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Comparison of vitamin D levels in primary infertility and infertile female patients

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Abstract

Aim: This study was conducted to compare vitamin D levels in primary infertile patients with vitamin D levels in fertile women.

Methods: This study was composed of 75 unexplained primary infertile and 75 fertile patients who applied to the hospital between 1 June 2022 and 31 March 2023 at Harran University Faculty of Medicine, Department of Obstetrics and Gynecology, prospectively. Two milliliters of blood samples were obtained from women with unexplained primary infertility and from fertile women. All venous blood specimens were centrifuged at 4000 rpm for 10 min. The plasma derived from the samples was evaluated for 25-hydroxy vitamin levels using LS-MS 8045 and subsequently compared. The plasma derived from the samples was examined for levels of AMH, TSH, prolactin, progesterone, estrogen, LH, and FSH.

Results: The mean of vitamin D in the unexplained primary infertile group was 5.35 ± 1.81 ng/mL while the mean of vitamin D in fertile women was 12.6 ± 4.44 ng/mL. It was determined that there was a significant difference between vitamin D levels according to the study groups.

Conclusion: We think that vitamin D supplementation is important in infertile women because the levels of vitamin D in the unexplained primary infertile group were lower than those in the fertile group. There is a need for more comprehensive multicenter studies showing the relationship between vitamin D levels and infertility related to the current study.

Keywords: Vitamin D level, infertility, infertile women, fertile women

Introduction

Infertility is a disease of the reproductive system defined as the inability of a couple to achieve pregnancy despite a year or more of unprotected sexual intercourse (1,2). Primary infertility is defined as the inability to experience any pregnancy, while secondary infertility is the inability of a woman to achieve a new pregnancy after a successful pregnancy (1, 3, 4).

Infertility has become an important global health problem affecting many people in the world today. According to the latest statistics of the World Health Organization; it has been reported that 186 million people in the world are infertile and 48 millions of them are couples (2). Infertility affects approximately 9-18% of couples of reproductive age in the world today (5) and while the rate is between 7.3-9.1% in women aged 15-34 years, it has increased to 25% in women aged 35-39 years and 30% in women aged 40-44 years (6). There are different rates of primary infertility in the literature. It has been reported to be less than 6% in China, Malawi, Tanzania, and Zambia; more than 9% in the Philippines; 10% in Finland, Sweden, and Canada; and 18% in Switzerland (1).

Since genetic causes of infertility cannot be explained, more attention is paid to environmental factors. The etiology of female infertility is diverse, and many factors are thought to be effective. These include genetic mutations, chromosomal abnormalities, lifestyle factors, ovulation disorders, tubal factors, endometriosis, and unexplained infertility (1, 4, 7). Recent studies have shown that individuals' dietary habits, stress level, alcohol use, smoking status, and obesity status affect female physiology in the long term (4, 8, 9).

Another important factor in female infertility is vitamin D deficiency. Vitamin D exerts its biological effects through the vitamin D receptor (VDR), a soluble protein. The vitamin D receptor (VDR) is a transcription factor in the nuclei of target cells that mediates the genomic effect of the active form of vitamin D. The presence of vitamin D receptor (VDR) in the female reproductive system suggests a role for vitamin D in infertility (10, 11).

The literature has shown that vitamin D is very important in female reproduction and infertility. Since vitamin D may be effective in female reproduction, this study aimed to investigate the comparison of Vitamin D levels in unexplained primary infertile patients with Vitamin D levels in fertile women.

Materials and Methods

The study was planned in accordance with the Declaration of Helsinki. Institutional permission was obtained from the hospital where the research was conducted. The research was conducted with the approval of the Non-Drug and Non-Interventional Clinical Research Ethics Committee of Harran University (decision dated 2022 and numbered HRU/22.09.28). Fertile and infertile women were informed about the study. Verbal consent and written informed consent were obtained from the women. They were informed that the results of the research would not be disclosed to third parties, companies or individuals.

This prospective study consisted of 75 unexplained primary infertile and 75 fertile women who were admitted to the Gynecology and Obstetrics Clinic of the Faculty of Medicine of Harran University between 1st June 2022 and 31st March 2023. A total of 150 women were included in the study. Unexplained primary infertile women and fertile women were informed about the study. Verbal and written informed consents were obtained from the women included in the study. To ensure standardization between patients, unexplained primary infertile patients and fertile patients were followed and treated by the same physician.

