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Original Article

Does Vitamin D Deficiency have a Role in Resistance to Ovulation Induction in Polycystic Ovary Syndrome Patients?

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Abstract:

Vitamin D deficiency strongly predisposes to a high chance of developing polycystic ovary (PCOS) women generally and specifically in resistance to ovulation induction. The frequency of vitamin D deficiency occurrence in women with PCOS ranges between 67% and 85%, with serum concentrations of 25(OH)D <20 ng/ml).

Aim and objectives: to detect the frequency of vitamin D deficiency occurrence in resistant cases of pcos, Association between body mass index and vitamin D deficiency and Impact of vitamin D supplementation on improvement of response to ovulation induction.

Patients and Methods: The research was a randomized prospective clinical trial that took place in (Obstetrics &Gynecology) department, Sohag University Hospital from November 2019 to February 2023.

Results: There was a statistically significant difference among two studied groups regarding Serum vitamin D, Signs of Ovulation, (Relation between ovulation and BMI), (Relation between pregnancy occurrence and Duration of infertility) and (Relation between pregnancy occurrence and AMH). There was no statistically significant difference among the two studied groups regarding Socio-demographics, (Period of infertility, type of infertility and phenotype of PCOS) and Laboratory investigations.

Conclusion: vitamin D deficiency occurs more frequently in resistant cases of polycystic ovary syndrome (PCOS) and represents about more than 80% of resistant cases while about 67% of nonresistant PCOS. Vitamin D supplementation shows improvements for patients with PCOS and vitamin D deficiency in the response to ovulation induction and pregnancy rate.

Key words: vitamin D deficiency, ovulation, polycystic ovary syndrome.

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Introduction

Polycystic ovary syndrome (PCOS) is the most popular endocrine disorder among women of reproductive age, with a rate of occurrence ranging between 6–10% in the general population .[1]

There are multiple regimens and lines of treatment and management of PCOS. First of all body weight reduction even 5% will improve outcome, oral ovulation induction specially letrozole which is considered first choice, gonadotropins and surgical ovarian drilling. [2, 3]

There are possible causes and risk factors associated with resistance of PCOS patients as obesity, insulin resistance, hyperandrogenism, insulin like growth factor, high free androgen index ,low serum FSH, high lutinzing hormone that stimulate theca cells to secrete more androgen that change

the milieu of the ovary , raised ratio of LH:FSH also affects the frequency and amplitude of gonadotrophin releasing hormone that affect the growth of follicles. [4]

Few clinical studies recently, focused on vitamin D supplementation as an adjuvant treatment for resistant cases of PCOS and its effect on ovulation and pregnancy. [5]

There is a recent meta-analysis published on February 2020 about vitamin D supplementation impacton PCOS by Chen-Yun Miao who gathered all the relevant studies regarding vitamin D in pcos. A total and more recent 11 studies involving 483 participants were included concluding more improvement in the levels of total testosterone, insulin resistance, the results failed to show positive effect on body mass index , further randomized controlled trial needed to assess the effect on improvement of pcos specially who show resistance to oral ovulation induction , and this point was be our point to evaluate addition of vitamin D as adjuvant therapy to ovulation induction drugs .[6]

This work focused on detecting the prevalence of vitamin D deficiency in resistant cases of pcos, Association between body mass index and vitamin D deficiency and Effect of vitamin D supplementation in improvement of response to ovulation induction.

PATIENTS AND METHODS

This study was a randomized prospective clinical trial that took place in (Obstetrics &Gynecology) department , Sohag University Hospital from November 2019 to February 2023 . 130 cases were recruited according to Sample size equation. Sample size justification by Using PASS II program for sample size calculation.

