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Effect of Vitamin D on Hormonal Factors in Women with Polycystic Ovary Syndrome (PCOS): Systematic Review and Meta-analysis

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ABSTRACT
Women with PCOS experience hormonal disorders, one of the causes is vitamin D deficiency. Total levels of vitamin D have a negative correlation with hormonal factors in women with PCOS.
Search for articles through the Science Direct, PubMed, and Pro Quest databases using the Boolean operators (AND and OR) system. The study inclusion criteria were publication with a randomized controlled trial design from 2015 to 2020, female study participants with PCOS aged 18 to 49 years, the intervention given was vitamin D compared to placebo, or other interventions. The results of the measurement of hormonal factors were the levels of LH, LH / FSH ratio, TT, FT, SHBG, and DHEAS.
Twelve studies met the eligibility criteria and were analyzed. Provision of vitamin D significantly affected LH levels (WMD,−2.16; 95% CI,−3.87 to−0.45, P = 0.01), and LH / FSH ratio (WMD,−0.28; 95% CI,−0.38 to−0.19, P <0.00001). Vitamin D intervention did not significantly affect the levels of TT, FT, SHBG, and DHEAS.
Vitamin D affects the levels of LH and LH / FSH ratio of hormonal factors in women with PCOS.

Keywords: Polycystic Ovarian Syndrome, Randomized Controlled Trial, Vitamin D

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BACKGROUND

Polycystic Ovary Syndrome (PCOS) is a disease that occurs due to an imbalance in the hormonal system or endocrine disorders. The prevalence of PCOS reaches 4-12% of women of reproductive age (Hestiantoro, Natadisastra, Wiweko, Sumapraja, & Harzif, 2014). PCOS is a disorder consisting of several clinical variants and genetic predispositions which are then grouped together into the PCOS phenotype (Sneeringer & Wright, 2018). PCOS diagnosis based on the Rotterdam criteria in 2003 includes two of three things, namely ovulatory dysfunction, hyperandrogenism, or polycystic ovaries. (Rausch & Dokras, 2012).

One of the main components of PCOS is hyperandrogenism which is characterized by clinical and biochemical symptoms. The clinical symptoms experienced by PCOS patients with hyperandrogenism include hirsutism, acne, and alopecia. Meanwhile, the biochemical symptoms experienced by PCOS patients include an increase in androgen hormones and a decrease in levels of Sex Hormone Binding Globulin (SHBG). The measured total testosterone (TT) and free testosterone (FT) hormone levels are usually within the normal range above or slightly above the normal range.

Women with PCOS have an estimated 90% increase in androgen levels. The androgen hormones measured include total testosterone (TT), free testosterone (FT), and dehydroepiandrosterone sulfate (DHEAS) levels. The marker for adrenal androgen production was also slightly elevated in women with PCOS (Rausch & Dokras, 2012). Androgen synthesis occurs in theca cells stimulated by Luteinizing Hormone (LH) and mediated by microsal P450c17. Changes in P450c17 activity at the transcription and posttranscription levels have been implicated in the etiology of PCOS. The excessive ovarian response to LH is further amplified by an increase in the amplitude and frequency of LH levels in PCOS.

Local ovarian androgens in patients with PCOS are converted to the stronger 5α reduced androgens which cannot be converted to estrogen. These androgens inhibit aromatase activity and induction of follicle stimulating hormone (FSH) from LH receptors on granulosa cells preventing follicle development. The development of the hyperandrogenic state in PCOS patients is also due to the presence of hyperinsulinemia and obesity which can also potentiate more LH activity. Circulating androgens are increased due to direct ovarian stimulation by hyperinsulinemia and by decreased SHBG production (Palomba, 2018).

