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Effect of Vitamin D Deficiency on Women with Polycystic Ovary Syndrome (PCOS)

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Abstract

The major an endocrine disorder in female is polycystic ovary syndrome (PCOS), characterized by anovulation, irregular menstruation, amenorrhea, hirsutism, and infertility. Recently, interest has increased in studying the effects of Vit. D in many diseases, including PCOS. The goal of this study was to estimate the link of Vit D and the risk of PCOS by measuring estrogen, progesterone, follicle-stimulating hormone (FSH), ovulation hormone, or called luteinizing hormone (LH), thyrotropin (TSH), total cholesterol, triacylglycerol TG, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very-low-density lipoprotein (VLDL), and vitamin D in serum of 100 infertile with PCOS, 60 infertile with non-PCOS, and 60 control group. The results showed increase significantly in the concentration of estrogen, LH, TSH, cholesterol, triacyclglycerol, VLDL, and LDL compared to healthy women at P=0.01 (82.1±33.1 pg/ml), P=0.01 (5.71±3.34 mlU/ml), P=0.001 (2.48±1.23 µlU/ml), P=0.001 (186.3±39.4 mg/dL), P=0.001 (166.3±86.7 mg/dL), P=0.01 (33.4±17.2 mg/dL), and P=0.01 (112.6±34.5 mg/dL), respectively. Further, there was indeed decrease significantly in the concentration of progesterone, FSH, Vit D, and HDL compared to healthy women at P=0.01(1.295±0.82 ng/ml), P=0.001 (4.83±2.9 mlU/ml), P=0.001(11.94±5.82 mg/dL), and P=0.01 (40.83±8.80 mg/dL), respectively. The study showed that there is a strong correlation between vitamin D and the hormonal and biochemical variables that were measured. Therefore, we conclude that Vit. D is a new indicates the increase of infertility with PCOS.

"Keywords: Vitamin D; Polycystic Ovary Syndrome; Infertility; Vitamin D Deficiency; Endocrine Disorder"

1. Introduction

PCOS would be a disease of endocrine glands and most common in fertile age, affecting 10% of women [1]. As the name suggests, ovaries with multiple cysts are involved in the disease. This is triggered by the imbalance of hormonal in adult females, which is further demonstrated by an abnormal menstrual cycle, multiple ovarian cysts, amenorrhea, and hirsutism PCOS is a multifactorial disorder that induces infertility mainly and therefore creates social inequality [2; 3].

(PCOS) It normally occurs in the teenage population combined with menstrual and hyperandrogenism dysfunction. Insulin secretion and action abnormalities, androgen production and action, relative gonadotropin ratios, ovulatory activity, and equilibrium of antioxidant systems are correlated with it. PCOS would be a life-long disorder and this disease has metabolic and reproductive effects. Ladies with PCOS are commonly obese with different abdominal or mass indexes, which will in turn increases insulin production. These women are at risk of having reduced insulin sensitivity (IGT), Obesity, dyslipidemia, heart disease, hypertension, and potentially metabolic disease in old age [4; 5], the most troubling associated morbidities are infertility, as it currently affects about 48.5 million women between the ages 20-44 years, with PCOS responsible for 6-15% of all these cases [6], while more than 70% could be undiagnosed [7]. Admittedly, due to its evident similarity with several other diseases, overweight as well as Cushing's syndrome, ovarian and gonads metabolic abnormalities, and renal dysfunction hyperplasia, its

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optimum diagnosis is sometimes impeded [8]. There is significant variability of clinical signs among PCOS women and these can change over time for a person [9]. Another common of PCOS feature is feedback disruptions in the hypothalamus-hypophysis-ovary axis (HHOA) [10].

Vitamin D role as a physiologic action in the reproductive system [11]. Recently, a series of studies have demonstrated that deficiency of vitamin D (VDD) is common in patients with PCOS and that VDD may be associated with metabolic and endocrine disorders in PCOS [12; 13]. Vit. D is a steroid hormone that is associated in calcium phosphate equilibrium and bone mineral deposits [14], and the receptor is found in ovary [15].

