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Original Research Article

Effect of Magnesium Supplementation on Insulin Resistance in Polycystic Ovary Syndrome: A Randomized, Single-blind, Placebo-Controlled Trial Study

Dr. Farhana Karim Satu[1*], Dr. Farzana Deeba[2], Dr. Jesmine Banu[3], Dr. Mitu Debnath[4], Dr. Fahmida Chowdhury[5], Dr. Kazi Shamim Ara[6], Dr. Asma Rumanaz Shahid[7]

[1]Consultant, Department of Reproductive Endocrinology and Infertility, Babgabandhu Shekih Mujib Medical University (BSMMU), Dhaka, Bangladesh
[2]Associate Professor, Department of Reproductive Endocrinology and Infertility, Babgabandhu Shekih Mujib Medical University (BSMMU), Dhaka, Bangladesh
[3]Professor & Chairman, Department of Reproductive Endocrinology and Infertility, Babgabandhu Shekih Mujib Medical University (BSMMU), Dhaka, Bangladesh
[4]Consultant, Department of Reproductive Endocrinology and Infertility, Babgabandhu Shekih Mujib Medical University (BSMMU), Dhaka, Bangladesh
[5]Consultant, Department of Reproductive Endocrinology and Infertility, Babgabandhu Shekih Mujib Medical University (BSMMU), Dhaka, Bangladesh
[6]Consultant, Department of Reproductive Endocrinology and Infertility, Babgabandhu Shekih Mujib Medical University (BSMMU), Dhaka, Bangladesh
[7]Assistant Professor, Department of Obstetrics & Gynecology, Kurmitola General Hospital, Dhaka, Bangladesh

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***Corresponding author:** Dr. Farhana Karim Satu **E-mail:** setufarhana76@gmail.com
Consultant, Department of Reproductive Endocrinology and Infertility, Babgabandhu Shekih Mujib Medical University (BSMMU), Dhaka, Bangladesh.

Abstract

Background: It has been revealed that low serum magnesium (Mg) is often associated with insulin Resistance (IR), cardiovascular problems, diabetes mellitus, and hypertension. Patients with polycystic ovary syndrome (PCOS) are known to have a high incidence of insulin resistance. ***Objective:*** To assess the effects of magnesium supplementation on insulin resistance in polycystic ovary syndrome. ***Methods:*** This randomized controlled study was conducted in the Department of Reproductive Endocrinology and Infertility, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, from January 2021 to December 2021. A total of 74 women diagnosed of infertility with PCOS were included in this study. Eligible women who gave their informed consent were allocated into either group A: (Magnesium oxide) or group B (placebo) on the basis of a computerized generated table system allocated into two groups 37 patients (group-A) and 37 patients (group -B). Group A received magnesium oxide & group B received a placebo for 12 weeks. After 12 weeks of treatment, both groups were advised to repeat biochemical assay for fasting glucose, fasting insulin, HOMA-IR, serum testosterone & serum lipid profile at the follow-up visit. ***Results:*** Magnesium supplementation for 12 weeks among women with PCOS had favorable effects on waist circumference (changes from baseline in the intervention group: -0.76±3.1 vs. -1.7±1.8 cm in the placebo group) and BMI (-2.13±0.98 vs. -0.32±0.52 kg/m^2) compared with the placebo group. Magnesium oxide led to a significant reduction in HOMA-IR (-1.49±0.95 vs. 0.09± 0.29) compared with placebo. Serum triglycerides was significantly decreased (-36.7±53.5 vs. 0.1±17.9 mg/d) in the magnesium group than placebo. HDL level was significantly increased in the magnesium group (2.3±5.9 mg/dl), while HDL level was decreased in the placebo group (-1.7±2.7 mg/dl). Significant mean change of total testosterone in magnesium group (0.43±0.35 ng/dL) than placebo group (-0.01±0.05 ng/dL). However, total cholesterol and LDL were also decreased in the magnesium group than in the placebo group, but the difference was not statistically significant (p>0.05) compared between the two groups. ***Conclusion:*** The present study provides evidence showing that magnesium supplementation resulted in reduced WC, BMI, HOMA-IR, total cholesterol, triglyceride, LDL, and testosterone levels in women with

PCOS. Also, magnesium supplementation might increase serum HDL levels. Though there was a significantly increased pregnancy rate of magnesium supplementation than in a placebo group.

Keywords: Serum magnesium (Mg), Insulin resistance (IR), Cardiovascular problems, Diabetes mellitus (DM).

