Bull. Egypt. Soc. Physiol. Sci. *38(1)*, 13-26

Bull. of Egyp. Soc. Physiol. Sci.

(Official Journal of Egyptian Society for Physiological Sciences)

(pISSN: 1110-0842; eISSN: 2356-9514)

Vitamin D Receptor Gene Polymorphism Taq in Egyptian Women With Polycystic Ovary Syndrome

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Received: 20 June 2017
Accepted: 5 August 2017
Available online: 1 Jan 2018

Keywords

- Gene polymorphism PCOS
- Vitamin-D
- Vitamin D receptor

Abstract

Background: Polycystic ovary syndrome (PCOS) is one of the commonest endocrine disorder affecting the women in childbearing period. Accumulating evidences from recent studies indicate that vitamin D receptor *(VDR)Taq1*(Tt) genetic variants may contribute to the pathogenesis of insulin resistance and polycystic ovary syndrome. The Vitamin-D receptor (VDR) regulates vitamin D levels and calcium metabolism in the body and these are known to be associated with insulin resistance and type-2 diabetes in polycystic ovarian syndrome (PCOS). This study aims to investigate the association of *VDR* polymorphism *Taq1*(Tt) and serum 25(OH)D level with PCOS. This study was carried out on140 subjects divided into 2 groups: 70 patients with PCOS (group I) and 70 healthy subjects served as controls (group II). All studied subjects were submitted to full history taking, general clinical examination and laboratory investigations for serum levels of fasting blood glucose, total cholesterol (TC),triglycerides (TG), HDL-c,LDL-c, fasting insulin and 25(OH)D. Also genotyping of *VDR* polymorphism *(Taq1)* was analyzed using the polymerase chain reaction–restriction fragment length polymorphism technique (PCR-RFLP). Results showed high significant statistical differences between the two studied groups regarding BMI (P value <0.001),SBP (P value <0.001) ,DBP (P value <0.001), fasting insulin (P value <0.001), fasting blood glucose (P value <0.001) ,insulin resistance (P value <0.001) ,triacylglycerol (P value <0.001), LDL cholesterol (P value <0.001), serum level of 25 (OH) Vit D (P value <0.001) and *VIT D R Taq1* genotype distribution (p value<0.001) with increased frequency of the tt andTt genotype in patients with PCOS and increased frequency of TT genotype in controls. Conclusion: Our results indicate that tt genotype and t allele of *VDR TaqI* polymorphism and serum level of 25OHD might be risk factors for PCOS.

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Introduction

Polycystic ovary syndrome (PCOS), is one of the commonest endocrine disorder affecting the women in childbearing period, it has a strong genetic background (1). The main causes of PCOS are not completely known. However, in addition to the menstrual disturbance and hyperandrogenism, PCOS patients demonstrate an increased occurrence of type 2 diabetes mellitus, impaired glucose tolerance, hyperinsulinemia, insulin resistance, and weight problems (2).

PCOS is due to a combination of genetic and environmental factors (3). The severity of PCOS symptoms appears to be largely determined by factors such as weight problems (4).The syndrome acquired its name due to the common sign on ultrasound examination of multiple (poly) ovarian cysts. These "cysts" are actually immature follicles not cysts (5).

Vitamin D deficiency may exacerbate symptoms of PCOS, with observational studies showing lower 25(OH)D levels were associated with insulin resistance, ovulatory and menstrual irregularities, lower pregnancy, weight problems and elevated cardiovascular disease risk factors (6). Obesity and insulin resistance are closely linked to the development of PCOS and its clinical features (7). Vitamin D3 is obtained from the diet or synthesized endogenously through sunlight-induced photochemical conversion of cholesterol to 7-dehydrocholesterol within the skin and subsequently hydroxylation inside the liver and kidney (8).

Vitamin D is thought to influence the development of PCOS through affecting gene transcription (9). Vitamin D is a prohormone which is converted into its active hormonal form 1, 25-(OH) 2D which activates its cellular receptor (VDR) which activate target genes to produce its biological actions (10). The vitamin D receptor (*VDR*) gene is considered to be an important candidate gene for PCOS (11). The association of vitamin D and *VDR* variants such as Taq1 with genetic aspects in PCOS has been reported indicating their strong functional role (12).