About 67-85% of women with PCOS have a vitamin D deficiency (Thomson, Spedding, & Buckley, 2012). Vitamin D can also decrease intrafollicular androgens and increase follicle sensitivity to FSH. This can be done because vitamin D can increase the production of Advanced Glycation End Products receptors and reduce AMH levels that are elevated abnormally in PCOS patients. Advanced Glycation End Products (AGEs) have a role in the pathogenesis of PCOS. Serum levels of AGEs are elevated in PCOS patients. AGEs accumulate in the theca cells and granulosa lining of the ovaries of women with PCOS resulting in worsening growth of ovarian follicles. Increased levels of vitamin D can increase the AGEs receptor (sRAGE) so that it can bind to circulating AGEs and inhibit the bad effects of the inflammatory process that occurs. Decreased levels of Anti Mullerian Hormone (AMH) due to vitamin D supplementation in PCOS patients have the potential to increase the ovulation process because it decreases intra follicular androgens and increases follicle sensitivity to FSH (Tehrani & Behboudi-gandevani, 2017).

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METHOD

Study design

This study uses a systematic review meta-analysis design based on the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) guide.

Inclusion and exclusion criteria

The inclusion criteria included articles using the 2015 to 2020 Randomized Controlled Trial study design focusing on women with PCOS who were either vitamin D deficient or not. Articles are presented in English and in full text. The intervention used vitamin D supplementation as compared to placebo or other interventions. The measurement results assessed were LH levels, LH / FSH ratio, TT, FT, SHBG, and DHEAS levels. Exclusion criteria included articles that did not review women with PCOS, interventions focused on pharmacological management and physical exercise, and did not measure hormonal factors in PCOS patients.

Search Strategy and Engines

The search for research articles was reviewed based on the ProQuest, ScienceDirect and PubMed databases from 2015 to 2020. The keywords used for the article search were "Polycystic Ovarian Syndrome", "vitamin D" and "Randomized Controlled Trial" combined with the Boolean operators (AND and OR) system and adjusted to the Medical Subject Heading (MeSH).

Study Selection and data extraction

The author evaluates the title and abstract of each article. If the article has the potential to meet the requirements, the author will screen it based on the eligibility criteria and full text. The authors document the reasons for the exclusion of articles based on the type of population, intervention, outcome or type of study design that was not appropriate. The author then extracts data on articles that meet the eligibility criteria based on: author's name, year of publication, country, study design, type and number of population, age, body mass index, type of intervention, comparator, duration of intervention, and measured outcome.

Risk of Study Bias

The authors assessed the risk of bias for each article using the Cochrane risk of bias tool to evaluate randomization performance and methods, allocation concealment, extent of blinding (participants, data collectors, outcome assessors, and data analysts), incomplete outcome data, selective reporting and other bias. The evaluations were scaled as low, unclear, and high risk of bias, according to criteria for judging risk of bias provided by the Cochrane handbook.

Statistical analysis

The data analyzed in the form of continuous data are presented in the form of the mean and standard deviation of each analyzed study. The results of continuous data analysis were weight mean differences (WMD) with 95% confident interval (CI). Continuous data were combined using the inverse variance model and the heterogeneity of the data for all articles was carried out using the Cochrane's Q-test with $p > 0.05$ and $I^2 < 50\%$ indicating statistical homogeneity. If the Cochrane's Q-test results show homogeneity data then a fixed effect model is used to calculate the total effect size, but if the test results show heterogeneous data, a random effect model is used. The results of the analysis were said to be statistically significant if the p value of 0.05 and 95% CI did not contain zero (0). The software used to perform the meta-analysis was RevMan 5.4.1 provided by Cochrane.

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RESULT

Study selection result

The flow chart for selecting research articles according to the inclusion and exclusion criteria is shown in Figure 1. Searching articles in three databases using predetermined keywords resulted in 1951 articles. The author conducted a duplication check and the remaining 1863 articles. Furthermore, the authors conducted a screening based on the title and abstract of the remaining 117 articles. The author conducted a screening based on the full text and the eligibility criteria for the remaining 12 articles to be analyzed quantitatively.

