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RESEARCH ARTICLE

Differences In Vitamin D Receptor Levels Between Women With Primary Infertility And Women Without Infertility

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Abstract

Introduction*: Many studies have been done on vitamin D, but there is still a lack of data regarding Vitamin D receptor (VDR) level and infertility, whereas VDR has a vital role in the action of vitamin D.*
Objective*: To determine the differences in VDR levels between women with primary infertility and women without fertility problems.*
Method*: This is a case-control study on women with and without fertility problems in Padang City, conducted from March 2023–January 2024. Serum VDR levels (ng/ml) were examined using the ELISA method in the Biomedicine Laboratory at Andalas University. Differences in VDR levels were calculated using the independent T-test.*
Results*: There were 60 subjects divided into 30 subjects with infertility and 30 subjects without fertility problems. Subjects with primary infertility had more 31-35 years and >35 years old patients (54.8% and 66.7%), more overweight and obese BMI patients (50% and 83.3%), and had experienced infertility for 1-4 years (73.3%). The mean VDR levels in infertility subjects were lower than in subjects without fertility problems (1.73±0.92ng/ml and 2.35±1.30ng/ml), where this difference was statistically significant (p=0.036).*
Conclusion*: VDR levels influence the incidence of primary infertility.*

Keywords: Vitamin D Receptors, Vitamin D, Infertility

INTRODUCTION

Vitamin D receptor (VDR) is widely expressed in the female reproductive system, including the ovaries, endometrium, fallopian epithelial cells, decidua, and placenta, indicating the role of vitamin D in the physiology of the reproductive system.[1] In the ovaries, VDR plays a role in helping the process of folliculogenesis by regulating the anti-Mulerrian Hormone (AMH). In addition, in the luteal phase, after two corpus luteum is formed, vitamin D binding with VDR will increase the process of progesterone production by the corpus luteum.[2]

Endometrial cells during the menstrual cycle and early pregnancy express vitamin D receptors. Vitamin D's action on the endometrium increases the phosphorylation of progesterone receptors needed for endometrial wall thickening.[3] Vitamin D also plays a role in the endometrium in expressing the homeobox 10 (HOXA 10) gene, which is important in endometrial receptivity.[4] Vitamin D receptors are also present in the myometrium to regulate calcium homeostasis to prevent excessive contractions during early pregnancy to prevent miscarriage.[5]

Vitamin D affects fertility through various actions, namely through effects on folliculogenesis, luteal phase, progesterone hormones, endometrial receptivity, and prevention of abortion. Vitamin D affects folliculogenesis and menstrual cycle regularity through its effects on granulosa cells' AMH and follicle-stimulating hormone (FSH) receptors. AMH inhibits the loss of the oocyte pool by inhibiting primordial follicles and slowing growth. By inhibiting AMH expression, vitamin D can counteract the repressive effects of AMH on granulosa cell differentiation, thus allowing follicles to reach terminal maturation and ovulation. In addition, the effect of vitamin D on follicle-stimulating hormone receptors (FSHR) may support the effect of FSH on follicular growth. FSH and FSHR binding is required to increase estradiol secretion by granulosa cells.[2,6] In the luteal phase, vitamin D plays a role in the release of activin A, Corticotropin-Releasing Factor (CRF), Vascular Endothelial Growth Factor (VEGF), and inhibits the synthesis of tumor necrosis factor-alpha (TNF-a), which supports the process of uterine preparation for implantation. Activin A and CRF are high during the luteal phase and pregnancy in normal women, indicating these two molecules' important role in fertility. VEGF is required in neovascularization to form the corpus luteum, which later secretes progesterone.