Taq1 polymorphism in exon 9 associated with rate of gene expression, is a T/C substitution (ATT to ATC) leading to a synonymous change at codon 352 (Isoleucine) (13). A number of predominantly restriction fragment length polymorphisms (RFLP) have been reported in the VDR gene and include a cluster towards the 30 end, Bsm1 (alleles Bb) and Apa1 (Aa) in intron 8, and *Taq1* (Tt) in exon 9, and a polyadenyl microsatellite length polymorphism (LS) in the terminal untranslated region. These polymorphisms are tightly linked (abTL) (14).

Aim of the work:

The aim of the present study was to investigate the distribution **of** *VDR* gene polymorphism *Taq1* (Tt) and its association with serum 25(OH)D level in patients with PCOS.

Subjects and Methods

Subjects:

This case–control study included (140) subjects: (70) PCOS and (70) healthy, age- and sex-matched subjects as a control group. Cases were selected from Obstetrics and Gynecology Department. Outpatient Clinic, Menoufia University Hospital, Egypt . All studied subjects

were subjected to complete history taking, physical examination including ultrasonography and anthropometric measurements. Estimation of body mass index [BMI] was done by dividing body weight in kilograms by (height in meter^2) (15). diagnosis of PCOS is based exclusively on reproductive criteria (hyperandrogenism, oligo/anovulation, and/or PCOS on ultrasound) (16).

Laboratory investigations including measuring total cholesterol(TC) , triglycerides (TG), low density lipoprotein (LDL-c), high density lipoprotein (HDL-c), triglycerides (TG), fasting blood glucose, fasting insulin, insulin resistance, serum 25(OH)D and VDR polymorphism (Taq1) genotypes were analyzed using the polymerase chain reaction–restriction fragment length polymorphism technique (PCR-RFLP).

Sample collection and assay:

Written consent forms (approved by the Committee of Human Rights in Research at Menoufia University) were obtained from all studied cases and control subjects. the study was conducted according to the World Medical Association (WMA) Declaration of Helsinki (17).

After 12 hours overnight fasting, 8 ml of venous blood were withdrawn from every subject by sterile vein-puncture and divided into three tubes. Two ml of blood were transferred into one EDTA tube: for DNA extraction and further molecular analysis.

One ml of blood was transferred into sodium flouride tube for enzymatic colorimetric determination of blood glucose.Blood glucose was determined by enzymatic colorimetric test, using Spinreactkit,SPAIN (18).

5ml of blood were was transferred into aplain tube and allowed to clot at 37° C, centrifuged for 10 minutes at 4000 r.p.m. The clear supernatant serum was separated from the clot and kept frozen at -80° C until determination of serum TC(19),HDL (20),LDL (21),TG (22), Serum 25(OH) (23) and serum fasting insulin (24).

Vitamin D was determined by enzyme linked immunosorbent assay method using DRG® 25-OH Vitamin D ELISA kit ,USA (23) and Serum insulin was determined by enzyme linked immunosorbent assay method,using DRG® Insulin ELISA kit ,GERMANY (24). Assessment of insulin resistance was done by homeostatic model assessment (HOMA) according to(25). HOMA- IR = fasting glucose (mg/dl) x fasting insulin (µIU/mL) / constant (405). *VDR* polymorphism *(Taq1)* genotypes were analyzed using the polymerase chain reaction–restriction fragment length polymorphism technique (PCR-RFLP).

DNA Extraction and amplification:

DNA was extracted from whole blood using Thermo Scientific Gene JET Genomic DNA purification kit,(Lithuania). DNA was eluted stored at -20^0 C for further PCR procedure.

PCR for the *VIT DR Taq1* gene was carried out to a total volume of 25 µl, containing 10 µl genomic DNA; 1 µl of each primer; 12.5 ul of master mix **(Genecraft; Germany)**; **(Stratagene; USA)** and .5 ul distal water (26).

***VIT DR Taq1* gene** was analyzed using the following designed primers **(Midland, Texas):**

Forward: **5′-CAGAGCATGGACAGGGAGCAAG-3′**

Reverse: **5′-CGGCAGCGGATGTACGTCTGCAG-3**

PCR amplification for **the *VIT DR Taq1* gene** gene was performed separately in using Applied Bio systems 2720 thermal cycler (Singapore).

* PCR condition consisted of: one cycle of amplification at 94 ºC for 3 minutes followed by 30 cycles at 94 ºC for 30 sec; 60ºC for 30sec; 72 ºC for 30sec; and one final cycle of extension at 72 ºC for 5 min The amplification products were separated by electrophoresis through 3% agarose gel stained with and visualized ethidium bromide with positive band at **345 bp**.

