Original Article

Effect of probiotic supplements in women with gestational diabetes mellitus on inflammation and oxidative stress biomarkers: a randomized clinical trial

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Background and Objectives: Very little is known about the use of probiotics among pregnant women with gestational diabetes mellitus (GDM) especially its effect on oxidative stress and inflammatory indices. The aim of present study was to measure the effect of a probiotic supplement capsule on inflammation and oxidative stress biomarkers in women with newly-diagnosed GDM. **Methods and Study Design:** 64 pregnant women with GDM were enrolled in a double-blind placebo controlled randomized clinical trial in the spring and summer of 2014. They were randomly assigned to receive either a probiotic containing four bacterial strains of Lactobacillus acidophilus LA-5, Bifidobacterium BB-12, Streptococcus Thermophilus STY-31 and Lactobacillus delbrueckii bulgaricus LBY-27 or placebo capsule for 8 consecutive weeks. Blood samples were taken pre- and post-treatment and serum indices of inflammation and oxidative stress were assayed. The measured mean response scales were then analyzed using mixed effects model. All statistical analysis was performed using Statistical Package for Social Sciences (SPSS) software (version 16). **Results:** Serum high-sensitivity C-reactive protein and tumor necrosis factor-α levels improved in the probiotic group to a statistically significant level over the placebo group. Serum interleukin-6 levels decreased in both groups after intervention; however, neither within group nor between group differences interleukin-6 serum levels was statistically significant. Malondialdehyde, glutathione reductase and erythrocyte glutathione peroxidase levels improved significantly with the use of probiotics when compared with the placebo. **Conclusions:** The probiotic supplement containing L.acidophilus LA- 5, Bifidobacterium BB-12, S.thermophilus STY-31 and L.delbrueckii bulgaricus LBY-2 appears to improve several inflammation and oxidative stress biomarkers in women with GDM.

Key Words: gestational diabetes mellitus, probiotics, inflammation, oxidative stress

INTRODUCTION

Gestational diabetes mellitus (GDM) is a common metabolic condition in pregnancy in which the pregnant woman has high serum glucose levels during gestation.[1] The American Diabetes Association states that the incidence of GDM is between 1% and 14%, and it entraps approximately 7% of pregnancies.[2] The incidence rate of GDM has exhibited quantized upward trend making it an increasing health threats.[3] It is characterized by maternal insulin resistance and is associated with inflammation throughout gestation.[4] Both mothers with GDM and their offspring have an increased risk of diabetes later in life and to other consequences of GDM[5] which could be prevented with appropriate treatment.[6-8] GDM is a growing health problem globally and is a common pregnancy-related complication.[9,10] Actually, the prevalence has growth by 10–100% in the most recent twenty years[11] and inflicts an important financial load with adverse maternal and neonatal consequences in pregnancy and long term well-being.[12,13]

The association of oxidative stress and inflammatory biomarkers with GDM in pregnant women has been dis-

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Manuscript received 03 January 2016. Initial review completed 07 February 2017. Revision accepted 22 February 2017.
doi: 10.6133/apjcn.082017.03

cussed. Oxidative stress is considered to be a common determinant of insulin resistance. It may present as inflammation that generates various inflammatory mediators.[9,14-16]

Several studies have confirmed the existence of an interaction between the host and gut microbiota. One clinical study on 91 pregnant women, by Koren et al showed a significant change in the composition of gut microbiota in the first trimester to the third trimester of pregnancy.[15] The flexibility of the intestinal microbial population and its fundamental role in the promotion of intestinal barrier function and control of local and systemic inflammatory response has drawn the attention of non-communicable disease specialists.[17]

Probiotics have been shown to affect oxidative stress and inflammation.[18-22] They have also been investigated for their impact on type 2 diabetes mellitus (DM) and the prevention and management of GDM.[19,23-27] Evidence on the effect of probiotics on inflammatory and oxidative stress biomarkers in GDM is quite limited. Some studies have examined the effect of probiotics on preventing GDM, some the general glucose patterns among normal pregnant populations and a few have assessed inflammatory and oxidative stress biomarkers. Randomized clinical trial studies investigating the effect of probiotics taken after diagnosis of GDM were rare, especially those assessing the effect on inflammatory and oxidative stress in women with GDM.[28]

