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Correlation of vitamin D with clinical, biochemical and anthropometrical parameters of PCOS patients

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Abstract---Objective: To find correlation of 25-hydroxy vitamin D (25OHD) levels with various clinical biochemicals, anthropometric parameters in patients of PCOS. Study design: Cross-sectional observational study. Materials and Methods: The study was conducted at Obstetrics and Gynaecological dept. of Jawaharlal Lal Nehru medical college, AMU, Aligarh, for a period of 2 years, 2018-2020. A total number of 100 PCOS patients satisfying inclusion criteria were enrolled for the study. Frequency of Vit-D deficiency in study group was estimated. Correlation of Vit-D levels with various clinical, anthropometrical, biochemical parameters was evaluated. Based on Vit -D levels the study population was divided in to Vit-D deficient and non-deficient individuals and Paired sample t-test was used to assess changes in quantitative variables. Pearson product moment correlation analysis was used to see correlation among different variables. Qualitative variables were interpreted using Chi square test. Statistical analysis was performed using computer program SPSS version 25.0. Observations and Results: The results of 100 PCOS patients were analysed. Vit-D deficiency was found in 74% of PCOS population. Vitamin D correlated inversely with LH, FSH Prolactin and Testosterone levels ($r = -0.213$, $p < 0.05$, $r = -0.176$; $p > 0.05$, $r = -0.101$; $p > 0.05$; $r = 0.246$, $p < 0.01$.) Though significant correlation was found with LH and testosterone only. Anthropometric parameters including BMI and waist circumference were inversely correlated with Vit-D levels ($r = -0.297$; $p < 0.01$, $r = -0.282$; $p < 0.01$) and the results were statistically

significant. Further study population was divided in to vit D deficient and non-deficient group and clinical manifestations were compared using chi square test and notable findings included significantly increased frequency of menstrual irregularities(m) ,infertility(i) and hirsutism (h) in Vitamin D deficient group ((m)ch²=26.298,(i) ch²=10.8, (h)ch²=23.17) with p<.001. Conclusion: Thus we conclude that Vitamin D deficiency exists with high frequency in PCOS patients and significant correlation exists between Vitamin D and various clinical, biochemical and anthropometric parameters in patients of PCOS suggesting the role of Vitamin D in pathophysiology of PCOS. Biochemical abnormalities and clinical features were more pronounced in vitamin D deficiency individuals and thus it emphasises the need of Vitamin D screening and supplementation in all the PCOS patients for formulating a better management protocol for PCOS.

Keywords---Obesity, BMI, Hirsutism infertility PCOS, Vitamin-D.

Introduction

PCOS is recognized as the most common endocrine disorder that affects women of reproductive age group. It is a heterogeneous disease characterized by symptoms of menstrual irregularity, anovulatory infertility, hirsutism as well as other metabolic manifestations including hyperandrogenaemia, dyslipidaemia, and insulin resistance^{1,2}. Worldwide, prevalence of PCOS is highly variable and estimated to be 5-10% ³. Various studies have been conducted on Indian population and they have found the prevalence to be 9.13% ⁴. Phenotypic manifestations of PCOS have been attributed to interaction of various genetic, familial and environmental factors which may function in utero or in early adolescent life, manifesting clinically a few years later as PCOS⁵.

Path physiology of PCOS is complex and several studies have demonstrated role of vitamin D in the pathogenesis of PCOS. Women with PCOS may also be at elevated risk of vitamin D deficiency. Prevalence of vit D deficiency in general population is 20-48%⁶ but is relatively higher in women with PCOS (approximately 67-85%) ⁷. Vitamin D responsive elements (VDREs) are considered to be the hallmark of vitamin D action. It is found in both the nuclei and cytoplasm of granulosa cells (GC) of human ovaries indicating that it is responsible for the physiologic functions of 1, 25(OH) 2D3 in ovarian follicles⁸ Vitamin D may play a role in glucose metabolism by enhancing insulin synthesis and release and increasing insulin receptor expression or suppression of pro-inflammatory cytokines and therefore its deficiency may contribute to the development of insulin resistance⁹. The effect of vitamin D deficiency on metabolic and reproductive dysfunctions in PCOS may be mediated by insulin resistance. Vitamin D deficiency is associated with an imbalance in hyperandrogenism markers¹⁰ which may be manifested as altered LH/FSH ratio and hirsutism which is an entity pathognomic to PCOS.

