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The Effects of Synbiotic Supplementation on Pregnancy Outcomes in Gestational Diabetes

Maryam Karamali[1] · Nabiollah Nasiri[1] · Naemeh Taghavi Shavazi[1] · Mehri Jamilian[2] · Fereshteh Bahmani[3] · Maryam Tajabadi-Ebrahimi[4] · Zatollah Asemi[3]

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Abstract Synbiotics are known to exert multiple beneficial effects, including anti-inflammatory and antioxidative actions. This study was designed to evaluate the effects of synbiotic administration on biomarkers of inflammation, oxidative stress, and pregnancy outcomes among gestational diabetic (GDM) women. This randomized, double-blind, placebo-controlled clinical trial was carried out among 60 subjects with GDM who were not on oral hypoglycemic agents. Patients were randomly assigned to consume either one synbiotic capsule containing *Lactobacillus acidophilus* strain T16 (IBRC-M10785), *L. casei* strain T2 (IBRC-M10783), and *Bifidobacterium bifidum* strain T1 (IBRC-M10771) (2×10^9 CFU/g each) plus 800 mg inulin (HPX) ($n = 30$) or placebo ($n = 30$) for 6 weeks. Compared with the placebo, synbiotic supplementation significantly decreased serum high-sensitivity C-reactive protein (hs-CRP) (-1.9 ± 4.2 vs. $+1.1 \pm 3.5$ mg/L, $P = 0.004$), plasma malondialdehyde (MDA) (-0.1 ± 0.6 vs. $+0.3 \pm 0.7$ μmol/L, $P = 0.02$), and significantly increased total antioxidant capacity (TAC) ($+70.1 \pm 130.9$ vs. -19.7 ± 124.6 mmol/L, $P = 0.009$) and total glutathione (GSH) levels ($+28.7 \pm 61.5$ vs. -14.9 ± 85.3 μmol/L, $P = 0.02$). Supplementation with synbiotic had a significant decrease in cesarean section rate (16.7 vs. 40.0%, $P = 0.04$), lower incidence of hyperbilirubinemic newborns (3.3 vs. 30.0%, $P = 0.006$), and newborns' hospitalization (3.3 vs. 30.0%, $P = 0.006$) compared with the placebo. Synbiotic supplementation did not affect plasma nitric oxide (NO) levels and other pregnancy outcomes. Overall, synbiotic supplementation among GDM women for 6 weeks had beneficial effects on serum hs-CRP, plasma TAC, GSH, and MDA; cesarean section; incidence of newborn's hyperbilirubinemia; and newborns' hospitalization but did not affect plasma NO levels and other pregnancy outcomes.

http://www.irct.ir: www.irct.ir: IRCT201704205623N108

Keywords Synbiotic supplementation · Gestational diabetes · Pregnant women

Introduction

Gestational diabetes (GDM) is defined as hyperglycemia, insulin resistance, and carbohydrate intolerance with the onset or first recognition during pregnancy [1]. The prevalence of GDM is 6–20% of pregnant women, and its prevalence is increasing in parallel with the obesity epidemic [2, 3]. GDM women have an increased risk of adverse maternal and perinatal complications, including preeclampsia, hydramnios, increased operative intervention and future type 2 diabetes mellitus, macrosomia, congenital anomalies, metabolic abnormalities, and subsequent childhood and adolescent obesity [4]. In addition, increased levels of inflammatory mediators and biomarkers of oxidative stress can induce maternal insulin resistance, DNA damage, and chromosomal aberrations [5, 6].