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a disease that affects 6-10% of females worldwide [1]. An ovulation constitutes 40% of all female subfertility [2]. Women with PCOS have an increased incidence of WHO-II an ovulatory infertility. COD is an endocrine disease of unknown etiology, most frequently associated with an ovulation. About 50% of PCOS patients suffer from infertility due to ovulation dysfunction [3]. Several hormonal disorders involve in this disease include abnormal gonadotropin secretion, elevated luteinizing hormone, depressed FSH, altered LH -SH ratio elevated androgen, elevated fasting insulin or insulin resistance & increased prolactin. The pathogenesis of PCOS is poorly understood. No single etiologic factor may be fully accounted for in the spectrum of abnormalities in PCOS. A great body of evidence has demonstrated the important rule of reduced insulin sensitivity in many patients with PCOS. Almost 50 to 75% PCOS women are insulin resistant. Hyperinsulinemia due to insulin resistance occurs in approximately 80% obese & 30 to 40% lean women with polycystic ovarian syndrome [4]. Signs and symptoms of PCOS may comprise of acne, excess of hair or hirsutism, obesity or abdominal fat, alopecia, and acanthuses Nigerians, as well as irregular menstrual cycles. Women with PCOS are at higher risk of developing obesity, Insulin resistance (IR), Type 2 diabetes mellitus, cardiovascular disease (CVD), cancer, infertility, and psychological disorders including depression and anxiety. An accurate diagnosis of PCOS and early intervention aids in the reduction of reproductive, metabolic and cardiovascular risks often noted this population. Although the exact pathogenesis of PCOS remains unknown researchers believe genetics, intrauterine factors such as androgen exposure and prenatal nutrition, environment and lifestyle and obesity are all risk factors. Some risk factors, such as prenatal environment and genetics cannot be changed however, lifestyle factors, including diet, physical activity, and obesity may play a role in disease progression and are modifiable. The treatment of PCOS is dependent upon the signs and symptoms but current recommendation includes weight loss as well as metformin for premenopausal women. Although weight loss, even as little as 5% has been noted to restore normal menstruation and improve ovulation. Main treatment modalities for an ovulatory infertile PCOS women are dietary and lifestyle modifications, insulin sensitizers, oral ovulation induction agents, gonadotropins & laparoscopic ovarian drilling. Given the association between PCOS and insulin resistance, several insulin sensitizing agents are recommended to ameliorate endocrine and metabolic abnormalities which facilitate ovarian folliculogenesis and minimizes long term health hazard of PCOS [5]. Hyperinsulinemia acts synergistically with luteinizing hormone to increase the androgen production of theca cells [6]. Therefore, administrations of insulin sensitizing agents increase the tissue sensitivity to insulin action in vivo and enhance insulin activity in PCOS women. Among insulin sensitizers, Metformine, an oral biguanide is commonly being used in clinical practice & has been studied most extensively in PCOS. In spite of being used for decades, metformine has not been able to show results in term of improved live birth rates in infertile PCOS women [7]. Magnesium (Mg^{+2}) is the fourth most abundant mineral in the body and the second most abundant intracellular cations after K^{+2}. Currently, enzymatic database list over 600 enzymes for which Mg^{+2}serves as a cofactor and additional 200 in which Mg^{+2} acts as an activator [8]. Only 1% of the total Mg^{+2} in the body is present in extracellular fluids and 0.3% is found in the serum [9] is a required nutrient for both energy production and nucleic acid synthesis [10]. The normal reference range for Mg^{+2} in the serum 0.76-1.15 mmol/L. Mg^{+2} deficiency (MGD) is a common condition where the serum concentration of Mg^{+2} in the body is <0.75 mmol/L (1.8 mg/dl) also considered as preclinical hypomagnesemia [8]. Signs and symptoms of hypo magnesemia occurs when serum Mg^{+2} is decreased below 0.5mmol/L (1.2 mg/dl) [11]. MGD is associated with an increased risk of multiple preclinical and clinical manifestation, including pancreatic beta-cell dysfunction, IR, increased risk of MetS and T2D [12]. Mg+2 directly influences glucose metabolism by acting as a cofactor for many involved in energy metabolism as well as being part of the Mg^{+2}-ATP complex [13]. Mg^{+2} is essential for both aerobic and anaerobic energy production by glycolysis and oxidative phosphorylation via the Mg-ATP complex or directly as an enzyme activator [14]. Mg^{+2} is required in the cellular absorption and conversion of glucose into energy, and essential for the autophosphoylation of the insulin receptor, tyrosine kinas [15,16], which play a crucial role in glucose uptake in muscular and adipose tissue the presence of Mg^{+2} ultimately increase the ATP receptor sensitivity by increasing tyrosine kina's activity. Mg+2 also required in cellular glucose transport via transport protein activity-4 (GLUT-4). Theautophosphorylation of tyrosine kinase influences the translocation of GLUT-4 to the plasma membrane by lower blood glucose concentrations [15]. It is believed IR is the main pathogenic factor related to the increased rate of metabolic disturbance among women with PCOS [17]. 14.5% to 81% population Worldwide-

Suboptimal magnesium deficiency and women with PCOS greater likelihood of low serum Mg and increased insulin resistance. Subclinical Mg deficiency is a clinical silent reduction in physiological, cellular and /biochemical functions of Mg^{+2}. Because serum Mg^{+2}does not reflect intracellular Mg^{+2}, most cases of Mg^{+2} deficiency are undiagnosed. Subclinical Mg^{+2} deficiency can lead to low level of vitamin D level because Mg^{+2} is required for synthesis and metabolism of Vit-D [8]. Research evaluating the dietary habits of women with PCOS indicate 1 out 4 women do not consume adequate amount of dietary Mg^{+2} [18]. Mg^{+2} may also aid in regulating oxidative stress, thus reducing inflammation and thereby stabilizing the endothelium. Management of PCOS typically includes lifestyle modification, including weight loss and dietary intervention with energy restriction and/or altered diet composition. Among others, insulin-lowering drugs, anti-androgen therapy, oral contraceptives pills, and dietary intake of minerals, including Mg might also play a key role in the pathogenesis of PCOS due to its contribution to insulin sensitivity. Low dietary intake and serum Mg concentration as well as an increased urinary output are frequently noted among individuals with IR [19]. In a 2016 systematic review and meta-analysis, researchers evaluated the effect of oral Mg supplementation doses ranged from 300 to 750mg/day duration>4months improved HOMA-IR (P=0.001) and fasting glucose (P=0.002) in PCOS patients with IR [20]. There is no evidence of adverse effects related to high intakes of naturally occurring Mg^{+2} in foods like almonds, spinach, black beans, avocado, yogurt etc. However, excess supplemental Mg intake can produce diarrhea and rarely more serious issue such as hypotension, weakness and confusion [21]. Therefore, an upper intake level of 350 mg/day from supplemented Mg for women aged>14 years was established to reduce the risk of adverse reactions from excess intake [22]. So we hypothesized that Magnesium supplementation might help improve metabolic profiles and clinical symptoms of PCOS through its role on insulin action.

OBJECTIVES

General objective:

To assess the effect so magnesium supplementation on insulin resistance in polycystic ovary syndrome.

Specific Objectives:

- To assess the HOMA-IR level in the magnesium-oxide group and placebo group.
- To assess the BMI and WC in both groups.
- To assess the serum lipid profile in both groups.
- To compare the effects of two drugs on insulin resistance.
- To compare the changes in BMI and WC in both groups.
- To observe the changes of serum lipid profile in both groups.
- To assess the serum testosterone in both groups.
- To assess changes of serum testosterone in both groups.

METHODOLOGY

This was a randomized controlled study conducted at the Department of Reproductive Endocrinology & Infertility, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh during the period from January 2021 to December 2021. The sample size was 74 Among them 37 of group A (Magnesium)and 37 of group B (Placebo), the age group was between 18 to 40 years. The patients were selected by purposive sampling method.