The most vital factors in being advantageous of probiotics intervention in inflammation and metabolism are genus and strains provided in the probiotics. Lactobacillus sp. and Bifidobacterium sp. have widely been studied and extensively used. Lactobacillus and in particular, Lactobacillus acidophilus (L.acidophilus) is recognized for its "anti-inflammatory", "anti-oxidant", "immune-modulatory", "anti-cancerous", "anti-diabetic", and "anti-arthritic" properties.[29-34] On the other hand, Bifidobacterium animalis subsp. lactis BB-12 is an extensively used probiotic species of Bifidobacterium.[35] Its "anti-inflammatory", "immune-modulatory", "antioxidant" and "anti-lipidemic" properties have been suggested in previous studies.[36-38] Beneficial properties of L.acidophilus and/or Bifidobacterium animalis in pregnancy have been shown previously in literatures.[39-44]

Treatment of patients with GDM provides an ideal basis for early interventional studies that are designed to prevent type II DM. It is expected that the benefits of probiotics in this critical period of growth will be obvious in terms of therapy and prevention and more importantly in planning for future disease.[45] The aim of present study was to measure the effect of a probiotic supplement capsule containing four bacterial strains in comparison with a placebo on inflammatory and oxidative stress biomarkers among women with newly-diagnosed GDM.

METHODS

Ethics

The study protocol was approved by the Ethics committee at Shahid Beheshti University of Medical Sciences (No. 116/449, date 1393/02/28). Written informed consent was obtained from all participants. The Clinical trial was registered in the Iranian Registry of Clinical Trials (no. IRCT201405181597N3) and is accessible through the World Health Organization database of clinical trial registries.

Study participants

The qualified subjects included all nulliparous women with GDM screened during 24-28 weeks of gestation using a two-hour 75-g oral glucose tolerance test, which referred to specialty and subspecialty gynecology or endocrinology clinics at Tabriz University of Medical Sciences.

Inclusion and exclusion criteria

The enrollment criteria were to be female aged 18-45 years, nulliparous, with a diagnosis of GDM between 24 and 28-weeks (+6 days) of gestation diagnosed by screening done by a gynecologist or internal medicine specialist, with a fasting blood sugar of 92 to 125 mg/dL early in diagnosis, a pre-pregnancy body mass index (BMI) above 18.5 kg/m2, no history of impaired glucose tolerance in early pregnancy, no history of type 2 DM, no history of chronic disease, no smoking or alcohol consumption, no ingestion of probiotics including probiotic yogurt, kefir and other fermented foods within two weeks of the intervention, no use of antibiotics for one month prior to intervention, lack of acute gastrointestinal problems for one month prior to intervention and no use of glucocorticoids (GCs) or immunosuppressive drugs. All subjects were appraised about the intervention and if they admitted to contribute in the study, they were asked to sign a consent form before participation.

The exclusion criteria were needs to insulin therapy or other diabetes medications (fasting plasma glucose >105 and blood sugar 2-hour postprandial >120 mg/dL) during intervention, the ingestion of other forms of probiotics including probiotic yogurt, kefir and other fermented foods, antibiotics, GCs or immunosuppressive drugs or any acute gastrointestinal problems during intervention and finally not taking an adequate number of capsules.

Sample size

The sample size was determined as 26 subjects per group, regarding to high sensitive C-reactive protein (hs-CRP) index in Asemi et al study[46] to detect the effect size of d=7.07 with the power of 90% and alpha of 5%. Considering 20 % as withdrawals, the study started with 32 subjects per group.

Study design

The effects of probiotic supplements on inflammatory and stress oxidative indices of nulliparous pregnant women with GDM were studied in a double blind placebo controlled randomized clinical trial. Sixty-four subjects with GDM who referred to Al-Zahra University Hospital in Tabriz, a city in northwestern Iran, were enrolled in the spring and summer of 2014. The patients were randomly allocated to receive either a probiotic supplement or a placebo capsule once in a day for eight weeks.