Study characteristics

The characteristics of the included studies are summarized in Table 1. The study was conducted among the same population of women with PCOS. 10 articles analyzing vitamin D interventions independently or in combination with other interventions such as a low-calorie diet, omega-3 fatty acids from fish oil, metformin, magnesium, zinc, calcium, probiotics, and vitamin K2 compared to placebo. 2 articles analyzed vitamin D intervention alone or in combination with metformin versus metformin. Vitamin D is given at a dose of 1µg / day - 50,000 IU / 1-2 weeks for 2 - 6 months. 10 studies used Rotterdam criteria for the diagnosis of PCOS. 1 study used the ESHRE / ASRM diagnosis and 1 used the Androgen Excess Society. The study was conducted in America, Iran, Syria, Jordan, Austria and Slovakia.

Figure 1. Flow diagram of the literature search for the effect of vitamin D on hormonal factors in PCOS patients

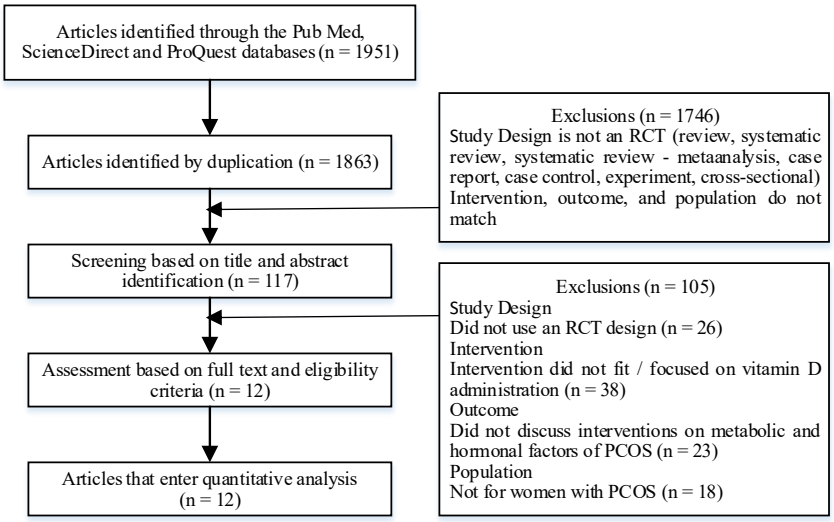
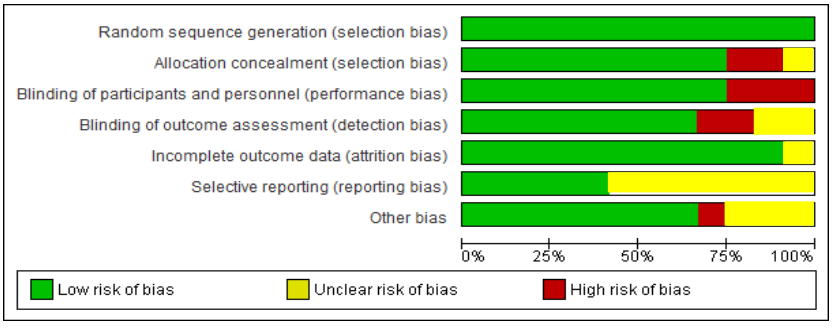
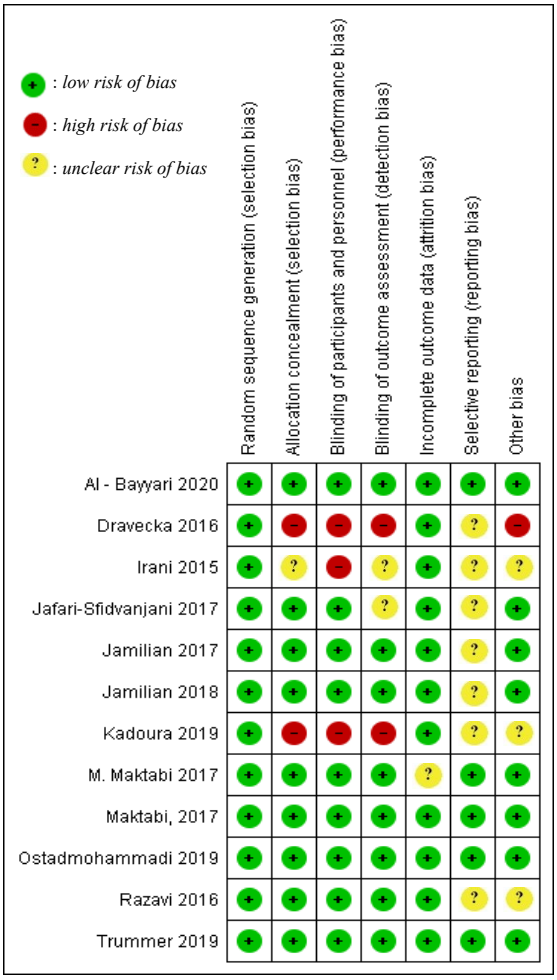