Endogenous vitamin D3 synthesis starts in the skin by the photolytic conversion of the 7-dehydrocholesterol present in dermal fibroblasts and epidermal keratinocytes to pro-vitamin D3 by the ultraviolet B (UVB) component of sunlight (290-29315nm). It is then subjected to two hydroxylation reactivity throughout the kidney and liver to give the vitamin a bioactive form; 1, 25-dihydroxy vitamin D3. Although the dominant vitamin D in humans is endogenous vitamin D3, it could also be derived from food components [14; 16]. Vitamin D for PCOS treatment had also gained attention as a consequence. Although a growing percentage of study designs have evaluated the relationship between both vitamin D deficiency and PCOS, there is an absence of persuasive evidence showing the effects of vitamin intake on PCOS. The research aim's is to investigate Vit D effects in the blood.

2. Subjects and Methods:

2.1. Sample collection

2.1.1. Polycystic Ovarian Syndrome Group (Inclusion Criteria)

A hundred infertile women also with polycystic ovaries & sixty infertile with non-polycystic ovary syndrome has been registered in this research. A cross-sectional, and hospital-based study was accomplished in the Educational Hospital of Azadi / Azadi Center for Infertility and IVF. Educational Hospital of Azadi / Azadi Center for Infertility and IVF. Specialists in Hospitals diagnosed it, and a laboratory test was done in the laboratories of the hospitals and external laboratories for the period from 3/9/2018 to17/5/2020. Their ages ranged from 19-45 years**,** body mass index BMI was about 20-58.6 kg/m^2. Clinical data as age, number of years of marriage, and number of abortions were obtained for each case. swish to thank Mosul University for its support.

2.1.1.1. Exclusion Criteria

Diabetes, blood pressure, thyroid disease, and heart disease were excluded.

2.1.2. Control Group (Reference Group)

This study was attended by sixty young fertile women non-PCOS (control group), ages ranging from 19-45 years. body mass index BMI was about 18-25 kg/m^2

2.1.3. Blood Collection

Both groups were blood tested over 12-hour fasting (values for 5ml), early follicular cycles (cycle days two or three) for estrogen (E2), progesterone, follicle-stimulating hormones (FSH) and lutein's hormone (LH), thyroid-stimulating hormone (TSH) and vitamin D. Luteal prolactin (cycle day 21). Into the test tube added to it Gels (gel & clot activator) by drawing (5ml), and centrifuged for serum separation within an hour of blood collection, and the serum was stored in a deep freezer at a temperature of -20°C for subsequent analysis. Samples were analyzed in batches of 100 to be omitted between analytical variations. Samples were permitted to achieve room temperature before the study

2.1.3.1. Materials

This study was performed using a special kit for each variable from the Minividas–France & Cobas c311-German to estimate the following hormonal and chemical variables: Estrogen hormone E2, progesterone, follicle-stimulating hormone (FSH), ovulation hormone, or called luteinizing hormone (LH), thyrotropin (TSH), vitamin D, and lipid profile were also measured, which include total cholesterol, triacylglycerol TG, and high-density lipoprotein (HDL).

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2.1.3.2. Procedures

The commercial kits for measured hormones and Vit. D was used (Bio Merieux Kits) and was then measured by Minividas–France. The lipid profile also included Total Cholesterol, TG, HDL analysis, and

was measured using commercial kits (Roche Kits) by Cobas c311.

2.1.3.3. Hormones and vitamin D measurement methods

The reagents used are ready and available in the device. After separating the serum in a centrifuge for five minutes at 3000 rpm, it was put the separated serum into a type test tube. by using commercial kits (Bio Merieux Kits). By using hormone analyzer (Minividas–France), through a fluorescent assay linked enzyme (ELFA) technique.

2.1.3.4. Lipid profile measurement methods

The reagents used are ready and available in the device. After separating the serum in a centrifuge for five minutes at 3000 rpm, we put the separated serum into a Hitachi tube. After that, the test tube was put on the required number inside the c311 Cobas device, then entered the clinical information, the required tests, and the number in which the serum was placed; then pressed the START button, and then the results appeared automatically.

