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The Effects of Magnesium and Vitamin E Co-Supplementation on Hormonal Status and Biomarkers of Inflammation and Oxidative Stress in Women with Polycystic Ovary Syndrome

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Abstract

Polycystic Ovarian syndrome is also an endocrine disorder that is common in women, has complex etiology and pathophysiology. Occurs in 8-13% of women of reproductive age, possibly those who are obese, have hyperandrogenism and irregular periods. Aim to assess the effect of Vitamin E and Magnesium co-supplementation on hormonal status, biomarkers of inflammation and oxidative stress in pcos patients. An interventional study was carried out in the Obstetrics and Gynecology Department and Outpatients infertility Unit of Maternity and Pediatrics Teaching Hospital, AL-Diwaniyah – Iraq for the period from February 2024 till December 2024. 40 women were included in our study, age of them 18-40 years old, BMI >25kg/m2 and all of them pcos patients. 20 patients were given Vitamin E and magnesium for 12 weeks and other group was not given any treatment. Cases were reassessed after 12 weeks of therapy for evaluation of changes in hirsutism score, hormonal profile, biomarkers of inflammation and oxidative stress. Significantly increased level of sex hormone binding globulin (SHBG) P<0.001, plasma nitric oxide (pNO) P=0.001 and total antioxidant capacity (TAC) P<0.001. Also resulted in significant decrease in high sensitivity c- reactive protein P<0.001 in study group after 12 week. E and Magnesium was effective in reducing oxidative stress andinflammation in PCOS.

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1. Introduction

Polycystic ovary syndrome (PCOS) is an endocrine disorder that occurs in 8-13% of women of reproductive age across the world [1,2]. It is a disorder which is heterogeneous in nature with reproductive, metabolic and psychological abnormalities, caused by a combination of genetic and environmental abnormalities [3]. The primary pathological manifestations of PCOS include hyperandrogenism, insulin resistance, and ovulatory dysfunction with resulting menstrual irregularities, infertility, obesity, and metabolic disturbances [4–5].

Women with PCOS have a higher risk of developing various long-term complications such as type 2 diabetes mellitus, dyslipidaemia, hypertension, cardiovascular disease, metabolic syndrome and infertility [6,7–8]. Besides these metabolic abnormalities, chronic low grade inflammation and oxidative stress have also been thought to have a significant role in the pathogenesis of PCOS. Steady increases in levels of inflammatory biomarkers (C-reactive protein (CRP), tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6)), as well as increased levels of oxidative stress markers have been reported in PCOS women [9–10].

Vitamin E is a fat-soluble antioxidant that helps prevent oxidative damage and decrease inflammation. Previous studies have shown that Vitamin E supplementation may help to improve insulin sensitivity, the hormonal profile, ovulatory function and inflammatory status in women with PCOS [11]. Magnesium is another essential mineral that plays a role in a variety of enzymatic and metabolic activities and has been reported to positively affect insulin sensitivity, inflammation and antioxidants, which may indicate it as a therapeutic agent for PCOS management [12-13].

Since inflammation and oxidative stress are important factors in PCOS, therapies that target these mechanisms could be beneficial for metabolic and reproductive function. In this interventional study, the aim was to investigate the effects of Vitamin E and Magnesium supplementation on hormonal factors, inflammatory markers and oxidative stress markers in women suffering from PCOS.

2. Method

The study was a prospective interventional study that was carried out in the Department of Obstetrics and Gynecology at Al-Diwaniyah Maternity and Pediatric Teaching Hospital, Iraq from February to December 2024. The Rotterdam criteria were used for the diagnosis of polycystic ovary syndrome (PCOS), and a total of 40 women aged 18-40 years were included in the study. Women who were pregnant or had thyroid disease, congenital adrenal hyperplasia, androgen secreting tumors, hyperprolactinemia, diabetes mellitus, recent infection, recent use of antioxidants or nutritional supplements in the last three months or were taking anticoagulant drugs (such as warfarin) were excluded.

The study protocol was accepted by the Iraqi Board for Medical Specializations and the Ethical Approval Committee of the Teaching Hospital. All subjects gave written informed consent to participate in the study.