Figure 2. The results of risk assessment of biased studies for systematic review – metaanalysis





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Study bias risk assessment

The risk assessment of study bias is presented in Figure 2. 12 studies had adequate randomization methods, and 9 studies had adequate hidden allocations throughout the research process. 9 studies had double blindness for participants and personnel. 8 studies had blinding in outcome rulers. 11 studies reported attrition during follow-up. 5 studies did not perform selective reporting. 8 studies had a homogeneous baseline characteristic value and a well-defined study design and 2 studies performed an intention to treat analysis.

Meta-analysis

Levels of LH and LH / FSH ratio

There are 4 studies analyzing the effect of vitamin D on LH levels. The results of the data heterogeneity test showed that the data were homogeneous ($p = 0.26$, $I^2 = 25\%$). The results of the fixed effect model analysis showed that vitamin D could significantly reduce LH levels (WMD,−2.16; 95% CI,−3.87 to−0.45, P = 0.01). The LH / FSH ratio was also significantly decreased after vitamin D intervention compared to the control group in the meta-analysis of 3 studies (WMD,−0.28; 95% CI,−0.38 to−0.19, P <0.00001) and the data were homogeneous ($p = 0.34$, $I^2 = 11\%$).

Table 1. Characteristics of included studies

No	Author & country	Study Design	Population (n)	Age	BMI	Intervention (n)	Control (n)	Time duration	Main Outcome
1	(Irani et al., 2015) USA	randomized, placebo-controlled trial.	PCOS (ESHRE/ ASRM) + VDD (n=53)	18 – 38 years	Group 1: 30 ± 1 kg/m^2 Group 2: 28 ± 1.6 kg/m^2	1. 50 000 IU vitamin D3 (n = 35)	2. Placebo (n = 18)	Once a week in 8 weeks	DHEAS, TT, SHBG, FT, LH/FSH, LH
2	(Sfidvajani et al., 2017) Iran	double-blind randomized placebo-controlled trial	PCOS (Rotterdam Criteria) with VDD + overweight/obese (n=54)	20 – 40 years	25–40 kg/m^2	1. 50,000 IU/weeks vitamin D3 + low calorie diet (n=26)	2. Placebo + low-calorie diet/weeks (n = 28)	12 weeks	TT, SHBG
3	(Jamilian et al., 2018) Iran	randomized, double-blinded, placebo-controlled clinical trial	PCOS (Rotterdam Criteria) (60)	18 – 40 years	Group 1: 27.4 ± 3.9 kg/m^2 Group 2: 27.1 ± 7.0 kg/m^2	1. 50,000 IU vitamin D/2 weeks + 2000 mg/day omega-3 fatty acids from fish oil (n=30)	2. placebo (n=30)	12 weeks	TT, SHBG
4	(Jamilian et al., 2017) Iran	randomized, double-blinded, placebo-controlled clinical trial	PCOS (Rotterdam Criteria) with insulin resistance (n=90)	18 – 40 years	Group 1: 33 ± 5 kg/m^2 Group 2: 31 ± 6 kg/m^2 Group 3: 30 ± 6 kg/m^2	1. 1000 IU/day + metformin (n=30) 2. 4000 IU/day +metformin (n=30)	3. Placebo (n=30)	12 weeks	TT, SHBG, DHEAS
5	(Kadoura, Alhalabi, & Nattouf, 2019) Syria	randomized, single-blinded, placebo controlled clinical trial	PCOS (Rotterdam Criteria) (n=34)	18 – 30 years	Group 1: 25.48 ± 4.97 kg/m^2 Group 2: 28.01 ± 4.41 kg/m^2	1. metformin 500 mg (1x/ day weeks to 1, 2x/day weeks to 2, 3x/day weeks to 3 – 8) + calcium carbonate 1000 mg/day + vitamin D3 6000 IU/day (n=18)	2. metformin (500 mg 1x/day in weeks I and 500 mg 2x/day in weeks II) and placebo (n=16)	8 weeks	LH, LH/FSH