Inclusion criteria

- Age 18 to 40 years.
- Primary or Secondary infertility.
- Diagnosed case of PCOS patients according to Rotterdam criteria.
- Insulin resistance by HOMA-IR >1.7 in women [23].

Exclusion criteria:

- Presence of any other endocrine disorders. (hyperthyroidism, hypothyroidism, hyperprolactinemia).
- Presents of any medical co -morbidity (renal, hepatic, and cardiovascular disease).
- Patients had taken any medication for at least three months that could influence hormonal metabolism & ovulation (metformin, myoinositol, OCP, anti-obesity).
- Patients with a high risk for hypomagnesaemia, such as using diuretics, alcoholism, persistent diarrhea.
- Known allergy to Magnesium-oxide.

Data collection Procedure

This Randomized controlled study was conducted in the Department of Reproductive Endocrinology and Infertility, BSMMU for 12 months from the day of IRB approval. Total 74 insulin resistant maintaining inclusion and exclusion criteria were considered as the study population. After a full explanation of the study, procedure informed written consent was taken. A detailed history and examination were obtained. Data was collected in a structured case report form to be filled as per the available records and laboratory results. The data was collected about age, parity, weight, height, BMI, waist circumference, hirsute, acanthuses Nigerians, and the result of basal investigations of fasting glucose, fasting insulin and fasting lipid profile, and serum testosterone. HOMA-IR was calculated by the formula HOMA-IR= Fasting glucose (mmol/L) × fasting insulin

(µIu/ml)/22.5HOMA-IR>1.7 in women [23] was accepted as insulin resistance. Participants were asked to routine a daily 500-1000 calorie deficit with a diet chart from B block room no-1004 and 150 minutes of exercise per week recorded. Eligible women who gave their informed consent were allocated into either group A: (Magnesium oxide) or group B (placebo) by on the basis of a computerized generated table system. Group A was treated with Magnesium Oxide 365 mg in single doses for 12 weeks & group B was treated with placebo. The appearance of the placebo such as color, shape, size and packaging were identical to the magnesium oxide tablets manufactured by square pharmaceutical in the same duration. All participants were instructed not to take any medications during the study except after consulting the physician. After 12 weeks of treatment, both groups observe for both physical and biochemical assays for fasting glucose, fasting insulin, HOMA-IR, serum testosterone & Serum lipid profile at the follow-up visit. Patients were advised for timed sexual intercourse and for pregnancy test in case of missed period either by pregnancy test kit or by βHCG as per patient's convenience. From each & every subject separate case report form was prepared. Data was collected from the patients on different visits on variables of interest using interview, observation, clinical examination, investigations & from the history sheet of the patients. The cumulative data was subjected to analysis.

Data analysis procedure

Statistical analyses were carried out by using the Statistical Package for Social Sciences version 23.0 for Windows (SPSS Inc., Chicago, Illinois, USA). The mean values were calculated for outcome variables and percentages for ovulation. Chi square test was used for categorical variables and paired t-test; unpaired t-test was used for continuous variables. P values <0.05 was considered as statistically significant.

Ethical implications

Written approval was taken from the concerned authority and the department with due procedure. The aims and objectives of the study along with its procedure, alternative diagnostic methods, risks, and benefits were explained to the patient in detail, in the easily understandable local language, and then voluntary informed written consent was taken from the patient before collecting data. Privacy, anonymity, and confidentiality were maintained during the procedure. It was assured that the procedure was helpful for both the physician and patients in making a rational approach regarding the management of the case. Ethical clearance was taken from the local Ethical Committee to perform the investigation and study.

RESULTS

This is a randomized controlled study, carried out in the outdoor Department of Reproductive Endocrinology and Infertilité, Bangabandhu Sheikh Mujib Medical University, from January 2021 to December 2021. A total of 74 diagnosed cases of PCOS patients with infertility were included in this study maintaining inclusion & exclusion criteria. Among them, 37 patients received magnesium oxide (Group A) & 37 patients received a placebo (Group B). During 12-week follow-up period, 1 patient was a dropout in magnesium oxide (Group A) and 2 patients in placebo (Group B).

Table 1: Socio-demographic characteristics of the study population, (N=74)

Socio-demographic characteristics	Group A (Magnesium) (n=37)		Group B (Placebo) (n=37)		P value
	n	%	n	%	
Age (years)					
18-24 yrs.	28	75.7	25	67.6	
25-30 yrs.	9	24.3	12	32.4	
Mean ±SD	23.2±2.3		23.6±2.1		0.398^{ns}
Range (min-max)	18.0-28.0		20.0-29.0		
Infertility					
Primary	37	100.0	37	100.0	-
Secondary	0	0.0	0	0.0	

Table 1 showed that the majority of patients belonged to the age group 18-24 years in both groups. The mean age was found 23.2±2.3 years in group A and 23.6±2.1 years in group B. Most of the patients were housewives in both groups. In group A, 20(54.1%) patients came from the urban area, and in group B 21(56.8%) came from the rural area. All patients had primary infertility in both groups. Age, occupational status, residence, and infertility were not statistically significant between the two groups.