All 64 pregnant participants were randomly allocated using block randomization techniques and were stratified according to the FBS and pre-pregnancy BMI groups. To ensure double blinding, a coder anonymously labeled the

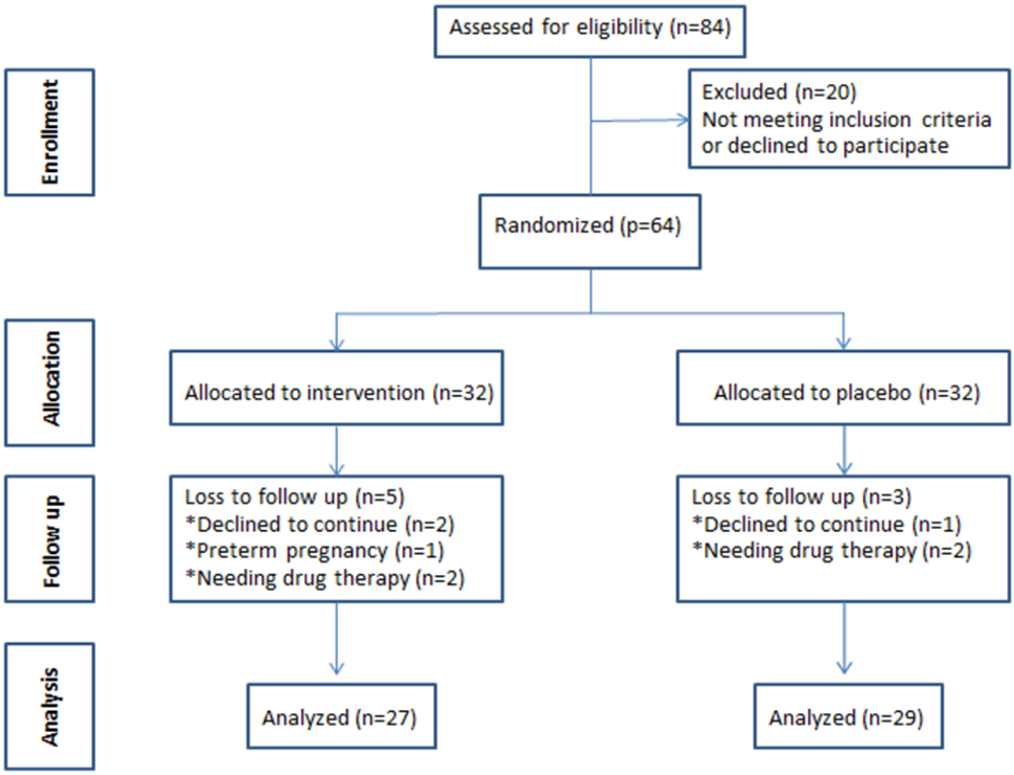

Figure 1. CONSORT 2010 flow diagram for the randomized placebo controlled clinical trial of the effect of probiotics in gestational diabetes mellitus

capsules packages as "A" or "B" and they were then assigned by the therapist according to the random sequence generated by a computer program.[47] Nutritional counseling by a trained nutritionist was provided equally to all subjects in both groups.[48,49]

Each probiotic capsule contained four bacterial strains (4 biocap >4*10^9 CFU) with standard freeze-dried cultures of L.acidophilus LA-5, Bifidobacterium BB-12, Streptococcus thermophilus (S.thermophilus STY-31) and Lactobacillus delbrueckii bulgaricus (L.delbrueckii bulgaricus LBY-27) plus dextrose anhydrous filler and magnesium stearate lubricant (CHR. HANSEN; Denmark) that were packed and gelatin covered by Tehran Darou. The Placebo capsules without bacteria had the same specifications as probiotic capsules and were identical in design, color and shape.

Patients received a 2-week supply of capsules every two weeks. During the study period, to control subjects in terms of supplement intake and to prevent attrition, telephone contact was established with each participant every week. Every two weeks, the mothers were asked to bring in the bubble packs of the capsules to count the number taken and confirm their compliance and decide whether or not to have the subject continue in the programs.

Study evaluation

During the initial interview, participants completed a general questionnaire and a dietary recall questionnaire. The general questionnaire was utilized to collect data on demographic information, pre-pregnancy weight, physical activity, medical history, drug history over the past month, and use of probiotics over the two weeks prior to intervention. Height was measured and additional information about their dietary habits and pre-pregnancy weight were also recorded to calculate the pre-pregnancy BMI. A Seca 206 wall-mounted stadiometer and Seca 813 digital scale were implemented to measure weight and height. BMI was then calculated and categorized according to the world health organization (WHO) guidelines.[50]

A 24-h dietary recall questionnaire was completed for three nonconsecutive days each (two weekdays and one weekend day, once at the baseline, after 4 weeks and post-intervention. To obtain the daily macro- and micronutrient intake of each subject, modified version of Nutritionist IV software (First Data-Bank, San Bruno, CA, USA) for Iranian food was used.