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6	(Maktabi, Jamilian, & Asemi, 2017) Iran	Randomized, Double-Blind, Placebo-Controlled Trial	PCOS (Rotterdam Criteria) (n=60)	18 – 40 years	Group 1: 24.2 ± 3.8 kg/m^2 Group 2: 25.6 ± 4.8 kg/m^2	1. 100 mg magnesium, 4 mg zinc, 400 mg calcium+ 200 IU vitamin D (n = 30),	2. Placebo (n = 30)	Twice a day for 12 weeks	TT, SHBG
7	(Maktabi, Chamani, & Asemi, 2017) Iran	Prospective, randomized, double-blind, placebo-controlled clinical trial	PCOS (Rotterdam Criteria) with VDD (n=70)	18 – 40 years	Data not showed	1. 50.000 IU vitamin D/2 weeks (n=35)	2. Placebo (n=35)	12 weeks	TT, FT, SHBG, DHEAS
8	(Ostadmohammadi, Jamilian, Bahmani, & Asemi, 2019) Iran	randomized, double-blinded, placebo-controlled clinical trial	PCOS (Rotterdam Criteria) with BMI; 17–34 kg/m^2 + insulin resistance: 1.4–4 (n=60)	18 – 40 years	Group 1: 24.3 ± 4.2 kg/m^2 Group 2: 25.1 ± 4.9 kg/m^2	1. 50,000 IU vitamin D /2 weeks + 8×109 CFU/day probiotic (n=30)	2. placebo (n=30)	12 weeks	TT, SHBG
9	(Al-Bayyari, Al-Domi, Zayed, Hailat, & Eaton, 2020) Jordan	Prospective, randomized, double-blind, placebo-controlled clinical trial	PCOS (Rotterdam Criteria) with BMI: 25 – 29.9 Kg/m^2 + VDD (58)	18 – 49 years	Group 1: 27.3 ± 1.9 kg/m^2 Group 2: 26.9 ± 1.6 kg/m^2	1. 50.000 IU vitamin D/weeks (n=29)	2. Placebo (n=29)	12 weeks	TT, SHBG
10	(Trummer et al., 2019) Austria	single-center, randomized, double-blind, placebo-controlled trial	PCOS (Rotterdam Criteria) with Vit.D insufficiency (n=180)	≥ 18 years	Group 1: 27.3 ± 7.4 kg/m^2 Group 2: 28.3 ± 7.8 kg/m^2	20,000 IU cholecalciferol/ weeks equals 50 drops of Oleovit D3/ weeks oil (n = 119)	1. Placebo (n=61)	6 months	TT, dan FT
11	(Dravecká et al., 2016) Slovakia	Randomized Clinical Trial	PCOS (AES) + VDD + HOMA-IR > 2.5 (n=32)	Group 1: 29.3±4.89 years Group 2: 29.2±5.42 years Group 3: 27.6±4.96 years	Group 1: 32.24±6.71 kg/m^2 Group 2: 33.15±7.11 kg/m^2 Group 3: 29.85±5.43 kg/m^2	1. alfacalcidiol 1 µg/day (n=9) 2. alfacalcidiol 1 µg/day + metformin 1700-2550 mg/day (n=11)	3. metformin 1700-2550 mg/day (n=12)	6 months	TT, FT, SHBG, DHEAS, LH, LH/FSH
12	(Razavi et al., 2016) Iran	Randomized, Double Blind, Placebo-Controlled Trial	PCOS (Rotterdam Criteria) + VDD (n=54)	18 – 40 years	Data not showed	1. 200 IU vitamin D, 90µg vitamin K2 menaquinone-7 (MK-7) and 500mg calcium (n=27)	2. placebo (n=27)	2x/day in 8 weeks	LH, FT, DHEAS