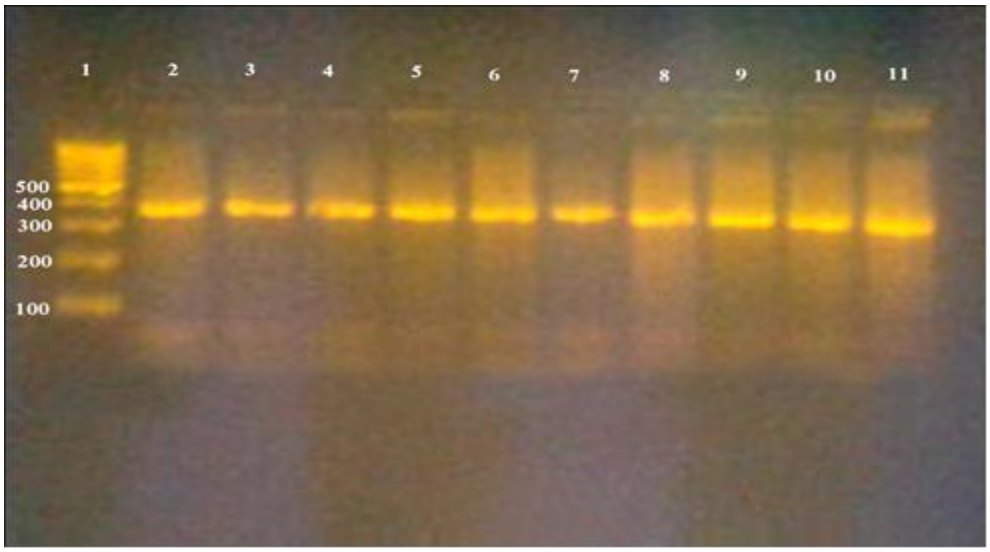

Figure (1): Shows the *VIT D R Taq1* gene, lanes from 2-11 show the length of the PCR amplicon which is 345 bp. ladder 100 bp was used in lane 1 .

The *VIT D R Taq1* gene polymorphism using the restriction fragment length polymorphism (the RFLP) technique:

15 ul of the PCR products for the *VIT D R Taq1* were mixed with 1ul (1 unit) of FastDigest® Taq1restriction enzyme (provided by Fermentas) with 6.5µl nuclease-free water and 2.5 of 10X FastDigest® Buffer.The mixture was good mixed and incubated for at 65 ºC for 30 minutes then 10µl of the products was loaded into a 3% agarose gel containing ethidium bromide for electrophoresis. The uncut fragment was 345 bp in

(TT) genotype and digestion products were 260 bp, 85 bp in (tt) genotype (26).

Statistical Analysis:

Results were collected, tabulated and statistically analyzed by IBM personal computer and statistical package SPSS version 20. Hardy-Weinberg equilibrium was computed to exclude any bias of results and we concluded that the genotype frequencies in this population are not significantly different than what would be expected as it was in Hardy-Weinberg frequencies with X^2<3.841.Student's t-test was used to compare quantitative data. Chisquare test ($\chi 2$): was used to study association between two qualitative variables. Mann whitney and Kruskal–Wallis tests for comparison two and three groups of not normally distributed variables respectively. Multiple regression analysis calculates the effects