2.1.3.5. Indirect Procedures

Low-Density-Lipoprotein (LDL) and Very-Low-Density-Lipoprotein (VLDL) were determined indirectly using the Friedewald equations:

$LDL_{(Con.)}(mg/dl) = Cholesterol_{(Con.)} – HDL_{(Con.)} – G/5$ (Eq.1)

$VLDL_{(Con.)}(mg/dl) = Triglyceride/5$ (Eq. 2)

2.1.3.6. Statistical analysis

SPSS software has been used to analyze the data. The T-test and Duncan-tests were already used to compare parameters between the total control number and patients based on occupancy at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$, respectively, and the test of Pearson correlation coefficients [17].

2.1.4 Ethical approval

The research has been carried out and agreed upon by the author's Institutional Review Board following all applicable national legislation, institutional policy, and the values of the Helsinki Declaration.

3. RESULTS AND DISCUSSION:

3.1. The level of parameters of infertile with PCOS compared with the group of control

The results in Table (1) showed increase a significantly in the concentration of estrogen, luteinizing hormone, and thyroid-stimulating hormone (TSH) at (P=0.01), (P=0.01), (P=0.001), respectively, when comparing the hormonal and biochemical level of PCOS women with the group of control. While there was a significant lower in the concentration of progesterone hormone (P4), FSH, and vitamin D concentration at (P=0.01), (P=0.001), and (P=0.001), respectively, in PCOS. Probably due to insulin resistance as it has a role, as the ovary responds to insulin by interacting with its receptors and thus its increase may stimulate steroid formation and increase the secretion of luteinizing hormone to increase androgen production from Thika cells [18; 19].

Ovarian response to ovulation hormone is the main source of androgen elevation in PCOS [20]. The production of the hormone estrogen in granule cells is stimulated by insulin. The unfavorable environment causes follicle growth to stop. Consequently, early luteinizing and follicular discontinuation develop and lead to menstrual disorders and obesity caused by lack of ovulation. A high concentration of luteinizing hormone leads to a rise in the concentration of estrogen, which leads to a decrease in the concentration of FSH through negative feedback. This results in a defect in the HPG axis [21]. The thyroid gland response by the ovaries can also be explained by the presence of thyroid hormone receptors on human eggs. TSH also affects estrogen metabolism and reduces the production of sex hormone-related globulin [22].

In PCOS progesterone levels are low. It may be due to an increase in the estrogen hormone that leads to a decrease in GnRH by negative feedback, and thus, the affected HPG axis causes irregular cycles or ovulation thus lowering progesterone levels [15]. Also, vitamin D deficiency may be related to its association with homeostasis regulation. Calcium and androgen hormones, the main characteristics of which are menopause, decreased metabolism, and an increased likelihood of miscarriage [23].

Also, there was a large increase in the concentration of cholesterol, triglycerides, LDL, and VLDL at (P=0.001), (P=0.001), (P=0.01), and (P=0.01), respectively. While there has been a marked decline in HDL concentration at (P=0.01) in the group of women with PCOS compared to healthy women. The cause of dyslipidemia in PCOS may be attributed to hyperandrogenemia and hyperinsulinemia. This

causes the adipose cells to experience enhanced access and utilization fatty acid and lipolysis composition release into circulatory system. Excessive fatty acids in the liver stimulate the secretion of VLDL, which eventually leads to dyslipidemia. By shifting the opposite path of cholesterol, a high blood concentration of TG leads to a decrease in HDL, and the rise in levels of LDL. It is also possible that hyperandrogenism also affects lipid metabolism by inducing hepatic lipase activity that has a role in the catabolism of HDL molecules [24].

3.2. The level of hormonal and biochemical parameters of infertile PCOS patients compared to infertile women without PCOS

The results in Table (2) when comparing hormonal levels and biochemistry of PCOS and without PCOS in women showed a high increase in the concentration of estrogen and thyroid-stimulating hormone (P=0.05), (P=0.01), and (P=0.05), respectively, in women with PCOS. It may be since leptin is, some reports regard the hyperleptinemia that appears in PCOS as only a by-product of this condition. From the other side, conclusions connecting leptin with estradiol, testosterone, as well as insulin in females with PCOS call for a far more critical landscape for leptin in pathophysiology [25].