VDD: Vitamin D Deficiency, BMI: Body Mass Index, LH: Luteinizing Hormone, FSH: Follicle Stimulating Hormone, TT: Total Testosterone, FT: Free Testosterone, SHBG: Sex Hormone Binding Globulin, DHEAS: Dehydroepiandrosterone Sulfate

Levels of Total Testosterone and Free Testosterone

The effect of vitamin D intervention on total testosterone levels was described by 10 studies and only 4 studies analyzed free testosterone levels between the vitamin D group compared with the control group. As depicted in Figure 5 and Figure 6, the results of the meta-analysis showed no significant effect of vitamin D supplementation on total testosterone and free testosterone levels.

SHBG and DHEAS levels

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There are total of 8 studies analyzed the effect of vitamin D on SHBG levels and only 5 studies analyzed DHEAS levels between the vitamin D group compared with the control group. The results of the meta-analysis showed no significant effect of vitamin D supplementation on SHBG and DHEAS levels compared to the control group, as depicted in Figure 7 and Figure 8.

Figure 3. Serum LH level (mIU/mL)

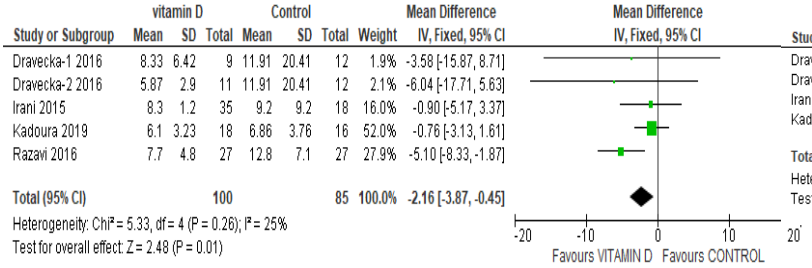


Figure 4. LH/FSH ratio

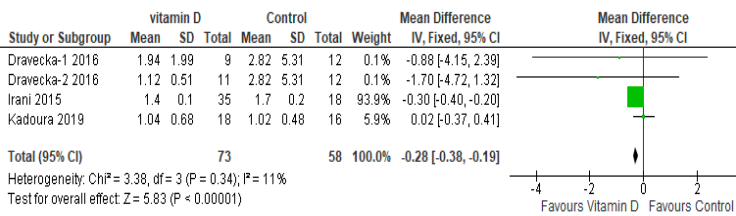


Figure 5. Serum TT level (ng/mL) (pg/mL)

