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Protocol

Probiotic Supplements in Gestational Diabetes Mellitus: Study Protocol for a Placebo-controlled Randomized Clinical Trial

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Abstract

A randomized clinical trial is reported in this protocol aiming to investigate the effect of a probiotic supplement capsule containing four bacterial strains in comparison compared with placebo on improving glucose metabolism, inflammation and oxidative stress among women with newly diagnosed gestational diabetes mellitus.

Introduction

Gestational diabetes mellitus(GDM) is characterized by maternal insulin resistance and is associated with inflammation through the gestation [1]. GDM is may lead to adverse pregnancy outcomes such as preeclampsia, abnormal delivery and neonatal suffering. Due to increasing rates of overweight and obesity, GDM rates are also increasing globally [2,3].

Probiotics are defined as live microorganisms that confer a health benefit on the host if given in adequate amounts and could affect the consumer's metabolism. Probiotics have been used

in various conditions including diabetes mellitus [4-9]. Studies have been conducting finding the probiotics to be beneficial in prevention of GDM, however, the evidence for use of probiotics after a GDM diagnosis is largely limited. A randomized clinical trial is reported in this protocol aiming to investigate the effect of a probiotic supplement capsule containing four bacterial strains in comparison compared with placebo on improving glucose metabolism, inflammation and oxidative stress among women with newly diagnosed gestational diabetes mellitus.

Setting & participating centers

The study was conducted in Alzahra University hospital which is a specialty obstetrics & gynecology teaching hospital. It is located in Tabriz city in East Azerbaijan province of Iran with a population of 3500000 people.

Study population and trial development

The effect of probiotic supplement on some health-related biomarkers among nulliparous pregnant women with gestational diabetes mellitus (GDM) will be investigated through a double blind placebo controlled randomized clinical trial.

These include four major types of assessments as follows

1- glucose metabolism indices
2- Body weight trend.
3- Inflammatory indices
4- Oxidative stress biomarkers

Sixty-four subjects with GDM referred to Alzahra University hospital in Tabriz, North-west of Iran, will be enrolled during the spring and summer months in 2014. The patients will be randomly allocated to receive either probiotic supplement or placebo capsules once daily for eight weeks. Each probiotic capsule of four bacterial strains (4 biocap > 4*109CFU) in standard freeze-dried culture will includ Lactobacillus acidophilus LA- 5, Bifidobacterium BB-12, Streptococcus Thermophilus STY-31 and Lactobacillus delbrueckii bulgaricus LBY-27 plus dextrose anhydrous filler and magnesium stearate lubricant produced by CHR HANSEN, Denmark, later packed and gelatin covered in Tehran Darou drug industries.

The sample size was estimated considering the HOMA-IR as the main outcome for ower estimation. Sample size was estimated using parameters from the study by Asemi et al. assuming a maximum type one error of 0.05 and 90% Statistical power, Ho-MA-IR index Standard nation equal to 31% and an effect size equal to 0.2, a total number of 32 subjects were estimated to be enrolled for each group taking into account 10% attrition rate [10].

The eligible subjects to be enrolled include all nulliparous women with gestational diabetes mellitus screened during 24-28 weeks of gestation who are referred to the specialty and subspecialty gynecology or endocrinology clinics of Tabriz University of medical Sciences.

The inclusion criteria are as following

1- Nulliparity
2- Gestational diabetes between 24-week and 28-week (+6 days) of gestation diagnosed through screening done by either a gynecologist or an internal medicine specialist.
3- Age range of 18 - 45 years
4- Fasting blood sugar range of 92 to 126 mg/dl early at the diagnosis
5- Body mass index(BMI) above 18.5 Kg/m^2
6- No history of type 2 diabetes mellitus
7- No history of chronic diseases
8- No smoking and alcohol consumption
9- Not using probiotic food products during the two weeks before intervention
10- Not using antibiotics during the month before intervention
11- Lack of acute gastrointestinal problems a month before trial
12- Not using Glucocorticoids (GCs) and immunosuppressive drugs