Sample size = $z^2 *P(1-P)/e^2 /1+(z^2 *p(1-p)$

The recruited participants went through a random distribution into two groups. Group I; involved (60) Patients with vitamin D deficiency who took **vitamin D. Patients were given vitamin D_2 (Oss Fortin 10000 I.U tablets Eva Pharma)** every other day for three months. Group II (n=70): Patients with vitamin D deficiency who weren't given vitamin D. Each group was given Letrozole (2.5mg two times a day for up to 5 days from the 2^{rd} day of cycle and folliculometry was done and triggering of ovulation by 5000iu HCG) for three consecutive cycles.

Inclusion criteria

Married women who have history of infertility of more than one year and seeking for fertility consistent with the diagnostic criteria fulfilling the diagnosis of polycystic ovary syndrome depending on Rotterdam-ASRM criteria with at least two criteria from the following chronic dis/anovulation and menstrual disturbances, hyperandrogenism either clinical assessment or biochemical methods and polycystic ovarian morphology and the antral follicle count is at least 12 follicle per ovary or and ovarian volume more than 10 cm^3 and their age ranges from (18-40) years. [7]

Those with a history of clomophine citrate or Letrozole resistance according to Legro et al, 2014 who mentioned that those patients who received Letrozole from 5mg up to 7,5 mg per day for five days for three consecutive cycles and failed to show signs of ovulation are considered to be Letrozole resistant.[8]

No drugs affecting metabolic parameters, including metformin, corticosteroid, vitamin D, calcium and multivitamin were administered for at least 6 months prior to study.

Exclusion criteria

Extremes of ages (<20 years or >40 years), Male factor infertility, Those who have congenital anomalies in the uterus, Structural abnormalities of the uterus as Fibroid, adenomyosis and endometriosis, Chronic diseases, Any endocrinopathy (hyperprolactinemia ,adult onset congenital adrenal hyperplasia, Cushing syndrome thyroid dysfunction), Tubal factor infertility and Premature ovarian insufficiency

Ethical Consideration

Approval was obtained by Medical Research Ethical Committee of Faculty of Medicine Sohag University, and all participants were asked to fill an informed written consent prior to participation.

All participants went through the followings

Informed consent was taken after comprehensive discussion and clarification of all of the steps in the study, history taking & demographic data collection, Complete physical & local examination, Husband semen analysis, Assessment & insurance of tubal patency, Trans vaginal ultrasound (TVS) and Laboratory Investigations(hormonal assay)