✉ Zatollah Asemi
asemi_r@yahoo.com

[1] Department of Gynecology and Obstetrics, School of Medicine, Iran University of Medical Sciences, Tehran, Iran

[2] Endocrinology and Metabolism Research Center, Arak University of Medical Sciences, Arak, Iran

[3] Research Center for Biochemistry and Nutrition in Metabolic Diseases, Kashan University of Medical Sciences, Kashan, I.R, Iran

[4] Faculty member of Science department, science faculty, Islamic Azad University, Tehran Central branch, Tehran, Iran

Prior studies have documented that throughout pregnancy, the gut microbiota undergoes significant changes [7, 8]. These changes in the gut microbiota can result in maternal inflammation, hyperglycemia, and insulin resistance [9]. Recently, few studies have reported beneficial effects of probiotics and synbiotics on metabolic profiles among pregnant women and diseases related to metabolic disorders. We have previously indicated that probiotic intake among GDM women for 6 weeks had beneficial effects on inflammatory and oxidative stress parameters but did not influence pregnancy outcomes [10]. Consumption of probiotic-rich food in pregnant women was associated with lower rates of preterm birth and preeclampsia [11], which may be attributed to the beneficial effects of probiotic administration on placental inflammatory responses [12]. In addition, synbiotic administration for 6 weeks among subjects with GDM improved markers of insulin metabolism, triglycerides, and VLDL-cholesterol values [13]. The positive effects of synbiotic administration for 8 weeks in overweight and obese children on inflammation markers, which were dependent to its effect on weight reduction, have also been reported [14].

To our knowledge, data on the effects of synbiotics on pregnancy outcomes in GDM women are scarce. In addition, data on the effects of probiotic supplementation on biomarkers of inflammation and oxidative stress are conflicting. Therefore, we hypothesized that synbiotic intake might affect pregnancy outcomes of GDM population. This study was, therefore, carried out to investigate the effects of synbiotic supplementation on pregnancy outcomes in GDM women.

Methods

Trial Design and Participants

This randomized, double-blind, placebo-controlled clinical trial, registered in the Iranian registry of clinical trials (http://www.irct.ir: IRCT201704205623N108), was performed among 60 patients with GDM aged 18–40 years old based on the American Diabetes Association guidelines [15] who were referred to the Akbarabadi clinic affiliated to Iran University of Medical Sciences (IUMS), Tehran, Iran, between April 2016 and December 2016. This research was carried out in accordance with the Declaration of Helsinki. Written and informed consent was obtained from all pregnant women. Patients with clinical characteristics at enrollment, such as preeclampsia, eclampsia, hypo- and hyperthyroidism; smokers; and those with kidney or liver diseases and required commencing insulin therapy during intervention and taking any probiotic and/or synbiotic products including probiotic yogurt and kefir during the trial were the exclusion criteria.

Study Design

Participants were randomly allocated into two treatment groups to intake either one synbiotic capsule containing *Lactobacillus acidophilus* strain T16 (IBRC-M10785), *L. casei* strain T2 (IBRC-M10783) and *Bifidobacterium bifidum* strain T1 (IBRC-M10771) (2×10^9 CFU/g each) plus 800 mg inulin (HPX) ($n = 30$) or placebo ($n = 30$) for 6 weeks. It is well known that it would be more appropriate if the strains used for human consumption originated from the human intestinal tract, well characterized, able to survive the rigors of the digestive tract and possibly colonize, biologically active against the target as well as to be stable and amenable to commercial production and distribution [16]. We used the above-mentioned doses of probiotic bacteria based on a previous study in GDM women [17] and dose of inulin based on a previous study in healthy pregnant women [18]. Both synbiotic and placebo capsules were produced by the Tak Gen Zist Pharmaceutical Company in Tehran, Iran, and approved by the Food and Drug Administration. To assess the compliance, patients were requested to bring the medication container. To ensure adherence, patients received a short message on their cell phones to intake the supplements daily. Randomization assignment was conducted using computer-generated random numbers. Randomization and allocation concealment were conducted by the researchers and patients and were done by a trained staff at the gynecology clinic. All participants based on standard protocol consumed 400 μg/day of folic acid starting at the beginning of pregnancy and 60 mg/day ferrous sulfate as of the second trimester. For assessment of dietary micro- and macro-nutrient intakes, patients were instructed to record their daily dietary intakes for 3 days, including one weekend day and two weekdays at weeks 1, 3, and 5. Dietary intakes were then analyzed using Nutritionist IV software (First Databank, San Bruno, CA) modified for Iranian foods.