Blood samples were taken and patients were randomly assigned in one of two study groups, to receive either a 2-week package of probiotic or placebo capsules. Patients were visited at the obstetrics and gynecology clinics of Tabriz Al-Zahra University Hospital. Fasting blood samples (10 mL) were taken at baseline and after 8-wk intervention in each separate group at Al-Zahra Hospital laboratory in early morning after an all-night fasting.

Tumor necrosis factor-alpha (TNF-α) was measured by immune-enzymatic assay using TNF-α EASIA kit no. KAP1751. This is an immune-enzymometric assay for quantitative measurement of human TNF-α in serum, plasma, cell culture medium or other biological fluids.[51-55] Interleukin-6 serum assay was done using an IL-6 human ELISA kit (IL-6-EASIA-CE; KAC1261) designed to quantify human IL-6 protein levels in serum, plasma, supernatant, and other biological fluids.[56-60] Hs-CRP was measured using a Monobind hs-CRP Elisa kit. Measurement of serum total antioxidant capacity (TAC) level was done using a LDN TAC Colorimetric Assay Kit.[61] Eryth-

rocyte Superoxide Dismutase (SOD) was assayed using a spectrum auto-analyzer (Abbott; model Alcyon 300; USA) and Biorex (BXC0531A) kits.[62] Erythrocyte glutathione peroxidase (GPX) was assayed using the spectrum auto-analyzer (Abbott ; model Alcyon 300 ; USA) and Biorex (BXC0551A) kits.[63] Glutathione reductase (GSHR) was assayed using an Eastbiopharm Human Glutathione Reductase ELISA Assay Kit based on sandwich enzyme immunoassay. Serum uric acid concentrations were assayed using uric acid kit (Pars Azmoon Inc., Tehran, Iran).

Statistical analysis

Data were analyzed using SPSS statistical software package version 16. Mean ± standard deviation (SD) for quantitative data and frequency and percent (%) for qualitative data were reported regarding study groups. Baseline characteristics were compared between two groups by chi square or exact test (Fishers exact test, for 2x2 contingency tables and Monte Carlo test for larger contingency tables) for categorical variables and independent t-tests for continuous variables. For all continuous data Shapiro-Wilk test and Levene's test was used to assess normality and Variances homogeneity, respectively.

Mean response scales measured over the 8-week study period were analyzed using mixed effects model including mixed effects model, in which participants were assumed to be random effects and treatment, sequence, and period were assumed to be estimable fixed effects. To avoid violation of the assumption of sphericity, Greenhouse-Geisser corrections were applied to correct the degrees of freedom. Log transformation was considered to TNF-α due to right skewedness of the data. We used transformed variables to our statistical inferences, and presented main findings in terms of statistics which have been back-transformed to usual scale.

Pairwise comparisons were corrected by Bonferroni adjustments. Details about the methodology of the study and energy intake analysis has been published elsewhere.[64] A p-value of <0.05 was regarded as statistically significant.

RESULTS

A total of 64 nulliparous pregnant women with GDM participated in the study. Data from 29 patients in the intervention group and 27 in the placebo group were finally analyzed (see CONSORT flow-diagram in Figure 1).

Generally, the compliance rate was high, such that 100% of capsules were taken during the study in two groups. No serious complications were reported following the consumption of the supplements in subjects with GDM throughout the study. The mean age of the subjects was 27.3 years (SD: 5.8). Of the 56 participants, 28 (50.0 %) had a positive family history of DM. The study subjects were predominantly Diploma (57.1 %), housewife (66.1%) and urban residence (57.1 %). Furthermore, approximately 70 % of the individuals reported low physical activity. As shown in Table 1, the Baseline characteristics (age, family history of DM, educational level, employment, residence, physical activity, weight, height and BMI) of the pregnant women under study were similar in both groups. Blood glucose levels at baseline showed no significant difference between groups in terms of classification of blood glucose at baseline.

The comparisons of inflammatory markers between groups in the same moment showed that at the baseline evaluation, there were no differences between groups concerning hs-CRP (p=0.551), TNF-α (p=0.703), serum IL-6 (p=0.496).

