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Effectiveness of Probiotics, Metformin and Their Combination Therapy in Ameliorating Dyslipidemia Associated With PCOS

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Authors' contributions

This work was carried out in collaboration among all the authors. Author UZ designed the study, wrote the protocol along with the first draft of the manuscript and did the sampling and statistical analysis. Authors ZM and KI managed the literature searches and final drafting. Author JAH provided the clinical facilities and helped in manuscript writing. Author SA and SB did all the final setting and helped in statistical analysis. All the authors read and approved the final manuscript.

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ABSTRACT

Background: Polycystic ovary syndrome (PCOS) is a heterogeneous metabolic disorder affecting women of the child-bearing age. Alteration in lipid profile is one of the troublesome features faced by PCOS patients that need to be treated.
Aim: The purpose of this study was to compare the effects of Probiotics, Metformin and their Combination therapy on lipid profile in women suffering from PCOS.
Study Design: Single-Centered Randomized Controlled Trial.
Setting: Gynecological clinic of Karachi at Tertiary care Hospital from December 2018 to May 2019.

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Patients and Methods: Total 78 PCOS patients of age between 18 to 40 years were involved in this trial rendering to Rotterdam criteria. After taking written and informed consent they were randomly allocated to three groups and were received: Metformin 500 mg BD (n = 26), Probiotic Capsule 180 mg BD (n = 26) and Combination of Metformin 500 mg BD / Probiotic Capsule 180 mg OD for 12-weeks. Lipid profile was measured at baseline and after 12-weeks of intervention along with the body weight. In the end, pre versus post therapy and comparison of different group's results were analyzed.

Results: Metformin and Met/Pro Combination groups resulted in a significant reduction in Total Cholesterol (TC) as compared to Probiotic group. Fall in Low-Density Lipoprotein Cholesterol (LDL-C) levels were noteworthy after Probiotics and Met/Pro treatment. Serum TG concentrations were significantly reduced in all the groups. High-Density Lipoprotein Cholesterol (HDL-C) levels were slightly elevated after Probiotics treatment, which was not statistically significant but Metformin and Met/Pro group increases it to the significant value. No substantial change was found in the Very Low-Density Lipoprotein Cholesterol VLDL-C levels in any of the three groups.

Conclusion: Overall, Probiotics treatment in PCOS women for 12 weeks exhibited noteworthy changes in TG, and LDL-C levels. Metformin markedly improve Cholesterol, TG and HDL-C. However, Met/Pro improved all the lipid changes to the significant value except for VLDL-C.

Keywords: Probiotics; metformin; ameliorating dyslipidemia; polycystic ovary syndrome.

1. INTRODUCTION

Dyslipidemia is defined as altered levels of cholesterol and other lipids or fats, in the blood. Lipids are important for life. They are the chief components of the living cells. However, when deranged, can increase risk of cardiovascular disease including stroke, myocardial infarction etc. [1]. Nearly 60-70% of patients with obesity are dyslipidemic [2].

Polycystic ovary syndrome is the most common type of endocrinopathy, associated with increased risk of infertility and metabolic syndrome [3]. The metabolic changes in PCOS have wide health consequences like obesity, insulin resistance and dyslipidemia in women of the reproductive ages [4]. Particularly, dyslipidemia is a foremost metabolic abnormality in these patients. The National Cholesterol Education Program guidelines remark that 70% of PCOS patients have lipid anomalies [5,6].

Pertaining to pathophysiology of dyslipidemia in PCOS, many factors may be involved such as ethnicity, family history of lipid irregularities, age, obesity etc. In addition, chronic testosterone stimulation is also associated with dyslipidemia though mechanism is yet not fully defined [7,8]. Similarly role of insulin resistance cannot be overlooked as dyslipidemia is more prevalent in patients of PCOS with poor glucose tolerance as compared to normal [9,10].