Assessment of Anthropometric Variables

At baseline and end-of-trial, all patients underwent standard anthropometric measurements: height and weight (Seca, Hamburg, Germany). BMI was calculated as weight in kilograms divided by height in meters squared. Weight and length of all newborns were measured in labor ward following the birth by a trained midwife by the use of standard methods (Seca 155 Scale, Hamburg, Germany). Infants' head circumference was calculated to the nearest 1 mm with a Seca girth measuring tape. We also determined infants' 1- and 5-min Apgar scores.

Outcomes

Primary outcomes were inflammatory markers. The secondary outcomes were biomarkers of oxidative stress and pregnancy outcomes.

Clinical Assessment

Polyhydramnios was diagnosed using the sonographic estimation method at post-treatment. On the basis of this measurement, polyhydramnios was defined as an amniotic fluid index (AFI) in excess of 25 cm [19]. Preterm delivery was described as delivery occurred at <37 weeks of pregnancy, and newborn's macrosomia was defined as birth weight of >4000 g.

Assessment of Biochemical Variables

Ten milliliters of fasting blood samples was obtained from each patient at baseline and the end of the treatment, at the IUMS reference laboratory. Serum high-sensitivity C-reactive protein (hs-CRP) levels were determined using ELISA kit (LDN, Nordhorn, Germany). Spectrophotometric methods were used to assess nitric oxide (NO) [20], total antioxidant capacity (TAC) [21], total glutathione (GSH) [22], and malondialdehyde (MDA) values [23]. The hs-CRP is a general marker for inflammation, so it can be used as a very rough proxy for heart disease risk [24]. The TAC considers the occurrence of a synergic function of all antioxidants present in organic fluids, providing an integrative system between such compounds [25]. Thus, TAC has a higher predictive capacity and biological relevance when compared to the activity of a single antioxidant. In addition, MDA is the breakdown product of the most important chain reactions leading to the oxidation of polyunsaturated fatty acids and therefore serves as a reliable oxidant marker of oxidative stress-mediated lipid peroxidation [26]. Newborns' hyperbilirubinemia was considered when the total serum bilirubin levels were at 15 mg/dL or more among infants who were 25 to 48 h old, 18 mg/dL in infants who were 49 to 72 h old, and 20 mg/dL in infants older than 72 h [27].

Sample Size

Using a formula suggested for clinical trials, having 25 patients in each group was adequate while considering a type one error (α) of 0.05 and type two error (β) of 0.20 (power = 80%), 0.44 μg/mL as SD and 0.35 μg/mL as the mean distinction (d) of hs-CRP as a primary outcome [10]. Assuming 5 dropouts in each group, the final sample size was determined to be 30 patients in each group.

Statistical Analysis

The Kolmogorov-Smirnov test was used to control the normal distribution of variables. Independent sample *t* test was used to establish changes in anthropometric measures and dietary intakes between the two groups. To determine the effects of synbiotic administration on biochemical variables, we used one-way repeated measures analysis of variance. To control some confounding variables including baseline values, maternal age and baseline BMI, we used ANCOVA test using general linear models. Differences in proportions were evaluated by Chi-square test. $P < 0.05$ was considered statistically significant. All statistical analyses were conducted using the Statistical Package for Social Science version 18 (SPSS Inc., Chicago, Illinois, USA).

Results

As revealed in the study flow diagram (Fig. 1), 60 pregnant women [synbiotic (n = 30) and placebo (n = 28)] completed the trial. On average, the compliance rate in the current study was high, such that 90% of synbiotic capsules were consumed throughout the study in both groups.

Mean age, height, baseline weight, and BMI as well as their means after the 6-week treatment were not significant between synbiotic supplements and placebo groups (Table 1).

Considering the 3-day dietary records obtained during the treatment, there was no significant difference in terms of dietary macro- and micro-nutrient intakes between synbiotic and placebo groups (Data not shown).