Vitamin D deficiency has higher prevalence and has multifaceted role at various levels in PCOS which is an area insufficiently explored therefore this study tries to establish a correlation of vitamin D with various parameters of PCOS for a thorough understanding of the disease pathophysiology and to further establish the role of vitamin D in PCOS which might obligate vitamin D screening in PCOS and hence may pave way for alternative management of the condition.

Material and Methods

Study population

This was an observational study conducted on 100 patients of PCOS in the Department of Obstetrics and Gynaecology, Jawaharlal Nehru Medical College and Hospital (JNMCH), A.M.U., Aligarh from November 2018 to December 2020. Study inclusion criteria consisted of women aged between 18-35 years diagnosed with PCOS according to the Rotterdam criteria (Rotterdam ESHRE/ASRM-sponsored PCOS Consensus Workshop Group, 2004). Exclusion criteria included women who were consuming supplemental vitamin D > 500 IU/day, pregnant women, and women having endocrine diseases including DM, thyroid disorder, elevated prolactin levels and women with a potential iatrogenic (e.g. ovarian surgery, gonadotoxic therapy).

Sample size

For a study with α error 5% and power of study 80%, sample size was calculated using formula,

$$N = (Z_{1-\alpha/2})^2 p (1-p) / L^2$$

Where: $Z_{1-\alpha/2} = 1.96$ (for α error 5% and β 20%)

P = expected prevalence of vitamin D deficiency in females with PCOS =

$$67\% = 0.67$$

L = allowable absolute error = 10%

$$= (1.96)^2 \times 0.67 \times 0.33 / (0.1)^2$$

$$= 85 \text{ (approx. 100)}$$

Adding 10% non-response rate, final sample size = 85 + 10 = 95

Procedure

Standard anthropometric data [height, weight (using Omron HBF- 375 Body Composition Monitor).waist (Waist circumference was measured in standing posture at the midpoint between the lower border of the rib cage and the iliac crest) and hip circumference (at the maximum circumference of the buttocks)] were obtained from each subject. The body mass index (BMI) was calculated as weight in kilograms divided by the square of the height in metres (kg/m^2).Waist to hip ratio(waist /hip circumference) was also evaluated which is an index of abdominal fat. Hirsutism was clinically evaluated using the modified Ferriman Gallwey scoring system. The scoring included evaluating nine body areas including the upper lip, chin, chest, upper back, lower back, upper arm, upper and lower abdomen, thighs.

Vitamin D levels

Serum was separated by centrifuging the blood at 3000 rpm for 20 minutes at 4°C and stored at -20°C until assayed. Estimation of 25-OH vitamin D was performed by using ELISA kit

Biochemical analysis

LH, FSH, Prolactin and testosterone were assessed from the sample collected from subjects on day 2 or 3 of cycle. Those who had amenorrhea/oligomenorrhea, the samples were collected after progesteron withdrawal. The kit used for analysis is based on immunoradiometric assay. In the kit, mouse monoclonal antibodies present are directed against two different epitopes.

Vitamin D levels were assessed in the study population and were correlated with biochemical and anthropometrical parameters; frequency of vitamin D deficiency was also calculated. On the basis of Vitamin D levels the study population was divided in to Vitamin D deficient and non-deficient groups and clinical manifestations namely menstrual irregularity, hirsutism, infertility, and acne were compared in both the groups thereby assessing the effect of vitamin D deficiency on the mentioned parameters.

Statistical analysis

Descriptive statistics including mean and standard deviation were calculated using the software SPSS version 25.0. Paired sample t-test was used to assess changes in quantitative variables. Pearson Product Moment Correlation analysis was used to see correlation among different variables.

Results

Characteristics of the study population

Mean age of study participants was 23.86 ± 4.4 years. More than half of the study subjects were graduate (60%) and only 2% were illiterate. More than half of the patients were unmarried (53%). Around one-third of the patients were working (35%) while more than one-third were students (38%).

In our study, 52% patients were having normal BMI¹¹, 32% patients were overweight and 16% patients were obese. Mean BMI of study subjects was 24.5 ± 4.51 kg/m². Mean waist circumference of study subjects was 86.1 ± 9.35 cm and 51% subjects were centrally obese¹² in our study having WC > 80 cm.