Individuals to be included and excluded from the study include the following criteria.

Inclusion criteria

- Between the ages of 20-40
- Unexplained primary infertile women
- Fertile women
- Women who accepted the study were included in the study.

Exclusion criteria

- Under 20 years of age
- Over 40 years of age
- Having diabetes
- Chronic hypertension
- Women with heart, liver, and kidney disease
- Pregnant women
- Women who did not agree to participate in the study were not included in the study.

A graphical illustration of the study design is shown in Figure 1.

Woman follow-up form

The female follow-up form was developed by the researcher by reviewing the literature. In the female follow-up form, the ages of the infertile women included in the study and the ages, gravidas, and parity numbers of the fertile women were recorded. Vitamin D, AMH (Anti Müllerian Hormone), TSH (Thyroid-Stimulating Hormone), Prolactin, Progesterone, Estrogen, LH (Luteinizing Hormone), and FSH (Follicle Stimulating Hormone) levels of women in both groups were recorded for follow-up.

Two ml of blood samples were collected from unexplained primary infertile women and fertile women aged 20-40 years. All venous blood samples were centrifuged at 4000 rpm for 10 min. The plasma obtained from the samples was analyzed for 25 hydroxy vitamin levels in LS-MS 8045 and compared. Then, the plasma obtained from the samples was analyzed for AMH level, TSH level, prolactin level, progesterone level, estrogen level, LH level, and FSH level in a Biochemistry and Hormone device.

Statistical analysis

Analyses were performed by using IBM SPSS 25.0 software (IBM Inc., Armonk, New York, USA).

The suitability of the data for normal distribution was evaluated based on the Kolmogorov Smirnov Test. The mean and standard deviation values of the demographic characteristics of all women (fertile and infertile) participating in the study are given. The distribution of the data was checked in order to compare the averages in two different groups. Independent Sample t test was used when the data fit the normal distribution. A value of $p<0.05$ was considered statistically significant.

