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# Correlation between vitamin D receptor gene polymorphisms and levels of some hormones in Iraqi infertile women with polycystic ovary syndrome

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**Abstract:** The polycystic ovary syndrome (PCOS) is a disorder that characterized by hyperandrogenism, ovulatory dysfunction, and polycystic ovarian morphologic features. As defined by the diagnostic criteria of the National Institutes of Health (i.e., hyperandrogenism plus ovulatory dysfunction). The purpose of this study was to investigate the correlation between *VDR* gene polymorphisms and level of LH, FSH, TSH and Prolactin hormones .This study was carried out in the Institute of Genetic Engineering and Biotechnology for Postgraduate Studies - University of Baghdad through the period from November 2016 - August 2017, The PCOS patients were taken from the Kamal Al-Samarraee Infertility Treatment Hospital in Baghdad.Women with PCOS (n=50) and apparently healthy control group (n=50) , were enrolled . Genotyping of *VDR* gene (rs2228570) (rs7975232) , as well as (rs731236) SNPS between groups were determined by using Taqman genotyping assay . Hormonal analysis for LH, FSH, TSH and Prolactin was performed by using Automated Immune Assay (AIA). the results of the present study indicate that serum LH , FSH and TSH concentrations were unaffected by the studied SNPs of *VDR* gene within carriers of genotypes of rs2228570 , rs7975232 and rs731236 SNPs in *VDR* gene, while serum prolactin levels were significantly ($p<0.05$) higher in PCOS patients *versus* controls.

**Keywords**: PCOS, VDR, Polymorphism, Infertile.

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## Introduction:

Polycystic ovary syndrome (PCOS) being considered the most common endocrine disorder in women of reproductive age (1,2) These disorders found to associate with hormone disturbances and genetic alterations (3,4,5,6). Prevalence estimates are highly variable, ranging from 2.2% to as high as 26% (1,7).

Vitamin D (calciferol) comprises a group of fat soluble seco-sterols found naturally only in a few foods, such as fish-liver oils, fatty fish, mushrooms, egg yolks, and liver. The two major physiologically relevant forms of vitamin D are D2 (ergocalciferol) and D3 (cholecalciferol). Vitamin D3 is photosynthesized in the skin of vertebrates by the action of solar ultraviolet (UV) B radiation on 7-dehydrocholesterol ,vitamin D2 is

produced by UV irradiation of ergosterol, which occurs in molds, yeast, and higher-order plants (8).

The calcitriol receptor, also known as the vitamin D receptor (VDR) and also known as NR1I1 (nuclear receptor subfamily 1, group I, member 1), is a member of the nuclear receptor family of transcription factors (9). Vitamin D (1,25(OH)2D) is the main ligand for the vitamin D receptor (VDR), a member of the nuclear receptor superfamily of transcriptional regulators, the 25(OH)D precursor is widely used in assessment of vitamin D repletion and has a slower rate of clearance from the circulation than 1,25(OH)2D. Because the VDR is expressed in a large number of tissues, it is not surprising that ligand-activated VDR modulates the expression of many genes (10,11).

Women with PCOS may also be at elevated risk of vitamin D deficiency (VDD). In contrast to a prevalence of 20%–48% among the general adult population (12,13,14) , a relative higher prevalence of VDD is observed among women with PCOS (approximately 67%–85% women with PCOS have VDD) (15).

*VDR* gene contains 14 exons and is mapped on chromosome 12cen-q12. Several allelic variations have been reported in the *VDR* gene such as the following restriction fragment length polymorphisms: FokI in exon 2 (C/T) (rs10735810), BsmI in intron 8 (G/A) (rs1544410), ApaI in intron 8 (C/A) (rs7975232), Tru9I in intron 8 (G/A) (rs757343) and TaqI in exon 9 (T/C) (rs731236). TaqI based restriction fragment length polymorphism (RFLP) is located at the 3' end of the *VDR* gene. The function of the TaqI-specific hypervariable polymorphism is unclear (16). *VDR* gene variants have been associated to breast cancer risk (17), prostate cancer progression (18), colorectal cancer (19), diabetes (20,21,22), primary hyperparathyroidism (23), coronary artery disease (24) and PCOS (25,26) .