Data related to pattern of dyslipidemia in PCOS revealed that elevation of LDL-C was found in almost all the PCOS women and reduced HDL-C levels were more prominent in PCOS having obesity even in 3rd decade of life. While triglycerides levels begin to rise from early adulthood that is not acceptable [6]. Due to these alterations, an increase in cardiovascular risk can be predicted in these patients. Moreover, dyslipidemia is often associated with obesity which causes further detrimental effects on metabolism and ultimate Cardio-vascular health consequences [11,12].

Life style modification is the cardinal approach, which includes restricted diet and energy consumption for its management particularly correction of obesity, dyslipidemia and insulin resistance which subsequently attenuate CVD risks. However, due to protracted duration of this ailment, the major confront is compliance of patients. In this anguish situation, medical treatment turn out to be indispensable for achieving aforementioned objectives [13].

Metformin is one of most comprehensively researched drug, which shows remarkable efficacy in lessening insulin resistance in patients with or without PCOS, but shows variable results in improving dyslipidemia associated with this syndrome, despite the fact that insulin resistance in some way is linked with the same [14,15].

In current researches and medical practice, Probiotics are gaining popularities owing to their diverse beneficial effects on health and relieving certain ailments including diarrhea [16], gingivitis [17], obesity [18] etc. Moreover, recently its protective role against insulin resistance and

dyslipidemia has been studied. The probable means through which Probiotics normalize dyslipidemia in particular include: de-conjugation and precipitation of bile by the action of enzymes; incorporating cholesterol in cytoplasm of microorganisms; inhibiting cholesterol transport in gut cells; inhibiting production of cholesterol in liver. Furthermore, role of individual host friendly micro-biota in reducing dyslipidemia has been elaborated and evidenced the abilities of certain strains of bacteria to reduce TC, LDL-C and TG [19].

Keeping in view the above particulars, this study was designed to elaborate the role of Probiotics in improving dyslipidemia either alone or in combination with Metformin in PCOS patients.

2. MATERIALS AND METHODS

2.1 Trial Design and Participants

This is an open label, parallel-arm, randomized controlled trial in which 78 newly diagnosed PCOS patients aged 18–40 years were enrolled from Out Patient Department of Gynaecology and Obstetrics clinic at Tertiary Care Hospital, Karachi. Subjects were enrolled via convenient sampling technique between January 2019 and June 2019. The sample size was calculated by Sealed Envelope calculator version 201: (Significance level (alpha) 1%, 99% confidence interval Power (1-beta) 90, Percentage success in control group 12%, Percentage success in experimental group 60%, the calculated sample size was 52).

2.2 Eligibility Criteria

Patients were included according to the Rotterdam European Society for Human Reproduction and Embryology/American Society for Reproductive Medicine sponsored a PCOS consensus criterion according to which any two of the following three features must be present for the patient to be diagnosed as a case of PCOS: Oligomenorrhea and/or amenorrhea (Oligomenorrhea>45 days or <8 cycles per year and amenorrhea >3 months in a women with pervious periodic menses) for a period of 6 months, clinical and/or biochemical hyperandrogenemia, presence of acne, hirsutism (FG score>8) and alopecia; and Polycystic ovaries on sonography (>12 follicles in one or both ovaries, 2-9 mm in diameter and/or increased ovarian volume >10 mL). While patients who were taking any medication or with history of chronic diseases, smokers and allergic to Probiotics or Metformin were excluded from the study.

2.3 Study Design

At beginning of the study, the procedure was carefully explained to all partakers before obtaining informed consent. After the agreement, patients were randomly allocated into three treatment groups. Group 1: Metformin 500 mg BD (n = 26), Group 2: Probiotic 180 mg BD (n = 26) and Group 3: Combination (Metformin 500 mg BD plus Probiotic 180 mg OD) for a period of 12 weeks.

Probiotic consisted of five viable and freeze-dried strains: *Lactobacillus Acidophilus* (1 X 109 CFU/g), *Lactobacillus Delbruekii* (1 X 109 CFU/g), *Bifidobacterium Bifidum* (1 X 109 CFU/g) *Lactobacillus Bulgaricus* and *Streptococcus Thermophilus*. At the onset of the study, subjects were requested to follow the proper and same diet plan. They were asked not to consume any medicine other than the one provided to them by the investigators for the 12-week of intervention. All partakers were asked to complete their daily food diaries.