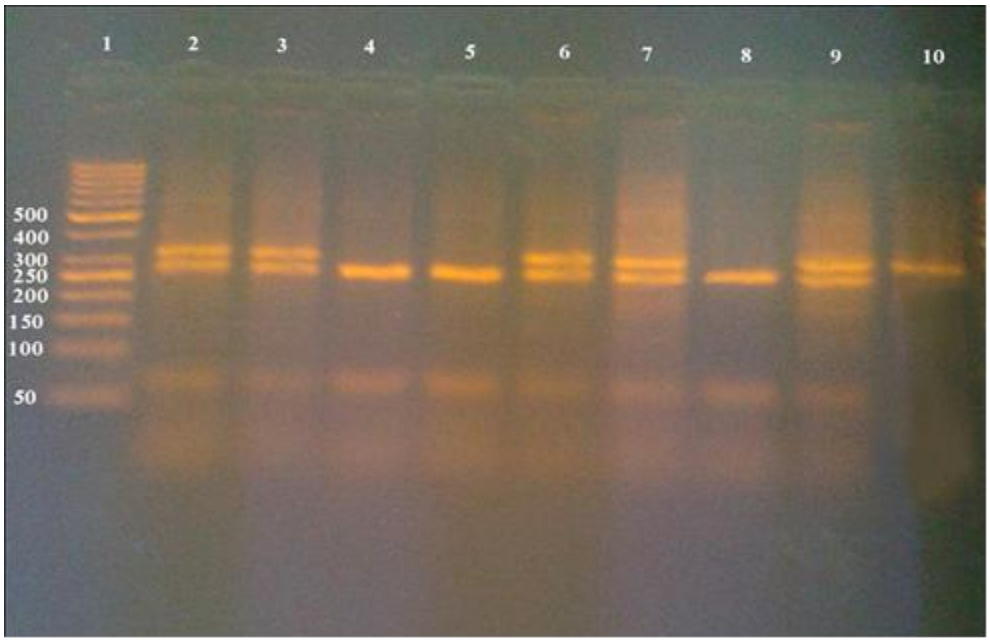

Figure (2): For the *VIT D R Taq1* gene polymorphism, the uncut fragment was 345 bp and digestion products were 260and 85 bp. ladder 50 bp was used in lane 1 .*Lanes 10 indicate TT genotype (345). *Lanes 2 , 3,6,7and 9 indicate Tt genotype (345 bp, 260and 85 bp).*Lane 4 , 5 and 8 indicates tt genotype (260and 85 bp).

of risk factors as independent Odds ratios with the effects of other confounders removed. P-value < 0.05 was considered statistically significant.

Results:

The study was conducted on a total number of 140 subjects divided into two groups as follows; 70 PCOS patients as group I and 70 healthy persons as group II. There was a statistically significant difference between the two studied groups regarding BMI,SBP,DBP, fasting insulin, fasting blood glucose and Insulin resistance ,triacylglycerol , LDL cholesterol, serum level of 25 (OH) Vit D and there was a significant decrease of HDL-c in PCOS group when compared to control group. While there is non-significant association between patients and controls regarding age (table 1).

As regards *VIT D R Taq1* genotype distribution between the two studied groups showed a significant difference, with increased frequency of the tt and Tt genotypes and t allele in the patient group and increased TT genotype and T allele frequency in the control group (P <0.001; Table 2 and Figure 3,4). The results also showed that the tt genotype of *VIT D R Taq1* increases the risk of PCOS by 7.1 fold and Tt genotype increases the risk by 4.1 fold, while the t allele increases the risk by 3.7 fold, as shown in (Table 2).

In group I, we compared the three different genotypes of *VIT D R Taq1(TT,Tt and tt) with* BMI, fasting insulin, fasting blood glucose and Insulin resistance ,triacylglycerol , LDL cholesterol, serum level of 25 (OH) Vit D and HDL-c. Patients with TT genotype showed higher levels of both HDL-c and serum level of 25 (OH) Vit D,while tt genotype

showed higher level of LDL-c ,TC, TG, higher insulin resistance and showed lower level of serum level of 25 (OH) Vit D (table 3).

tt genotype is associated with higher insulin resistance and lower level of serum 25 (OH) Vit D while TT genotype showed lower level of insulin resistance with higher level of serum level of 25 (OH) Vit D (figure 5,6). There was significant negative correlation between Serum 25(OH) D and insulin resistance (figure 7).

Multivariate logistic regression for risk of PCOS showed that the BMI was the most significant risk OR; 696.8 (40.3-867.9), followed by HDL OR; 127 (5.1-224.3), TG OR; 90 (3.9-191.7), HOMA-IR OR; 81.9 (4.8-397.3), Cholesterol OR; 76.2 (2.4-119.4), tt genotype OR; 19.02 (1.3-279.3) and Serum 25(OH) Vitamin D OR; 15.01 (1.3-76) (Table 4).

Table 1: Demographic and clinical characteristics in PCOS (group1) and control (group2)

	Case (PCO) N=70	Control N=70	T test	P value
Age (years)	29.3±2.8	29.7±2.9	0.745	0.458
BMI(kg/m2)	31.6±3.1	23.2±1.5	20.6	<0.001
Systolic BP(mm.Hg)	133.6±11.5	112.6±8.4	12.3	<0.001
Diastolic BP(mm.Hg)	86.3±7.6	74.1±6.2	10.3	<0.001
Fasting glucose (mg/dl)	99.2±11.3	86.9±8.2	7.4	<0.001
Fasting insulin (µIU/ml)	20.2±13.7	4.8±3.5	9.1*	<0.001
25 (OH) Vit D (nmol/L)	20.1±3.04	32.9±3.1	24.7	<0.001
TAG(mg/dl)	165.5±9.9	92.9±4.9	55.05	<0.001
Cholesterol (mg/dl)	257.1±25.5	172.3±8.9	26.3	<0.001
HDL-c (mg/dl)	31.9±1.4	48.5±1.2	76.6	<0.001
LDL-c (mg/dl)	190.3±52.7	112.4±9.8	12.1	<0.001
Insulin resistsnce (HOMA-IR)	4.8±3.3	0.9±0.09	9.8*	<0.001