The findings of Ranjzad *et al*. (25) demonstrated that there is a significant association between VDR TaqI CC genotype and serum concentrations of LH in women with PCOS. Whereas there is a association between VDR TaqI CC genotype with serum level of LH and LH with insulin sensitivity in PCOS (26,27) .

Dasgupta *et al*. (28) analyzed Cdx2, FokI, ApaI, and TaqI polymorphisms in a case–control study, in the Indian population and verified the polymorphism's influence in PCOS parameters. When the ApaI polymorphism was analyzed, the carrier of the ‘CC’ genotype presented a higher follicle-stimulating hormone (FSH), thyroid-stimulating hormone (TSH), and cholesterol levels were found in carriers of the variant ‘AA.’ For TaqI, the variant ‘CC’ showed higher mean testosterone levels, and higher FSH, LH, and cholesterol levels were found in the heterozygous carrier women. Concerning Cdx2, heterozygotes presented higher levels of mean testosterone and TSH levels, and ‘CT’ FokI carriers presented higher testosterone, FSH, LH, and prolactin levels.

Hong *et al.*, (29) suggests vitamin D supplementation studies have revealed an important approach in the management of PCOS manifestations, reinforcing the contribution of this hormone's deficiency in the pathogenesis.

The aim of the present study was to study the effect of some variant of *VDR* gene on some hormones levels in Iraqi women with PCOS .

**Materials and methods:**

In this study blood samples were collected from PCOS patients (n=50) and apparently healthy subjects as controls (n=50) . The PCOS patients were taken from the Kamal Al-Samarraee Infertility Treatment Hospital in Baghdad , To confirm the subjects with PCOS should include at least two of the following three features according to the Rotterdam 2003 criteria (Rotterdam ESHRE/ASRM Consensus, 2004): (1) Oligo and /or anovulation. (2) Clinical and /or biochemical features of hyperandrogenism.(3) The presence of polycystic ovary morphology(30).

Apparently healthy control group consists of 50 healthy fertile women of different ages, all of them were chosen depending on the Regular menstrual cycle (26 to 30 days) , Age 15 to 45 years No history of endocrine disease and no use of medication or oral contraceptives (31).

Every participant woman was interviewed and asked to answer information including, menstrual history, gynecological surgery , obstetric , sociodemographic data and PCOS family histories. They were also subjected to medical checkup for signs of hyperandrogenism and polycystic ovary **.**

The blood samples were collected during the follicular phase (day 3, 4) of the menstrual cycle from each women of both patients and healthy control. five ml of venous blood samples were collected and divided into two portions :

A. First portion: EDTA containing tubes for DNA isolation ( genotyping analyses were evaluated by Real Time PCR in else were (32).

B. Second portion: The serum obtained by putting the blood samples in a vacuum sterile glasses gel and clot activator 6ml tube and allowed to clot at 37 ºC for 30 minutes before centrifugation. The tubes centrifuged at 6000 rpm for 5 minutes, serum was collected and kept in freezer until used for hormonal assays.

Hormonal analysis for LH, FSH, TSH and Prolactin was performed by using Automated Immune Assay (AIA) by the VIDAS auto analyzer, (bioMérieux Company ) France. VIDAS hormonal assay is an automated quantitative test for use on the VIDAS instrument for the quantitative measurement of human serum using the ELFA (enzyme linked fluorescent assay) technique.the solid phase receptacle (SPR) serves as the solid phase as well as the pipetting device for the assay. Reagents for the assay are ready-to-use and predisposed in the sealed reagents strips. All of the assay steps are performed automatically by the instrument. The reaction medium is cycled in and out of the SPR several times.the principle of FSH and LH , TSH and Prolactin estimation combine a one step enzyme immunoassay sandwich method with a final fluorescent detection by ELFA .