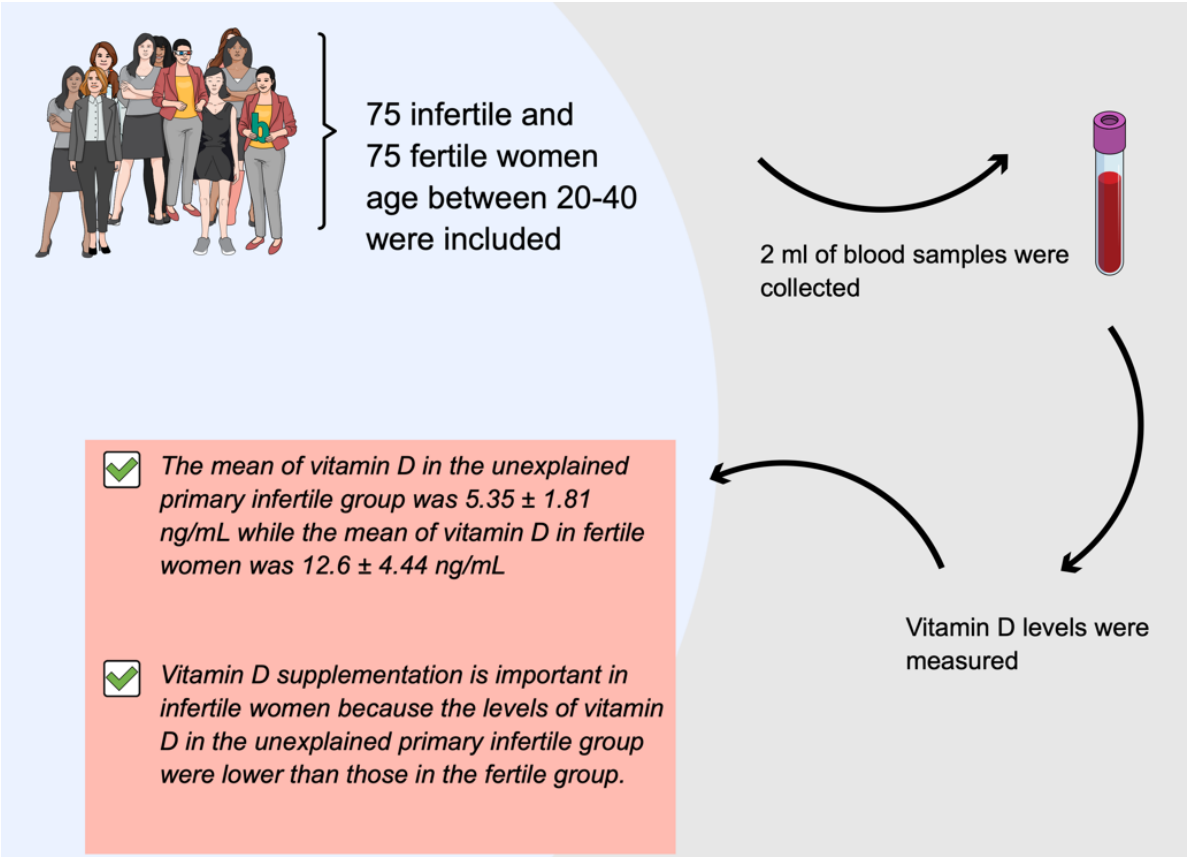

Figure 1. Graphical illustration of the study design and the obtained results.

Results

The mean age of all women who participated in the study was 30.2 ± 5.89 years, mean gravidity was 1.85 ± 2.37, mean parity was 1.57 ± 1.95 and mean number of pregnancies was 1.56 ± 1.94. The mean vitamin D level of the women was 8.97 ± 4.96 ng/mL, FSH level was 7.26 ± 6.84 mIU/mL, LH level was 6.1 ± 5.94 mIU/mL, estrogen level was 87.29 ± 85.95 pg/mL and progesterone level was 2.97 ± 4.31 ng/mL Prolactin level was 10.73 ± 6.82 ng/mL, TSH level was 2.64 ± 4.76 µIU/mL and AMH level was 2.04 ± 2.13 ng/mL.

The mean endometrial thickness of the women was 6 ± 0 mm (Table 1).

Table 2 shows the comparison of vitamin D levels according to the study groups. The mean vitamin D level in the unexplained primary infertile group was 5.35 ± 1.81 ng/mL, while the mean vitamin D level in fertile women was 12.6 ± 4.44 ng/mL. It was determined that there was a significant difference between vitamin D levels according to the study groups. Vitamin D levels were found to be significantly lower in women in the unexplained primary infertile group (Table 2).

Table 1. Descriptive characteristics of women participating in the study

Sociodemographic characteristics (n=150)	Mean±SD	Median (Min-Max)
Age (year)	30.2 ± 5.89	29.5 (20 - 46)
Gravide	1.85 ± 2.37	0 (0 - 9)
Parity	1.57 ± 1.95	0 (0 - 7)
Number of Living Children	1.56 ± 1.94	0 (0 - 7)
Vitamin D Level	8.97 ± 4.96	7.2 (2.2 - 24)
FSH (Follicle Stimulating Hormone) (mIU/mL)	7.26 ± 6.84	5,7 (1,4 - 50,5)
LH (Luteinizing Hormone) (mIU/mL)	6.1 ± 5.94	4.9 (0.1 - 36.5)
Estrogen Level (pg/mL)	87.29 ± 85.95	61.4 (3.9 - 748)
Progesterone Level (ng/mL)	2.97 ± 4.31	0.8 (0.1 - 19.5)
Prolactin Level (ng/mL)	10.73 ± 6.82	9 (0.4 - 35.8)
TSH; Thyroid stimulating hormone (µIU/mL)	2.64 ± 4.76	1.7 (0.1 - 39.3)
AMH; Anti Müllerian hormone (ng/mL)	2.04 ± 2.13	1.15 (0.1 - 11.3)
Endometrial thickness on ultrasound (mm)	6 ± 0	6 (6 - 6)

Table 2. Serum vitamin D levels in unexplained primary infertile and fertile women

	Unexplained primary infertility group	Fertile group	Test statistic	P*
Vitamin D Level (ng/mL)	5.35 ± 1.81	12.6 ± 4.44	13.106	*p*<0.001

Mean ± Standard Deviation; *: Independent samples t-test

FSH levels were analyzed in Table 3. The mean FSH level in the unexplained primary infertile group was 6.94 ± 6.14 mIU/mL, while the mean FSH level in fertile women was 7.58 ± 7.5 mIU/mL. It was determined that FSH levels in women in the unexplained primary infertile group were lower. There was no clinically significant difference between FSH levels according to the study groups (Table 3).