Hypothyroidism and PCOS Perform a significant function in linking the two disorders. There is sufficient Proof that TSH is increased in females as higher BMI. It primarily acts on the hypothalamus, resulting in increased TRH secretion. High TSH levels, with both of these two paths, behave on adipose tissue to increase their spread [26; 27].

Low concentrations of Vit. D could aggravate the associated symptoms of PCOS, with insulin resistance, ovulation, irregular menstruation, infertility, hyperandrogenism, obesity, and an increased risk of cardiovascular disease. This is consistent with another study in which it was shown that women with both the lowest concentrations of vitamins in the blood also had ovarian endometriosis or ovarian cysts. In relation to immune response, mood, and power, Vit D acts a role in the control of blood glucose metabolism [28], sex hormones synthesis [29], and ovulatory development [30; 31; 32].

There has been no substantial difference in the levels of progesterone, LH, FSH, prolactin, cholesterol, triglycerides, HDL, LDL, and VLDL, between the two groups.

3.3. The relationship of vitamin D with the regularity of period in PCOS

The results in Figure (1) showed a significant decrease in Vit. D in PCOS patients with repeated miscarriages compared to the control group at (P=0.01), highlighting the fact of lack of vitamin D increases thyroid hormone production. As T3 & T4 is controlled by the concentration of the vitamin and calcium in the blood, an increase in thyroid hormone is independently associated with PCOS and ovarian infertility [33]. Besides, lack of vitamin D is associated with an imbalance in calcium regulation, which contributes to the development of follicle cessation in PCOS and leads to poor menstruation and fertility. Plus, it may be delayed by 2-3 months [34; 35]. From this, we conclude that deficiency of the vitamin appears as a role for irregular menstruation [35].

3.4. Vitamin D correlation for infertile PCOS patients with hormonal and biochemical parameters

Infertile PCOS and vitamin D correlation is shown in Table 3, which confirms that vitamin D, is a sign that increases risk factors of PCOS. The relationship between Vit. D and fertility problems are highly associated with progesterone, FSH, TSH, total cholesterol, HDL, and HDL, especially in FSH. On the other hand, the same correlation has a significant negative correlation with estrogen, LH, TSH, TG, LDL, and VLDL. Especially in LH and TG. This proves the affiliation of PCOS with metabolic disorders like dyslipidemia, obesity, and hyperandrogenism.

Table (1): *The level of parameters of infertile with PCOS compared with the group of control.*

Hormonal and biochemical parameters	PCOS group Mean ± SD	Control group Mean ± SD	P-value
Estrogen (E2) (pg/ml)	82.1 ± 33.1	55.93 ± 26.4	0.01**
Progesterone (ng/ml)	1.295 ± 0.82	3.93 ± 2.1	0.01**
FSH (mlU/ml)	4.83 ± 2.9	6.81 ± 1.7	0.001***
LH (mlU/ml)	5.71 ± 3.34	3.86 ± 1.5	0.01**
TSH (µlU/ml)	2.48 ± 1.23	1.56 ± 0.60	0.001***
Vit. D (ng/dL)	11.94±5.82	39.59±5.39	0.001***
Total Cholesterol (mg/dL)	186.3 ± 39.4	153.91 ± 33.5	0.001***
Triglyceride (TG) (mg/dL)	166.3 ± 86.7	110.1 ± 55.4	0.001***
VLDL (mg/dL)	33.4 ± 17.2	21.87 ± 10.9	0.01**
HDL (mg/dL)	40.83 ± 8.80	68.1 ± 16.84	0.01**
LDL (mg/dL)	112.6 ± 34.5	85.51 ± 25.8	0.01**

**Significant differences at P≤0.01; ***Significant differences at P≤0.001

Table (2). *A comparison of the level of hormonal, biochemical parameters, and Vit. D in the two groups of infertile with PCOS and* Non PCOS.