The Statistical Analysis System-SAS (2012) program was used to evalute effect of studied factors on parameters . Chi-square test was used to

compare between percentage and Least significant difference –LSD test was used to significant compare between means in this study(33) .

## Results and Discussion:

### Serum luteinizing hormone (LH):

Serum LH concentrations as affected by the genotypes of rs731236, rs2228570 and rs7975232 SNPs of *VDR* gene in Iraqi women with PCOS and controls are presented in table 1.

Generally, the results of the present study indicate that serum LH concentrations were unaffected by the studied SNPs of VDR gene. Although, serum LH concentrations were in PCOS patients significantly ($p<0.05$) lower than those of apparently healthy subjects (2.10 *versus* 5.07, respectively). Overall, the present results of serum LH concentrations are unreliable because of the small sample size and since the increased levels of LH is a usual sign of PCOS (34, 35, 25). High levels of LH not only has an effect on oocyte maturity and human reproduction , but also on lower fertility and higher miscarriage prevalence (36).

Previous studies were found that PCOS patients show higher levels of LH than constant as compared with controls (37,38,25). High levels of LH leads to an elevated of LH: FSH ratio in PCOS patients (37). Ranjzad *et al.* (2011) demonstrated that there is a significant association between VDR genotype CC of rs731236 and elevated concentrations of serum LH in PCOS women (25). In addition, Bagheri *et al*. (39) found that LH concentrations were significantly higher in PCOS patients with CC genotype of *VDR* gene at rs731236 *versus* controls. Some limitations to be considered for the present study include low sample size and lack of data regarding vitamin D status in contributors. Therefore, studies in large numbers of PCOS are necessary to validate our results.

### Serum follicle stimulating hormone (FSH):

Serum FSH concentrations as affected by the genotypes of rs731236, rs2228570 and rs7975232 SNPs of VDR gene in Iraqi women with PCOS and controls are presented in table 2.

Generally, the results of the present study indicate that serum FSH concentrations were unaffected by the studied SNPs of *VDR* gene. Although, serum FSH concentrations were in PCOS patients significantly ($p<0.05$) lower than those of apparently healthy subjects (5.72 *versus* 9.04, respectively). Overall, the present results of serum FSH concentrations are disagree with previous studies that patients with PCOS show lower level of FSH as compared with controls (37,38,25)**.** Schmidt *et al.* (40) found lower FSH concentrations in postmenopausal women with PCOS than apparently healthy controls . Al-Mulhim *et al.* (41) found that Saudi women with PCOS had lower FSH concentrations *versus* controls. Recently, Al-Tamimi (42) found that Iraqi women with PCOS and PCOS plus T2DM had lower FSH levels than apparently healthy subjects.

**Table (1): Serum LH concentrations as affected by the genotypes of rs731236, rs2228570 and rs7975232 SNPs of *VDR* gene in Iraqi women with PCOS and controls.**

| VDR SNPs | Genotypes | LH (mIU/ml) | | *p*- value |
|---|---|---|---|---|
| | | Control[1] | Patients[2] | |
| rs2228570 T > C | TT | - | 6.00 ± 4.10 | - |
| | TC | 5.59 ± 0.48 | 4.58 ± 0.55 | 0.739 NS |
| | CC | 4.76 ± 0.75 | 6.22 ± 1.56 | 0.405 NS |
| | *p*- value | 0.353 NS | 0.458 NS | - |
| *rs7975232 C>A* | CC | 6.11 ± 1.23 | 10.10 ± 0.00 | 0.335 NS |
| | CA | 5.26 ± 0.63 | 4.47 ± 0.47 | 0.684 NS |
| | AA | 5.26 ± 0.60 | 5.37 ± 1.09 | 0.696 NS |
| | *p*- value | 0.785 NS | 0.301 NS | - |
| rs731236 T>C | TT | 5.07 ± 0.93 | 2.10 ± 0.00 | 0.047 * |
| | TC | 5.02 ± 0.43 | 4.86 ± 0.63 | 0.812 NS |
| | CC | 7.76 ± 1.48 | 5.65 ± 1.03 | 0.362 NS |
| | *p*- value | 0.096 NS | 0.641 NS | - |