*U (Mann whittney test)

Table 2: Comparison of VITDR Taq1 genotypes between the studied groups

	Case (PCOS)	Control	X2	P value	OR
***VITDR Taq1* polymorphism**			21.7	<0.001	
TT*	14(20%)	40(57.1%)			
Tt	26(37.1%)	18(25.7%)			4.1(1.7-9.7)
tt	30(42.9%)	12(17.1%)			7.1(2.9-17.6)
***VITDR Taq1* alleles**			27.9	<0.001	
T*	54(38.6%)	98(70%)			
t	86(61.4)	42(30%)			3.7(2.3-6.1)

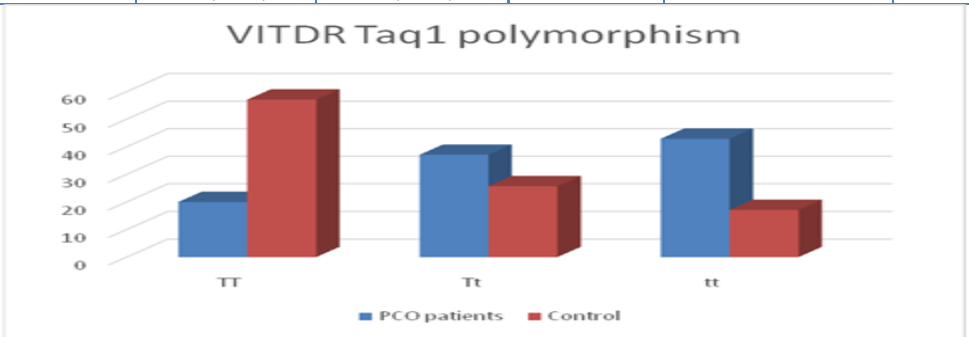

Figure 3:Genotype distribution of the VITDR Taq1 polymorphism between two studied groups.

Table 3: Biochemical parameters of the studied patients with PCOS in different genotypes of VITDR Taq1

	TT	Tt	tt	F test	P value
Age (years)	29.7±2.8	29.1±3.1	29.7±2.6	0.547	0.58
BMI(kg/m2)	25.4±4.4	28±4.8	29.3±4.7	8.4	<0.001
Systolic BP (mm.Hg)	118.3±11.6	124.5±15.3	127.6±15.7	5.4	0.005
Diastolic BP (mm.Hg)	78.1±7.5	81.8±9.9	81.2±10.2	2.3	0.106
Fasting glucose (mg/dl)	90.5±10	93.1±9.8	96±14.2	2.9	0.059
Fasting insulin (µIU/ml)	7.8±9.1	10.2±9.8	21.1±14.8	17.6*	<0.001
25 (OH) Vit D (nmol/L)	30.3±5	26.2±6.2	21.8±7.4	22.3	<0.001
TAG(mg/dl)	112.3±32.9	135.1±37.2	144.7±34.5	11.2	<0.001
Cholesterol (mg/dl)	194.5±38.6	219.7±40.7	235.5±51.9	10.9	<0.001
HDL-c (mg/dl)	44.3±7.5	38.8±8.2	36.4±7.7	13.4	<0.001
LDL-c (mg/dl)	134.5±45.7	146.7±41	177.9±66.6	8.6	<0.001
Insulin resistsnce (HOMA-IR)	1.5±1.3	2.4±2.1	5±3.7	21*	<0.001