The mean LH level in the unexplained primary infertile group was 5.64 ± 5.49 mIU/mL, while the mean LH level in the fertile group was 6.6 ± 6.36 mIU/mL. Although LH levels in the fertile women were higher than those in the unexplained primary infertile group, there was no clinically significant difference between the LH levels (Table 3).

The mean estrogen level in the unexplained primary infertile group was 63.19 ± 50.3 pg/mL, while the mean estrogen level in fertile women was 70.39 ± 50.67 pg/mL. Although estrogen levels were found to be lower in the unexplained primary infertile group, there was no clinically significant difference between estrogen levels (p=0.080) (Table 3).

When the progesterone levels of the study groups were compared, it was found that the mean progesterone level in women in the unexplained primary infertility group was 2.93 ± 4.94 ng/mL, while the mean progesterone level in fertile women was 3.0 ± 3.62 ng/mL. It was determined that progesterone levels were lower in women in the primary infertility group. There was no clinically significant difference between the mean progesterone levels according to the study groups (p=0.590) (Table 3).

The mean prolactin level in women in the unexplained primary infertility group was 11.41 ± 6.74 ng/mL, while the mean prolactin level in fertile women was 12.05 ± 6.68 ng/mL. There was no significant difference between the mean prolactin levels of the study groups (p=0.240) (Table 3).

The mean TSH level in the unexplained primary infertility group was 2.48 ± 2.78 µIU/mL, while the mean TSH level in fertile women was 1.4 ± 1.75 µIU/mL. Although TSH levels in infertile women were found to be slightly higher than those in fertile women, no significant difference was found between the mean TSH levels according to the study groups (p=0.382) (Table 3).

The mean AMH level in the unexplained primary infertility group was 2.69 ± 2.28 ng/mL, while the mean AMH level in fertile women was 1.4 ± 1.75 ng/mL. Although the mean AMH levels were slightly higher in the unexplained primary infertile group, there was no significant difference between the mean AMH levels of the study groups (p=0.112). This difference was not clinically significant (Table 3).

Table 3. Serum FSH (Follicle Stimulating Hormone), LH (Luteinizing Hormone), Estrogen, Progesterone, Prolactin, TSH, and AMH levels in unexplained primary infertile and fertile women

	Unexplained Primary Infertility Group	Fertile Group	Test Statistic	P*
FSH (mIU(mL)	6.94 ± 6.14	7.58 ± 7.5	2800.000	0.963
LH (mIU(mL)	5.64 ± 5.49	6.6 ± 6.36	2137.500	0.111
Estrogen (ng/mL)	63.19 ± 50.3	70.39 ± 50.67	1721.500	0.080
Progesterone (ng/mL)	2.93 ± 4.94	3.0 ± 3.62	2669.500	0.590
Prolactin (ng/mL)	11.41 ± 6.74	12.05 ± 6.68	164.450	0.240
TSH (µIU/mL)	2.48 ± 2.78	1.4 ± 1.75	2580.000	0.382
AMH (ng/mL)	2.69 ± 2.28	1.4 ± 1.75	1070.000	0.112

Mean ± Standard deviation; *: Independent samples t-test

Discussion

In our study, we aimed to evaluate vitamin D levels in unexplained primary infertile and fertile patients and the effect of these levels on fertility. In recent studies, low vitamin D levels have been explained as an important factor causing infertility and poor pregnancy outcomes (12). The effect of vitamin D on reproduction has been described in murine models. Vitamin D was reported to be effective on uterine hypoplasia, decreased aromatase gene expression, gonadal insufficiency, impaired folliculogenesis, and infertility (11, 12). Similarly, in studies conducted in the literature: The presence of vitamin D levels in many tissues, including the pituitary, uterus, ovary, and placenta, reveals that it is an important regulator of the female reproductive system (13). At the same time, the fact that vitamin D receptor (VDR) and vitamin D metabolizing enzymes are found in human reproductive tissues also supports that it is an important regulator of the female reproductive system (11, 14-16).