**[1] apparently healthy subjects. [2] Patients with polycystic ovary syndrome.**

**NS: No significant. *: Significant at 0.05 level.**

**Table (2): Serum FSH concentrations as affected by the genotypes of rs731236, rs2228570 and rs7975232 SNPs of *VDR* gene in Iraqi women with PCOS and controls.**

| VDR SNPs | Genotypes | FSH (mIU/ml) | | *p*- value |
|---|---|---|---|---|
| | | Control[1] | Patients[2] | |
| rs2228570 T > C | TT | - | 5.30 ± 1.10 | - |
| | TC | 6.05 ± 0.35 | 5.15 ± 0.26 | 0.548 NS |
| | CC | 9.04 ± 2.32 | 5.72 ± 0.73 | 0.047 * |
| | *p*- value | 0.066 NS | 0.692 NS | - |
| *rs7975232 C>A* | CC | 6.13 ± 0.94 | 5.40 ± 0.00 | 0.552 NS |
| | CA | 6.32 ± 0.51 | 5.21 ± 0.28 | 0.781 NS |
| | AA | 7.73 ± 1.58 | 5.34 ± 0.47 | 0.712 NS |
| | *p*- value | 0.605 NS | 0.970 NS | - |
| rs731236 T>C | TT | 6.26 ± 0.86 | 6.60 ± 0.00 | 0.894 NS |
| | TC | 7.03 ± 0.91 | 5.28 ± 0.29 | 0.306 NS |
| | CC | 6.63 ± 0.96 | 5.06 ± 0.51 | 0.721 NS |
| | *p*- value | 0.932 NS | 0.728 NS | - |

**1 apparently healthy subjects. [2] Patients with polycystic ovary syndrome.**

**NS: No significant. *: Significant at 0.05 level.**

## Serum thyroid stimulating hormone (TSH):

Serum TSH concentrations as affected by the genotypes of rs731236, rs2228570 and rs7975232 SNPs of *VDR* gene in Iraqi women with PCOS and controls are presented in table 3.

The results of the present study indicate that serum TSH concentrations were unaffected by the studied SNPs of VDR gene. These results are in agreement with Al-Tamimi (2015) and Al-Deresawi (2012) who found no significant differences in TSH levels between Iraqi patients with PCOS and apparently healthy controls(42,43) . The results of this study are disagree with Dahiya *et al*. (2012) and GulabKanwar *et al*. (2015) who found increased levels of TSH in PCOS women compared with controls (44,45) .

**Table (3): Serum TSH concentrations as affected by the genotypes of rs731236, rs2228570 and rs7975232 SNPs of *VDR* gene in Iraqi women with PCOS and controls.**

| VDR SNPs | Genotypes | TSH (mIU/ml) | | *p*- value |
|---|---|---|---|---|
| | | Control[1] | Patients[2] | |
| rs2228570 T > C | TT | - | 1.980 ± 0.60 | - |
| | TC | 1.734 ± 0.12 | 2.268 ± 0.49 | 0.367 NS |
| | CC | 1.485 ± 0.24 | 1.760 ± 0.27 | 0.695 NS |
| | *p*- value | 0.312 NS | 0.873 NS | - |
| *rs7975232 C>A* | CC | 1.733 ± 0.11 | 1.270 ± 0.00 | 0.672 NS |
| | CA | 1.590 ± .18 | 1.738 ± .14 | 0.706 NS |
| | AA | 1.725 ± 0.17 | 2.75 ± 0.88 | 0.351 NS |
| | *p*- value | 0.826 NS | 0.436 NS | - |
| rs731236 T>C | TT | 1.316 ± 0.18 | 1.400 ± 0.00 | 0.283 NS |
| | TC | 1.781 ± 0.13 | 2.311 ± 0.48 | 0.359 NS |
| | CC | 1.273 ± 0.22 | 1.620 ± 0.12 | 0.872 NS |
| | *p*- value | 0.177 NS | 0.755 NS | - |

**[1] apparently healthy subjects. [2] Patients with polycystic ovary syndrome.**

**NS: No significant. *: Significant at 0.05 level.**

## Serum prolactin:

Serum prolactin concentrations as affected by the genotypes of rs731236, rs2228570 and rs7975232 SNPs of *VDR* gene in Iraqi women with PCOS and controls are presented in table 4.