Hormonal and biochemical parameters	PCOS group Mean ± SD	Non PCOS group Mean ± SD	P-value
Estrogen (E2) (pg/ml)	82.1 ± 33.1	66.7 ± 28.4	0.05*
Progesterone (ng/ml)	1.295 ± 0.82	2.40 ± 0.43	N
FSH (mlU/ml)	4.83 ± 2.9	5.33 ± 0.6	N
LH (mlU/ml)	5.71 ± 3.34	6.48 ± 4.37	N
TSH (µlU/ml)	1.70 ± 1.20	1.87 ± 0.86	0.01**
Vit. D (ng/dL)	186.3 ± 39.4	11.94±4.82	0.05*
Total Cholesterol (mg/dL)	166.3 ± 86.7	187.8 ± 31	N
Triglyceride (TG) (mg/dL)	33.4 ± 17.2	160.4 ± 82.6	N
VLDL (mg/dL)	40.83 ± 8.80	32.1 ± 16.4	N
HDL (mg/dL)	112.6 ± 34.5	41.77± 8.67	N
LDL (mg/dL)	82.1 ± 33.1	114.2 ± 34	N

*Significant differences at P≤0.05; **Significant differences at P≤0.01, N=No significant differences.

Table (3). *Vitamin D correlation for infertile PCOS patients with biochemical and hormonal parameters.*

Biochemical and hormonal parameters	r-value	p-value
LH (mlU/mL)	-0.075	0.05*
FSH (mlU/mL)	0.39	0.01**
Progesterone (ng/mL)	0.0078	0.05*
Estrogen (E2) (pg/mL)	0.0391	0.05*
TSH (µlU/ml)	-0.053	0.05*
Total cholesterol (mg/dL)	0.093	0.05*
Triglyceride (TG) (mg/dL)	-0.135	0.05*
HDL (mg/dL)	0.199	0.01**
LDL (mg/dL)	0.097	0.05*
VLDL (mg/dL)	-0.150	0.01*

*Significant differences at P≤0.05; **Significant differences at P≤0.01

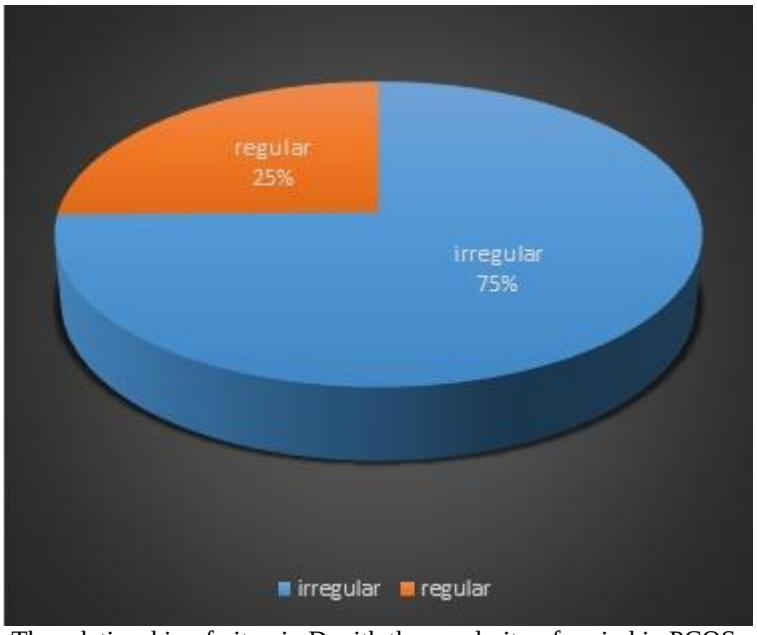

Figure (1): The relationship of vitamin D with the regularity of period in PCOS

4. Conclusions:

PCOS is a disorder of the endocrine, and this study revealed that there is a significant decrease in the level of the vitamin in PCOS with infertile compared to the healthy group of women. It was clearly proven there is a clear inverse relationship between the irregularity of period in polycystic ovary syndrome and the vitamin level of healthy women.