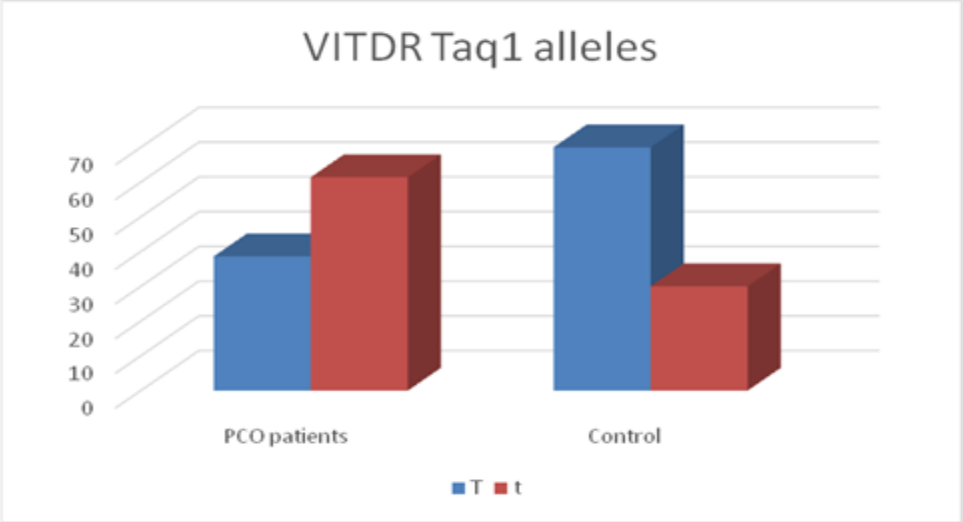

Figure 4: Allelic distribution of the VITDR Taq1 polymorphism between two studied groups.

Table 4 : Multivariate logistic regression for risk of PCOS.

	B	P value	OR	CI
BMI (kg/m2)	7.4	<0.001	696.8	40.3-867.9
FBS (mg/dl)	0.658	0.564	1.9	0.207-18
25 (OH) Vit D (nmol/L)	2.7	0.03	15.01	1.3-76
Cholesterol (mg/dl)	5.1	0.018	76.2	2.4-119.4
HDL-c (mg/dl)	-6.6	0.009	127.7	5.1-224.3
TG(mg/dl)	4.5	0.005	90	3.9-191.7
Insulin (µIU/ml)	1.2	0.401	3.2	0.209-50.1
HOMA-IR	4.4	0.002	81.9	4.8-397.3
Genotype				
Tt	1.3	0.415	3.6	0.162-82.5
tt	4.6	0.036	19.02	1.3-279.3

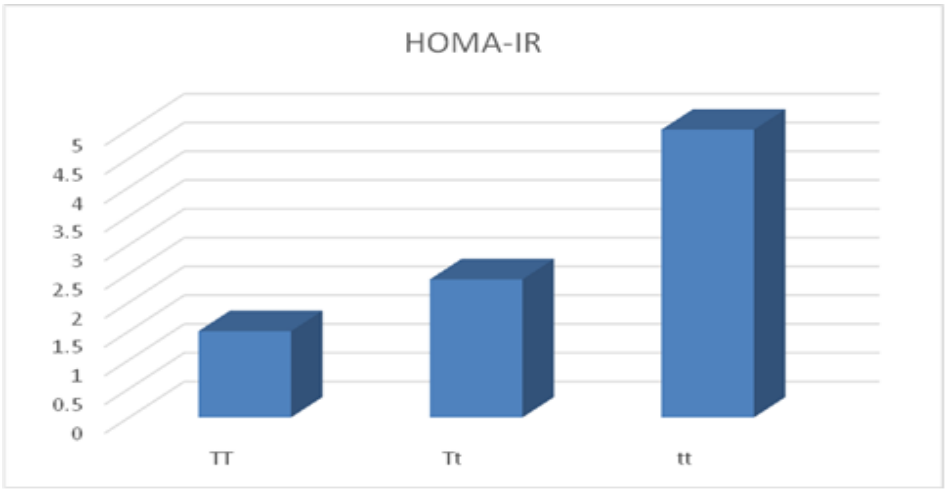

Figure 5: Association between VITDR Taq1 polymorphism & HOMA-IR in group I.