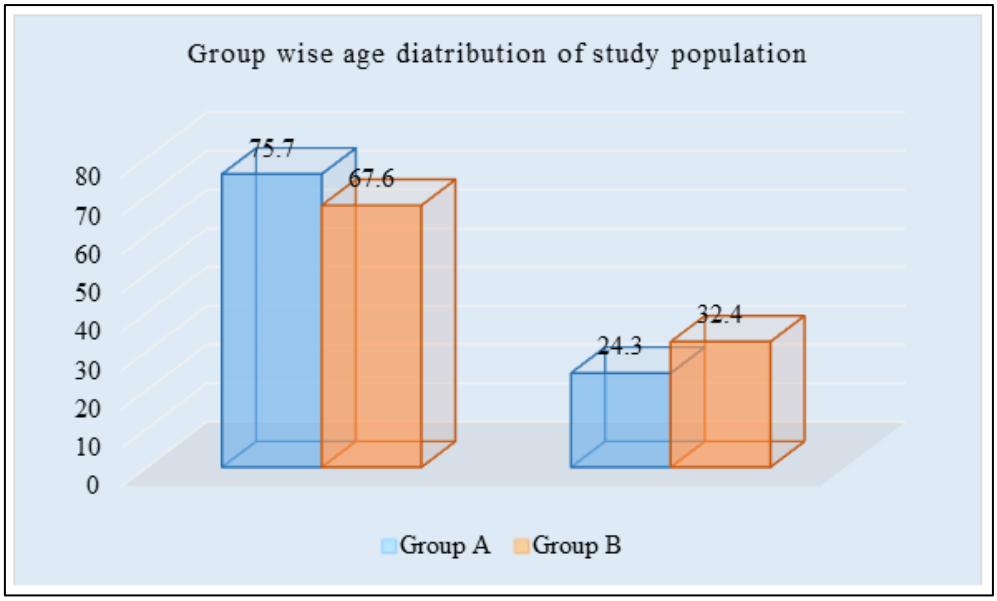

Figure I: Group wise age distribution of patient's, (N=74)

Table 2: Distribution of the study patients by clinical characteristics of the study populations in two groups, (N=74)

Clinical characteristics	Group A (Magnesium) (n=37)		Group B (Placebo) (n=37)		P value
	n	%	n	%	
Oligo anovulation	37	100.0	37	100.0	-
Hirsute	24	64.9	21	56.8	0.475^{ns}
Acne	26	70.3	28	75.7	0.601^{ns}
Acanthuses Nigerians	37	100.0	37	100.0	-

Table 2 showed that all patients had oligoanovulation in both groups. Hirsute was found 24(64.9%) in group A and 21(56.8%) in group B. All patients had acanthuses Nigerians in both groups. The difference was not statistically significant (p>0.05) between the two groups.

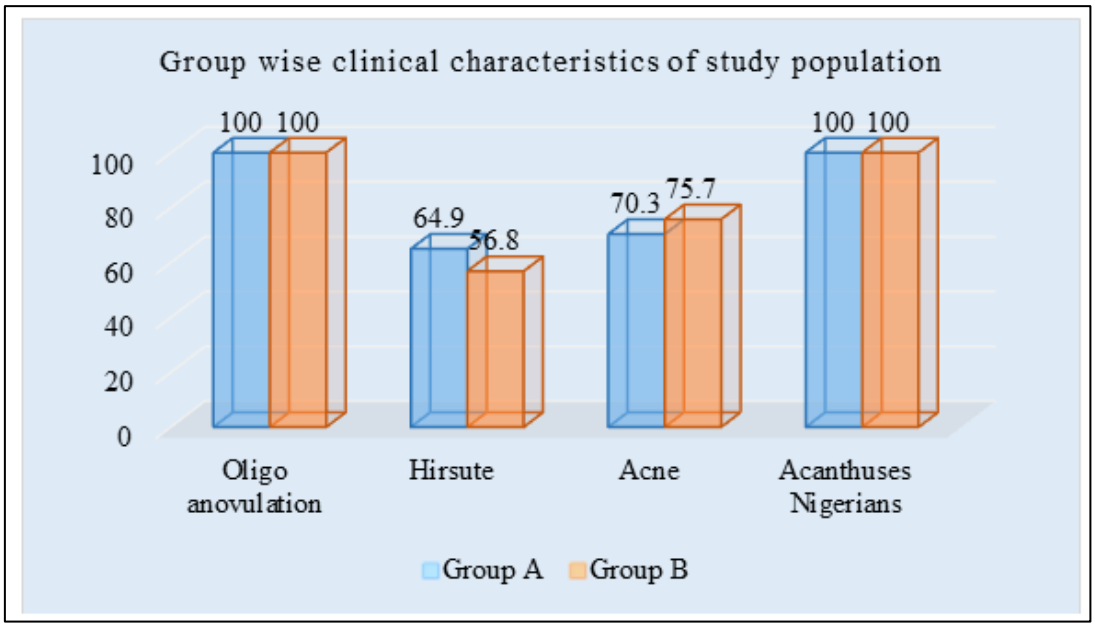

Figure II: Distribution of the patients by clinical characteristics in two groups, (N=74)

Table 3: Comparison of baseline insulin resistance between two groups, (N=74)

Baseline parameters	Group A (Magnesium) (n=37)	Group B (Placebo) (n=37)	P value
	Mean ±SD	Mean ±SD	
Fasting glucose (mg/dl)	5.2±0.5	5.2±0.4	0.487^{ns}
Fasting insulin (µIU/ml)	15.5±5.9	13.9±4.6	0.187^{ns}
HOMA-IR	3.6±1.5	3.2±1.1	0.181^{ns}

Table 3 showed that at baseline, fasting glucose, fasting insulin, HOMA-IR mean value which were not statistically significant (p>0.05) between the two groups.

Table 4: Comparison of baseline physical and biochemical parameters between two groups, (N=74)

Baseline parameters	Group A (Magnesium) (n=37)	Group B (Placebo) (n=37)	P value
	Mean ±SD	Mean ±SD	
Waist circumference (cm)	99.9±8.7	102.7±5.5	0.105^{ns}
BMI (kg/m^2)	29.0±3.0	29.6±1.5	0.302^{ns}
Total cholesterol (mg/dl)	187.8±37.2	195.2±39.3	0.404^{ns}
Triglyceride (mg/dl)	145.3±76.9	134.8±29.3	0.439^{ns}
HDL (mg/dl)	38.2±8.6	36.2±9.7	0.340^{ns}
LDL (mg/dl)	133.7±37.3	146.5±34.3	0.130^{ns}
Serum testosterone (pg/mL)	2.02±0.81	2.00±0.59	0.929^{ns}

Table 4 showed that at baseline, mean waist circumference, BMI, total cholesterol, triglyceride, HDL, LDL, and serum testosterone were not statistically significant (p>0.05) between the two groups.

Table 5: Comparison of pre and post-treatment insulin resistance parameters in group A, (n=37)

Physical and biochemical parameters	Baseline (n=37)	At 12 weeks (n=36)	P value
	Mean ±SD	Mean ±SD	
Fasting glucose (mg/dl)	5.2±0.5	4.8±0.3	0.001^{s}
Fasting insulin (µIU/ml)	15.5±5.9	9.8±3.9	0.001^{s}
HOMA-IR	3.6±1.5	2.1±0.9	0.001^{s}

Table 5 showed that in group A, fasting glucose (5.2±0.5 vs 4.8±0.3 mg/dl, fasting insulin (15.5±5.9 vs 9.8±3.9 µIU/ml), and HOMA-IR (3.6±1.5 vs 2.1±0.9).