This study prove that vitamin D is an increased risk factor for developing PCOS.

5. CONFLICT OF INTERESTS:

There is no-conflict of interest in the publishing of this article.

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7. References

[1] Khan MJ, Ullah A, Basit S. (2019). Genetic Basis of Polycystic Ovary Syndrome (PCOS): Current Perspectives. Appl Clin Genet., 12,249-260.

[2] Nelson VL, Legro RS, Strauss JF, McAllister JM. (2014). Augmented androgen production is a stable steroidogenic phenotype of propagated theca cells from polycystic ovaries. Mol Endocrinol, 13:946–957.

[3] Villarroel C, Merino PM, López P, et al. (2012), Polycystic ovarian morphology in adolescents with regular menstrual cycles is associated with elevated anti-Mllerian hormone. Hum Reprod. 26,2861–2868. doi:10.1093/humrep/der223

[4] Głuszak O., Stopińska-Głuszak U., Glinicki P., et al., (2012). Phenotype and metabolic disorders in polycystic ovary syndrome. ISRN Endocrinology, 2012, 7., Article ID 569862.

[5] Teede H, Deeks A, Moran L. (2010). Polycystic ovary syndrome: a complex condition with psychological, reproductive and metabolic manifestations that impacts on health across the lifespan. BMC Med., 2010;8. doi:10.1186/1741-7015-8-41

[6] Fauser B.C.J.M., Tarlatzis B. C., Rebar R. W., et al. (2012). Consensus on women's health aspects of polycystic ovary syndrome (PCOS): the Amsterdam ESHRE/ASRM-sponsored 3rd PCOS consensus workshop group. Fertility and Sterility, 97(1),28.e25–38.e25.

[7] Boyle J., Teede H. J. (2012). Polycystic ovary syndrome—an update. Australian Family Physician, 41(10),752–756.

[8] Aljawadi, Zena A.M. (2020). Effect of selenium level of serum and semen in infertile Iraqi, Biochemical and Cellular Archives, 10(2),3619-3622.

[9] Balen A H. (2017). Polycystic ovary syndrome (PCOS). TOG, 19(2),119-129.

[10] Blank K., McCartney C. R., Marshall J. C. (2006). The origins and sequelae of abnormal neuroendocrine function in polycystic ovary syndrome. Human Reproduction Update, 12(4),351–361.

[11] MOHAMMED N.S., AL-JAWADI Z.A.M. (2020). THE ROLE OF VITAMIN D AS A NEW MARKER ON THE WOMEN INFERTILITY. Periódico Tchê Química, 17(36), 1099-1109.

[12] Wild RA, Rizzo M, Clifton S, Carmina E. (2011). Lipid levels in polycystic ovary syndrome: Systematic review and meta-analysis. Fertil Steril. 95:1073–1079.

[13] Trummer C, Pilz S, Schwetz V, Obermayer-Pietsch B, Lerchbaum E (2018). Vitamin D, PCOS and androgens in men: A systematic review. Endocr Connect., 7, R95–R113.

[14] Holick MF. (2007). Vitamin D deficiency. N Engl J Med., 357,266–281.

[15] Gatti D, Idolazzi L and Fassio A. (2016). Vitamin D: Not just bone, but also immunity. Minerva Med., 107,452–460.

[16] Ricardo LB. (2011). VDR activation of intracellular signaling pathways in skeletal muscle. Mol Cell Endocrinol, 347(1-2),11-16.

[17] Kirkpatrick L. and Feeney B.C. (2012). A Simple Guide to IBM SPSS Statistics for Version 18.0 and 19.0 11th Edn., pp:115, Wadsworth Cengage Learning, Belmont, ISBN-10:1111352550.

[18] Kumar A.N., Naidu J.N., Satyanarayana U., Ramalingam K., Anitha, M. (2016). Metabolic and Endocrine Characteristics of Indian Women with Polycystic Ovary Syndrome. Int J Fertil Steril., 10(1),22–28.

[19] McFarland C. (2012). Treating polycystic ovary syndrome and infertility. MCN Am J Matern Child Nurs, 37(2),116–121.