The participants were given drugs for 6 weeks and were instructed to bring back the written schedule of the medicinal intake. On the second visit i.e. at sixth week, they were given next 6 weeks supplements. To increase the compliance, all participants' received short messages daily on their cell phones as a reminder. All the patients were examined in detail before and after the treatment and so their blood samples were taken at week 0 and 12.

Weight: A portable weighing machine with a 125 kg maximum capacity was used.

Height: Subjects were asked to stand with their scapula, buttocks and heels resting against a wall; the neck was held in a natural non-stretched position. Then the height was measure in inches with help of wall chart.

2.4 Assessment of Biochemical Parameters

The blood samples were collected after twelve to fourteen hours of fasting at second day of the menstrual cycle by venipuncture, before and at

the end of the intervention i.e. at 0 and 12 week at Dow Laboratory collection center.

The serum lipid concentrations were evaluated spectrophotometrically by means of commercial kits in which evaluation of Total cholesterol (TC) and triglycerides (TG) was enzymatical, while the high-density lipoprotein cholesterol (HDL-C) was measured by using precipitation technique. According to Friedewald equation, very low-density lipoprotein cholesterol (VLDL-C) and low-density lipoprotein cholesterol (LDL-C) was calculated with the help of analyzer using respective kits [20].

2.5 Statistical Analysis

The data was analyzed using SPSS software 20.The numeric variables were expressed as mean ± standard deviation. The pre and post results of all the groups were compared by using paired t test. The difference among the treatment groups were assessed by ANOVA, the results were further analyzed by post hoc tukey's test. The *p* value of <0.01 were considered statistically significant.

3. RESULTS

Altogether 90 patients were enrolled in this trial, out of them 12 participants were excluded during the eligibility stage (n=5), those for not meeting the inclusion criteria (n= 3) while remaining participant lost the follow-up (n=4). All the partakers were allocated to three groups either to Metformin, Probiotics or Combination group randomly. Overall, the rate of compliance in our study was high i.e. approximately 95% consumption in all three groups. As far as side effects are concern, In the Metformin group, 11 patients complained of headache, abdominal pain or diarrhea while no side effects were reported in the other groups.

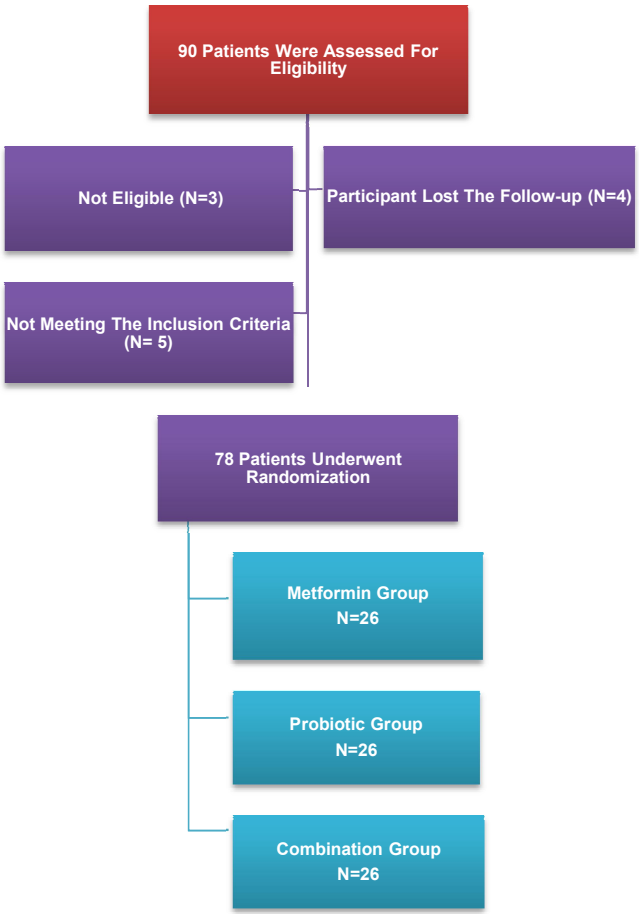

Fig. 1. Schematic diagram of patients flow

Baseline differences in the mean age, height, weight, and lipid profile between the groups were non-significant among all three groups (Table 1).