In our study, vitamin D levels of women in the unexplained primary infertile group were found to be significantly lower than fertile women. This result suggests that vitamin D deficiency may be the cause of infertility in unexplained primary infertile women. In cohort studies conducted in Italy, vitamin D deficiency was reported to be the first among the independent risk factors for infertility in women (11, 17, 18). It has also been confirmed by studies that vitamin D level has a significant effect on fertility (16, 17).

Recent studies have focused on the role of vitamin D levels in the endometrium (12). It has been reported that women with high vitamin D levels have an increase in the probability of pregnancy with assisted reproductive techniques and this effect may be through the endometrium (16, 17, 19). In studies conducted in the literature, it has been reported that vitamin D deficiency in women is effective in infertility by reducing the number and quality of embryos (11, 20). According to the meta-analysis conducted by Shen et al., vitamin D deficiency causes low IVF pregnancy success (21). Since a large proportion of women presenting to infertility centers show insufficient vitamin D levels in serum, it is recommended that physicians working in IVF centers monitor serum 25(OH)D levels during any assisted reproductive technique intervention. Especially in women with vitamin D deficiency, it has been explained that vitamin D supplements given externally are effective on reproduction and have a more important effect on a reproductive role other than calcium hemostasis (12).

Anti-Müllerian hormone (AMH) in the serum of women of reproductive age is known to be secreted by the granulosa cells of ovarian follicles and regulates

early follicle development (22). This function of anti-Müllerian hormone (AMH) makes it recognized as one of the most important indicators of female fertility (16, 22, 23). Studies in the literature have indicated that vitamin D has a direct effect on AMH production. It is claimed that the ovarian reserves of people with high levels of vitamin D are preserved longer. It has also been reported that vitamin D is a positive regulator of AMH production (22, 24). However, in our study, it was determined that there was no significant difference between the AMH levels of women in the unexplained infertile group and women in the fertile group. Similarly, in a study conducted in Italy in which vitamin D levels in infertile women applying assisted reproductive techniques were analyzed, it was observed that AMH values in the infertile group were similar to each other (17). Similarly, in a study by Arabian and Raoofi in Iran, in which the effect of serum vitamin D level on endometrial thickness and follicle growth parameters in infertile women undergoing ovulation induction was investigated; although vitamin D levels of infertile women were measured at different levels, it was observed that there was no difference between FSH, TSH, endometrial thickness and AMH values (25). In our study, there was no significant difference between infertile and fertile patient groups in terms of AMH levels, but vitamin D levels were lower in the infertile patient group compared to the fertile patient group, suggesting that vitamin D deficiency is associated with fertility.

In our study, although FSH, LH, estrogen, progesterone and prolactin levels of the women in the unexplained primary infertile group were lower than the women in the fertile group, no statistically significant difference was found and it was determined that these values were not clinically important. This situation once again emphasized the importance of vitamin D levels between the study groups. High prolactin levels cause anovulation, irregular ovulation, galactorrhoea, oligomenorrhoea or amenorrhoea, and infertility in non-pregnant women. In our study, prolactin levels of all patients in the unexplained primary infertile group were within the normal range, suggesting that the cause of infertility may be due to vitamin D deficiency (26, 27).

In our study, vitamin D levels of fertile women were found to be in accordance with the literature compared to infertile women. We think that the main reasons for vitamin D deficiency are the frequent pregnancies of women in the region, dietary patterns, and clothing styles in the region. The findings obtained from the study reveal that it may have important implications for both infertile patients and national health policy.

Conclusion

Since vitamin D levels in the unexplained primary infertile group were lower than those in the fertile group, we suggest that vitamin D supplementation is important in infertile women.

With some mechanistic evidence supporting the potential role of vitamin D in supporting optimum reproductive function, we conclude that our study is in agreement with the literature and that vitamin D supplementation should be used in the treatment and follow-up of infertile couples. In addition, we suggest that more comprehensive multicenter studies showing the importance and relationship of vitamin D levels in infertility should be conducted.

On the other hand, there was no significant difference between FSH, LH, Estrogen, Progesterone, Prolactin, TSH and AMH values in primary infertile and fertile women. It is recommended that the study should be carried out with larger sample groups.

Disclosures

Ethical Approval: Ethics committee approval was received for this study from the Harran University, Non-Drug and Non-Interventional Clinical Research Ethics Committee, in accordance the World Medical Association Declaration of Helsinki, with the approval number: 2022- HRU/22.09.28.

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