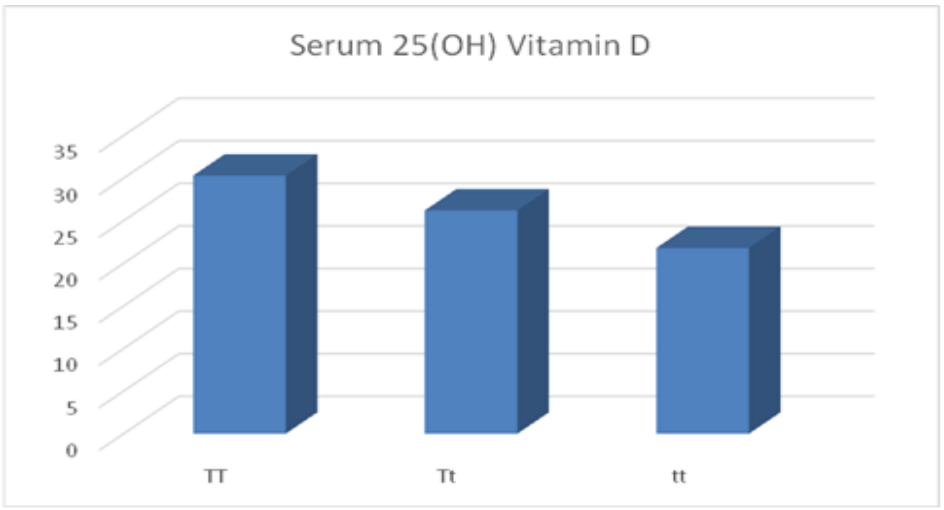

Figure 6: Association of serum 25 (OH) Vit D with VITDR Taq1 polymorphism in group I

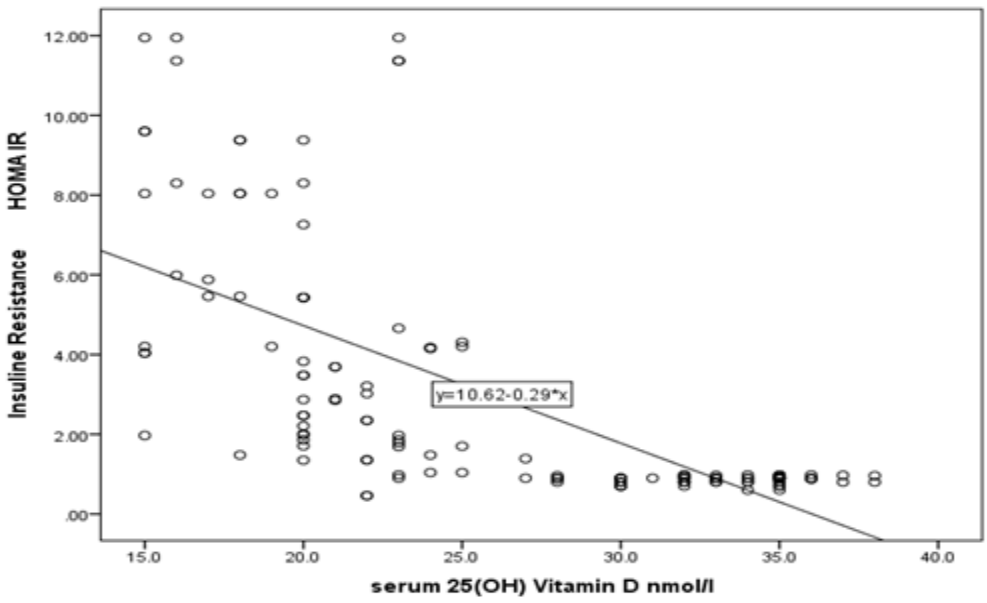

Figure 7: Correlation coefficient between serum 25 (OH) Vit D & HOMA-IR in group I. r = -0.685 p value <0.001

Discussion:

Polycystic ovary syndrome (PCOS) is the most common endocrine-metabolic disorder affecting 5-10 per cent of women in childbearing period and is a common cause of anovulatory infertility. It is a heterogeneous disease characterized by oligomenorrhoea due to increased ovarian and adrenal androgen secretion, acne and/or alopecia, menstrual irregularity, and polycystic ovaries (27).

The vitamin D receptor (*VDR*) gene, is considered to be an important candidate gene for PCOS(28). It is a ligand-activated transcription factor that mediates the genomic actions of vitamin D regulating several endocrine functions and cell functions including bone metabolism and calcium-phosphate homeostasis (29).

In our study, there was a significant statistical difference between PCOS group and controls as regarding systolic and diastolic blood pressure. This in agree with Li et al (30), who show that, there was significant elevation of systolic and diastolic blood pressure in PCOS group when compared with controls. The present study reported that BMI were significant higher in PCOS than the controls. This result was explained by Susan (31), who demonstrated that, chronic exposure to higher testosterone levels in women with PCOS may modify body fat distribution in these women.

The present study reported that, the fasting glucose was significant higher in PCOS group than the controls. This result was in agreement with Bhattacharya (32). This result was explained by Dunaif (33) who demonstrated that, there was abnormalities in insulin secretion and action in PCOS patients. In our study, fasting insulin and insulin resistance were significantly higher in PCOS group than the controls .This is in agreement with the results obtained by Phelan et al., (34).