After 6 weeks of intervention, compared with the placebo, synbiotic supplementation significantly decreased serum hs-CRP (− 1.9 ± 4.2 vs. + 1.1 ± 3.5 mg/L, P = 0.004) and plasma MDA (− 0.1 ± 0.6 vs. + 0.3 ± 0.7 μmol/L, P = 0.02) and significantly increased TAC (+ 70.1 ± 130.9 vs. − 19.7 ± 124.6 mmol/L, P = 0.009) and GSH levels (+ 28.7 ± 61.5 vs. − 14.9 ± 85.3 μmol/L, P = 0.02) (Table 2). Supplementation of synbiotic showed no detectable change in plasma NO levels.

There was a significant difference in baseline levels of MDA (P = 0.01) between the two groups. Therefore, we adjusted the analysis for baseline values of biochemical variables, maternal age, and BMI at baseline. When we adjusted the analysis for these variables, findings were not altered (Table 3).

Supplementation with synbiotic had a significant decrease in cesarean section rate (16.7 vs. 40.0%, P = 0.04) and lower incidence of hyperbilirubinemic newborns (3.3 vs. 30.0%, P = 0.006) and newborns' hospitalization (3.3 vs. 30.0%, P = 0.006) compared with the placebo. Synbiotic supplementation did not affect other pregnancy outcomes (Table 4).

Discussion

This study demonstrated that the 6-week intervention with synbiotic supplements among women with GDM had beneficial effects on serum hs-CRP, plasma TAC, GSH, and MDA; cesarean section; and incidence of newborn's hyperbilirubinemia and newborns' hospitalization, but did