The two groups of women each had 20 participants. All were subjected to detailed history, clinical examination, anthropometric measurements and laboratory investigations at baseline. Vitamin E supplementation (400IU/day; Nature Plus®) was administered in the morning and Magnesium supplementation (250mg/day; 21st Century®) was given at bedtime for 12 weeks in the intervention group. The control group were not supplemented. All subjects were asked to continue with their normal physical activity and diet, and not to initiate any extra nutritional supplements during the study. The efficacy of magnesium supplementation was evaluated by serum magnesium levels. All clinical, biochemical and hormonal parameters were repeated after 12 weeks.

Standardized procedures were used to obtain anthropometric measurements. Participants were evaluated in their natural footwear and wearing light clothing. Measurements were taken using a stadiometer and recorded in centimetres and using a scale for body weight which was recorded in kilograms. BMI was defined as weight/height2 (kg/m2) and categorized using World Health Organization (WHO) classification. Sitting position blood pressure measured after 10 minutes rest, with a standard sphygmomanometer.

Clinical parameters assessed included the modified Ferriman–Gallwey (mFG) score for hirsutism. This technique is used for estimating terminal hair growth across nine body sites that are sensitive to androgen, such as the upper lip, chin, chest, upper and lower back, upper and lower abdomen, upper arms and thighs. A score of 0 to 4 was assigned to each area and a total score of 8 or higher was defined as indicative of hirsutism.

Blood samples were obtained after an overnight fast of 8–12 hours at baseline and at the end of intervention period of 12 weeks. Under Aseptic condition about 5 mL of blood was withdrawn from Anticubital vein. The samples were centrifuged and serum separated and stored at −20°C until analyzed. Total testosterone was measured in serum using an immunofluorometric assay and the sex hormone-binding globulin (SHBG) was measured in serum by electrochemiluminescence immunoassay. Enzyme-linked immunosorbent assay (ELISA) kits were used to measure high-sensitivity C-reactive protein (hs-CRP). Oxidative stress biomarkers such as plasma nitric oxide (NO) levels (measured by Griess method) and total antioxidant capacity (TAC) (measured by Ferric reducing antioxidant power (FRAP) assay) were used.

The Statistical Package for the Social Sciences (SPSS) version 26 was used for the statistical analysis. Data for continuous variables was reported as mean ± standard deviation (SD) and for categorical variables as frequencies and percentages. Independent samples t-test was used to compare between groups while paired samples t-test was used to compare within groups before and after intervention. A p value of < 0.05 was regarded as statistically significant.

3. Results

Comparison of serum magnesium (Mg) levels before and after treatment is shown in table 3.1. Regarding study group, there was significant rise in mean serum mg from (p <0.001), but in the control group, there was no significant change in mean serum mg level (p = 0.197).

Table 1. Comparison of serum magnesium (Mg) levels before and after treatment in study group and control group

Mg (mg/dl)	**Study group** ***n* = 20**	**Control group** ***n* = 20**	***p***
Baseline			
Mean ±SD	2.03 ±0.30	2.05 ±0.33	0.799 I NS
Range	1.51 -2.54	1.5 -2.6	
After 6 months			
Mean ±SD	2.26 ±0.30	2.01 ±0.35	0.021 I *
Range	1.8 -2.91	1.31 -2.5	
p-value	<0.001 Pa ***	0.197 Pa NS	

Mg: magnesium; **I**: independent samples *t*-test; **Pa**: paired-test; **SD**: standard deviation**;** ***n***: number of cases; **NS**: not significant; *: significant at $p \leq 0.05$; ***: significant at $p \leq 0.001$

Comparison of sex hormone binding globulin (SHBG) levels before and after treatment is shown in table 3.2. Regarding study group, mean SHBG showed significant rise (p <0.001); however, in the control group, there was no significant change in mean SHBG level (p = 0.812).