The anthropometric and biochemical data is depicted in table 1

Table I
Anthropometric and biochemical parameters of study subjects

S.No.	Variables	Mean $\pm$ SD
1	Age (years)	23.86 $\pm$ 4.38
2	BMI (kg/m ²)	24.58 $\pm$ 4.51
3	WC (cm)	86.10 $\pm$ 9.35
4	WHR	0.85 $\pm$ 0.06
5	S. Testosterone	3.85 $\pm$ 0.88
6	LH (IU/L)	11.16 $\pm$ 6.16
7	FSH (IU/L)	5.04 $\pm$ 2.22
8	PRL (ng/ml)	13.23 $\pm$ 7.22
9	Vitamin D (ng/ml)	17.27 $\pm$ 5.46

Frequency of Vitamin D deficiency in study subjects:

In our study, frequency of vitamin D deficiency (<20ng/ml) ¹³ PCOS was estimated to be 74% (95% confidence interval 64.6% to 81.6%).

Frequency of vitamin D deficiency in different BMI groups

PCOS patients were classified into three BMI groups¹¹ according to WHO classification and vitamin D deficiency was assessed in each group as shown in table II. Among normal weight PCOS individuals, 67% were vitamin D deficient while of vitamin D deficiency among overweight and obese PCOS patients was 75% and 93.7% respectively. This frequency distribution came out to be statistically significant using χ^2 test (χ^2 -8.135; p-0.017)

Table II
Frequency of vitamin D deficiency in different BMI groups (N=100)

BMI	Vitamin D level				Total
	Normal		deficient		
	No.	%	No.	%	
Normal	17	33	35	67	52
Overweigh	8	25	24	75	32
Obese	1	6.3	15	93.7	16

Correlation of Vitamin D with Biochemical parameters (LH, FSH, Prolactin and Testosterone)

Biochemical parameters were assessed and on applying correlations statistics, we found that vitamin D was having inverse correlation with LH, FSH and prolactin ($r = -0.213$, $p < 0.05$; $r = -0.176$, $p > 0.05$; $r = -0.1$, $p > 0.05$; $r = -0.283$, $p < 0.05$) However,

result was found statistically significant for LH. And testosterone As shown in table III

Table III
Correlation between Vitamin D and LH, FSH and Prolactin and testosterone levels in PCOS patients:

Correlation- Coefficient	Vitamin D	Vitamin D	LH	FSH	PRL	Testosterone
		1	-0.213	-0.176	-0.1	-0.283
P- Value			P <0.05	P > 0.05	P >0.05	P<0.05

Correlation of Vitamin D with anthropometrical parameters of PCOS (BMI, Waist circumference)

Correlation of Vitamin D with BMI was evaluated using Pearson correlation analysis. It was found that both the variables had inverse relation ($r = -0.297$, $p < 0.01$) and the result was statistically significant.

Table IV
Correlation between vitamin D levels and BMI

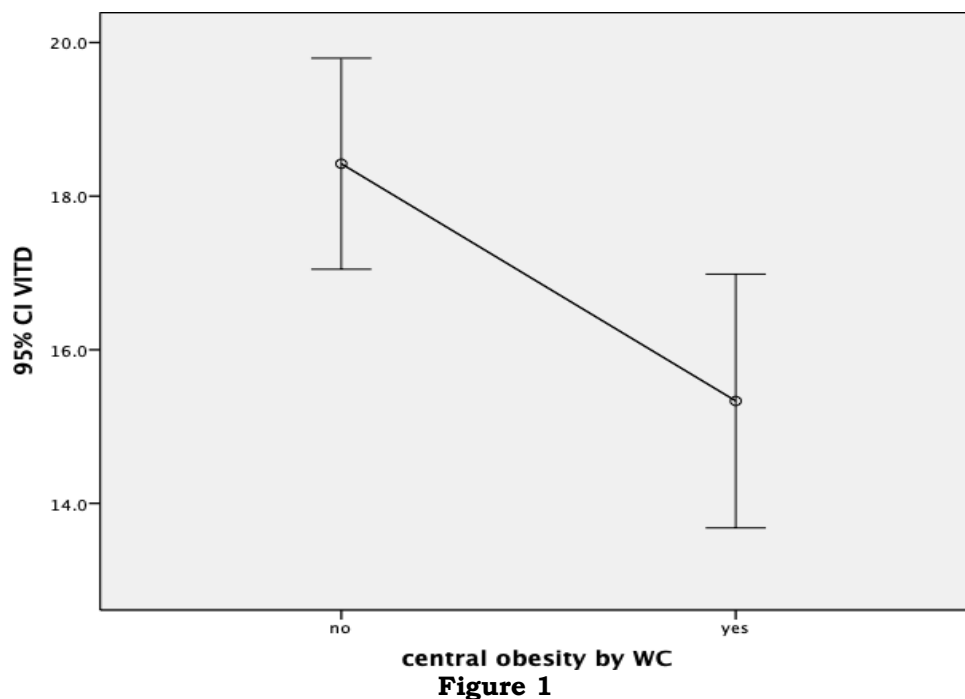
Correlation coefficient	BMI	Vitamin D (pre)
		-0.297
p-value		$p < 0.01$