Table 6: Comparison of pre and post-treatment physical and biochemical parameters in group A, (n=37)

Physical and biochemical parameters	Baseline (n=37)	At 12 weeks (n=36*)	P value
	Mean ±SD	Mean ±SD	
Waist circumference (cm)	99.9±8.7	92.0±7.4	0.001^{s}
BMI (kg/m^2)	29.0±3.0	26.6±2.4	0.001^{s}
Total cholesterol (mg/dl)	187.8±37.2	186.5±36.6	0.001^{s}
Triglyceride (mg/dl)	145.3±76.9	109.3±44.5	0.001^{s}
HDL (mg/dl)	38.2±8.6	40.8±6.3	0.029^{s}
LDL (mg/dl)	133.7±37.3	127.3±34.9	0.001^{s}
Serum testosterone (pg/mL)	2.02±0.81	1.55±0.65	0.002^{s}

* 1 patient was dropout in follow up period

Table 6 showed that in group A (magnesium oxide), waist circumference (99.9± 8.7 vs 92.0±7.4 cm), BMI (29.0±3.0 vs 26.6±2.4 kg/m^2), total cholesterol (187.8±37.2 vs 186.5±36.6 mg/dl), triglyceride (145.3±76.9 vs 109.3±44.5 mg/dl), LDL (133.7±37.3 vs 127.3±34.9 mg/dl) and serum testosterone (2.02±0.81 vs 1.55±0.65 ng/dL) were significantly reduced at 12 weeks than baseline. However, HDL (38.2±8.6 vs 40.8±6.3 mg/dl) was significantly increased at 12 weeks than baseline.

Table 7: Comparison of pre and post-treatment insulin resistance parameters in group B. (n=37)

Physical and biochemical parameters	Baseline (n=37)	At 12 weeks (n=35*)	P value
	Mean ±SD	Mean ±SD	
Fasting glucose (mg/dl)	5.2±0.4	5.2±0.3	0.858^{ns}
Fasting insulin (µIU/ml)	13.9±4.6	13.4±4.4	0.040^{s}
HOMA-IR	3.2±1.1	3.1±1.1	0.089^{ns}

Table 7 showed that in group B, fasting insulin (13.9±4.6 vs 13.4±4.4 µIU/ml) and HDL (36.2±9.7 vs 34.5±9.4 mg/dl) were significantly reduced at 12 weeks than baseline. Fasting glucose, HOMA-IR, were not significant compared with baseline vs at 12 weeks.

Table 8: Comparison of pre and post-treatment physical and biochemical parameters in group B, (n=37)

Physical and biochemical parameters	Baseline (n=37)	At 12 weeks (n=35*)	P value
	Mean ±SD	Mean ±SD	
Waist circumference (cm)	102.7±5.5	100.5±5.7	0.001^{s}
BMI (kg/m^2)	29.6±1.5	29.2±1.5	0.001^{s}
Total cholesterol (mg/dl)	195.2±39.3	194.1±41.6	0.431^{ns}
Triglyceride (mg/dl)	134.8±29.3	133.6±31.8	0.993^{ns}
HDL (mg/dl)	36.2±9.7	34.5±9.4	0.001^{s}
LDL (mg/dl)	146.5±34.3	143.2±30.2	0.157^{ns}
Serum testosterone (ng/dL)	2.00±0.59	1.97±0.58	0.332^{s}

*2 patients were dropout in follow up period

Table 8 showed that in group B, waist circumference (102.7±5.5 vs 100.5±5.7 cm), BMI (29.6±1.5 vs 29.2±1.5 kg/m^2), total cholesterol, triglyceride, LDL and serum testosterone level were not significant compared with baseline vs at 12 weeks.

Table 9: Comparison of mean changes after treatment insulin resistance parameters between two groups, (N=74)

Mean changes after treatment	Group A (Magnesium)	Group B (Placebo)	P value
	Mean ±SD	Mean ±SD	
Fasting glucose (mg/dl)	-0.4±0.49	0.01±0.21	0.001^{s}
Fasting insulin (µIU/ml)	-5.62±3.82	-0.34±0.95	0.001^{s}
HOMA-IR	-1.49±0.95	-0.09±0.29	0.001^{s}

Table 9 showed that magnesium supplementation after the 12-week intervention group, compared with the placebo, magnesium oxide lead to a significant reduction in fasting glucose (-0.4±0.49 vs. 0.01±0.21), fasting insulin (-5.62±3.82 vs. -0.34±0.95) and HOMA-IR (-1.49±0.95 vs. 0.09± 0.29).

Table 10: Comparison of mean changes after treatment physical and biochemical parameters between two groups, (N=74)

Mean changes after treatment	Group A (Magnesium)	Group B (Placebo)	P value
	Mean ±SD	Mean ±SD	
Waist circumference (cm)	-7.6±3.1	-1.7±1.8	0.001^{s}
BMI (kg/m^2)	-2.13±0.98	-0.32±0.52	0.001^{s}
Total cholesterol (mg/dl)	-2.72±2.09	-0.82±6.14	0.085^{ns}
Triglyceride (mg/dl)	-36.7±53.5	0.1±17.9	0.010^{s}
HDL (mg/dl)	2.3±5.9	-1.7±2.7	0.010^{s}
LDL (mg/dl)	-7.9±5.8	-3.9±15.8	0.147^{ns}
Serum testosterone (pg/mL)	-0.43±0.35	-0.01±0.05	0.001^{s}

Table 10 showed that magnesium supplementation for 12 weeks among women with PCOS had favorable effects on waist circumference compared with the placebo group (changes from baseline in the intervention group: -0.76±3.1 vs. -1.7±1.8 cm in the placebo group) and BMI compared

with the placebo group (changes from baseline in the intervention group: -2.13±0.98 vs. -0.32±0.52 kg/m2 in the placebo group). Serum triglycerides were significantly decreased (-36.7±53.5 vs. 0.1±17.9 mg/d) in group A (magnesium supplementation) than in group B (Placebo). HDL level was significantly increased in group A (2.3±5.9 mg/dl), while HDL level was decreased in the placebo group (-1.7±2.7 mg/dl). The mean change (baseline vs at 12 weeks) of total testosterone was -0.43±0.35 ng/dL in group A and -0.01±0.05 ng/dL in the placebo group, which was significant (p=0.001). However, total cholesterol and LDL were also decreased in the magnesium group than in the placebo group, but the difference was not statistically significant (p>0.05) compared between the two groups.