Table 2. Comparison of sex hormone binding globulin (SHBG) levels before and after treatment in study group and control group

SHBG (n mol/L)	**Study group** ***n* = 20**	**Control group** ***n* = 20**	***p***
Baseline			
Mean ±SD	56.29 ±7.02	56.26 ±6.10	0.320 I NS
Range	49 -71	46 -67	
After 6 months			

Mean ±SD	63.04 ±9.32	54.21 ±6.26	0.001 I ***
Range	49 -83	45 -66	
p-value	0.009 Pa **	0.812 Pa NS	

SHBG: sex hormone binding globulin; **SD**: standard deviation; **Pa**: paired-test; ***n***: number of cases; **NS**: not significant; ; *: significant at $p \leq 0.05$; ***: significant at $p \leq 0.001$

Comparison of serum testosterone levels before and after treatment is shown in table 3.3. Regarding both study group and control group, there was no significant changes in mean serum testosterone levels (p = 0.269 and p = 0.276, respectively).

Table 3. Comparison of testosterone levels before and after treatment in study group and control group

Testosterone	**Study group** ***n* = 20**	**Control group** ***n* = 20**	***p***
Baseline			
Mean ±SD	3.11 ±0.43	3.04 ±0.49	0.656 I NS
Range	2.4 -4	2 -4	
After 6 months			
Mean ±SD	3.08 ±0.38	3.07 ±0.51	0.939 I NS
Range	2.4 -3.7	2 -4	
p-value	0.269 Pa NS	0.272 Pa NS	

I: independent samples *t*-test; **Pa**: paired-test; **SD**: standard deviation**;** ***n***: number of cases; **NS**: not significant;

Comparison of serum hirsutism score before and after treatment is shown in table 3.4. Regarding both study group and control group, there was no significant changes in mean hirsutism scores (p = 0.065 and p = 0.061, respectively).

Table 4. Comparison of hirsutism score before and after treatment in study group and control group

Hirsutism score	**Study group** ***n* = 20**	**Control group** ***n* = 20**	***p***
Baseline			
Mean ±SD	12.07 ±1.64	12.16 ±2.59	0.247 I NS
Range	9.8 -16	5 -15	
After 6 months			
Mean ±SD	12.48 ±2.06	11.53 ±2.53	0.198 I NS
Range	7 -15.4	5 -15	
p-value	0.065 Pa NS	0.061 Pa NS	

SD: standard deviation; **I**: independent samples *t*-test; **Pa**: paired-test; ***n***: number of cases; **NS**: not significant; *: significant at $p \leq 0.05$

Comparison of C-reactive protein (CRP) before and after treatment is shown in table 3.5. There was significant reduction in mean CRP levels in both study and control groups, but the reduction in study group was more significant than that reported in control group, p <0.001 and p = 0.036, respectively.

Table 5. Comparison of C-reactive protein (CRP) before and after treatment in study group and control group

CRP	Study group *n* = 20	Control group *n* = 20	*p*
Baseline			
Mean ±SD	4.15 ±0.69	4.29 ±0.91	0.608 I NS
Range	3.09 -5.6	3.08 -6.23	
After 6 months			
Mean ±SD	3.82 ±0.62	4.35 ±0.89	0.037 I *
Range	3.1 -5	3.18 -6.3	
p-value	<0.001 Pa ***	0.036 Pa *	

CRP: C-reactive protein; **SD**: standard deviation; **I**: independent samples *t*-test; **Pa**: paired-test; ***n***: number of cases; **NS**: not significant; *: significant at $p \leq 0.05$; ***: significant at $p \leq 0.001$

Comparison of markers of nitric oxide levels before and after treatment is shown in table 3.6. Regarding study group, there was significant rise in mean serum nitric oxide level ($p = 0.001$). However, in the control group, there was no significant change in mean nitric oxide level ($p = 0.590$).