The role of vitamin D in endometrium-promoting pregnancy implantation has been demonstrated in a cohort study conducted by Chu et al. (2019) that women with vitamin D sufficiency were more likely to achieve a live birth through in vitro fertilization (IVF) treatment than women with vitamin D deficiency. Hong et al. (2016) also conducted experiments in animal models where granulosa cells from pig ovaries were taken to inject vitamin D. The results of this study concluded that vitamin D3 increases the expression of genes that synthesize progesterone.[5] Some literature found that progesterone-regulated osteopontin, which mediates implantation and decidualization, increases endometrial cells' expression in

response to 1,25(OH)2D3. The response to high estrogen and progesterone during implantation is seen in the expression of HOXA10 regulated by 1,25(OH)2D3 in human endometrial stromal cells.[7]

Hou et al. (2020) researched 20 placental and decidual samples after abortion and 22 normal pregnancy samples after termination to see the effect of vitamin D on spontaneous abortion. In this study, there was a decrease in the expression of the enzyme 1α-hydroxylase in spontaneous abortion samples, indicating the role of vitamin D in the prevention of abortion. This study suggests that women undergoing reproductive technology should have adequate vitamin D levels before pregnancy.[8] Ersahin et al. (2022) conducted a study on 31 women with PCOS who were divided into groups of vitamin D deficiency, insufficiency, and normal levels. Endometrial samples were taken to test the expression of messenger ribonucleic acid (mRNA) HOXA10. The results showed that HOXA10 expression was higher in the group with normal vitamin D levels. This indicates a higher level of endometrial receptivity in patients with normal vitamin D levels.[9] Given the importance of the role of vitamin D receptors on fertility, especially in terms of influencing vitamin D expression, researchers are interested in proving the importance of vitamin D on the expression of vitamin D receptors in the blood in infertile women.

METHODS

This type of research is analytic observational using a case-control study design. In case-control studies, researchers look for differences between independent variables (independent variables), namely serum Vitamin D receptor levels, by taking instantaneous measurements with dependent variables (dependent variables), namely women with and without infertility. This research was conducted in March 2023-January 2024 at the Biomedical Laboratory of the Faculty of Medicine, Andalas University. The population of this study were all women with infertility and women without infertility in Padang. The sample of this study is a population that meets the inclusion and exclusion criteria set. Based on the calculation results, the number of samples used was 60 samples, and the samples were taken using consecutive sampling techniques. The data that has been collected and processed is then carried out through data analysis activities. To assess the difference in mean vitamin D receptor levels in the infertility and fertility groups, data analysis was carried out using the independent T-test with a significance limit of p=0.05. A normality test was performed using the Kolmogorf test before conducting the significance test. An independent T-test can be performed if found to be normally distributed. However, if the data is not normally distributed, an alternative test is Mann Whitney U. The results are declared significant if $p<0.05$ is obtained.

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RESULT

In Table 1, the mean age of infertility subjects was 31.73 ± 3.20 years, while in fertility subjects, the mean age was 30.63 ± 3.29 years. In the age group of 25-30 years, most subjects were fertile (60.9%), and 39.1% experienced infertility. In the age group of 31-35 years and above 35 years, most subjects were infertile, with a proportion of 54.8% and 66.7%, respectively.

The mean body mass index (BMI) in infertility subjects was higher than fertility subjects at 24.44 ± 2.40 and 22.58 ± 1.80 kg/m2, respectively. One subject with an underweight BMI was infertile. Most subjects were fertile in the norm weight group (73.7%). In the overweight group, the number of infertile and fertile subjects was found to be similar. Meanwhile, in the obese subjects, most of the subjects were infertile (83.3%). In subjects who experienced infertility, the average length of infertility was 3.40 ± 1.71 years, with most experiencing 1-4 years of infertility (73.3%) and the rest more than five years (26.7%).

Table 1. Characteristics of Research Subjects

Characteristics	Infertile subject	Fertile Subject	Total
Age (years)	**31,73 ± 3,20**	**30,63 ± 3,29**	**23 (100)**
25-30 years old	9 (39,1)	14 (60,9)	31 (100)
31-35 years old	17 (54,8)	14 (45,2)	6 (100)
>35 years old	4 (66,7)	2 (33,3)	
BMI (kg/m2)	**24,44 ± 2,40**	**22,58 ± 1,80**	**1 (100)**
Underweight	1 (100)	0 (0)	19 (100)
Normoweight	5 (26,3)	14 (73,7)	28 (100)
Overweight	14 (50)	14 (50)	12 (100)
Obesity	10 (83,3)	2 (16,7)	
Length Of Infertility (years)	**3,40 ± 1,71**		
1-4 years	22 (73,3)		
≥5 years	8 (26,7)		

Table 2 shows the results of the normality test with Shapiro-Wilk. A value of p>0.05 indicates that the data is normally distributed. Therefore, the independent T-test can be carried out.