The present study reported that, the fasting triglyceride was significantly higher in PCOS group compared with the control group and HDL-c was significantly low in PCOS group. This is in agreement with the results obtained by Stojkovic et al., and wild et al., 35,36). The present study reported that LDL-c was significantly higher in PCOS group compared with the control group. This result in agreement with the results obtained by Sarama et al., and wild et al., (36,37) and in contrast with the results obtained by Li et al., (30).

In our study, the 25(OH) vit D was significantly lower in PCOS group when compared with controls. This result in agreement with the results obtained by John et al and Sahar et al., (38,39). This result is explained by Hahn et al., (40) who reported that, vitamin D might be a causal factor in the pathogenesis of metabolic syndrome in PCOS. In our study, there was significant negative correlation between Serum 25(OH) D and insulin resistance in PCOS. This is in agreement with the results obtained by Hahn et al (40,41).

Rebecca L et al (6) reported that, Vitamin D deficiency is common in women with polycystic ovary syndrome (PCOS), with the 67–85% of women with PCOS having lower serum concentrations of 25-hydroxy vitamin D (25OHD) than controls. Vitamin D deficiency may exacerbate symptoms of PCOS, several studies were showing that lower 25OHD levels were associated with insulin resistance, ovulatory and menstrual irregularities, lower pregnancy success,

hyperandrogenism, weight gain and increased cardiovascular disease risk factors.

The current study as regards *VIT D R Taq1* genotype distribution between the two studied groups showed a significant difference, with increased frequency of the tt and Tt genotypes and t allele in the patient group and increased TT genotype and T allele frequency in the control group . The results also showed that the tt genotype of *VIT D R Taq1* increases the risk of PCOS by 7.1 fold and Tt genotype increases the risk by 4.1 fold, while the t allele increases the risk by 3.7 fold, tt genotype showed higher insulin resistance and lower level of serum 25 (OH) Vit D while TT genotype showed lower level of insulin resistance with higher level of serum level of 25 (OH) Vit D.

This is in contrast with Touraj Mahmoudi (42) who reported that, No significant difference was observed for the *VDR TaqI,* gene polymorphism between the women with PCOS and controls. A study by Hahn *et al* (40) also found that when grouping the women with PCOS according to 25OHD levels, lower levels of 25OHD were associated with insulin resistance and obesity. It has been suggested that obesity may have a confounding role in the relationship between 25OHD and insulin resistance in women with PCOS. Women with PCOS with severe vitamin D deficiency were more insulin resistant.

Oh and Barrett-Connor (43) suggest that VDR gene variant may be associated with glucose intolerance independent of defective insulin secretion and with IR. Mahmoudi (9) indicated that VDR gene variant may affect PCOS development as well as IR in women with PCOS.

In our study Multivariate logistic regression for risk of PCOS showed that the BMI was the most significant risk OR; 696.8 (40.3-867.9), followed by HDL OR; 127 (5.1-224.3), TG OR; 90 (3.9-191.7), HOMA-IR OR; 81.9 (4.8-397.3), Cholesterol OR; 76.2 (2.4-119.4), tt genotype OR; 19.02 (1.3-279.3)and Serum 25(OH) Vitamin D OR; 15.01 (1.3-76).

Dyslipidemia is the most common metabolic abnormality in PCOS. Polycystic ovary syndrome is the leading cause of dyslipidemia in reproductive-age women . Overall, studies of PCOS patients report slightly decreased levels of cardioprotective HDL-C, with slightly elevated levels of TG, VLDL-C, and LDL-C. PCOS women display the lipid profile observed in insulin resistant states such as DM2 and characterized specifically by elevated TG and lowered HDL-C (44). There is emerging evidence that women with PCOS have an elevated risk of being overweight and obese and have increased weight gain with increased BMI compared with community controls (45).

Rebecca L et al (6) reported that, (tt) genotype of *VDR TaqI* in exon 9 (rs731236) was significantly higher in PCOS cases versus controls also suggests that the (tt) genotype of *VDR TaqI* in exon 9 (rs731236) is arisk factor for PCOS.

Conclusion: Based on the previous results, we demonstrated that serum level of 25OHD has a significant positive association with insulin sensitivity, and that the tt genotype and t allele of the *VDR TaqI* in exon 9 (rs731236) gene as well as low serum 25OHD levels might be considered as genetic risk factors for PCOS. and might be used for screening of the early detection of PCOS.

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