[20] Truerhaug T.G., Hertel J.K., Nordstrand N., Dale P.O., Hofso D., Hjelmesæth, J. (2015). The Association Between Hyperandrogenemia and The Metabolic Syndrome in Morbidly Obese Women. Diabetology & Metabolic Syndrome., 7,46-56.

[21] Lal R.Z., Biyani S., Lodha R. (2016). Correlation of Thyroid Hormones with FSH, LH and Prolactin in Infertility in th Reproductive Age Group Women. International Archives of Integrated Medicine., 3(5),146-150.

[22] Chen L., Tan Y., Cao P. (2017). Relationship of thyroid stimulating hormone with sex hormones in patients with polycystic ovary syndrome: a single-center, retrospective observational study. Int J Clin Exp Pathol., 10(5),5989-5993.

[23] Voulgaris N, Papanastasiou L, Piaditis G, Angelousi A, Kaltsas G, Mastorakos G Mastorakos G and Kassi E. (2017). Vitamin D and aspects of female fertility. Hormones (Athens, Greece), 16(1),5-21.

[24] Swetha R., Ravi B.V., Nalini K.S. (2015). Serum Lipoprotein(a) and Lipid Profile In Polycystic Ovarian Syndrome. J Clin Sci Res., 4,2-6.

[25] Rojas J., Chávez M., Olivar L., Rojas M., Morillo J., Mejías J., Calvo M., Bermúdez, V. (2014). Polycystic Ovary Syndrome, Insulin Resistance, and Obesity: Navigating the Pathophysiologic Labyrinth. International Journal of Reproductive Medicine., 2014,1-19. DOI: 10.1155/2014/719050.

[26] Yu Q., Wang J.B. (2016). Subclinical Hypothyroidism in PCOS: Impact on Presentation, Insulin Resistance, and Cardiovascular Risk. BioMed Research International., 2016,1-7.

[27] Singla R., Gupta Y., Khemani M., Aggarwal S. (2015). Thyroid disorders and Polycystic Ovary Syndrome: An Emerging Relationship. Indian Journal of Endocrinology and Metabolism., 19(1),25-30.

[28] Alvarez J. A., Ashraf, A. (2010). Role of vitamin d in insulin secretion and insulin sensitivity for glucose homeostasis. International journal of endocrinology, 2010,351385.

[29] Irani M., Merhi, Z. (2014). Role of vitamin D in ovarian physiology and its implication in reproduction: a systematic review. Fertility and Sterility, 102(2),460-468.

[30] vkan S., Jindal S., Greenseid K. *et al.* (2010). Replete vitaminD stores predict reproductive success following in vitro fertiliza-tion. Fertility and Sterility, 94,1314–1319.

[31] Oostingh EC, Hall J, Koster MPH, Grace B, Jauniaux E and Steegers-Theunissen RPM. (2019). The impact of maternal lifestyle factors on preconception outcomes: a systematic review of observational studies. Reproduce Biomed Online, 38,77–94.

[32] Kokanalij D., Karaca M., Ozakşit G., Elmas B.

and Üstün Y.E. (2019). Serum Vitamin D Levels in Fertile and Infertile Women with Polycystic Ovary Syndrome, Geburtshilfe Frauenheilkd, 79(5),510–516.

[33] Panidis C B., Farmakiotis D., Rousso D, Kourtis A, et al. (2005). Serum parathyroid hormone concentrations are increased in women with polycystic ovary syndrome. Clin. Chem., 51(9),1691-1697.

[34] Nissou A G., Zenga C, Berger F, Issartel JP, et al. (2014). Additional clues for a protective role of vitamin D in neurodegenerative diseases: 1,25-dihydroxyvitamin D3 triggers an anti-inflammatory response in brain pericytes. J Alzheimers Dis., 42(3),789-799.

[35] Moini N Sh., Ahmadzadeh M., Hosseini R., Hosseini L., et al. (2015). Comparison of 25-hydroxyvitamin D and calcium levels between polycystic ovarian syndrome and normal women. Int J Fertil Steril, 9,1–8.