Correlation of Vitamin D with waist circumference

Vitamin D levels were also compared in PCOS group with central obesity ($WC > 80\text{cm}$) and without central obesity and it was found that Vitamin D levels were significantly lower in centrally obese group ($MSD1\ 18.42 \pm 5.45$, $MSD2\ 15.33 \pm 4.95$) on application of chi2 test as shown in table V and fig1

Table V
Correlation of Vitamin D with Waist circumference

Vit D	Central obesity by WC	N	Mean	Std. Deviation
	No	63	18.42	5.45
	Yes	37	15.33	4.95



Comparison of clinical manifestations between vitamin D deficient and non-deficient group

Clinical manifestations of PCOS in vitamin D deficient and non-deficient groups were compared using chi square test and it was observed that vitamin D deficient PCOS subjects had significantly higher frequency of menstrual complaints (82%), hirsutism (54%) and infertility (32%) compared to non-vitamin D deficient population [m=61%,h=26%,in=23%] and the frequency distribution was statistically significant ($p < 0.001$) as shown in table V.

Table VI
Comparison of presenting complaints in vitamin D deficient and non-deficient groups

S.No.	Complaints	Deficiency	No Deficiency	p-value
1.	Menstrual complaints	61	16	<0.001
2.	Infertility	24	6	<0.001
3.	Hirsutism	40	7	<0.001
4.	Acne	15	8	>0.05

Discussion and Conclusion

This study shows the frequency of Vitamin D deficiency (VDD) was estimated to be 74% whereas reported prevalence in Indian subcontinent is nearly 76% and between 67% to 85% in PCOS¹⁴, but this was not a population based study so true prevalence could not be estimated, however other observational studies studies^{14, 15, 16, 17} mentioned greater prevalence of Vitamin D deficiency in PCOS.

Vitamin D acts through VDR (vitamin D receptors) genes involved in vitamin D metabolism and polymorphisms in the VDR gene may determine susceptibility to PCOS¹⁸, specifically alteration in protective VDR gene, Cdx2 plays an influential role on insulin secretion and sensitivity in PCOS women¹⁹ and thus the genetic linkage could be the possible cause of higher frequency of vitamin D deficiency in PCOS.

The frequency of VDD in different BMI groups was analysed to find correlation between vitamin D deficiency and obesity. The study shows statistically significant difference in frequency of vitamin D deficiency in different BMI groups with higher frequency in obese PCOS. In addition, inverse and significant correlation of Vitamin D with BMI and waist circumference was obtained symbolising the association particularly with abdominal obesity which is a greater risk factor for cardiovascular disease. A possible explanation for this may be sequestration of lipophilic vitamin D molecules by adiposities²⁰ or volumetric dilution. The causal relationship is still not established as some studies showed lower VD concentration predisposes an individual to obesity and thus vit D deficiency may be involved in pathogenesis of disease²¹. Consistent findings were reported by Asheim et al, who found lower levels of vitamin D in morbidly obese individuals²². A meta-analysis of 15 studies showed increased prevalence of vitamin D in obese vs non obese controls ²³.