Table 6. Comparison of nitric oxide (NO) level before and after treatment in study group and control group

NO (µmol/L)	Study group *n* = 20	Control group *n* = 20	*p*
Baseline			
Mean ±SD	38.03 ±4.21	36.71 ±7.88	0.511 I NS
Range	31 -45	23 -50.1	
After 6 months			
Mean ±SD	40.84 ±6.41	36.78 ±7.83	0.089 I NS
Range	31 -58	23.5 -50	
p-value	0.001 Pa ***	0.590 Pa NS	

NO: nitric oxide; **SD**: standard deviation; **I**: independent samples *t*-test; **Pa**: paired-test; ***n***: number of cases; **NS**: not significant; ***: significant at $p \leq 0.001$

Comparison of markers of total anti-oxidant capacity (TAC) level before and after treatment in study group and control group is shown in table 3.7. In the study group, there was significant increase in mean TAC (p <0.001), but in the control group, the change in mean TAC was not significant ($p = 0.051$).

Table 7. Comparison of markers of total anti-oxidant capacity (TAC) level before and after treatment in study group and control group

TAC (µmol/L)	Study group *n* = 20	Control group *n* = 20	*p*
Baseline			
Mean ±SD	422.54 ±80.56	426.62 ±46.91	0.361 I NS
Range	300 -538	289 -460	
After 6 months			
Mean ±SD	449.71 ±84.34	348.99 ±45.45	<0.001 I

Range	300 -570.3	290 -460	***
p-value	<0.001 Pa ***	0.051 Pa NS	

TAC: total anti-oxidant capacity; **SD**: standard deviation; **I**: independent samples *t*-test; **Pa**: paired-test; ***n***: number of cases; **NS**: not significant; ***: significant at $p \leq 0.001$

4. Discussion

Polycystic ovarian syndrome (PCOS) is a heterogeneous, chronic disorder of the ovary which is hormonally and metabolically significant. It is a common endocrine disorder which directly leads to anovulatory infertility in women of reproductive age [6].

We evaluated the effect of co-administration of magnesium and vitamin E. From the findings of our study, it was concluded that there is significant difference in SHBG [p<0.001] in study group. The finding is also in line with the study of Izadi et al. (2019) which showed that 8 weeks of coenzyme Q10(200mg/day) with vit E (400 mg/day) supplementation to women with pcos was associated with significant increase in
SHBG(P<0.008) [14]. Also agree with that obtained by Jiaqi zhang et al .(2021), co enzyme Q10 combined with vit E and inositol in pcos patients was associated with significant increase in SHBG(P<0.03) [15] And Junhui He et al.(2024),efficacy of antioxidant supplementation in
A study by shokrpour, M. et al. found that women with pcos who took this combination of vitamin E (400mg/day) and magnesium (250mg/day) for 12 w did not experience a significant change in SHBG [17]. Also disagree with a study by maktabi et al., which demonstrated that magnesium –zinc-calcium-vit D co-supplementation for 12 w to pcos women cause no significant change in SHBG [18] and In Ebrahimi. FA et al., vit E (400IU/day) and omega
3(1000mg/day) co- supplementation for 12 w to pcos women was not affect SHBG [19].
As for the hirsutism aspect, my study revealed there to be no significant difference in hirsutism score after treatment (p=0.065). The finding is in accordance with Botros et al. who found no significant change in hirsutism score after magnesium supplementation in pcos women, Ebrahimi,FA et al.(2017) who found no significant change on hirstutism score after melatonin with magnesium co-supplementation in pcos women and Alizadeh et al.(2022) found no significant change on iron deficiency anemia after supplementation of vitamin E (400IU/day) and omega 3 (1000mg/day) in iron deficient anemia women.
There was no significant difference in hirsutism score among the pcos women[21]. This result is disagree with a study by shokrpour,M .et al.(2019),vit E (400 mg/day) with magnesium(250mg/day) for 12 w to women with pcos was associated with significant decreased in hirsutism score[17] and
maktabi et al.,which demonstrated that magnesium-zinc-calcium vit D co-supplementation after 12 weeks to pcos women significantly decreased hirsutism score[18].
There were no significant changes in serum testosterone seen in our study after treatment [p=0.269].
The result is in agreement with a study by shokrpour, M .et al.(2019),vit E (400 mg/day) with magnesium(250mg/day) for 12 w to women with pcos was associated with no significant change in serum testosterone[17], Also in agree with maktabi et al .(2018),which demonstrated that
Weeks: Magnesium, zinc, calcium, and vitamin D co-supplementation for 12 weeks in PCOS women without significant changes in serum testosterone level[18] This was in contrast to a study by Ebrahimi, FA et al. (2017) where vitamin E (400 iu/day) and omega 3 (1000mg/day) co-supplementation for 12 weeks.
to pcos women significantly improved total and free testosterone[19] and Izadi A et al . (2019) concluded that: Coenzyme Q10 (200mg/day) with or without Vit E (400iu/day) supplementation for 8 weeks to pcos women had beneficial effect on total testosterone (p<0.OO1) [16].
Low dose of magnesium and vit E and short duration of therapy may be responsible for lack of benefit for total T and hirsutism.Some of the possible mechanisms involved in the beneficial effects of Mg and vit E on ovarian functions are the antioxidant and anti-inflammatory properties of both. Regarding serum hs-CRP,the co-supplementation of magnesium and vitamin E for 12 weeks significantly decrease serum hs-CRP (p<0.001).