Within carriers of CC genotype of rs2228570 SNP in *VDR* gene, serum prolactin levels were significantly ($p<0.05$) higher in PCOS patients *versus* controls (40.19 *versus* 13.14, respectively).

Also, as related with rs7975232 SNP of *VDR* gene, serum prolactin levels of CC and CA genotypes carriers were significantly ($p<0.05$) higher in PCOS patients *versus* controls (44.80 *versus* 13.0 and 32.53 *versus* 12.39, respectively).

Whereas, serum prolactin levels in AA genotype were significantly ($p<0.05$) lower in PCOS patients *versus* controls (14.93 *versus* 30.26, respectively). Moreover, AA genotype of rs7975232 led to significantly ($p<0.05$) increase in serum prolactin levels of apparently healthy controls, while, in PCOS patients led to significantly ($p<0.05$) decrease in serum prolactin levels.

Generally, the results of serum prolactin in the present study are in agreement with Al-Mulhim *et al.* (2014) who found that Saudi women with PCOS had higher prolactin levels compared with apparently healthy controls (41). Al-Tamimi (2015) observed that prolactin levels were significantly higher in PCOS and diabetic PCOS patients compared with control subjects(42) .

In carriers of CC genotype of rs731236 SNP in *VDR* gene, serum prolactin levels were significantly ($p<0.05$) higher in PCOS patients *versus* controls (22.34 *versus* 12.21, respectively). Also, TC genotype of rs731236 led to significantly ($p<0.05$) increase in serum prolactin levels in both PCOS patients and apparently healthy subjects when compared with TT and CC genotypes (22.72 *versus* 10.29 and 12.21, respectively, in apparently healthy subjects and 26.61 *versus* 8.30 and 22.34, respectively, in PCOS patients).

**Table (4): Serum prolactin concentrations as affected by the genotypes of rs731236, rs2228570 and rs7975232 SNPs of *VDR* gene in Iraqi women with PCOS and control**

| VDR SNPs | Genotypes | Prolactin (ng/ml) | | *p*- value |
|---|---|---|---|---|
| | | Control[1] | Patients[2] | |
| rs2228570 T > C | TT | - | 33.40 ± 0.60 | - |
| | TC | 22.62 ± 10.79 | 21.07 ± 2.13 | 0.663 NS |
| | CC | 13.14 ± 1.11 | 40.19 ± 23.4 | 0.025 * |
| | *p*- value | 0.597 NS | 0.290 NS | - |
| *rs7975232 C>A* | CC | 13.00 ± 2.01 | 44.80 ± 0.00 | 0.257 * |
| | CA | 12.39 ± 0.96 | 32.53 ± 8.39 | 0.033 * |
| | AA | 30.26 ± 18.4 | 14.93 ± 1.83 | 0.038 * |
| | *p*- value | 0.051 * | 0.049 * | - |
| rs731236 T>C | TT | 10.29 ± 1.38 | 8.30 ± 0.00 | 0.315 NS |
| | TC | 22.72 ± 10.17 | 26.61 ± 6.19 | 0.553 NS |
| | CC | 12.21 ± 0.60 | 22.34 ± 4.07 | 0.041 * |
| | *p*- value | 0.038 * | 0.039 * | - |