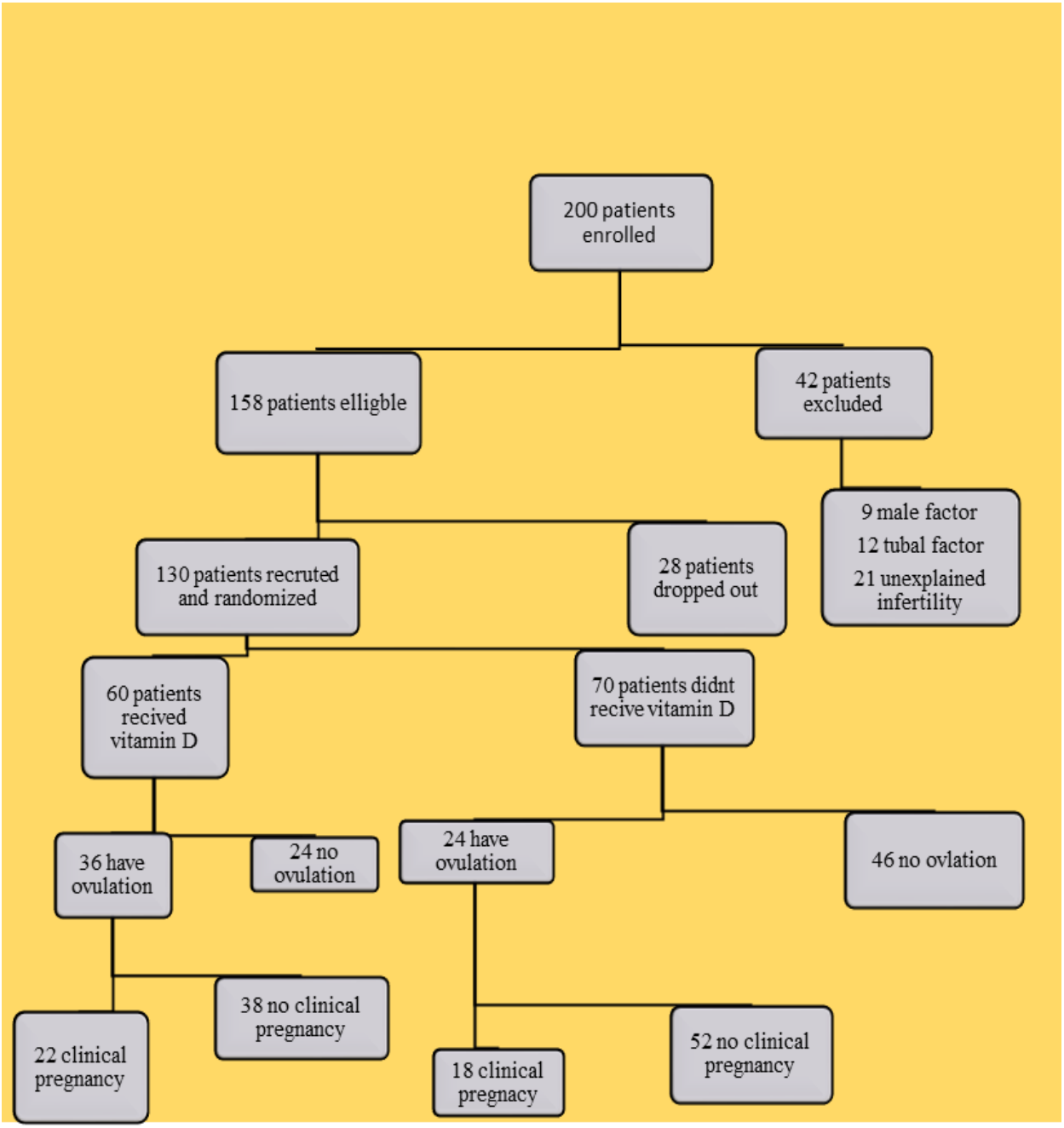
Biochemical assessment of serum vitamin D for all enrolled patients was done using Mini VIDAS

device (made in Italy 2015, serial number: IVD5206613).

Statistical analysis

Statistical analysis was completed using SPSS v26 (IBM Inc., Chicago, IL, USA). The quantitative variables were expressed as the mean and standard deviation (SD) and compared among the two groups using an unpaired Student's t-test. The qualitative variables were displayed as frequency and percentage (%) and analyzed using either the Chi-square test or Fisher's exact test, depending on the situation. A Pearson correlation analysis was conducted to assess the extent of correlation between two quantitative variables. A two-tailed P value less than 0.05 was deemed to be statistically significant.

Results:



Flow chart of recruited cases in the study

Table 1: Sociodemographics of the studied groups

		Group D (n=60)	**Group N (n=70)**	**P value**
Age (years)	**Mean ± SD**	27.65 ± 4.65	28.41 ± 4.04	0.318
	Range	20 – 38	21 – 37	
BMI (Kg/m^2)	**Mean ± SD**	26.45 ± 5.32	27.46 ± 6.27	0.53
	Range	17 – 40	16 – 45	
Family history of PCOS	**PCOS**	10 (16.67%)	13 (18.57%)	0.777
	No	50 (83.33%)	57 (81.43%)	

This table showed that both groups were comparable as regards age, BMI, family history of PCOS. About 77% of recruited cases were younger than 35 years old cases and mean of age in both groups was 27.65±4.65 and 28.41±4.04 respectively

Table 2: shows the different prevalence of normal, insufficient and deficient serum vitamin D in polycystic ovary syndrome patients and resistant PCOS and serum level of vitamin D in both groups