Hs-CRP value showed decrease in probiotic group an increase in placebo group at the after-treatment evaluations, where a generalized linear mixed model analysis for two factors (group and time of evaluation) revealed

Table 1. Baseline comparison of demographic and anthropometric characteristics of the participants

Variables	Intervention (n=29)	Placebo (n=27)	p-value
Maternal age, y	28.1±6.25	26.5±5.24	0.369
Family history of DM, n (%)			
Yes	16 (55. 2)	12 (44.4)	0.593
No	13 (44.8)	15 (55.6)	
Educational level, n (%)			
Under diploma	4 (13.8)	5 (18.5)	0.893
High school diploma	17 (58.6)	15 (55.6)	
Academic education	8 (27.6)	7 (25.9)	
Employment, n (%)			
Employed	10 (34.5)	9 (33.3)	0.998
Unemployed or housewife	19 (70.4)	18 (66. 7)	
Residence, n (%)			
Urban	17 (58.6)	15 (55.6)	0.997
Rural	12 (41.4)	12 (44.4)	
Physical activity, n (%)			
Low	22 (75.9)	17 (62.9)	0.386
Moderate	7 (24.1)	10 (37.1)	
High	0 (0.0)	0 (0.0)	
Weight, kg	83.3±12.1	78.7±11.1	0.143
Height, cm	162.7±5.6	162.1±5.9	0.728
BMI, kg/m^2	31.4±3.9	29.9±3.4	0.126

BMI: body mass index; DM: diabetes mellitus.
Numeric scales are reported as mean±standard deviation, and categorical measures are reported as frequency (percent).

Table 2. Inter and Intra-group changes in serum inflammatory indices compared for participants

Inflammatory indices	Probiotic (n=29)	Placebo (n=27)
Serum hs-CRP (μg/mL)		
Before Intervention	8.16 (0.934)	8.93 (0.878)
After Intervention	7.46 (0.559)	9.76 (0.792)
Mean differences	-0.704 (0.622)†	0.823 (0.945)†
p- value	0.364	0.307
lnTNF-α (pg/mL) ‡		
Before Intervention	1.96 (0.0752)	1.85 (0.086)
After Intervention	1.92 (0.0971)	2.25 (0.124)
Mean differences	-0.0412 (0.103)†	0.379 (0.123)†
p- value	0.703	0.001
Serum IL-6 (pg/mL)		
Before Intervention	3.11 (0.551)	3.72 (0.711)
After Intervention	2.68 (0.529)	3.19 (0.504)
Mean differences	-0.421 (0.622)	-0.522 (0.732)
p- value	0.526	0.448

hs-CRP: high sensitivity C-reactive protein; IL-6: interleukin 6; TNF-α: tumor necrosis factor alpha.
The data are presented as man (SE).
†In the same row means intragroup differences.
‡To ensure normal distribution logarithmic transformation was applied.

Table 3. Inter and Intra-group changes in oxidative stress indices compared for participants

Oxidative stress indices	Probiotic (n=29)	Placebo (n=27)
Serum TAC (mmol/L)		
Before study	1.51 (0.0403)	1.56 (0.0424)
After study	1.66 (0.0542)	1.55 (0.0691)
Mean differences	0.147 (0.0701) †	- 0.00741 (0.0706) †
p- value	0.038	0.992
Serum MDA (nmol/mL)		
Before study	4.84 (0.438)	4.11 (0.139)
After study	3.89 (0.245)	4.96 (0.323)
Mean differences	- 0.942 (0.429)†	0.848 (0.339)†
p- value	0.017	0.038
Serum GSHR (ng/mL)		
Before study	24.003 (2.02)	24.8 (2.95)
After study	28.38 (2.13)	22.6 (1.81)
Mean differences	4.38 (1.83)†	-2.21 (2.91)†
p- value	0.068	0.368
Erythrocyte GPx (U/gHb)		
Before study	42.3 (2.01)	44.7 (3.38)
After study	39.63 (2.12)	32.7 (1.91)
Mean differences	-2.67 (2.17)†	-12.002 (3.71)†
p- value	0.368	< 0.011
Serum uric acid (mg/dL)		
Before study	2.65 (0.111)	2.49 (0.118)
After study	2.46 (0.108)	2.67 (0.116)
Mean differences	-0.193 (0.114) †	0.178 (0.100) †
p- value	0.075	0.113
Erythrocyte SOD (U/gHb)		
Before study	2243.8 (80.3)	2290.9 (66.7)
After study	2278.5 (91.3)	2089.3 (86.5)
Mean differences	34.7 (66.5) †	-201 (98.7) †
p- value	0.673	0.021

GPx: glutathione peroxidase; GSHR: glutathione reductase; MDA: malondialdehyde; SOD: superoxide dismutase; TAC: total antioxidant capacity.
The data are presented as Mean (SE).
†In the same column means intragroup differences.

that adding probiotics to the routine diet results in significantly lower mean hs-CRP values (p=0.019) (Table 2).