**[1] apparently healthy subjects. [2] Patients with polycystic ovary syndrome.**

**NS: No significant. *: Significant at 0.05 level.**

## References:


1. Azziz, R.; Woods, K.S.; Reyna, R.; Key , T.J.; Knochenhauer, E.S. and Yildiz, B.O. (2004). The prevalence and features of the polycystic ovary syndrome in an unselected population. *J. Clin. Endocrinol Metab*, 89:2745-2749.
2. Kauffman, R.P.; Baker, T.E.; Baker , V.M.; Marino, P. and Castracane, D. (2008). Endocrine and metabolic differences among phenotypic expressions of polycystic ovary syndrome according to the 2003 Rotterdam consensus criteria. *Am. J. Obstet Gynecol*, 198:670.e1-670.e10.
3. AL-Faisal A.H.M. and Mahdi Saber G. Al-Deresawi. 2012. The correlation between thyroid hormones, reproductive hormones, body mass index (BMI) and hirsute in Iraqi women with polycystic ovary syndrome (PCOS). Anbar University,The Second Scientific Conference for Pure Science.22-24 November.
4. Al-Deresawi,M.S.G., Al Najar,A.F. and Abdul AlFaisal,A.H.M. 2013. Detection of five substitution TPO mutations in Polycystic Ovary Syndrome (PCOS) and thyroid hormones disturbance patients. *J.Biotechnol.Resaerch Center*, 8(2):5-10.
5. AL-Faisal, A.H.M. and Tabark Sabah Al – Rubiay (2014). Association of body mass index (BMI) and reproductive hormones with polycystic ovary syndrome in Iraqi patients. *Int.J.Advance Res*.,2(11): 788-791.
6. Al-Deresawi, M.S. and AlFaisal, A.H.M.2015.Two novel missense mutations in exon 9 of *TPO* gene in Polycystic Ovary Syndrome patients with hypothyroidism. *Journal of Biotechnology Research Center* 9(1):30-37.
7. Asuncion, M.; Calvo, R.M.; San Millan, J.L.; Sancho, J.; Avila, S. and Escobar-Morreale, H.F. (2000) . A prospective study of the prevalence of the polycystic ovary syndrome in unselected Caucasian women from Spain. *J Clin Endocrinol Metab* , 85:2434-2438.
8. Fieser, L.F. and Fieser, M. (1959) . Vitamin D. In: Steroids. 1st ed. New York: *Reinhold Publishing Corporation* , 90-168.
9. Bollag, W.B. (2007). Differentiation of human keratinocytes requires the vitamin d receptor and its coactivators. *J. Invest. Dermatol*. 127 (4):74850.
10. DeLuca, H.F. (1984). The metabolism, physiology, and function of vitamin D. In: Kumar R, editor. Vitamin D: basic and clinical aspects. *Boston (MA): M. Nijhoff Publishers*, 1-68.
11. MacDonald, P.N. (1999). Molecular biology of the vitamin D receptor. In Vitamin D. Physiology, *Molecular Biology, and Clinical Applications*, 109–128.
12. Tangpricha, V.; Pearce, E.N.; Chen, T.C. and Holick, M.F. (2002) . Vitamin d insufficiency among free-living healthy young adults. *Am. J. Med.*, 112: 659–662.
13. Forrest, K.Y. and Stuhldreher, W.L. (2011) . Prevalence and correlates of vitamin d deficiency in us adults. *Nutr. Res.* , 31: 48–54.
14. Hovsepian, S.; Amini, M.; Aminorroaya, A.; Amini, P. and Iraj, B. (2011). Prevalence of vitamin d deficiency among adult population of Isfahan city, Iran. *J. Health Popul. Nutr.*, 29:149–155.
15. Thomson, R.L.; Spedding, S. and Buckley, J.D. (2012) . Vitamin D in the aetiology and management of polycystic ovary syndrome. *Clin. Endocrinol.* , 77: 343–350.
16. Uitterlinden , A.G.; Fang, Y.; Van Meurs, J.B.; Pols, H.A. and Van Leeuwen, J.P. (2004). Genetics and biology of vitamin D receptor polymorphisms. *Gene.* ,338(2):143–156.
17. Guy, M.; Lowe, L.C.; Bretherton-Watt, D.; Mansi, J.L.; Peckitt, C.; Bliss, J. *et al*. (2004). Vitamin D receptor gene polymorphisms and breast cancer risk. *Clin Cancer Res.*, 10(16):5472–5481.
18. Xu, Y.; Shibata, A.; McNeal, J.E.; Stamey, T.A.; Feldman, D. and Peehl, D.M. (2003). Vitamin D receptor start codon polymorphism (FokI) and prostate cancer progression. *Cancer Epidemiol Biomarkers Prev*., 12(1):23-27.
19. Yaylim-Eraltan, I.; Arzu Ergen, H.; Arikan, S.; Okay, E.; Oztürk, O.; Bayrak, S. and Isbir, T. (2007) . Investigation of the *VDR* gene polymorphisms association with susceptibility to colorectal cancer. *Cell Biochem Funct*., 25(6):731–737.
20. Pani, M.A.; Knapp, M.; Donner, H.; Braun , J.; Baur, M.P.; Usadel, K.H. *et al*. (2000). Vitamin D receptor allele combinations