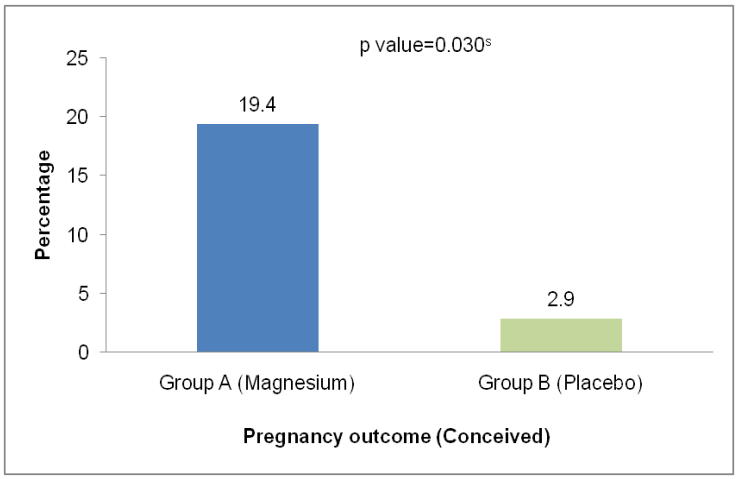

Figure III: Pregnancy outcome between two groups, (N=74)

Table 11: Side effects of the study population, (N =71)

Side effects	Group A (Magnesium) (n=36)		Group B (Placebo) (n=35)		P value
	n	%	n	%	
Diarrhea	4	11.1	1	2.9	0.187^{ns}
Nausea	2	5.6	1	2.9	0.385^{ns}
Weakness	1	2.8	0	0.0	0.507^{ns}

Table 11 showed that in group A, majority 4(11.1%) patients had diarrhea, while in group B 1(2.9%). Nausea was 2(5.6%) in group A and 1(2.9%) in group B. Weakness was 1(2.8%) in group A, not found in group B patients. Side effects was not statistically significant between two groups (p>0.05).

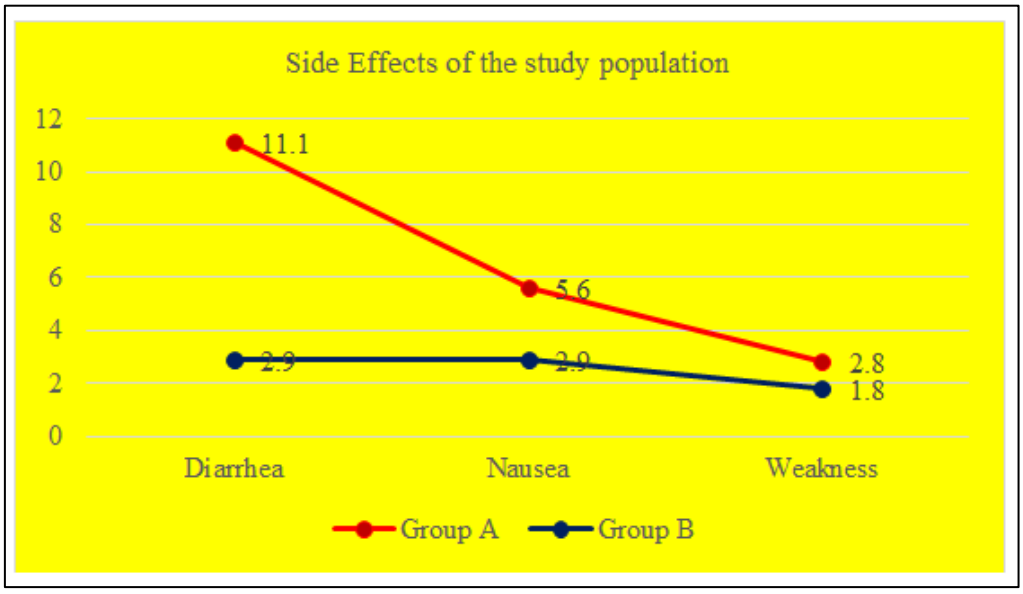

Figure IV: Side effects of two groups, (N=71)

