Effects of Daily Consumption of Probiotic Yoghurt on Inflammatory Factors in Pregnant Women: A Randomized Controlled Trial

Zatollah Asemi, Shima Jazayeri, Mohammad Najafi, Mansoreh Samimi, Vahid Mofid, Farzad Shidfar, Abbas Rahim Foroushani and Mohamad Esmaeil Shahaboddin
Department of Nutrition and Biochemistry, School of Public Health, Tehran University of Medical Sciences, Iran

Department of Gynecology and Obstetrics, Kashan University of Medical Sciences, Iran
Department of Biochemistry, Tehran University of Medical Sciences, Tehran University, Iran
Department of Food Science, Tehran University of Medical Sciences, Iran
Department of Biostatic, Tehran University of Medical Sciences, Iran
Department of Nutrition and Biochemistry, School of Public Health, Kashan University of Medical Sciences, Iran

Abstract: Previous studies have shown that inflammatory factors increases in pregnancy and is associated with several complications of pregnancy. The aim of this study was to assess effects of daily consumption of probiotic yoghurt on inflammatory factors in pregnant women. In a randomized clinical trial, seventy primigravid (the first pregnancy) and singleton pregnant women aged 18-30 years were assigned to two groups. Subjects consumed daily 200 g probiotic yoghurt containing Lactobacillus acidophilus La5 and Bifidobacterium animalis BB12 (10⁶ CFU g⁻¹ for each) or 200 g conventional yoghurt for 9 weeks. Fasting blood samples were collected at baseline (28 weeks of gestation) and after intervention (37 weeks of gestation). Inflammatory factors, hs-CRP and TNF-α were measured by Enzyme-linked Immunosorbent Assay (ELISA). Independent t-test was used to compare the two groups after intervention and paired-sample t-test compared variables before and after treatment. The results showed that the probiotic yoghurt brought about a decrease in the serum hs-CRP level, from 10.44±1.56 to 7.44±1.03 μg mL⁻¹ (p = 0.041). There was no significant change in the conventional yoghurt group in the serum hs-CRP level (12.55±1.57 to 14.51±1.62 μg mL⁻¹, p = 0.202). The probiotic yoghurt had no effect on TNF-α (from 73.75±6.39 to 77.91±5.61 pg mL⁻¹, p = 0.633). Serum TNF-α did not change in the conventional yoghurt group (p = 0.134). In conclusion probiotic yoghurt significantly decreased hs-CRP in pregnant women but had no effect on TNF-α.

Key words: Probiotic yoghurt, inflammatory factors, pregnant women, hs-CRP, TNF-α

INTRODUCTION

Pregnancy is associated with increased production of pro-inflammatory factors. Elevated biosynthesis of these factors- including tumor necrosis factor alpha (TNF-α) and high sensitivity C reactive protein (hs-CRP) is due to increased adipose tissue (Kirwan et al., 2002) and placenta, especially in the third trimester (Jahromi et al., 2011). Increased pro-inflammatory factors have been associated with insulin resistance in obesity, aging, sepsis and muscle damage (Del Aguila et al., 1999). Furthermore, in pregnancy have been associated with insulin resistance and Gestational Diabetes Mellitus (GDM) (Melczer et al., 2002; Szarka et al., 2010), pre-eclampsia (Kirwan et al., 2001), preterm delivery (Pitiphat et al., 2005), intrauterine growth restriction (Tjøa et al., 2003), increased risk of preterm low birth weight (Offenbacher et al., 1996) low birth weight (Dasunayake, 1998) and preterm birth (Jeffcoat et al., 2001).

It is estimated that pre-eclampsia, leading cause of maternal and perinatal mortality and morbidity in the Western world, occurs in 2 to 7% of all pregnancies (Pipkin, 2001; Sibai et al., 2005; Mistry et al., 2008). It is noticeable that pre-eclampsia is responsible for about 60,000 deaths worldwide (Poston et al., 2006).