Vitamin D levels showed inverse and significant correlation with LH and testosterone levels($r=-0.213, p<0.05, r=-.283, p<0.05$) which subsidizes the role of VDD in hyperandrogenism. Vitamin D deficiency is associated with an imbalance in hyperandrogenism markers¹⁰. Research has reported that Vitamin D receptor polymorphisms and altered aromatase gene expression in VDD, might dictate higher LH levels²⁴. Studies have also reported a correlation between 25(OH) D levels with testosterone and DHEAS levels, in addition to the LH/FSH ratio¹⁰ similar to our study.

There was statistically significant difference in frequency of menstrual irregularity (82%), hirsutism (54%) and infertility (32%) in vitamin D deficient group than non-deficient group [menstrual irregularity (61%), hirsutism (26%) and infertility (23%)]. In concordance with our study, Thys-Jacobs et al²⁵ reported improvement in menstrual complaints following vitamin D supplementation. However Reza Ghadimi and coworkers found no correlation between clinical manifestations and vitamin D deficiency²⁶. Further a study in Middle East found inverse correlation between hirsutism and acne with vitamin D levels ²⁷. Vitamin D has receptors in granulosa cells of ovary²⁸ and is responsible for multiple facets of reproductive functions and thereby result in menstrual abnormalities and infertility.

This study supports the hypothesis that hypovitaminosis D is prevalent in PCOS and strong correlation exists between Vitamin D and hyper androgenic markers as well as obesity which are salient features of PCOS and thus it may have a significant contribution in the pathogenesis of the disease. Furthermore clinical manifestations are exaggerated in vitamin D deficient PCOS subjects signifying its role in the disease process. Thus the study highlights the importance of vitamin D screening in all the PCOS subjects and possible role of Vit D supplementation in relief of symptoms and improvement in fertility and metabolic disturbances in

addition to existing medical therapy. Also it accentuates role of vitamin D in weight reduction along with life style modification in PCOS. Therefore it is suggested that vitamin D fortified products should be supplied specially in indian subcontinent which may affect the prevalence of the disease and may alter the obesity pandemic along with life style changes. The strength of the study is detailed evaluation and follow up of the patients and limitations are lack of controls, small sample size and lack of insulin resistance evaluation. Furthermore studies with large sample sizes are required to correlate vitamin D with various parameters of PCOS and thereby support the role of vitamin D in pathophysiology of PCOS

Conflict of Interest

There is no conflict of interest associated with the research work.

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References

1. Sirmans SM, Pate KA. Epidemiology, diagnosis, and management of polycystic ovary syndrome. *ClinEpidemiol.* 2013;6:1-13
2. Azziz, R. et al. The Androgen Excess and PCOS Society criteria for the polycystic ovary syndrome: the complete task force report. *Fertil. Steril.* 2009;91, 456–488
3. Dumont A, Robin G, Catteau-Jonard S, Dewailly D. Role of anti-Müllerian hormone in pathophysiology, diagnosis and treatment of polycystic ovary syndrome: a review. *ReprodBiolEndocrinol.* 2015; 13:137
4. Nidhi R, Padmalata V, Nagarathna R, Amritanshu R. Prevalence of polycystic ovarian syndrome in Indian adolescents. *J PediatrAdolesc Gynecol.* 2011; 24:223–7.
5. Kshetrimayum C, Sharma A, Mishra VV, Kumar S. Polycystic ovarian syndrome: Environmental/occupational, lifestyle factors; an overview. *J Turk GerGynaecol Assoc.* 2019;20(4):255-263.
6. Amrein, K., Scherkl, M., Hoffmann, M. et al. Vitamin D deficiency 2.0: an update on the current status worldwide. *Eur J ClinNutr* 2020;74, 1498–1513.
7. Lin MW, Wu MH. The role of vitamin D in polycystic ovary syndrome. *Indian J MedRes.* 2015;142(3):238-240.
8. Jones G. Pharmacokinetics of vitamin D toxicity. *American Journal of Clinical Nutrition.* 2008;88(2):582S–6S.
9. Chih- Chien Sung, Min- Tser Liao, Kuo-Cheng Lu, Chia-Chao Wu, “Role of Vitamin D in Insulin Resistance”, *BioMed Research International*, vol. 2012, Article ID 634195
10. Yildizhan R, Kurdoglu M., Adali E. et al., Serum 25-hydroxyvitamin D concentrations in obese and non-obese women with polycystic ovary syndrome, *Arch. Gynecol. Obstet.* 280 (2009) 559–563
11. WHO Expert Consultation. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. *Lancet.* 2004 Jan 10;363(9403):157-63