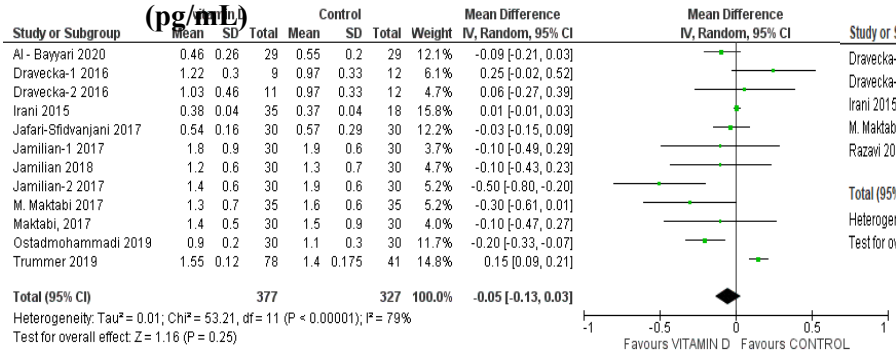


Figure 6. Serum FT level

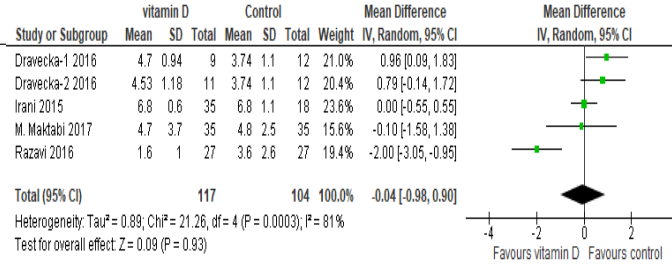


Figure 7. Serum SHBG level (nmol/L) (µg/ml)

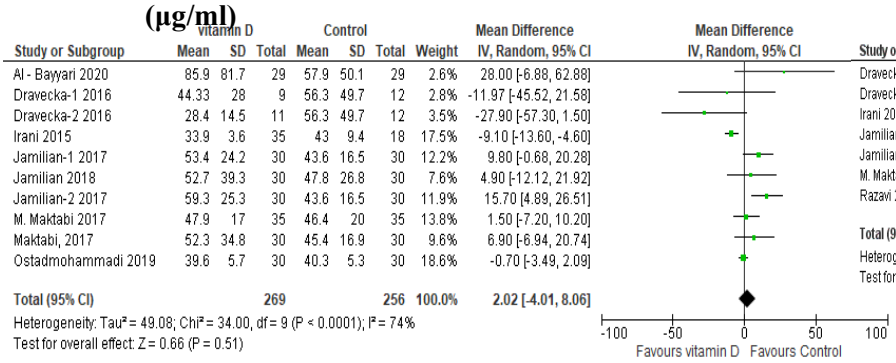
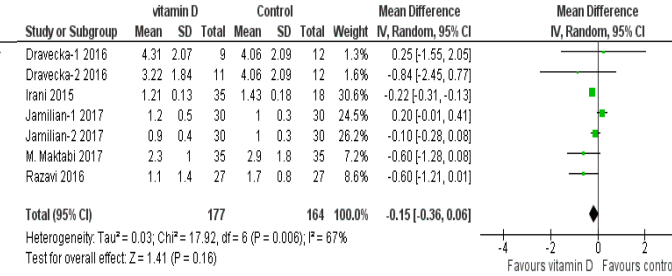


Figure 8. Serum DHEAS level



DISCUSSION

We conducted a systematic review and meta-analysis of RCTs to determine the effect of vitamin D supplementation on hormonal factors in women with PCOS. The results of this analysis showed that vitamin D supplementation could not reduce androgen hormones such as levels of TT, FT, and DHEAS and could not significantly increase SHBG levels. However, the results of this meta-analysis show that vitamin D supplementation can significantly reduce LH levels and the LH / FSH ratio.

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PCOS is one of the most common causes of irregular menstruation, and excess androgen in women, which is characterized by hyperandrogenic, chronic anovulation, infertility, irregular menstruation and hirsutism (Ross et al., 2011). Hyperandrogenism that occurs in women with PCOS is characterized by an increase in androgen hormones such as TT, FT, and DHEAS and a decrease in SHBG levels (Goldrat & Delbaere, 2018; Rosenfield, 2015). In addition to an increase in androgen hormones, hormonal disturbances that are experienced are also in the form of gonadotropin hormone imbalances characterized by an increase in the LH hormone and the LH / FSH ratio (Goldrat & Delbaere, 2018). The state of hyperandrogenism experienced by women with PCOS is closely related to the incidence of insulin resistance and hyperinsulinemia. This occurs because insulin acts as a cogonadotropin in the ovaries, facilitates the secretion of androgens from the adrenal glands and modulates the pulsality of the hormone LH. A decrease in insulin sensitivity can lead to a decrease in SHBG levels (Thaler, Seifert-Klauss, & Luppa, 2015) which causes an increase in androgen hormones. Hyperandrogensime can indicate insulin resistance and hyperinsulinemia, as well as insulin resistance and hyperinsulinemia can stimulate excessive androgen secretion in the ovaries and adrenal glands of women with PCOS (Escobar-Morreale, 2018).