It has been shown that the enhanced insulin resistance may lead to abnormal blood glucose, fetal macrosomia, increased obstetric complications and, in some cases, could increase the risk of stillbirth (Kirwan et al., 2002). Various treatments to decrease inflammatory factors have been suggested, including use of antioxidants (Kamiya et al., 2005), cytokines, including Interleukin-10 (Rachmawati et al., 2011), adiponectin...
(Lin et al., 2010) and aldose reductase (Srivastava et al., 2011; Yadav et al., 2011). Recently, clinical trials in non-pregnant women have shown that probiotics can decrease pro-inflammatory factors (Araki et al., 2004; Shimosato et al., 2006; Meyer et al., 2007; Lin et al., 2008; Fleige et al., 2009) and immunomodulatory effects (Timmerman et al., 2007; Van Minnen et al., 2007). Probiotics are live bacteria that could exert beneficial health effects (Liong and Shah, 2006; Liong, 2008). The anti-inflammatory effects of probiotics may result from decreased expression of IL-6 (Hegazy and El-Bedawy, 2010). To our knowledge, up to now, there are not reports about decreasing effects of probiotic yoghurt on pro-inflammation factors in pregnant women. Hence, the current study was dedicated to assess the effects of daily consumption of probiotic yoghurt on inflammatory factors including hs-CRP and TNF-α, in Iranian pregnant women.

MATERIALS AND METHODS

Methods: This prospective, randomized, single-blinded clinical trial was carried out in Kashan, Iran during October 2010 to March 2011. The participants were recruited from 18-30 year-old pregnant women who were primigravid and singleton selectively and visited in maternity clinics (Naghai, Shaheedi Beheshti Specialties' and Subspecialities' Polyclinic and ten antenatal centers) affiliated to Kashan University of Medical Sciences. A total of seventy pregnant women provided blood samples and completed interviews. Pregnant women were excluded if one of the followings were diagnosed: Multipara, pre-eclampsia, liver or renal disease, gestational diabetes, taking antibiotics and Complete Bed Rest (CBR). The ethical committee of Tehran University of Medical Sciences approved the study and written consent was obtained from all participants. They completed a questionnaire administered by a trained interviewer at or near enrollment.

Women provided detailed health, reproductive, supplement use and lifestyle information via a standardized face-to-face interview and questionnaire administered by trained interviewers at enrollment. Gestational age was assessed from the date of last menstrual period and concurrent clinical assessment (Gupta et al., 2004).

Samples: Two weeks (26 and 27 weeks of gestation) as wash-out period was designated, all subjects had to refrain from taking probiotic yoghurt or any other probiotic food. Subjects were selectively assigned into two groups, conventional group consisting of thirty three and probiotic group consisting of thirty seven pregnant women. The first and second groups from the beginning of the trial to the end of trial (from 28 to 37 weeks of gestation) consumed 200 g daily of probiotic and conventional yoghurt, respectively. The pregnant women were told not to alter their exercise routine or regular diet and not to consume any yoghurt other than the one provided to them by the researcher. They were also asked to refrain from consuming any other probiotic and fermented products. Necessary arrangements were made so that every week the subjects would receive a week's supply of their probiotic or conventional yoghurts directly from the factory. Sample size in this study is considered eighteen pregnant women. Of the subjects, three had to be excluded from the study because of taking antibiotics and pre-eclampsia in the probiotic group and seven because of CBR, gestational diabetes and pre-eclampsia from the control group.

Yoghurt: The probiotic yoghurt was a commercially available product prepared with the starter cultures Streptococcus thermophilus and Lactobacillus bulgaricus, enriched with the probiotic culture two strains of lactobacilli (Lactobacillus acidophilus LA5) and bifidobacteria (Bifidobacterium animalis BB12) with a total of min 1×10⁷ colony-forming units (Chr Hansen, Denmark). The control yoghurt contained the starter cultures S. thermophilus and L. bulgaricus. Direct Vat Starter (DVS) cultures were used. The yoghurts’ pH was 4.3-4.5. The fat content was 1.5%, comparable in both yoghurt types.