Table 2. VDR data normality test

	Fertility	Kolmogorov-Smirnov			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
VDR	Infertile	0.108	30	0.200	0.972	30	0.596
levels	Fertile	0.180	30	0.015	0.950	30	0.172

In Table 3, the mean level of vitamin D receptor is 1.73 ± 0.92 ng/ml, with the smallest level being 0.36 ng/ml and the highest level being 4.55 ng/ml. Meanwhile, in the group of women

without infertility, the mean vitamin D receptor level was 2.35 ± 1.30 ng/ml, with the smallest level being 0.67 ng/ml and the highest level being 5.35 ng/ml, resulting in lower vitamin D receptor levels in infertility subjects. Independent T-test showed that this difference was statistically significant with p=0.036 (<0.05).

Table 3. Differences in Vitamin D Receptor Levels in Infertility and Fertility Subjects

	N (60)	VDR (ng/ml)			p-value
		Mean ± SD	Max	Min	
Infertile subject	30	1.73 ± 0.92	0.36	4.55	0.036
Fertile subject	30	2.35 ± 1.30	0.67	5.35	

DISCUSSION

The mean age of infertility subjects is almost similar to fertility subjects, namely 31.73 ± 3.20 years and 30.63 ± 3.29 years. Based on the proportion in each age group, infertility occurs more in the age group above 30 years. Fertility decreases with age where. This decline begins after the age of 30 years and accelerates after the age of 35 years. This is also associated with reduced number and quality of eggs.[10]

The mean body mass index (BMI) in infertility subjects is higher than fertility subjects, which are 24.44 ± 2.40 and 22.58 ± 1.80 kg/m2, where most obese subjects experience infertility (83.3%). The study by Aghadov found an increase in infertility in overweight patients where excess weight is also at risk for PCOS, and this study conveyed a decrease in vitamin D receptor expression, which also plays a role in fertility.[11]

In subjects who experienced infertility, the average duration of infertility was 3.40 ± 1.71 years, with most experiencing 1-4 years of infertility (73.3%) and the rest five years or more (26.7%). Research by Zhang et al. in 2022 found a link between the duration of infertility and the success of in vitro fertilization, where women who have experienced infertility for more than 4.8 years will reduce the success of in vitro fertility. This was also associated with decreased oocyte quality.[12]

Today, a growing body of evidence suggests that vitamin D may regulate female reproductive processes. Biologically, vitamin D function is mainly mediated by the vitamin D receptor (VDR), expressed in many reproductive tissues, including the ovaries, uterus, testes, and placenta. VDR is a nucleophile protein belonging to the steroid hormone receptor superfamily that mediates the expression of vitamin D's active component, 1,25-dihydroxy vitamin D.[13] Unexplained infertility refers to the absence of an explainable cause of reproductive failure. The vitamin D receptor (VDR) acts as a transcription factor. It regulates several vitamin D-responsive genes, including genes involved in the immune system that are likely to affect implantation success.[14]

In this study, the mean level of vitamin D receptor in infertility subjects was 1.73 ± 0.92 ng/ml, with the smallest level being 0.36 ng/ml and the highest level being 4.55 ng/ml. A study by Farooqui et al (2021) in 8 infertile patients found a lower mean serum VDR of 0.1 ± 0.09 ng/ml.[15] The study by Syrkasheva et al. (2022) involving 100 infertile patients who will undergo assisted reproductive therapy found in its multivariate analysis that clinical pregnancy rates were influenced by vitamin D deficiency and genetic characteristics of the VDR gene, suggesting a role for VDR in infertility.[16]