12. Zimmet PZ, Alberti KG. Introduction: Globalization and the non-communicable disease epidemic. *Obesity* (Silver Spring), 2006, 14(1):1-3.
13. El-Hajj Fuleihan G, Nabulsi M, Tamim H, Maalouf J, Salamoun M, Khalife H, et al. Effect of Vitamin D replacement on musculoskeletal parameters in school children: A randomized controlled trial. *J Clin Endocrinol Metab.* 2006;91:405–12
14. Van Schoor N.M., Lips P. Worldwide Vitamin D Status. *Best Pract. Res. Clin. Endocrinol. Metab.* 2011;25:671–680. doi: 10.1016/j.beem.2011.06.007
15. M. Irani, H. Minkoff, D.B. Seifer, Z. Merhi, Vitamin D increases serum levels of the solublereceptor for advanced glycation end products in women with PCOS, *J. Clin.Endocrinol. Metab.* 99 (2014) E886–E890.
16. Alvarez JA, Ashraf A. Role of vitamin D in insulin secretion and insulin sensitivity for glucose homeostasis. *International Journal of Endocrinology.*2010;351385.
17. R.L. Thomson, S. Spedding, J.D. Buckley, Vitamin D in theaetiology and management of polycystic ovary syndrome, *Clin.Endocrinol. (Oxf)* 2012;77.
18. Moini A, Shirzad N, Ahmadzadeh M, Hosseini R, Hosseini L, Sadatmahalleh SJ. Comparison of 25-hydroxyvitamin D andCalcium Levels between Polycystic Ovarian Syndrome and NormalWomen. *IntJ FertilSteril.* 2015;9(1):1-8.
19. Al-Daghri NM, Al-Attas OS, Alkharfy KM, Khan N, Mohammed AK, Vinodson B, et al. Association of *VDR*-gene variants with factors related to the metabolic syndrome, type 2 diabetes and vitamin D deficiency. *Gene.* 2014;542:129–33.
20. Dasgupta S, Dutta J, Annameneni S, Kudugunti N, Mohan Reddy B. Association of vitamin D receptor gene polymorphisms with polycystic ovary syndrome among Indian women. *Indian J Med Res.* 2015;142:276–85
21. Wortsman J., Matsuoka L.Y., Chen T.C., Lu Z., Holick M.F. Decreased bioavailability of vitamin D in obesity. *Am. J. Clin. Nutr.* 2000;72:690–693. doi: 10.1093/ajcn/72.3.690.
22. Ashiem ET, Hofso D, Hjelmesaeth J, Birkeland KI, Bohmer T.Vitamin status in morbidly obese patients: a cross sectionalstudy. *Am J ClinNutr.* 2008;87(2):362–369.
23. Yao Y, Zhu L, He L, et al. A meta-analysis of the relationshipbetween vitamin D deficiency and obesity. *Int J ClinExp Med.*2015;.
24. Wehr E, Trummer O, Giuliani A, Gruber HJ, Pieber TR, Obermayer-Pietsch B. Vitamin D-associated polymorphisms are related to insulin resistance and Vitamin D deficiency in polycystic ovary syndrome. *Eur J Endocrinol.* 2011;164:741–9
25. Thys-Jacobs, S., Donovan, D., Papadopoulos, A. et al. (1999) Vitamin D and calcium dysregulation in the polycystic ovarian syndrome. *Steroids*, 64, 430–435.
26. Ghadimi R, Esmaeilzadeh S, Firoozpour M, Ahmadi A. Does vitamin D status correlate with clinical and biochemical features of polycystic ovarysyndrome in high school girls? *Caspian J Intern Med.* 2014;5(4):2028
27. Middle East Fertility Society Journal volume 24, Article number: 5 (2020).
28. M. Thill, S. Becker, D. Fischer, T. Cordes, A. Hornemann, K. Diedrich, et al., Expression of prostaglandin metabolising enzymes COX-2 and 15-PGDH and VDR in human granulosa cells, *Anticancer Res.* 22009(29);3611–3618.