Data collection: Fasting blood samples and anthropometric measurements were collected before (28 weeks of gestation) and after intervention (37 weeks of gestation). Compliance with the yoghurt consumption guidelines at home was monitored once a week through phone interviews. At each of the two intervals, body weights were measured (digital floor scale; Seca, Hamburg, Germany) with 0.1 kg accuracy without shoes and with minimal clothing. Weight was measured in the fasting state. The subjects’ heights were measured, with 0.1 cm accuracy, with non-stretchable tape (Seca). BMI was determined by dividing body weight by height squared (kg m²). The subjects were directed to report to Kashan reference laboratory at the end of each interval by blood tests. Fasting venous blood samples were obtained (after a 12 h fast), early morning blood (10 mL) before and after intervention.

Safety: There were no serious adverse reactions reported throughout this study in the pregnant women.
Biochemistry analysis: Serum hs-CRP was assayed by ELISA (IBL, Germany Ref No EU 59131; it is based on the direct sandwich technique, in which two monoclonal antibodies are directed against human CRP). Serum samples were analyzed for concentrations of TNF-α and CRP. Serum TNF-α was assayed by ELISA (Boster, China Ref NO: EK 0525; It is based on the direct sandwich technique with avidin-biotin-peroxidase, in which two monoclonal antibodies are directed against human TNF-α).

Statistical analysis: The data was analyzed using Independent t-test and paired-sample t-test. Independent t-test was used to identify any differences between the two groups and paired-sample t-test was used to identify any differences into groups in the beginning and the end of the trial. Using ANCOVA test, potential confounding factors were identified, also. The following confounding variables were considered: weight and BMI difference at baseline with in the end study. A difference with p<0.05 between the groups was considered statistically significant. Calculations were performed using the SPSS 17 statistical package (SPSS Inc., Chicago, Illinois, USA). The present study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Ethics Committee at TUMS. Written informed consent was obtained from all subjects.

RESULTS

The number of seventy pregnant women who were primigravid and were 18-30 years old participated in the study. The mean values of maternal age, weight and BMI at the pre-pregnancy, 13, 28 and 37 weeks of gestation, did not indicate any significant differences among the two study groups. Also, the mean values of height indicate significant differences between the two study groups (in probiotic group 158.37±5.54 cm vs in conventional group 160.81±4.52 cm; p = 0.049) (Table 1).

Independent t-test showed that there was no statistically significant difference between the two groups in serum inflammatory markers at baseline (28 weeks of gestation). Also independent t test showed that there was no statistically significant difference between the two groups in serum TNF-α at the end of the study (37 weeks of gestation) but was significant changes between the two groups in serum hs-CRP (in probiotic group 7.44±1.03 μg mL⁻¹ vs. in conventional group 14.51±1.62 μg mL⁻¹; p = 0.001) (Table 2).

Paired-sample t-test showed that there was no statistical significant difference in groups in serum TNF-α and also, in serum hs-CRP of Conventional yogurt group subjects. But, there was significant difference in serum hs-CRP in Probiotic yogurt group at baseline 10.44±1.56 μg mL⁻¹ with the end study 7.44±1.03 μg mL⁻¹ (p = 0.041) (Table 2).

In addition, ANCOVA test showed that there was statistically significant difference in groups in serum hs-CRP after deletion of weight confounding variable (p = 0.018) (Table 3).

Also ANCOVA test showed that there was statistically significant difference in groups in serum hs-CRP after deletion of BMI confounding variable (p = 0.023) (Table 4).

<table>
<thead>
<tr>
<th>Table 1: Subjects’ maternal age, height, weight and BMI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variables</td>
</tr>
<tr>
<td>Maternal age (year)</td>
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<tr>
<td></td>
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<tr>
<td>Height (cm)</td>
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<tr>
<td></td>
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<tr>
<td>Weight in the pre-pregnancy (kg)</td>
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<td></td>
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<tr>
<td>Weight in the 13 weeks of gestation (kg)</td>
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<td></td>
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<tr>
<td>Weight in the 28 weeks of gestation (kg)</td>
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<td></td>
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<tr>
<td>Weight in the 37 weeks of gestation (kg)</td>
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<td></td>
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<tr>
<td>BMI in the pre-pregnancy (kg m⁻²)</td>
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<tr>
<td>BMI in the 13 weeks of gestation (kg m⁻²)</td>
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<tr>
<td>BMI in the 28 weeks of gestation (kg m⁻²)</td>
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<tr>
<td>BMI in the 37 weeks of gestation (kg m⁻²)</td>
</tr>
</tbody>
</table>

