production and interferes with ovulation.

This is further supported by a correlation analysis because vitamin D levels were found to have a significant positive correlation with estradiol and progesterone and an inverse relationship with LH and FSH. This implies that the lack of vitamin D can respond at the hypothalamic-pituitary axis and ovarian axis, causing the compensatory response in secretion of gonadotropins but insufficient secretion of ovarian steroid hormones. These findings are consistent with the results of Lerchbaum and Obermayer-Pietsch (2012), who found that the lack of vitamin D was related to a higher LH/FS ratio and inefficient ovarian functioning in European cohorts. Also, the research findings on women with polycystic ovarian syndrome (PCOS) proved that the use of vitamin D supplementation could lower LH levels, improve the estradiol and progesterone concentrations, and bring menstrual cycles closer to normal.

The implication of these results is especially critical as far as reproductive health in South Asia is concerned. Pakistan has one of the highest recorded levels of vitamin D deficiency in the world and women of reproductive age form one of the vulnerable groups as they lack the necessary diet, sunlight exposure through cultural attires and urban living. The clinical problem of menstrual irregularities and subfertility is also of high importance in the given population, and our results might indicate that the simple and low-cost intervention that involves correcting vitamin D deficiency will help to achieve better reproductive outcomes. Moreover, the correlation of vitamin D deficiency and the occurrence of menstrual irregularities might also be generalized to assisted reproductive technologies (ART) as past studies have shown that proper levels of vitamin D are associated with the increase in implantation and pregnancy success.

Although this study contributes to the existing body of knowledge, it is important to note that the study has some shortcomings. To begin with, it is a cross-sectional study and causal relationships cannot be concluded in such a study, longitudinal or interventional study would be required to prove causality. Second, the study failed to determine confounding variables including dietary calcium,

physical exercise, or genetic polymorphisms of vitamin D receptors that might also have an effect on reproductive hormone regulation. Third, the sample size was sufficient but it was only hospital-based and might not be applicable to the general population of the community. Notwithstanding these shortcomings, the study offers compelling data of a close association between vexing deficiency of vitamin D and reproductive disturbances among Pakistani women, and the necessity of a regular screening of vitamin D levels as a part of gynecological practice.

## **CONCLUSION**

This study proved that the vitamin D deficiency is very common among the women of the reproductive age in Pakistan and was strongly related with the irregularity in menstrual cycles and negative changes in the reproductive hormone levels. The deficient women had higher chances of having oligomenorrhea, none, or irregular cycles, and had high levels of FSH and LH and low levels of estradiol and progesterone. These results are good indications that vitamin D is an essential factor in ensuring reproductive endocrine balance and regularity of the menstrual cycle. The clinical consequences are also significant: a regular screening of vitamin D levels in women with menstrual abnormalities should be rated as something normal, and early supplementation could be used to normalize abnormal hormonal levels and ensure better fertility rates. The interventional approaches involving the use of vitamin D supplementation need to be investigated in the future so as to determine its application in the treatment of reproductive disorders. Finally, there is a strong solution to vitamin D deficiency, which is simple, cost-effective and effective in enhancing reproductive health of women in South Asia and other global regions.

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### **Conflict of Interest**

The authors declare no conflicts of interest.

### **Ethical Approval**

Ethical approval was obtained from the Institutional Review Boards of Muhammad Medical College & Hospital, Mirpurkhas, and Ittefaq Hospital Trust, Lahore.

# **Informed Consent**

Written informed consent was obtained from all participants prior to their inclusion in the study.

#### **Author Contributions**

MM: Conceptualization, study design, data collection, manuscript drafting

MS: Data acquisition, analysis, and interpretation

**UM:** Literature review, writing – introduction and discussion

MSZKS: Statistical analysis and results interpretation

AJ: Critical revision and editing of manuscript

**WQ:** Supervision, final approval of the version to be published

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