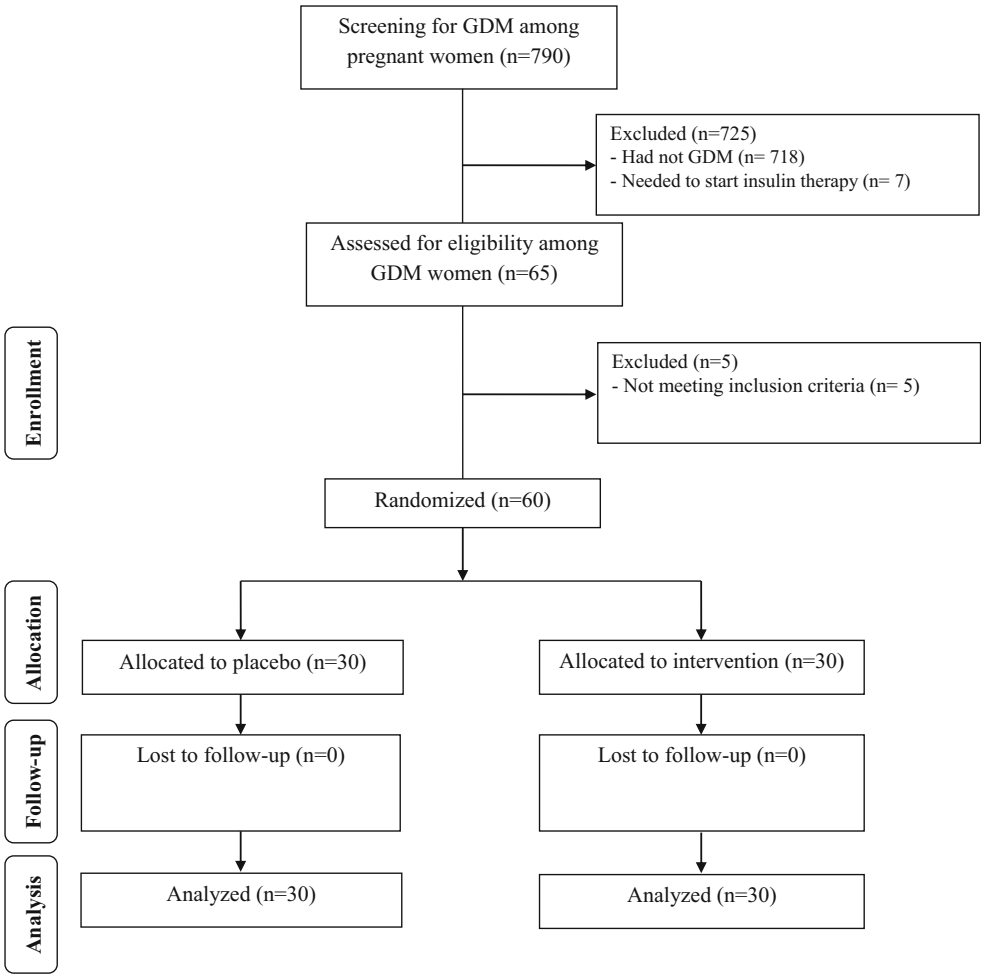

Fig. 1 Summary of patient flow diagram

not affect plasma NO levels and other pregnancy outcomes. To our knowledge, this is the first to evaluate the effects of synbiotic supplementation on biomarkers of inflammation and oxidative stress and pregnancy outcomes in GDM women.

Pregnancy and GDM are associated with several adverse outcomes for mothers and newborns in the short and long term [28, 29]. The current study supported that synbiotic supplementation compared with the placebo for 6 weeks among GDM women resulted in significant decreases in serum hs-CRP and plasma MDA concentrations and significant elevations in TAC and GSH levels, but did not affect plasma NO levels. In line with our results, treatment with synbiotics in subjects with multiple injuries resulted in a significant reduction in CRP concentrations in patients who either did or did not develop sepsis [30]. Jafarnejad et al. [31] exhibited that a mixture of probiotic (VSL no.3) in women with GDM after

Table 1 General characteristics of the study participants[a]

	Placebo group (n = 30)	Synbiotic group (n = 30)	P value[b]
Maternal age (year)	26.2 ± 3.1	27.2 ± 5.9	0.39
Height (cm)	160.9 ± 3.2	161.8 ± 4.3	0.36
Weight at study baseline (kg)	73.1 ± 5.8	74.7 ± 10.5	0.47
Weight at end-of-trial (kg)	75.2 ± 5.8	77.0 ± 10.4	0.40
Weight change (kg)	2.1 ± 0.9	2.3 ± 0.7	0.23
BMI at study baseline (kg/m^2)	28.3 ± 2.5	28.6 ± 4.3	0.72
BMI at end-of-trial (kg/m^2)	29.1 ± 2.5	29.5 ± 4.2	0.65
BMI change (kg/m^2)	0.8 ± 0.3	0.9 ± 0.3	0.28

[a] Data are means ± standard deviation

[b] Obtained from independent sample t test

Table 2 Biomarkers of inflammation and oxidative stress at baseline and after the 6-week intervention in women with gestational diabetes that received either synbiotic supplements or placebo[a]

	Placebo group (n = 30)			Synbiotic group (n = 30)			P value[b]
	Wk0	Wk6	Change	Wk0	Wk6	Change	
hs-CRP (mg/L)	7.3 ± 3.8	8.4 ± 3.9	1.1 ± 3.5	7.8 ± 5.7	5.9 ± 4.3	−1.9 ± 4.2	0.004
NO (μmol/L)	40.7 ± 5.7	39.8 ± 7.1	− 0.8 ± 3.8	41.6 ± 1.8	41.8 ± 2.9	0.2 ± 3.0	0.23
TAC (mmol/L)	930.9 ± 124.3	911.2 ± 100.2	− 19.7 ± 124.6	970.1 ± 86.9	1040.2 ± 129.6	70.1 ± 130.9	0.009
GSH (μmol/L)	470.5 ± 77.9	455.6 ± 96.3	− 14.9 ± 85.3	486.2 ± 71.0	514.9 ± 68.9	28.7 ± 61.5	0.02
MDA (μmol/L)	2.9 ± 0.9	3.2 ± 1.2	0.3 ± 0.7	2.4 ± 0.5	2.3 ± 0.6	− 0.1 ± 0.6	0.02