Serum Vitamin D	**Pcos cases (n=525)**	**%**	**Resistant pcos (n=158)**	**%**	**P value**
Normal >30ng/ml	168	32%	28	17.72%	**0.01**
Insufficient 20-30ng/ml	148	28.19%	34	21.5%	0.427
Deficient <20ng/ml	209	39.81%	96	60.7%	**0.003**
Serum vitamin D mean±SD range	22.7±6.78 11.4-42.5		15.5±5.54 3.4-36		**0.005**

This table showed that serum level of vitamin D concentration was significantly less in resistant PCOS women than in non-resistant PCOS. In addition, the frequency rate of vitamin D deficiency occurrence and insufficiency were significantly more in resistant cases than non-resistant PCOS. Furthermore, the prevalence of normal serum vitamin D in resistant PCOS was significantly less in non-resistant PCOS.

3: Duration of infertility, type of infertility and phenotype of PCOS of the studied groups

		GroupI (n=60)	GroupII (n=70)	P value
Duration of infertility (years)	**Mean ± SD**	4.07 ± 2.65	4.74 ± 2	0.103
	Range	1.5 – 13	2 – 10	
Type of infertility	**Secondary**	11 (18.33%)	13 (18.57%)	0.972
	Primary	49 (81.67%)	57 (81.43%)	
Phenotype of PCOS	**A**	24 (40%)	24 (34.29%)	0.857
	B	9 (15%)	14 (20%)	
	C	13 (21.67%)	16 (22.86%)	
	D	14 (23.33%)	16 (22.86%)	
Score of hirsutism	**Mean ± SD**	8.88 ± 3.18	9.3 ± 3.15	0.456

This table showed that whether 1ry or 2ry infertility and phenotype of polycystic ovary syndrome and score of hirsutism detected by modified Ferriman Gallwey Score

The 2012 National Institutes of Health–sponsored Evidence-Based Methodology PCOS Workshop categorized PCOS into four phenotypes as follows:

-Phenotype A, hyperandrogenism, ovulatory dysfunction, and polycystic ovary morphology;
-Phenotype B, hyperandrogenism and ovulatory dysfunction;
-Phenotype C, hyperandrogenism and polycystic ovary morphology; and
-Phenotype D, ovulatory dysfunction and polycystic ovary morphology

Table 4: Laboratory investigations of both groups as a method for detection of clinical hyperandrogenism and were statically insignificant. About 81% of the cases had primary infertility.

Variable	Group 1 (n=60)	Group 2 (n=70)	P value
3rd day FSH level (mIU/L): Range **Mean±S.D**	3.5-10 6.34±1.7	3.3-9.1 5.62±1.5	0.205
3rd day LH level (mIU/L): **Range** **Mean+-S.D**	7.2-15 9±2.5	7.1-14.3 11.65±2.7	0.361
H/FSH ratio: Range **Mean+-S.D**	1.5-3 2.2±0.3	1.86-4.1 2.48±0.5	0.315
Prolactin (ng/ml): Range **Mean+-S.D**	2.3-20 10.75±3.6	6.4-21.2 10±2.3	0.218
Free testosterone pg/ml Range Mean+_SD	2.64-4.8 3.66±0.5	0.8-8 3.24±1	0.412

This table showed that there was no statistically significant variation among both groups regarding the biochemical hormonal assessment.
All the studied cases showed high level of serum LH

Table 5: detection of signs of Ovulation and clinical pregnancy percentage in both groups

		GroupI (n=60)	GroupII (n=70)	P value
Signs of Ovulation	**Positive**	36 (60%)	24 (34.29%)	**0.003***
	Negative	24 (40%)	46 (65.71%)	
Clinical Pregnancy	**Yes**	22 (36.67%)	18 (25.71%)	0.177
	No	38 (63.33%)	52 (74.29%)	

*Significant as P value ≤0.05

This table that Ovulation was statically significant higher in the first group who were given vitamin D than the other group not given vitamin D (P value=0.003), yet, clinical pregnancy rate is heigher in the first group but not statistically significant.