TNF-α serum levels (logarithmic scale) decreased in the intervention group and increased in the placebo group. Intergroup comparison indicated that the serum TNF-α levels were significantly lower in probiotic group as compared to placebo (-0.04±0.10 vs 0.38±0.12; p=0.009). The serum IL-6 levels had decreased in both groups and there was not a significant difference between two groups in the term of serum IL-6 levels at final evaluation (-0.42±0.62 vs -0.53±0.73; p=0.915) (Table 2).

The baseline measures of serum TAC (p=0.465), serum malondialdehyde (MDA) (p=0.131), serum GSHR (p=0.813), erythrocyte GPx activity (p=0.536), serum uric

acid (p=0.318) and erythrocyte SOD activity levels (p=0.656) were not significantly different between groups.

A generalized linear mixed model analysis for two factors (group and time of evaluation) revealed that serum MDA, serum GSHR and erythrocyte GPx levels improved in the intervention group over the placebo group at statistically significant levels (p=0.002, p=0.047 and p=0.032 respectively (Table 3). Serum TAC (p=0.144), serum uric acid (p=0.148) and erythrocyte SOD activity (p=0.055), however, did not change significantly between groups.

Details on glucose control in groups, insulin levels, insulin resistance, sensitivity and gestational weight gain pre and post-treatment have been published elsewhere.[28]

DISCUSSION

The present study found that both inflammatory and oxidative stress biomarkers improved after use of the probiotic supplement when compared to the placebo. The differences for two of the three inflammatory biomarkers, hsCRP and TNF-α were statistically significant. Serum IL-6 levels did not change significantly between groups.

Despite the fact that a few probiotic clinical trials have been conducted among pregnant women, randomized clinical trials investigated the effect of probiotics taken after diagnosis of GDM are rare, especially those that evaluated the effect of L.acidophilus LA-5, Bifidobacterium BB-12, S.thermophilus STY-31 and L.delbrueckii bulgaricus LBY-27 on both inflammatory and oxidative stress in women with GDM.

It has been generally established that the microbiota has an essential role in health and disease. How probiotics influence healthy and unhealthy animals and humans is a field of serious investigation and attention. The most extensively applied probiotic bacteria pertain to Bifidobacterium and Lactobacillus spp. Those species have been widely examined for their health advancing effects where intestinal dysbiosis is concluded to have a causal role.[65,66] L. acidophilus, one of the most common probiotics, has been established its role in inflammatory regulation.[67,68] An animal study examined the effect of administration of probiotic Lactobacillus acidophilus on the inflammatory response to enterotoxigenic Escherichia coli (ETEC) K88 in piglets and found that L.acidophilus arranged inflammatory response via harming both nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and mitogen-activated protein kinase (MAPK) signaling pathways.[69] In another study, Bifidobacterium animalis (10^9 CFU/day) in high-fat diet-induced obese mice, reduced glucose intolerance, bacterial attachment to the gut mucosa, plasma lipopolysaccharide concentrations and expression of inflammatory markers (TNF-α and IL-1β) in liver.[70] Secretion metabolites of S.thermophilus were capable of preventing LPS induced TNF-α production by immune cells.[72] There are growing evidences demonstrating that probiotics have valuable benefits on intestinal inflammation.[71,72] They also inhibit the NF-κB pathway, which therewith decreases oxidative stress.[73,74]

In a recent randomized controlled clinical trial by Jafarnejad et al[75] the effect of probiotic VSL#3 supplementation on glycemic status and inflammatory markers among pregnant women with GDM were studied over the course of 8 weeks. Probiotic supplements contained eight strains of lactic acid bacteria (S.thermophilus, Bifidobacterium breve, Bifidobacterium longum, Bifidobacterium infantis, L.acidophilus, L.plantarum, L.paracasei, and L.delbrueckii subsp. Bulgaricus). They found a significant decrease in levels of TNF-α and hsCRP in the probiotic group compared to the placebo which was parallel to the results of the present study. Unlike the results of current study, however, the levels of serum IL-6 decreased significantly.