DISCUSSION

Polycystic ovary syndrome (PCOS) is an endocrine disorder affecting 6-10% of reproductive-aged women [24, 25]. Individuals with PCOS are at increased risk of metabolic syndrome, infertility, diabetes, and cardiovascular diseases [26]. Dietary intake of minerals, including magnesium, might also play a key role in the pathogenesis of PCOS due to its contribution to insulin sensitivity [27]. Inadequate intake of magnesium has been shown to be linked with insulin resistance through its influence on tyrosine-kinas activity, enhancing oxidative stress and inflammation [6, 28]. 12-week magnesium and vitamin E co-supplementation had beneficial effects on parameters of insulin metabolism and serum triglycerides, VLDL, and total cholesterol in PCOS women [29]. Another trial indicated that magnesium-zinc-calcium-vitamin D co-supplementation for 12 weeks in PCOS women led to a significant reduction in total testosterone compared with the placebo [30]. This study showed the majority of patients belonged to the age group 18-24 years in both groups. The mean age was found 23.2±2.3 years in group A (Magnesium oxide) and 23.6±2.1 years in group B (Placebo). The difference was not statistically significant between the two groups. A study done by Farsinejad-Marj *et al*., (2020) [31] found mean age was 26.32±3.92 years in the intervention (magnesium supplementation) and 26±5.0 years in the placebo group. No significant differences were found between the two groups in terms of mean age. Maktabi *et al*., (2018) [30] reported that the mean age was 23.8±5.7 years in the Magnesium-zinc-calcium-vitamin D group and 24.8±4.8 years in the placebo group. No significant difference was found between the two groups. Mousavi *et al*., (2021) [32] observed that the mean a was 25.57±4.88 years in the Mg group and 26.20±5.72 years in the placebo group. No significant difference was found between the two groups. The above findings were almost similar in this study. In this present study observed that at baseline, mean serum LH, FSH, TSH, and AMH were not statistically significant ($p>0.05$) between the two groups. All patients had bilateral PCOin both groups. We found that in group A (magnesium oxide), waist circumference (99.9± 8.7 vs 92.0±7.4 cm) and BMI (29.0±3.0 vs 26.6±2.4 kg/m2) were significantly reduced at 12 weeks than baseline. In a study conducted by Farsinejad-Marj *et al*., (2020) [31] showed that magnesium supplementation for 8 weeks resulted in a significant reduction in BMI (changes from baseline in intervention, the above findings were similar to my study. Makati *et al*., (2018) [30] described that at baseline mean BMI was found 24.2±3.8 kg/m2 and at end-of-trial BMI was 24.0±3.7 kg/m2. Jamilian *et al*., (2018) [29] demonstrated that in Magnesium plus vitamin E group, at baseline mean BMI was found 25.5±3.5 kg/m2 and at the end-of-trial BMI was 25.5±3.3 kg/m2. Mousavi *et al*., (2021) [32] also found WC was also significantly different in the magnesium group compared to baseline values. Their study findings were almost similar in this study. The current study showed that in group A (magnesium oxide), fasting glucose (5.2±0.5 vs 4.8±0.3 mg/dl, fasting insulin (15.5±5.9 vs 9.8±3.9 µIU/ml), and HOMA-IR (3.6±1.5 vs 2.1±0.9), total cholesterol (187.8±37.2 vs 186.5±36.6 mg/dl), triglyceride (145.3±76.9 vs 109.3±44.5 mg/dl), LDL (133.7±37.3 vs 127.3±34.9 mg/dl) and serum testosterone (2.02±0.81 vs 1.55±0.65 ng/dL) were significantly reduced at 12 weeks than baseline. However, HDL (38.2±8.6 vs 40.8±6.3 mg/dl) was significantly increased at 12 weeks than baseline. In a study conducted by de Souza *et al*., (2014) [33] observed their study in the magnesium group, fasting insulin was higher (12.3±7.0) before treatment than after treatment (10.7±6.3 mU/L) was not statistically significant. HOMA-IR was higher (3.2±2.0) before treatment than after treatment (2.8±1.9) that was not statistically significant. Fasting glucose, insulin, HOMA-IR, total cholesterol, triglyceride, HDL, and LDL were not significant compared with baseline vs at 12 weeks compared with before treatment than after treatment. Their study findings were not similar with my study. This study found in group B (Placebo), waist circumference (102.7±5.5 vs 100.5±5.7 cm) and BMI (29.6±1.5 vs 29.2±1.5 kg/m2) were significantly reduced at 12 weeks than baseline. de Souza *et al*., (2014) [33] described that mean waist circumference and BMI were statistically significant ($p<0.05$) when compared with baseline vs after treatment, that was support with my study. Mousavi *et al*., (2021) [32] had observed their study in the placebo group, at baseline mean WC was 85.75±7.23 cm, and after treatment was 86.85±7.37 cm. At baseline mean BMI was 26.94±3.83 kg/m2 and after treatment was 26.95±3.72 kg/m2 that was not significant. Maktabi *et al*., (2018) [30] reported that in the Placebo group, at baseline mean BMI was found 25.6±4.8 kg/m2 and at end-of-trial BMI was 25.0±4.9 kg/m2. Jamilian *et al*., (2018) [29] also found in the Placebo group, at baseline mean BMI was 26.0±4.7 kg/m2 and at end-of-trial BMI was 26.0±4.7 kg/m2. Their study findings were not correlated with my study. In my study, it was observed that in group B (Placebo), fasting insulin (13.9±4.6 vs 13.4±4.4 µIU/ml) and HDL (36.2±9.7 vs 34.5±9.4 mg/dl) were significantly reduced at 12 weeks than baseline. Fasting glucose, HOMA-IR, total cholesterol, triglyceride, LDL, and serum testosterone level were not significant compared with baseline vs at 12 weeks. de Souza *et al*., (2014) [33] demonstrated that in the placebo group, fasting insulin was higher (14.4±6.2) before treatment than after treatment (12.6±6.5 mU/L) was not statistically significant. HOMA-IR was higher (3.6±1.9) before treatment than after treatment (3.2±1.8) was not statistically significant. Fasting glucose, insulin, HOMA-IR, total cholesterol, triglyceride, HDL and LDL were not significant compared with baseline vs at 12 weeks. Their study also supports with my study.