*p-values were determined by student T test, 2p value indicates a significant difference (p<0.05) between both groups.
Table 2: Changes of serum inflammation markers in pregnant women after 9 weeks intervention

<table>
<thead>
<tr>
<th>Variables</th>
<th>Groups</th>
<th>Week 0</th>
<th>Week 9</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>hs-CRP† (µg mL⁻¹)</td>
<td>Probiotic yogurt</td>
<td>10.44±1.56</td>
<td>7.44±1.03</td>
<td>0.041</td>
</tr>
<tr>
<td></td>
<td>Conventional yogurt</td>
<td>12.55±1.57</td>
<td>14.51±1.62</td>
<td>0.292</td>
</tr>
<tr>
<td>TNF-α† (µg mL⁻¹)</td>
<td>Probiotic yogurt</td>
<td>73.75±6.59</td>
<td>77.91±4.61</td>
<td>0.633</td>
</tr>
<tr>
<td></td>
<td>Conventional yogurt</td>
<td>92.94±8.18</td>
<td>75.41±7.5</td>
<td>0.134</td>
</tr>
</tbody>
</table>

*p-values were determined by student T test; †p-values were determined by paired t test; ‡p-value indicates a significant difference (p<0.05) between both groups; ‡C-reactive protein; †Tumor necrosis factor alpha

Table 3: Effect of weight difference on change of serum hs-CRP in pregnant women after 9 weeks intervention

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III sum of squares F</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight difference</td>
<td>322.505</td>
<td>4.55</td>
</tr>
<tr>
<td>Groups (probiotic</td>
<td>416.89</td>
<td>5.88</td>
</tr>
<tr>
<td>and conventional)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>4747.63</td>
<td></td>
</tr>
<tr>
<td>Corrected total</td>
<td>5499.77</td>
<td></td>
</tr>
</tbody>
</table>

*p-values were determined by ANCOVA test; ‡p-value indicates a significant difference (p<0.05) between both groups; ‡Weight difference at baseline with the end study

Table 4: Effect of BMI difference on change of serum hs-CRP in pregnant women after 9 weeks intervention

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III sum of squares F</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI difference</td>
<td>171.82</td>
<td>2.35</td>
</tr>
<tr>
<td>Groups (probiotic</td>
<td>393.9</td>
<td>3.38</td>
</tr>
<tr>
<td>and conventional)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>4898.31</td>
<td></td>
</tr>
<tr>
<td>Corrected total</td>
<td>5160.77</td>
<td></td>
</tr>
</tbody>
</table>

*p-values were determined by ANCOVA test; ‡p-value indicates a significant difference (p<0.05) between both groups; ‡BMI: Body mass index difference at baseline with the end study

DISCUSSION

It is found that Elevated basal levels serum hs-CRP and TNF-α in late pregnant women during mild inflammation and due to maternal weight gain, placenta. The potential role of the intestinal microflora in modulating immune responses has led to an interest in using probiotics as preventive and therapeutic interventions (Mandel et al., 2010).

In the present study there were no statistical significant differences between the two groups in terms of weight and BMI. The present study showed that probiotic yogurt administration for 9 weeks had significant statistical difference in hs-CRP within any group throughout the study in pregnant women. Up on our knowledge, our study is the first study to reveals that probiotics may reduce serum hs-CRP levels in pregnant women in a randomized, single-blind setting. It revealed that in the L. acidophilus La5 and Bifidobacterium lactis Bb12 treated groups, the hs-CRP level tended to be lower during the intervention.

Present results confirm the results a number of previous studies among non-pregnant women. The effect of probiotics on hs-CRP has been studied in immunocompromised patients (Anderson et al., 2004; McNaught et al., 2005), allergic children (Viljanen et al., 2005) and patients with rheumatoid arthritis (Hatakka et al., 2003). In patients suffering from immunocompromised problems, a combination of L. casei, B. breve and probiotic galactooligosaccharides (Sugawara et al., 2006) and B. longum (Furrie et al., 2005) have reduced serum hs-CRP levels and also resulted in the improvement of clinical appearance of chronic inflammation (Furrie et al., 2005). It has been revealed that Bifidobacterium probiotic consumption in patients colorectal cancer can reduce serum hs-CRP concentrations, significantly (Zhang et al., 2010). Also, oral probiotic dietary consumption in patients with Chronic Kidney Disease (CKD) can do the same (Ranganathan et al., 2009).