GSH total glutathione, *hs-CRP* high-sensitivity C-reactive protein, *MDA* malondialdehyde, *NO* nitric oxide, *TAC* total antioxidant capacity

[a] All values are means ± SDs

[b] P values represent the time × group interaction (computed by analysis of the repeated measures ANOVA)

8 weeks affected the inflammatory cytokines, including hs-CRP. In addition, in a meta-analysis study conducted by Liu et al. [32], a significant decrease in CRP levels without any change in other inflammatory cytokines was observed following supplementation with probiotics in subjects with colorectal cancer after operation. Furthermore, we have previously documented that synbiotic intake for 8 weeks among subjects with rheumatoid arthritis (RA) had beneficial effects on hs-CRP and GSH levels, but did not change other inflammatory and oxidative stress parameters [33]. Moreover, several studies have documented antioxidant properties of special strains of lactic acid bacteria [34, 35]. Synbiotic administration for 30 days also had positive effects on TAC and MDA concentrations in breastmilk [36]. However, no significant change of *L. casei* supplementation for 8 weeks was seen on parameters of oxidative stress of subjects with RA [37]. Changes in the gut and vaginal microbiome [38] might influence maternal biomarkers of inflammation and oxidative stress, which in turn would result in metabolic and immunological disorders of the offspring [39]. GDM is associated with increased concentrations of oxidative stress, due to overproduction of reactive oxygen species (ROS) and/or defects in antioxidant defenses [40]. Overproduction of ROS induces oxidative damage in membrane lipids, proteins, and DNA, such as purine and pyrimidyne bases and, as a consequence, single-strand breaks, double-strand breaks, and DNA-DNA or DNA-proteins cross-links [41, 42]. In addition, increased oxidative stress during embryonic, fetal, and placental development may cause several pregnancy-related disorders, including embryopathies, spontaneous abortions, preeclampsia, preterm labor, and low birth weight [43]. Therefore, synbiotic supplementation in GDM women may decrease complications related to oxidative stress due to its antioxidative effects. However, increased levels of inflammatory markers were reported in GDM women [44], the importance of reducing systemic inflammation in these patients is still incompletely understood. The up-regulation of gene expression of interleukin-18 by produced short-chain fatty acids (SCFA) [45] and increased production of methylketones family in the colon following intake of synbiotic [46] might justify its anti-inflammatory effects. In addition, synbiotic intake may improve oxidative stress via improved inflammatory factors resulting from produced SCFA in the gut [47] and its effect in decreased variables, such as oxidized LDL and 8-isoprostanes [48].

This study documented that synbiotic supplementation among GDM women decreased cesarean section rate and incidence of newborn's hyperbilirubinemia and newborns' hospitalization compared with the placebo, but did not affect

Table 3 Adjusted changes in biomarkers of oxidative stress and inflammation in women with gestational diabetes that received either synbiotic supplements or placebo[a]

	Placebo group (n = 30)	Synbiotic group (n = 30)	P value[b]
hs-CRP (mg/L)	1.0 ± 0.6	− 1.8 ± 0.6	0.002
NO (μmol/L)	− 0.9 ± 0.6	0.3 ± 0.6	0.20
TAC (mmol/L)	− 29.9 ± 19.9	80.3 ± 19.9	< 0.001
GSH (μmol/L)	− 17.4 ± 13.0	31.3 ± 13.0	0.01
MDA (μmol/L)	0.3 ± 0.1	− 0.1 ± 0.1	0.02

GSH total glutathione, *hs-CRP* high-sensitivity C-reactive protein, *MDA* malondialdehyde, *NO* nitric oxide, *TAC* total antioxidant capacity

[a] All values are means ± SE

[b] Obtained from ANCOVA-adjusted based on maternal age, BMI at baseline, and baseline values of biochemical parameters