Weight was significantly reduced in all the groups i.e. around 3.1 kg in the Metformin group (p <0.001), 1.7 kg on average in Probiotics (p <0.01) and 5 kg in the Combination group (p <0.001) after 12 weeks of intervention. Hence, there was no significant difference at the end of the trial between them (*p*-0.97), though Metformin and the combination group showed more promising results than Probiotics alone.

Serum Cholesterol showed a decline in the Metformin group (p<0.01) as well as in the Combination group but no major change was observed in Probiotics group when set against baseline. However, a significant reduction was observed between the groups at the end of the trial (*p*-0.009).Therefore, a noteworthy difference was observed between Metformin and Combination group.

In all the three groups, TG was reduced and found to be statistically significant against baseline. So, no significant change was observed when three groups were compared.

There was an increase in HDL-C levels in the Metformin group and Combination group which were statistically significant versus baseline (*p*<0.01), as compared to Probiotic (*p*-0.54). Though, after the intervention no significant change was observed between the groups. Also, no substantial change was noted when all the three groups were associated (Table 2).

Lastly, no change was observed in the VLDL-C levels in the three groups as well as when they were compared.

4. DISCUSSION

Polycystic ovarian syndrome is strongly associated with different metabolic syndrome and has serious health concerns. Weight gain, insulin resistance and lipid abnormalities, like elevated cholesterol, low-density lipoprotein (LDL), triglyceride levels and decreased high-density lipoprotein (HDL), are often found in PCOS women [7].

The present study validated a small change in weight on average after Probiotics supplementation. Investigation on Probiotics is still quite new and growing. Researchers believe that there is an association between reduced bacterial gut diversity and obesity. Probiotics may also play an important role in reducing weight through release of GLP-1 satiety (appetite-reducing) hormone [21] or else through increase of ANGPTL4 (Angiopoietin-like 4) which might cause decline in fat storage [22].

Moreover, some evidence from clinical trials has confirmed that some Probiotics, containing *Lactobacillus* strains, may help to lose apparent weight and adiposity. However, to date Probiotics are not a guaranteed weight loss strategy and this could be one part of a comprehensive weight loss program [23].

Our study noticed a significant change in TG and LDL-C in the probiotic group but not on TC, VLDL and HDL. To elaborate their effects, all

Table 1. Baseline comparison of metformin, probiotics and the combination (Met/Pro) therapy on lipid profile in PCOS patients

Parameters	Metformin	Probiotic	Combination	p-value
Anthropometric				
Age (years)	27.2 ± 4.6	24.2 ± 4.8	25.1 ± 5.3	0.08
Height (inches)	5.3 ± 0.2	5.1 ± 0.8	5.4 ± 0.2	0.08
Weight (kg)	79.2 ± 15.8	76.8 ± 18.8	80.1 ± 17.2	0.78
Metabolic Parameters				
Cholesterol (mg/dl)	159.7 ± 20.7	163.8 ± 23.5	157.2 ± 22.4	0.56
TG (mg/dl)	117.9 ± 19.1	117.6 ±16.8	119.4 ± 17.9	0.91
LDL (mg/dl)	97.9 ± 14.7	98.8 ± 10.9	100.8 ± 14.8	0.73
HDL (mg/dl)	40.3 ± 6.4	38.8 ± 8.4	41.0 ± 5.5	0.52
VLDL (mg/dl)	30.2 ± 8.9	33.9 ± 6.7	34.2 ± 8.6	0.15

Data are shown as mean ± SD; Obtained from ANOVA; Statistically significant p-value < 0.01; TG: Triglyceride, HDL-cholesterol: high-density lipoprotein cholesterol, LDL cholesterol: low-density lipoprotein-cholesterol, VLDL-cholesterol: very low-density lipoprotein cholesterol