Table 6: show the ovulation rate between the two groups through three cycles after vitamin D supplementation in both groups

	Group 1	Percentage %	Group 2	Percentage %	P value
First cycle	N=60		N=70		
Ovulation	10/60	16.66%	6/70	8.57%	0.075
Second cycle	N=51		N=63		
Ovulation	22/51	43.14%	9/63	14.29%	**0.03**
Third cycle ovulation	N=43 33/43	76.74%	N=51 13/51	25.49%	**0.003**

Table 7: Relation between pregnancy occurrence and (BMI, duration of infertility and AMH) of the studied groups

Variables		No clinical pregnancy (n=90)	Detection of pregnancy (n=40)	P value
BMI (kg/m^2)	**Mean ± SD**	28.08 ± 6.54	26.3 ± 3.96	0.114
	Range	16 – 45	19 - 41	
Duration of infertility (years)	**Mean ± SD**	4.71 ± 2.45	3.79 ± 1.95	**0.039***
	Range	1.2 – 13	1.3 - 9	
AMH (ng/ml)	**Mean ± SD**	5.69 ± 2.23	4.49 ± 1.08	**0.002***
	Range	3.1 – 13	1.3 2.9 - 7.8	

*Significant as P value ≤0.05, BMI: body mass index, AMH: anti-mullerian hormone

BMI was insignificant between both groups while, Duration of infertility and AMH were significantly higher in No Pregnancy occurrence than pregnancy occurrence.

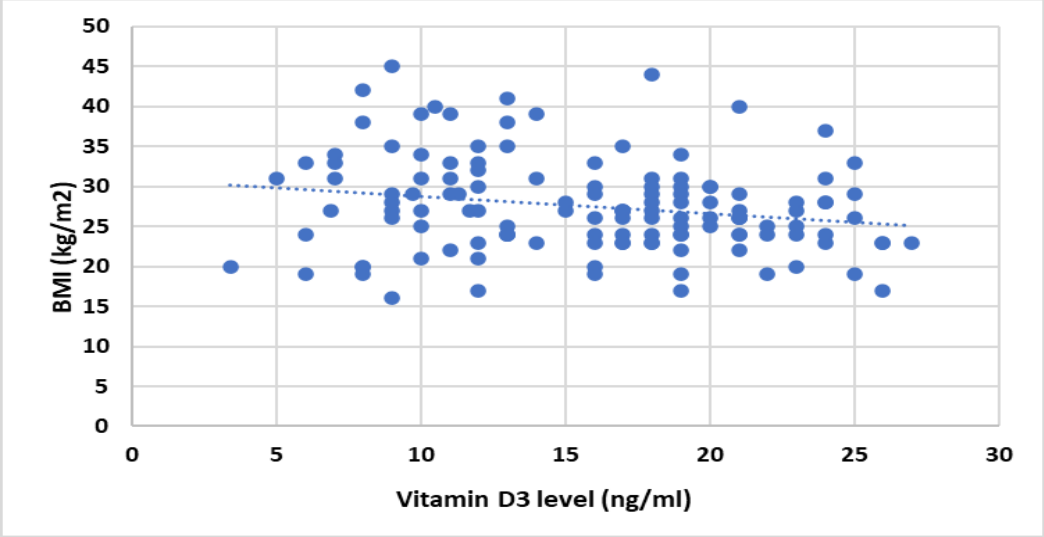


Figure 1: Correlation between Vitamin D 3 level and BMI of the studied groups

There was negative correlation between vitamin D and MBI.