Another study by Aghadavod et al. (2017) in 80 women aged 20-35 years who will undergo in vitro fertilization found that vitamin D receptor gene expression in granulosa cells measured using PCR decreased in PCOS / overweight patients compared to non-PCOS / norm weight patients where PCOS / overweight is a risk factor for infertility. In addition to providing research results in the form of an association between VDR and infertility, the study also includes knowledge about infertility management through weight loss, which has an impact on increasing VDR concentrations.[11] This study found several infertility subjects with vitamin D receptor levels similar to fertility subjects, which suggests that despite the normal number of receptors, other factors affecting vitamin D action can still contribute to infertility, including the concentration of vitamin D itself or abnormalities in vitamin D receptor function due to gene polymorphisms that were not examined in this study.[14,17]

Previous research by Ashraf et al. (2023) assessing VDR polymorphisms in infertile women found that the presence of gene polymorphisms encoding vitamin D receptors causes changes in the performance of available vitamin D receptors. The study found that the FokI SNP gene polymorphism was most influential in increasing the incidence of infertility.[17] Djurovic et al. (2018) conducted a case-control study to examine the association of polymorphisms in the VDR gene with reproductive success where DNA from 117 female patients with idiopathic infertility and 130 fertile controls were isolated from peripheral blood samples and VDR genotypes (FokI, BsmI, ApaI, and TaqI) were detected by PCR-RFLP. This study found a significant association between FokI and BsmI polymorphisms that alter the role of vitamin D receptors in infertility.[14]

Based on the examination results, the mean vitamin D receptor level in fertility subjects was 2.35 ± 1.30 ng/ml, with the smallest level being 0.67 ng/ml and the highest level being 5.35 ng/ml. This average is higher than in the infertile group, as well as those revealed by Farooqui et al. (2021), where in vitro fertilization subjects with successful outcomes found higher VDR levels.[15] Another study by Guo et al. also found that VDR and HOXA10 protein expression increased significantly in pregnant women compared to non-pregnant women. VDR protein levels correlated positively with HOXA10 levels, which play a role in the implantation process. The study supports the hypothesis that the Vitamin D-VDR system plays a role during the development of endometrial receptivity. [18]

1α-hydroxylase (vitamin D enzyme) and vitamin D receptors are expressed on human decidua during the first trimester. Vitamin D receptor and 1,25(OH)2D3 regulate the transcription of

HOXA10, a major target gene associated with implantation. Increased clinical pregnancy rates may be related to implantation success as binding between vitamin D and the receptor exerts important effects on early embryo implantation, early trophoblast invasion, and endometrial decidualization.[19]

As a marker of endometrial receptivity, HOXA10 is an important molecule involved in embryo implantation. Alterations in HOXA10 expression can lead to impaired endometrial receptivity and failure of the embryo to implant. 1,25(OH)2D plays a role in increasing HOXA10 mRNA and protein manifestation in human endometrial stromal cells, where the role of 1,25(OH)2D depends on VDR.[18]

This study found significant differences between vitamin D receptor levels of infertility subjects and fertility subjects, with lower levels found in infertility subjects. Farooqui et al. (2021) also found similar results when examining patients' vitamin D and vitamin D receptor levels from serum and follicular fluid after 34 hours of intramuscular HCG injection. In this study, it was found that Vitamin D and VDR were detected highest in women who experienced pregnancy compared to women who experienced abortion or pregnancy failure. This study also found that vitamin D and VDR levels were associated with the number of oocytes, proving that high concentrations of VDR contribute to successful conception after successful assisted reproductive techniques. In addition, high VDR levels in serum also indicate high VDR levels in follicular fluid.[15]

CONCLUSION

1. Primary infertility subjects mainly were over 30 years old, had overweight BMI, and experienced infertility for 1-4 years. Subjects without infertility were mainly under 30 years old and had mostly normal weight.
2. The mean serum vitamin D receptor level in subjects with primary infertility was 1.73 ± 0.92 ng/ml.
3. The mean serum vitamin D receptor level in Subjects without fertility was 2.35 ± 1.30 ng/ml
4. There is a significant difference between serum vitamin D receptor levels in subjects with infertility, where the value is lower than that of subjects without infertility.

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