Several clinical trial studies have been conducted to assess the anti-inflammatory and antioxidant properties of probiotics. Some were conducted with healthy participants, including a randomized clinical trial on 62 volunteers who were randomized to receive a milk-based drink containing either L.rhamnosus GG (LGG), Bifidobacterium animalis ssp. lactis Bb12 (Bb12) and Propionibacterium freudenreichii ssp. shermanii JS (PJS) or a placebo drink for three weeks.[76] The aim was to assess serum hsCRP, in vitro production of TNF-α from in vitro cultured peripheral blood mononuclear cells and IL-2 production. The study revealed that probiotic bacteria produced strain-specific anti-inflammatory effects in healthy adults. The effect of the consumption of a yogurt containing Bifidobacterium animalis subsp. lactis BB-12 at a dose of $\log_{10}$±0.5 CFU/day on immune responses was investigated in a randomized, partially blinded, 4-period crossover study among young adults and the findings supported the anti-inflammatory properties of Bifidobacterium animalis subsp. lactis Bb-12.[36]

The association between subclinical inflammation and GDM can be explained through different mechanisms. The progressive increase in insulin resistance that occurs among women with GDM is caused by the anti-insulin effects of accumulated adipose tissue and placental hormones (cortisol and human placental lactogen). Glycated end-products produced hyperglycemia increase oxidative stress. They also activate macrophages, and increase serum IL-1, IL-6 and TNF levels, which increases production of CRP. Increased CRP levels in GDM patients can also be attributed to adipose tissue cytokines.[15-17,77]

The level of four out of five oxidative stress serum biomarkers (MDA, GSHR, GPx and SOD) improved significantly when compared with the placebo. TAC was the only stress biomarker that did not change significantly. Oxidative stress management is a major challenge for researchers and clinicians. Hypoglycemia and oxidative stress are closely associated with the complications of diabetes. Abnormally high levels of blood glucose increase free oxygen, hydrogen peroxide and superoxide radical production.[78]

Growing evidence indicates the importance of oxidative stress in the pathogenesis of GDM[79] and complications of diabetic pregnancies for both mother and fetus.[80,81]

Evidence suggests that, probiotics exert biological effects through different mechanisms. One of the most controversial is their antioxidant activity.[82] Previous animal and human studies have reported findings that are analogues with those of present study. An animal study used bacterial strains to prepare a potentially antioxidative probiotic formulation, which was then administered to

rats for 18 days.[59] Analysis of plasma MDA, GSHR, GPx and SOD activity showed that the antioxidant mixture effectively reduced doxorubicin-induced oxidative stress.

The mechanism of action of lactic acid bacteria and bifidobacteria in the terms of antioxidative function is not fully understood, but prevention of spontaneous oxidation of ascorbate, chelating of the metal ions, reducing activity, scavenging the free radicals of superoxide anion and hydrogen peroxide and reactive oxygen species which are continuously produced in the food and human body and preventing peroxidation of plasma lipids are some of probable mechanisms.[83-85] A potential mechanism of improved antioxidative activities by probiotics may be an increasing expression of MDA, GSHR, GPx and SOD.[86] Enhanced SOD and GPx with probiotics have been described in neonatal rats.[20] Another description might be the additional production of inflammatory cytokines and increased oxidative stress during gestation because of weight gain.[87,88] Obesity, or increased fat repletion, is known to promote oxidative stress.[89,90] The impact of probiotic supplements containing L.acidophilus LA- 5, Bifidobacterium BB-12, S.thermophilus STY-31 and L.delbrueckii bulgaricus LBY-2 in controlling weight gain during pregnancy has been shown previously.[28]

The authors concluded that probiotic supplements containing L.acidophilus LA-5, Bifidobacterium BB-12, S.thermophilus STY-31 and L.delbrueckii bulgaricus LBY-2 may help prevent and control diseases associated with oxidative stress.

The findings of present study contrast with those of Soleimani et al[91] who observed a substantial increase in TAC plasma levels in diabetic patients on hemodialysis after taking L.acidophilus, L.casei and Bifidobacterium bifidum for 12 weeks when compared with the placebo. Probiotic supplementation also resulted in significant reductions in plasma MDA. Jamilian et al[92] reported that multispecies probiotic supplements containing L.acidophilus, L.casei and Bifidobacterium bifidum in healthy pregnant women from 9 weeks of gestation for a duration of 12 weeks, increased plasma TAC significantly compared to the placebo after adjusting for baseline values, age and BMI at the trial baseline.