This study found that magnesium supplementation for 12 weeks among women with PCOS had favorable effects on waist circumference compared with the placebo group (changes from baseline in intervention group: -0.76±3.1 vs. -1.7±1.8 cm in placebo group) and BMI compared with the placebo group (changes from baseline in intervention group: -2.13±0.98 vs.0.32±0.52 kg/m2 in placebo group). In a study done by Farsinejad-Marj *et al*., (2020) [31] showed that that magnesium supplementation for 8 weeks among women with PCOS had favorable effects on BMI compared with the placebo group (changes from baseline in intervention group: -0.31±0.07 vs. 0.07±0.09 kg/m2 in placebo group). In addition, this supplementation leads to preventing the increase in waist circumference in intervention group compared with the placebo group (0.02 vs. 1.15 cm), their study findings were similar to my study. Jamilian *et al*., (2018) [29] reported that BMI at end-of-trial was found 25.5±3.3 kg/m2 in Magnesium plus vitamin E group and 26.0±4.7 kg/m2 in placebo group. Mean change was -0.03±0.1 kg/m2 and –0.05±0.3 kg/m2 in magnesium plus vitamin E group and placebo group spectively, however, difference was not statistically significant (p>0.05) between two groups. Mousavi *et al*., (2021) [32] described that after adjustment for baseline values, no significant differences were observed among the different groups (P>0.05); only an insignificant marginal difference was seen in WC between groups (P=0.085). The above findings were not similar in my study. We found that after the 12-week intervention group, compared with the placebo, magnesium oxide led to a signify care diction in fasting glucose (-0.4±0.49 vs. 0.01±0.21), fasting insulin (-5.62±3.82 vs. -0.34±0.95) and HOMA-IR (-1.49±0.95 vs. 0.09±0.29). In a study done by Farsinejad- Marj *et al*., (2020) [31] reported that plasma concentrations of fasting blood glucose and insulin as well as HOMA-IR were significantly changed during 8 weeks in either group. However, when compared changes from baseline between the two groups, we failed to find a significant difference; their study finding was similar with this study. Jamilian *et al*., (2018) [29] consisted that mean change of FPG was -1.5±1.0 mg/dl in magnesium plus vitamin E co-group and 1.0±1.0 mg/dl in placebo group, which was not significant. Mean change of insulin was -1.0±0.5 mg/dl in magnesium plus vitamin E co-group and 1.5±0.5 mg/dl in placebo group, that was significant (p=0.002), which was similar with my study. After the 12-week intervention, compared with the placebo, magnesium, and vitamin E co-supplementation lead to a significant reduction in HOMA-IR (-0.2±0.7 vs.+0.4 ±0.9, p=0.002), which was consisted in this study. Another study done by de Souza *et al*., (2014) [33] also found fasting blood glucose, insulin; HOMA-IR did not reach statistically significant reduction in the both groups, which was inverse in this study. Regarding lipid profiles in this study observed that serum triglycerides was significantly decreased (-36.7±53.5 vs. 0.1±17.9 mg/d) in group A (magnesium supplementation) than group B (placebo). HDL level was significantly increased in group A (2.3±5.9 mg/dl), while HDL level was decrease in placebo group (-1.7±2.7 mg/dl). However, total cholesterol and LDL were also decreased in magnesium group than placebo group, but the difference was not statistically significant (p>0.05) compared between two groups. In a study conducted by Jamilian *et al*., (2018) [29] found magnesium plus vitamin E supplementation significantly decreased serum triglycerides (-15.0±24.4 vs. +6.7±22.2 mg/dl, p=0.001) and VLDL-cholesterol concentrations (-3.0±4.9 vs.+0.6±2.4 mg/dl, p=0.01) compared with the placebo. A trend toward a greater decrease in total cholesterol levels was observed in magnesium plus vitamin E group compared to placebo group (-7.0±32.6 vs.+8.1±26.6 mg/dl, p=0.05). Their study findings were similar with my study. Farsinejad -Marj *et al*., (2020) [31] demonstrated their study did not observe any significant effect of the supplementation on levels of serum triglyceride, total cholesterol, LDL, and HDL cholesterol in either the crude or adjusted model. Although magnesium supplements resulted in a decrease in serum triglyceride levels and total and LDL-cholesterol concentrations, comparing the two groups, this finding was not statistically significant. de Souza *et al*., (2014) [33] also observed mean change of triglycerides and HDL level were not statistically significant when compared between magnesium and placebo group. Their study findings were not correlated with my study. In this current study it was observed that the mean change (baseline vs at 12 weeks) of total testosterone was -0.43±0.35 ng/dL in group A and -0.01±0.05 ng/dL in placebo group, that was statistically significant when compared between two groups (p=0.001). A randomized, placebo-controlled trial indicated that magnesium-zinc-calcium-vitamin D co-supplementation for 12 weeks in PCOS women led to a significant reduction in total testosterone (-0.2±0.1 ng/mL in magnesium-zinc-calcium-vitamin D group and 0.03±0.1 ng/mL in placebo group) reported by Makati *et al*., (2018) [30]. Farsinejad-Marj *et al*., (2020) [31] study showed a significant decrease in serum testosterone levels in intervention and placebo groups, comparing the changes between the two groups, a marginally significant difference in serum testosterone levels was found (51.65 vs. 47.80 in intervention, 43.41 vs. 39.46 in placebo), that support with my study. Regarding pregnancy outcome, it was observed that pregnancy rate was significantly higher in group A than group B (19.4 vs 2.9%). Regarding side effects in this study found that in group A (magnesium), majority 4(11.1%) patients had diarrhea, while in group B (Placebo) 1(2.9%). Nausea was 2(5.6%) in group A and 1(2.9%) in group B. Weakness was 1(2.8%) in group A, not found in group B patients. Side effects was not statistically significant between two groups (p>0.05). de Souza *et al*., (2014) [33] reported that the Mg supplement was well tolerated, and there were no relevant side effects, which occur in same proportion in patients taking Mg or placebo: slight epigastria pain

(5.4% vs. 8.6%), nausea (2.7% vs. 5.7%), and diarrhea (2.7% vs. 2.9%), which did not require treatment or interruption of medications. There is no evidence of adverse effects related to high intakes of naturally occurring Mg+2 in foods like almonds, spinach, black beans, avocado, yogurt etc. However, excess supplemental Mg intake can produce diarrhea and rarely more serious issue such as hypotension, weakness and confusion [21]. The above mentioned study findings were almost similar with my study.

CONCLUSION

In conclusion, the present study provides the evidence showing that magnesium supplementation resulted in reduced WC, BMI, HOMA-IR, triglyceride, and testosterone levels in women with PCOS. Also, magnesium supplementation might increase serum HDL levels. Though there was a significantly increased pregnancy rate of magnesium supplementation than the placebo group.

Limitations of the Study

The study was conducted during the COVID pandemic situation and locked down period. The relatively short duration of the study and the fact that no attempt was made to measure ionized and urinary magnesium. The applied dose of elemi magnesium in the present study was rather modest, and it may not be high enough to achieve the desired outcomes of our research. Furthermore, since our results were obtained in subjects with normal serum magnesium levels, the present findings cannot be generalized to conditions associated to magnesium depletion this study was done with small sample size.

RECOMMENDATIONS

Further community-based or multicenter studies can be undertaken by including a large number of patients. Further studies are necessary with single supplementation for each comparison with co-supplementation to determine the beneficial effects of each on hormonal profiles, biomarkers of inflammation, and oxidative stress. Future studies with a longer duration of the intervention and a bigger sample size are needed to confirm the validity of our findings.

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