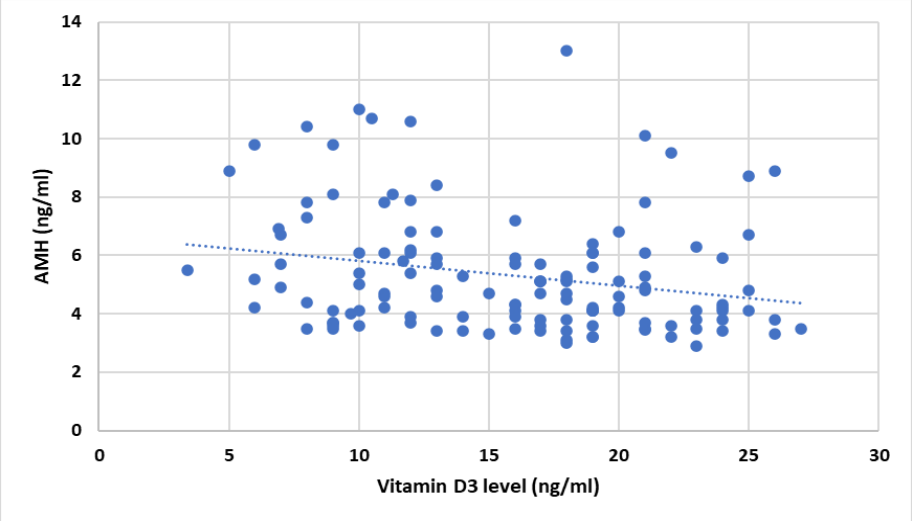


Figure 2: Correlation between Vitamin D 3 level and AMH of the studied groups

There was negative correlation between serum vitamin D and AMH

Discussion

Polycystic Ovary Syndrome (PCOS) is a popular endocrine disorder that occurs in more than 10% of women of reproductive age. PCOS is the primary etiology of anovulatory infertility with clinical symptoms, including anovulation or disovulation, infertility, menstrual irregularity, and hyperandrogenism**.** [(9)]

Vitamin D enhance the ovulation response and increase the number of mature follicles through its effect on improvement of hyperinsulinemia and insulin resistance in those patients and decrease the hyperandrogenism and its effect on steroid hormones and aromatase enzyme and to some extent its effect upon AMH hormone that recently highlights on its role in ovulation resistance and prognosis of PCOS and its role as a substitution for polycystic ovarian morphology in some patients. [(10)]

In our findings, the frequency of vitamin D deficiency and insufficiency occurrence reached about 81% of resistant cases of PCOS to ovulation induction while only 67% of abnormal serum vitamin D was detected in PCOS patients in whom no history of ovulation resistance. There was statistically significant difference among the resistant and nonresistant cases of serum level vitamin D (22.7±6.78 versus 15.5±5.54, P value =0.005)

Baseline sociodemographic data of the studied groups of patients:

Age: seventy seven of cases were less than 35 years. It was proved that age is an important factor in determining response to ovulation induction as the quantity and quality of oocytes are changing in PCOS patients . The symptoms improved with age this is according to Malgorzata et al who performed a small cohort observational study on 35 patients with PCOS and evaluated all aspects of PCOS and found that there was improvement in forth decade than third decade in symptoms of PCOS and response to ovulation **.** [(11)]

Body Mass Index: in our study the recruited cases included all types of body built and BMI range from 16-45kg\m^2, more than 37% of patients were obese, more than 21% of patients overweight, while morbid obese about 13% and less than 15% are lean ie, more than 70% of PCOS patients in this study had increase in body mass index and obesity was more common, this was in agreement with Yonggang et al. who detected the prevalence of obesity in PCOS was about 67%. [(12)]

In our findings, a negative association between serum level vitamin D and BMI (p = 0.018) was noted. Explanation of the association between obesity and vitamin D deficiency, those with high body mass index vitamin D will be sequestered in adipocyte .[(13)]

Period of infertility lies between 1.5 and 13 years and the mean of infertility period was about 4.5 years and more than of 80% of patients were suffering from primary infertility this agreed with Seyedoshohadaei et al**.** [(3)]

Phenotypes distribution of PCOS in our study were as following: about 37% of cases were phenotype (A), about 17% of cases were (B), about 22% were (c) and about 23% of cases were phenotype (D) this was in agreement with Gluszak et al. in which phenotype A was the most prevalent , (A) was the worst prognosis of all phenotypes of PCOS. [(14)]

In our study all of the patients exhibited a raised LH level, and 22% of them had low level FSH , 58% of them had elevated LH/FSH ratio greater than 3 and about 12% of them had l elevation of serum prolactin from 25 to 32ng/ml.