Table 2. Comparison of metformin, probiotic and the combination (Met/Pro) treatment on lipid profile of PCOS patients

Parameters	Metformin	Probiotic	Combination	*p*-value
Anthropometric				
Weight (kg)				
0 Week	79.2 ± 15.8	76.8 ± 18.8	80.1 ± 17.2	0.78
12 Week	76.1 ± 15.0	75.1 ±18.4	75.1 ± 17.1	0.97
p-value	<0.01	<0.01	<0.01	
Metabolic Parameters				
Cholesterol (mg/dl) [$]				
0 Week	159.7 ± 20.7	163.8 ± 23.5	157.2 ± 22.4	0.56
12 Week	150.3 ± 21.3	162.7 ± 22.6	143.9 ± 21.6	0.009
p-value	<0.01	0.49	<0.01	
TG (mg/dl)				
0 Week	117.9 ± 19.1	117.6 ± 16.8	119.4 ± 17.9	0.91
12 Week	111.6 ± 14.1	112.5 ± 16.9	109.5 ± 17.6	0.79
p-value	<0.01	<0.01	<0.01	
LDL-C (mg/dl)				
0 Week	97.9 ± 14.7	98.8 ± 10.9	100.8 ± 14.8	0.73
12 Week	96.8 ± 14.4	96.6 ± 10.8	97.0 ± 13.7	0.99
p-value	0.03	<0.01	<0.01	
HDL-C (mg/dl)				
0 Week	40.4 ± 6.4	38.8 ± 8.5	40.9 ± 5.0	0.52
12 Week	42.7 ± 6.1	39.5 ± 8.2	50.9 ± 8.3	0.017
p-value	<0.01	0.03	<0.01	
VLDL-C (mg/dl)				
0 Week	30.2 ± 8.9	33.9 ± 6.7	34.2 ± 8.6	0.15
12 Week	29.3 ± 9.3	33.5 ± 6.9	33.2 ± 7.5 / .013	0.12
p-value	0.2	0.11	0.01	

Data are shown as mean ± SD; Pre and post are obtained from paired t test; Comparison among the groups are obtained from ANOVA; Difference exits between Metformin and Combination group obtained from post hoc tuckey's [$]; Statistically significant p-value < 0.01; TG: Triglyceride, HDL-cholesterol: high-density lipoprotein cholesterol, LDL cholesterol: low-density lipoprotein-cholesterol, VLDL-cholesterol: very low-density lipoprotein cholesterol

RCT published from 2000 to 2014 were systematically searched by Sun and Buys, comprising of 15 studies and 788 subjects. The study concluded, Total Cholesterol (TC) and LDL-C levels were reduced that is found to be statistically significant after Probiotics intervention. They also mentioned that the significant decrease in LDL was found in the clinical trials that had *Lactobacillus Acidophilus* strain when compared to other types of strains [24].

A study done in Iran by Ahmadi et al. in PCOS women reported that Probiotic supplementation containing *Lactobacillus Acidophilus*, *Lactobacillus Casei* and *Bifidobacterium Bifidum* species for 12 weeks had promising effects not only on weight loss and markers of insulin resistance but also on triglycerides and VLDL-C [25]. Rajkumar et al. (2015) found that Probiotic supplementation among hale and hearty young individuals for 6 weeks resulted in a significant reduction in total-cholesterol, LDL-C, triglycerides and a significant increase in HDL-cholesterol concentrations [26].

Moreover a study demonstrated that *Bifidobacterium* containing Probiotic supplements along with diet therapy improved dyslipidemia (especially TC and LDL-C) in children [27].

The results of the meta-analysis by Guo et al. indicated that Probiotics rich-diet reduces TC and LDL-C levels in plasma for partakers with high and borderline high cholesterol levels [28].

The some inconsistencies between results of our study and those of earlier reports might be clarified by variability in strains and prescribed quantity of Probiotics used, the duration of the treatment, clinical features of the participants as well as the quality of the supplements.