The difference in the results may be due to differences in plasma levels of TAC, the probiotic strain used and/or the prevailing dose and pathological conditions of the subjects.

Probiotics exert a biological effect by various mechanisms; one of the most debated is antioxidant activity. Among the beneficial effects of probiotics, their ability to protect against oxidative stress in humans has been reported by several authors.[82]

A clinical trial on a healthy population confirmed the anti-oxidative effect of probiotic L.fermentum ME-3 in volunteers on oxidative stress markers in the blood and urine after 3 weeks of probiotic consumption. Total anti-oxidative activity, total anti-oxidative status, and glutathione red-ox ratio were assayed. They showed improvement, but the change was not statistically significant.[93] Asemi et al[86] evaluated the effect of probiotic yoghurt enriched with probiotic culture of two strains of lactobacilli (L.acidophilus LA5) and bifidobacteria (Bifidobacterium lactis BB12) on oxidative stress. They concluded that compared to the conventional yogurt, the consumption of probiotic yogurt among pregnant women increased serum erythrocyte GSHR levels, but they inferred no other effect of other indices of oxidative stress.

Other studies on healthy populations primarily showed significant results in favor of probiotics use, to improve anti-inflammatory or antioxidant biomarkers. Outcome biomarkers including levels of reactive plasma oxygen metabolites, biological antioxidant potential, TAC, MDA, vitamin E and biological antioxidant potential (BAP) were studied.[94-96]

In general, probiotics appear to affect inflammatory and oxidative stress biomarkers; however, it has been suggested that antioxidant activity reported for probiotics is both stain-specific and dose-dependent.[97]

In the terms of the underlying mechanisms of probiotics on systemic inflammation and oxidative, evidence has increasingly demonstrated that probiotics have a positive impacts on gastrointestinal inflammation. Probiotics also can create a barricade of the NF-kB pathway without direct contact and attenuates oxidative stress. In addition, these anti-oxidative and anti-inflammatory effects defend gut barrier wellbeing against inflammation and oxidative stress.

Probiotics could be the missing ingredient in dietary intervention that focuses on the interaction of the food matrix and dietary contents with gut microbiota. Specific probiotics and dietary intervention, may control intestinal gut barrier function, systemic and local inflammation and reverse the vicious cycle of abnormal metabolic control during pregnancy.[98]

The results of present study provide new horizons for researcher and clinicians in the management of GDM. Few studies have investigated the potential benefits of antioxidant agents in patients with GDM. Alternative approaches including the use of flavonoids and peroxisome proliferator-activated receptor (PPAR) agonists can be useful,[99] however, it has been shown that the administration of vitamins in the perinatal period, increases blood glucose levels in patients with GDM.[100] This has been linked with the risk of metabolic syndrome in male infants in their adulthood.[101]

Some of the strengths of the present study are the evaluation of inflammatory and oxidative indices as a double-blind clinical trial and the randomized design. This study has also some limitations that should be considered. Failure to investigate the effect of probiotic supplementation on pregnancy outcomes, short duration of study, Lack of a control group of healthy pregnant women and lack of generalizability due to nulliparous subjects are some of these limitations. Future studies with bigger sample sizes and longer duration of the intervention are needed to endorse these findings.

Conclusion

The present study concluded that a probiotic supplement containing L.acidophilus LA-5, Bifidobacterium BB-12, S.thermophilus STY-31 and L.delbrueckii bulgaricus LBY-2 improves several inflammatory and oxidative stress biomarkers in women with GDM.

ACKNOWLEDGEMENTS

We are grateful to all participants who helped us to conduct this study and Tehran Darou Pharmaceuticals, Tehran, Iran, for providing 4- Biocap (a probiotic supplement). The authors would like to thank the staff of Al-Zahra Obstetrics and Gynecology Clinic (Tabriz, Iran) for their assistance during the study.

AUTHOR DISCLOSURES

The authors declare that they have no competing interests.

This study (the design of the study and collection, analysis, and interpretation of data and writing the manuscript) was supported financially by Shahid Beheshti University of Medical Sciences.

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