Table 4 The association of synbiotic supplementation with pregnancy outcomes

	Placebo group (n = 30)	Synbiotic group (n = 30)	P value[a]
Cesarean section (%)	12 (40.0)	5 (16.7)	0.04[b]
Preterm delivery (%)	1 (3.3)	0 (0)	0.31[b]
Preeclampsia (%)	4 (13.3)	4 (13.3)	>0.999[b]
Polyhydramnios (%)	2 (6.6)	1 (3.3)	0.55[b]
Maternal hospitalization (%)	2 (6.7)	1 (3.3)	0.55[b]
Macrosomia > 4000 g (%)	3 (10.0)	0 (0)	0.07[b]
Gestational age (weeks)	39.0 ± 1.1	39.4 ± 1.5	0.24
Newborns' weight (g)	3373.3 ± 412.1	3181.6 ± 459.8	0.09
Newborns' length (cm)	50.1 ± 1.9	50.0 ± 2.5	0.77
Newborns' head circumference (cm)	35.4 ± 2.0	35.2 ± 2.1	0.72
1-min Apgar score	8.90 ± 0.30	8.86 ± 0.34	0.69
5-min Apgar score	9.90 ± 0.30	9.86 ± 0.34	0.69
Newborns' hyperbilirubinemia (%)	9 (30.0)	1 (3.3)	0.006[b]
Newborns' hospitalization (%)	9 (30.0)	1 (3.3)	0.006[b]
Newborns' hypoglycemia (%)	2 (6.7)	3 (10.0)	0.64[b]

Values are means ± SDs for continuous measures and are number (%) for dichotomous variables

[b] Obtained from Pearson Chi-square test

[a] Obtained from independent t test

other pregnancy outcomes. In a study by Dugoua et al. [49], it was seen that Lactobacillus and Bifidobacterium had no detectable effect on the incidence of cesarean section, newborns' weight, and gestational age. In another study, Demirel et al. [50] showed that *Saccharomyces boulardii* supplementation at a dosage of 250 mg in infants with a gestational age of ≤32 weeks and a birth weight of ≤1500 g decreased their serum bilirubin levels and the duration of phototherapy. However, we have previously indicated no significant change in the incidence of newborns' hyperbilirubinemia and cesarean section rate after supplementation with a synbiotic food containing *L. sporogenes* and inulin in healthy pregnant women [18]. In addition, in a meta-analysis study conducted by Taylor et al. [51], no significant effects on gestational weight gain, delivery method or neonatal outcomes, and metabolic profiles except insulin resistance were documented following supplementation with probiotic for 6–8 weeks in GDM women. It has been shown that supplementation with some species of probiotics could change the intestinal flora, suppress the activity of β-glucuronidase, and might accelerate the bilirubin metabolism, which in turn leads to decreasing the enterohepatic circulation [52]. The aim of controlling the hyperbilirubinemia is to prevent the indirect bilirubin levels from reaching the point at which neurotoxicity may occur [53].

The current study had few limitations. Firstly, we did not evaluate the effects of synbiotic supplementation on other pregnancy outcomes, such as the infant respiratory status and the time in neonatal intensive care unit. Due to funding limitations, we did not assess the compliance to synbiotic intake through quantifying fecal bacteria loads and SCFA. Therefore, this should be taken into account in the interpretation of our findings. Although the effect of synbiotic supplementation on other inflammatory cytokines and biomarkers of oxidative stress, including tumor necrosis factor alpha, interleukins, and superoxide dismutase in GDM women is interesting, its performance is suggested in next studies.

Overall, synbiotic supplementation among GDM women for 6 weeks had beneficial effects on serum hs-CRP, plasma TAC, GSH, and MDA; cesarean section; and incidence of newborn's hyperbilirubinemia and newborns' hospitalization, but did not affect plasma NO levels and other pregnancy outcomes.

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Compliance with Ethical Standards

Conflicts of Interest The authors declare that they have no conflict of interest.

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