Multiple set of studies have been conducted to establish the importance of improving dyslipidemia in relation to diabetes [29,30] and so effects of metformin on the same [31]. Our data too, revealed the beneficial effects of metformin on serum cholesterol, TG and HDL but in the setting of PCOS.

Karimzadeh et al indicated that Metformin is not only suggested for PCOS women because of its worthwhile efficacy in ovulation induction and overcoming insulin resistance , but also for its probable effectiveness against disturbed lipid metabolism [32]. Zhang et al. in the same way evaluated lipid parameters after 3 months of metformin treatment in which they remarked that serum total cholesterol and triglycerides were significantly decreased [33]. In 2017 Singh et al. evaluated different lipid markers in PCOS after 6 months of Metformin treatment and found that there was significant reduction in serum total cholesterol, LDL-C and TG ($p<0.01$) [34].

Another study by Kailka et al. mentioned the beneficial effects of Metformin on lean women with polycystic ovarian syndrome with displayed significant decrease in the TG and LDL-C levels [35].

Metformin therapy improves cardiometabolic possibilities related to elevated LDL-C and non-HDL-C, therefore, the study highlighted the cardio protective part of Metformin in youngsters with metabolic syndrome and offers extra explanation to consider the use of Metformin in PCOS [36].

The current study also demonstrated significant changes in the total cholesterol, TG, LDL-C and HDL-C in combination group. There is a scarcity of literature regarding the efficacy of combination therapy in improving lipid profiles and to the best of our data search there is only single study by Shavakhi et al. that showed the combination of Metformin and Probiotic improved liver aminotransferases superior than metformin alone in participants with non-Alcoholic Steatohepatitis. Moreover, it demonstrated that total cholesterol changed significantly relative to baseline after 6 months in Metformin/Probiotic group when compared to Metformin/placebo group [37].

There is no significant difference among three groups in modifying different parameters of dyslipidemia and more or less all the three treatment regimen showed variable improvement, except for Total Cholesterol which was significantly improved in Combination (Met/Pro) as compared to Metformin group. As mentioned earlier this was the first study which evaluated the efficacy of combined Metformin and Probiotic in ameliorating altered lipid profile so, further studies are warranted in this regard to confirm the results.

5. CONCLUSION

All the three groups i.e. Metformin, Probiotics and Combination have potential to reduce weight gain and mitigate the lipid imbalances associated with PCOS. Further multi-centered clinical trials must be conducted to validate our results before commercialization of Probiotics in favor of this disease with different proportions of Metformin and the same. Moreover, the efficacy of Combination therapy must be evaluated in alleviating different symptoms of PCOS including insulin resistance, so as to support or substitute the Metformin alone, in whom it is insufficient for improvement of clinical outcomes of PCOS.

6. LIMITATIONS

- It was a single-centered study.
- The sampling technique was non-probability convenient sampling.