Vitamin D supplementation may increase insulin sensitivity by stimulating the expression of insulin receptors in peripheral tissues (Von Hurst, Stonehouse, & Coad, 2010) and maintaining intracellular and extracellular calcium balance in the process of insulin secretion (Ardabili, Gargari, & Farzadi, 2012). Vitamin D deficiency leads to insulin resistance and diabetes which results in hyperandrogenism followed by menstrual irregularities (Firouzabadi, Aflatoonian, Modarresi, Sekhavat, & MohammadTaheri, 2012). Vitamin D receptors are located on the ovaries, fallopian tubes, and endometrium (Parikh et al., 2010). Genetic polymorphisms associated with vitamin D receptors have been associated with serum LH, SHBG, and testosterone levels (Ranjzad et al., 2011; Thomson et al., 2012; Elisabeth Wehr et al., 2011). Vitamin D may be involved in ovarian physiology by regulating the activity of genes involved in follicle development, steroidogenesis, and androgen production (Shahrokhi, Ghaffari, & Kazerouni, 2016). Low circulating 25 (OH) D, insulin resistance and hyperinsulinemia can lead to overproduction of the hormones LH and androgens resulting in worsening ovulatory dysfunction in women with PCOS.

A study in 100 women with PCOS reported a correlation between 25 (OH) D levels and testosterone, DHEAS, and LH / FSH levels (Yildizhan et al., 2009). The significant correlation between vitamin D and LH / FSH ratio suggests that vitamin D status may contribute to hormone dysregulation in women with PCOS (Kozakowski, Kapuścińska, & Zgliczyński, 2014). However, another study also states that 25 (OH) D levels do not correlate with TT, FT, and Free Androgen Index, but have a significant relationship with SHBG levels (E. Wehr et al., 2009). A study conducted by Kyei showed that vitamin D3 supplementation could reduce the levels of oxidative stress in the ovaries of DHEA-induced PCOS-model mice. The reduction in oxidative stress can increase the steroidogenesis signaling pathway of vitamin D3 which causes a decrease in estradiol, progesterone, LH, FSH, and LH / FSH ratio, thereby increasing the folliculogenesis and histomorphology of ovarian tissue (Kyei et al., 2020). A meta-analysis study showed vitamin D supplementation did not influence hormonal factors in women with PCOS (He, Lin, Robb, & Ezeamama, 2015).

The strengths of this study include a comprehensive literature search. The study with an RCT design from three databases was identified using a comprehensive search strategy. However, studies also have limitations, due to the limited number of studies, small

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population size, and varying doses of vitamin D supplementation from one study to another. In addition, some studies come from different countries, continents and cultures. Most of the studies were conducted in the Muslim country namely Iran where the women mostly wear closed clothes so as to prevent sunlight to stimulate vitamin D production, which may be linked to vitamin D deficiency. Despite the limitations of this study, vitamin D supplementation is a safe and effective treatment. it may be used as an adjuvant therapy or in combination with first-line treatment therapy which may benefit women with PCOS.

CONCLUSION

This meta-analysis study shows that vitamin D supplementation can reduce LH levels and the LH / FSH ratio, but cannot reduce levels of TT, FT, SHBG, and DHEAS. Therefore, further studies with adequate sample size, longer duration of follow-up are needed to confirm the effect of vitamin D supplementation on biomarkers of hyperandrogenism in PCOS disorders and to determine the optimal dose of vitamin D supplementation.

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