In contrast to mentioned studies and our results in the present study, Lactobacillus rhamnosus GG increased serum hs-CRP levels in comparison to placebo in infants with IgE-associated atopic eczema dermatitis syndrome (Viljanen et al., 2005). But, L. rhamnosus GG had no effect on serum CRP levels in patients suffering from rheumatoid arthritis (Hatakka et al., 2003), consumption of L. plantarum in critically ill patients (McNaught et al., 2005) and consumption of Lactobacillus rhamnosus GG on rheumatoid arthritis (Hatakka et al., 2003) were not shown a significant reduction of serum hs-CRP concentrations.

In the present study, possible mechanisms could be responsible for the reduced hs-CRP effect when probiotics settle in the gut; they ferment indigestible carbohydrates from food. Their action raises the short Chain Fatty Acid (SCFA) in the gut. Produced SCFA entered into the blood circulation, then into the liver (Sadrzadeh-Yeganeh et al., 2010). Probably, SCFA can lower the serum hs-CRP in the blood by enzymatic synthesis blocking in hepatic hs-CRP.

CRP is synthesized by the liver (Pepys and Hirschfield, 2003) in response to factors released by fat cells (adipocytes) (Lau et al., 2005). It is an important First-line host defense molecule, that recognizes pathogens and damaged cells and promotes their eliminations by activating the complement system and mediating their phagocytic clearance (Boutsikou et al., 2010). It’s synthesis increment is due to a rise in the plasma concentration of IL-6 which is produced predominantly by macrophages (Pepys and Hirschfield, 2003) as well as adipocytes (Lau et al., 2005). Moreover, using high-sensitivity assays for hs-CRP, several studies.
have shown elevation of CRP levels in obesity, since adiposity resembles a low grade systemic inflammatory state and hs-CRP is released by adipose tissue (Ouchi et al., 2003). Also, Hegazy and El-Bedewy (2010) showed that probiotic use for 8 week significantly ameliorated the inflammation due to decreasing expression concentration of IL-6 in ulcerative colitis. Likely, decreasing expression concentration of IL-6 indirectly causes decrease of CRP production.

This study showed that probiotic yogurt administration for 9 week has no statistical significant difference in TNF-α within any group throughout the study in pregnant women. Our study results are in contrast to similar studies including of Cianchi et al. (2004) who have found that a symbiotic preparation that contained Lactobacillus paracasei B 20160 restored the serum level and mRNA expression of TNF-α in UC patient. Significant reduction of serum TNF-α concentrations was not shown with consumption of Lactobacillus rhamnosus GG humans with nonalcoholic fatty liver disease (Vajro et al., 2011), Lactobacillus delbrueckii and Lactobacillus fermentum in patients ulcerative colitis (Hegazy and El-Bedewy, 2010) and L. casei Cryptosporidium parvum infection in neonatal rats (Gutard et al., 2006). Other studies have shown that Lactobacillus HY 7801 blocks the expression of TNF-α (Konishi et al., 2005). Significant reduction of serum TNF-α concentrations was seen with bifidobacterium, lactobacillus and enterococcus capsules consumption for colitis in rats (Wan et al., 2010) and with L. acidophilus consumption for ulcerative colitis in rats (Abdin and Saeid, 2008).

CONCLUSION

It appears that probiotics effect on TNF-α is controversial and it is the different patient materials (various diseases) and the different probiotic strains that have been used. It seems that age, the immunological status of the individual and the probiotic strain used in the study has a great impact on the immunomodulatory effects. Our results support the hypothesis that probiotic yogurt consumption after intervention 9 weeks significantly increased hs-CRP in pregnant women but had no effect on TNF-α. Limitation of this study is that we were unable to get fasting another blood sample from any of participants.

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