Chen et al. and Yuan et al. found a significant decrease of serum FSH and an increase in the level of serum LH levels. [(15, 16)]

In their study they recruited 58 cases of PCOS and evaluated them as regards hormonal profile and their results showed that an elevation of serum LH and more than 30% presented by mild elevation of serum prolactin and 60% showed elevation of LH/FSH ratio more than 3, and so their results aligned with our results.

Yonggang et al. reported high level of LH and increase in LH: FSH ratio like our study.

In The Last Evidence Based Medicine, serum AMH have a role in diagnosis of polycystic ovary syndrome. [(12)]

High level of AMH may be used instead of polycystic ovarian morphology in diagnosis of PCOS in adults. Also, its role as prognostic factor to ovulation induction and in certain critical level is considered as one of the factors of resistance to ovulation induction and failure of response. [(10)]

In our study, high level of AMH affect the response of ovulation induction in PCOS and

there is growing evidence that AMH increase the resistance to ovulation induction.

This was in agreement with Irani et al. observational pilot study was to investigate whether baseline serum AMH levels predicted the development of ovulation after LZ therapy in infertile PCOS patients. In this study women with PCOS and AMH levels≥ 4.53 ng/mL had lower chances to develop ovulation .[(5)]

Our study documented a negative association between serum vitamin D and AMH, which is consistent with the finding of Butts et al. who observed a considerable association between vitamin D deficiency and mean AMH since a negative correlation between serum vitamin D and AMH was noted. [(17)]

Our findings addressed a statistically significant difference increase in detection of ovulation rate in cumulative cycles. The cumulative ovulation rate detected in group (I) was about (60%)of cases versus (34.3%) of cases in the other group with p-value(0.003).

Our results agree with Yang et al. who found that the ovulation rate & pregnancy rate improved with vitamin D supplementation . They hypothesized that administering vitamin D supplements to people with polycystic ovary syndrome (PCOS) could enhance their chances of getting pregnant, which could be achieved by reducing inflammation in the granulosa cells, improving ovulation, and increasing the thickness of the endometrial lining.. [(18)]

Abedi et al. in their clinical study performed at Isfahan University of Medical Sciences and their candidate had PCOS prepared for ICSI. Their age ranged from 18 to 38 years and with Vitamin D deficiency. They proved that Vitamin D supplementation in PCOS patients with vitamin D deficiency could improve the clinical result of ICSI as regards the mean diameter of the growing follicles and number and its effect upon endometrial thickness .[(19)]

Conclusion

vitamin D deficiency occurs more frequently in resistant cases of polycystic ovary syndrome (PCOS) and represents about more than 80% of resistant cases while about 67% of non resistant PCOS. Vitamin D supplementation in patients with PCOS and vitamin D deficiency improves the response to ovulation induction and pregnancy rate.

Limitations

- The sample size was relatively small, which may limit the generalizability of the findings to a larger population of PCOS patients.
- The study was conducted at a single center, which may introduce bias and limit the external validity of the results.
- The study did not assess long-term outcomes, such as live birth rates or pregnancy complications, which could provide a more comprehensive understanding of the impact of vitamin D supplementation.
- The study did not evaluate other potential confounding factors, such as dietary habits, sunlight exposure, or
- other comorbidities, which may influence the relationship between vitamin D deficiency and PCOS outcomes.
- The study did not investigate the mechanism by which vitamin D supplementation affects ovulation response, and further research is needed to elucidate the underlying pathways involved.
- The study did not consider different dosages or durations of vitamin D supplementation, and it would be valuable to explore optimal regimens in future studies.

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