CONSENT AND ETHICAL APPROVAL

The research was approved by the Ethics Review committee of Ziauddin University. It was conducted in accordance with the Declaration of Helsinki and informed written consent form was taken from all individuals The present clinical trial was registered in US National Library of Medicine at clinical trial.gov (identifier: NCT04009603, Unique Protocol ID: 651118UZPHA).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Oliveira GB, Avezum A, Roever L. Cardiovascular disease burden: Evolving knowledge of risk factors in myocardial infarction and stroke through population-based research and perspectives in global prevention. Frontiers in Cardiovascular Medicine. 2015;2:32.
2. Feingold KR, Grunfeld C. Obesity and dyslipidemia. Endotext [Internet]: MDText. com, Inc.; 2018.
3. Zafar U, Memon Z, Moin K, Agha S, Hassan JA, Zehra D. Prevalence of PCOS with associated symptoms and complications at Tertiary Care Hospital of Karachi. Journal of Advances in Medicine and Medical Research. 2019:1-9.
4. Ayhan ME, Durmaz SA, Carlioglu A, Demirelli S, editors. Dyslipidemia in young women with polycystic ovary syndrome. 18th European Congress of Endocrinology; BioScientifica; 2016.
5. Kim JJ, Choi YM. Dyslipidemia in women with polycystic ovary syndrome. Obstetrics & Gynecology Science. 2013;56(3):137-42.
6. Macut D, Bjekić-Macut J, Savić-Radojević A. Dyslipidemia and oxidative stress in PCOS. Polycystic Ovary Syndrome. 40: Karger Publishers; 2013;51-63.
7. Diamanti-Kandarakis E, Papavassiliou AG, Kandarakis SA, Chrousos GP. Pathophysiology and types of dyslipidemia in PCOS. Trends in Endocrinology & Metabolism. 2007;18(7):280-5.
8. Castelo-Branco C, Steinvarcel F, Osorio A, Ros C, Balasch J. Atherogenic metabolic profile in PCOS patients: Role of obesity and hyperandrogenism. Gynecological Endocrinology. 2010;26(10):736-42.
9. Wild RA. Dyslipidemia in PCOS. Steroids. 2012;77(4):295-9.
10. El-Mazny A, Abou-Salem N, El-Sherbiny W, El-Mazny A. Insulin resistance, dyslipidemia, and metabolic syndrome in women with polycystic ovary syndrome. International Journal of Gynecology & Obstetrics. 2010;109(3):239-41.
11. Hernández-Mijares A, Bañuls C, Gómez-Balaguer M, Bergoglio M, Víctor VM, Rocha M. Influence of obesity on atherogenic dyslipidemia in women with polycystic ovary syndrome. European Journal of Clinical Investigation. 2013;43(6):549-56.
12. Studen KB, Sever MJ, Pfeifer M. Cardiovascular risk and subclinical cardiovascular disease in polycystic ovary syndrome. Polycystic Ovary Syndrome. 40: Karger Publishers; 2013;64-82.
13. Bates GW, Legro RS. Longterm management of polycystic ovarian syndrome (PCOS). Molecular and cellular endocrinology. 2013;373(1-2):91-7.
14. Kocer D, Bayram F, Diri H. The effects of metformin on endothelial dysfunction, lipid metabolism and oxidative stress in women with polycystic ovary syndrome. Gynecological Endocrinology. 2014;30(5): 367-71.
15. Kialka M, Milewicz T, Wajda A, Czekanska P, Zdzierak B, Mrozinska S, editors. Metformin and changes in serum lipid profile in lean patients with polycystic ovary syndrome (PCOS). 19th European Congress of Endocrinology; BioScientifica; 2017.
16. Guarino A, Guandalini S, Vecchio AL. Probiotics for prevention and treatment of diarrhea. Journal of Clinical Gastroenterology. 2015;49:S37-S45.
17. Morales A, Bravo-Bown J, Bedoya J, Gamonal J. Probiotics and Periodontal Diseases. Insights into Various Aspects of Oral Health: IntechOpen; 2017.
18. Borgeraas H, Johnson L, Skattebu J, Hertel J, Hjelmesaeth J. Effects of probiotics on body weight, body mass index, fat mass and fat percentage in subjects with overweight or obesity: A systematic review and meta-analysis of randomized controlled trials. Obesity reviews. 2018;19(2):219-32.
19. Fortes PM, Marques SM, Viana KA, Costa LR, Naghettini AV, Costa PS. The use of probiotics for improving lipid profiles in dyslipidemic individuals: an overview protocol. Systematic reviews. 2018;7(1): 165.
20. Ali A. Polycystic ovary syndrome and metabolic syndrome. Ceska gynekologie. 2015;80(4):279-89.
21. Yadav H, Lee J-H, Lloyd J, Walter P, Rane SG. Beneficial metabolic effects of a probiotic via butyrate-induced GLP-1 hormone secretion. Journal of Biological Chemistry. 2013;288(35):25088-97.
22. Aronsson L, Huang Y, Parini P, Korach-André M, Håkansson J, Gustafsson J-Å, et al. Decreased fat storage by Lactobacillus paracasei is associated with increased