The result is in agreement with a study by shokrpour,M .et al.(2019),vit E (400 mg/day) with magnesium(250mg/day) for 12 w to women with pcos was associated with significant decrease in hs-CRP (p=0.001) [17], Also agree with Faraneh Af.Ebrahimi et al[2018],magnesium(250mg)

The significant decrease in serum hs-CRP was observed with Omega 3 and Vit E (400 IU) co-supplementation in 1000 mg for 12 weeks in pcos women [18] and Magnesium-Zinc-Calciumvit D co-supplementation in 220 mg for 12 weeks in pcos women [21]. A study by Reihana,M et al. (2021) has found that there was no significant difference in the level of hs-CRP after 12w of co-supplementation with melatonin and magnesium in women with pcos (p>0.35) [23]. In our study, the plasma No significantly increased [p=0.001]. The findings concur with another study by Shokrpour,M .et al.(2019),where 12 weeks of vit E (400mg/day) and magnesium (250mg/day) to women with pcos showed a significant increase in plasma NO (p=0.001) [17] , and another study by maktabi et al. (2018), where plasma NO in women with pcos was significantly increased after 12 weeks of Mg-Zn-Ca-vit D co-supplementation (p<0.001) [18] and another study by Heidari et al.(2022), 400mg/day of vit E with 250mg/day of magnesium in pcos women led to significant increase in plasma NO (p<0.001) [24] .

This finding is challenged by Ebrahimi, FA et al. who found that there was no significant difference between plasma [19] of pcos patients receiving vit E (400 iu) + omega 3 (1000 mg) co-supplementation and the control group.Ebrahimi et al. found that there were no significant changes in plasma as a result of magnesium(250mg) and zinc (220 mg) co-supplementation in women with PCOS [22]. Regarding TAC levels significantly increase in our study [p<0.001]. The result is in agreement with a study by Shokrpour,M .et al.(2019),vit E (400 mg/day) with magnesium(250mg/day) for 12 w to women with pcos was associated with significant increase in TAC level (p=0.001) [17]. Also agree with maktabi et al. (2018), which demonstrated that magnesium-zinc-calcium-vit D co-supplementation after 12 weeks to pcos women significantly increase TAC level (p<0.001) [18] and Sadeghi, F.et al.(2019), omega 3 and vit E co- supplementation in pcos women was significantly increase TAC level [25].

This result is disagreed with study by Faraneh Af.Ebrahimi et al. (2018), magnesium(250mg) and zinc (220 mg) co- supplementation in pcos women not significantly change TAC level [22]. This study had a few limitation,we did not evaluate it vit E level before and after treatment also did not assess gene expression related to inflammatory cytokines and biomarkers of oxidative stress.

5. Conclusion

In the present study, the effect of the administration of Vit E and Magnesium supplements for a period of 12 weeks was observed on the patients suffering from pcos; the following parameters were observed: SHBG, hs-CRP, plasma NO and TAC; no significant change was observed in the hirsutism score and the level of Testosterone.

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