influence genetic susceptibility to type 1 diabetes in Germans. *Diabetes*., 49(3):504–507.

21. Oh, J.Y. and Barrett-Connor, E. (2002). Association between vitamin D receptor polymorphism and type 2 diabetes or metabolic syndrome in community-dwelling older adults: *the Rancho Bernardo Study. Metabolism.* , 51(3):356-359.
22. Motohashi ,Y.; Yamada, S.; Yanagawa, T.; Maruyama, T.; Suzuki, R.; Niino , M. *et al.* (2003). Vitamin D receptor gene polymorphism affects onset pattern of type 1 diabetes. *J. Clin Endocrinol Metab.* ,88(7):3137–3140.
23. Carling, T.; Kindmark, A., Hellman, P.; Lundgren, E.; Ljunghall , S.; Rastad, J. *et al.* (1995) . Vitamin D receptor genotypes in primary hyperparathyroidism. *Nat Med.*, 1(12):1309–1311.
24. Van Schooten, F.J.; Hirvonen, A.; Maas, L.M.; De Mol, B.A. ; Kleinjans, J.C.; Bell, D.A. *et al*. (1998). Putative susceptibility markers of coronary artery disease: association between VDR genotype, smoking, and aromatic DNA adduct levels in human right atrial tissue. *FASEB J.* ,12(13):1409–1417.
25. Ranjzad, F.; Mahban, A.; Shemirani, AI.; Mahmoudi, T.; Vahedi, M.; Nikzamir, A. *et al*. (2011) . Influence of gene variants related to calcium homeostasis on biochemical parameters of women with polycystic ovary syndrome. *J. Assist Reprod Genet*, 28(3): 225-232.
26. Lerchbaum, E. and Obermayer-Pietsch, B. (2012). Vitamin D and fertility: a systematic review. *Eur J. Endocrinol*. , 166(5):765–778.
27. Grulet, H.; Hecart, A.C.; Delemer, B.; Gross, A.; Sulmont, V. ; Leutenegger, M. *et al*. (1993) . Roles of LH and insulin resistance in lean and obese polycystic ovary syndrome. *Clin Endocrinol (Oxf)* ,38(6):621–626.
28. Dasgupta, S.; Dutta, J.; Annamaneni, S.; Kudugunti, N. and Battini, M.R. (2015). Association of vitamin D receptor gene polymorphisms with polycystic ovary syndrome among Indian women. *Indian J Med Res* , 142: 276–285.
29. Hong, Y.; Chen, D.; Li, Y.; Li, C. ; Liu, Y. and Xie, M. (2013). Serum sex hormone binding globulin profile and its association with insulin resistance in Chinese peri-menopausal women. *Endokrynol Pol*., 64(3):197–202.
30. Rotterdam ESHRE/ASRM Sponsored PCOS Concensus Workshop Group. (2004). Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome. *Fertil Steril*, 81: 19-25.
31. Macklon, N.S. and Fauser, B.C. (2000). Regulation of follicle development and novel approaches to ovarian stimulation for IVF. *Hum. Reprod. Update*, 6(4):307-312.
32. Kadhim, S.J.; Abdul-Hassan I.A. and Badr, A.H. (2017). Association of vitamin d receptor gene polymorphism at three snps and their haplotypes with polycystic ovary syndrome risk in Iraqi women.
33. SAS. (2012) . Statistical Analysis System, User's Guide. Statistical. *Version 9.1$^{th}$ ed. SAS. Inst. Inc. Cary. N.C. USA.*
34. Sam, S. and Dunaif, A. (2003) . Polycystic ovary syndrome: syndrome XX? *Trends Endocrinol Metab* ,14:365–370.
35. Asgharnia, M.; Mirblook, F. and Ahmad Soltani, M. (2011). The Prevalence of Polycystic Ovary Syndrome (PCOS) in High School Students in Rasht in 2009 According to NIH Criteria. *Int. J. of Fertility and Sterility*, 4(4): 156–159.
36. Balen, A.H.; Schachter, M.E.; Montgomery, D.; Reid, R.W. and Jacobs, H.S. (1993). Polycystic ovaries are a common finding in untreated female to male transsexuals. *Clinical Endocrinology*, 38: 325–329.
37. Franks, S. (1989) . Polycystic ovary syndrome: A changing perspective E. *Clinical Endocrinology*, 31: 87–120.
38. Lane, D.E. (2006). Polycystic ovary syndrome and its differential diagnosis. *Obstetrical and Gynecological Survey* ,61(2):125–135.
39. Bagheri, M.; Rad, I.A.; Jazani, N.H. and Nanbakhsh, F. (2013) . Vitamin D receptor TaqI gene variant in Exon 9 and polycystic ovary syndrome risk. *Int. J. Fertil Steril* , 7: 116–121.
40. Schmidt, J.; Landin-Wilhelmsen, K.; Brännström, M. and Dahlgren, E. (2011). Cardiovascular disease and risk factors in PCOS women of postmenopausal age: a 21-