levels of angiopoietin-like 4 protein (ANGPTL4). PloS one. 2010;5(9):e13087.
23. Guarner F. The gut microbiome: What do we know? Clinical Liver Disease. 2015; 5(4):86.
24. Sun J, Buys N. Effects of probiotics consumption on lowering lipids and CVD risk factors: a systematic review and meta-analysis of randomized controlled trials. Annals of medicine. 2015;47(6):430-40.
25. Ahmadi S, Jamilian M, Karamali M, Tajabadi-Ebrahimi M, Jafari P, Taghizadeh M, et al. Probiotic supplementation and the effects on weight loss, glycaemia and lipid profiles in women with polycystic ovary syndrome: a randomized, double-blind, placebo-controlled trial. Human Fertility. 2017;20(4):254-61.
26. Rajkumar H, Kumar M, Das N, Kumar SN, Challa HR, Nagpal R. Effect of probiotic Lactobacillus salivarius UBL S22 and prebiotic fructo-oligosaccharide on serum lipids, inflammatory markers, insulin sensitivity, and gut bacteria in healthy young volunteers: a randomized controlled single-blind pilot study. Journal of Cardiovascular Pharmacology and Therapeutics. 2015;20(3):289-98.
27. Guardamagna O, Amaretti A, Puddu PE, Raimondi S, Abello F, Cagliero P, et al. Bifidobacteria supplementation: effects on plasma lipid profiles in dyslipidemic children. Nutrition. 2014;30(7-8):831-6.
28. Guo Z, Liu X, Zhang Q, Shen Z, Tian F, Zhang H, et al. Influence of consumption of probiotics on the plasma lipid profile: A meta-analysis of randomised controlled trials. Nutrition, Metabolism and Cardiovascular Diseases. 2011;21(11): 844-50.
29. Panahi Y, Khalili N, Sahebi E, Namazi S, Reiner Ž, Majeed M, et al. Curcuminoids modify lipid profile in type 2 diabetes mellitus: A randomized controlled trial. Complementary Therapies in Medicine. 2017;33:1-5.
30. Bhowmik B, Siddiquee T, Mujumder A, Afsana F, Ahmed T, Mdala I, et al. Serum lipid profile and its association with diabetes and prediabetes in a rural Bangladeshi population. International Journal of Environmental Research and Public Health. 2018;15(9):1944.
31. Garimella S, Seshayamma V, Rao HJ, Kumar S, Kumar U, Saheb SH. Effect of metformin on lipid profile of type II diabetes. Int J Intg Med Sci. 2016; 3(11):449-53.
32. Karimzadeh MA, Eftekhar M, Taheripanah R, Tayebi N, Sakhavat L, Zare F. The effect of administration of metformin on lipid profile changes and insulin resistance in patients with polycystic ovary syndrome. Middle East Fertility Society Journal. 2007; 12(3):174.
33. Zhang E, Wang P, Li X, Xing W, Tao X. Effects of metformin on the blood lipid profile and insulin sensitivity in obese women with polycystic ovary syndrome. Heart. 2012;98(Suppl 2):E155-E6.
34. Singh G, Afroz N, Saeed N, Siddiqi SS, Ehsan A, Rafey M. Study of lipid profile in patients of polycystic ovarian syndrome before and after metformin therapy. Annals of Pathology and Laboratory Medicine. 2017;4(4).
35. Kiałka M, Gałuszka-Bednarczyk A, Wajda A, Czekańska P, Zdzierak B, Mrozińska S, et al. Metformin and changes in serum lipid profile in lean patients with polycystic ovary syndrome. Przeglad lekarski. 2017; 74(4):144-6.
36. Luong DQ, Oster R, Ashraf AP. Metformin treatment improves weight and dyslipidemia in children with metabolic syndrome. Journal of Pediatric Endocrinology and Metabolism. 2015;28(5-6):649-55.
37. Shavakhi A, Minakari M, Firouzian H, Assali R, Hekmatdoost A, Ferns G. Effect of a probiotic and metformin on liver aminotransferases in non-alcoholic steatohepatitis: A double blind randomized clinical trial. International Journal of Preventive Medicine. 2013;4(5):531.

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