year controlled follow-up study. *J. Clin. Endocrinol. Metab.*, 96: 3794-3803.

41. Mulhim, A.A.; Abul‑Heija, A.A.; Al‑Talib, A.A.; Al−Turki, H.A. and Gasim, T.G. (2014). Hormonal, Metabolic and Clinical Profile of Saudi Women with Polycystic Ovary Syndrome. *Saudi Journal of Medicine and Medical Sciences*, 1(1): 30-34.
42. Al-Tamimi, M.T.; Abdul-Hassan, I.A.; Al-dujaily S.S.; and Al-musawi, B.J.M. (2015). Impact of the transcription factor 7-like 2 (TCF7L2) gene polymorphisms on the risk of polycystic ovary syndrome in Iraqi patients . *World J. of Pharmaceutical Research*, 4(2).
43. Al-Deresawi, M.S.G. (2012). Hormonal-Genetical Study on Women with Polycystic Ovary Syndrome and Thyroid Disorders. A thesis Submitted to the Counsel of Genetic Engineering and Biotechnology Institute for Postgraduate Studies University of Baghdad In partial fulfillment of the requirements for the Degree of Master in Genetic Engineering and Biotechnology., 86.
44. Dahiya, K.S.; Achdeva, A.; Singh, V.; Dahiya, P.; Singh, R.; Dhankhar, R.; Ghalaut, P. and Malik , I. (2012) . Reproductive Hormone and Thyroid Hormone Profile in Polycystic Ovarian Syndrome. *WebmedCentral ENDOCRINOLOGY* , 3(6):WMC003455.
45. Gulab, K. ; Monika, S.; Nidhi, S.; Rinki, H.; Rahul, K. and Juber, A. (2015). Hypothyroidism- A Risk Factor for Menstrual Disorders among Nulliparous Females . *IOSR Journal of Dental and Medical Sciences*. 14 (12) : 78-81.