The exclusion criteria are as follows

1- Needing to use insulin of other diabetes drugs through the study period
2- Use of antibiotics through the study period
3- Use of GCs and immunosuppressive drugs through the study period

At baseline, the purpose and method of study will be described in detail for the patients and a trained practitioner will provide similar diet recommendations for patients in both groups. Written informed consent will be obtained from all the patients for being enrolled into this study. Then, during an interview with the participants, general questionnaire and a dietary recall questionnaire will be completed. The general questionnaire is used to collect data on demographic information, weight before pregnancy, physical activity, past medical history, drug history over the past month, and use of probiotic food products over the past two weeks before the enrollment. A 24-hour dietary recall questionnaire will be completed at sessions of three nonconsecutive days each(two normal and one weekend day) once at the baseline, secondly after four weeks and also at the end of study. To obtain the nutrient intakes of participants based on these 3-day food diaries, we used Nutritionist IV software (First Databank, San Bruno, Calif., USA) modified for Iranian foods. Weight, height and blood pressure will be measured and some information about their dietary habits and weight before pregnancy will also be taken. Seca 206 wall-mounted stadiometer and Seca 813 digital scale will be used to measure weight and height. Body Mass Index (BMI) will then be calculated and categorized according to the world health organization guidelines [11].

Laboratory assessments

Blood samples will be taken. Fasting blood samples (10 ml) before and after the intervention will be collected by the laboratory technician for measurement of fasting blood glucose and fasting insulin at Alzahra Hospital laboratory. Plasma glucose levels will be assessed using a glucose oxidase/peroxidase method as an enzymatic colorimetric (GOD-PAP) methodology [12] by Pars Azmoon test kits (Pars Azmoon Inc, Tehran, Iran). Serum insulin levels will be measured by ELISA method using Monobind kit [13]. Homeostasis Model Assessment Insulin Resistance (HOMA-IR) will be used to assess insulin resistance [14]. A HOMA-IR value above 3.8 is defined as insulin resistance [15].

QUICKI index (Quantitative Insulin Sensitivity Check) will be used in present study to assess insulin sensitivity [16].

Tumor necrosis factor-alpha (TNF-α) will be measured by an immunoenzymatic assay using the TNF-α EASIA kit no. KAP1751 which is an immunoenzymometric assay for the quantitative measurement of human TNF-a in serum, plasma, cell culture medium or other biological fluids [17]. The interleukin-6 serum assay was done using IL-6 Human ELISA Kit(IL-6-EASIA-CE KAC1261) which is designed to quantify human IL-6 protein levels in serum, plasma, supernatant, and other biological fluids. Interleukin-6 (IL-6) regulates growth and differentiation on the immune system, hematopoiesis, and inflammation [18]. Highsensitivity C-reactive protein (hs-CRP) will be measured using Monobind Hs-CRP Elisa kit. Measurement of serum total antioxidant capacity (TAC) level will be done using LDN TAC Colorimetric Assay Kit [19]. Erythrocyte Superoxide Dismutase(SOD) will be assayed using Abbott Spectrum autoanalyzer(Abbott, model Alcyon 300, USA) and Biorex(BXC0531A) kits [20]. Erythrocyte Glutathione peroxidase (GPX) will be assayed using Abbott Spectrum autoanalyzer(Abbott, model Alcyon 300, USA) and Biorex(BXC0551A) kits [22]. Glutathione Reductase(GSH-R) will be assayed using Eastbiopharm Human Glutathione Reductase ELISA Assay Kit based on sandwich enzyme immunoassay.

Randomization & blinding

A total of 64 pregnant women with GDM will be randomly allocated using block randomization techniques stratified according to the pre-pregnancy FBS and BMI groups. To ensure double blinding, a coder will anonymously label the capsules packages as “A” or “B” and therapist will assign them according to the random sequence generated through computer program [21].

Statistical analysis

Data will be analyzed using SPSS statistical software package. Mean response scales measured over the 8-week study period will be analyzed using appropriate statistical methods including independent samples t-test, repeated measurements analysis of variance, one-way analysis of variance and analysis of covariance. A p-value below 0.05 is considered as statistically significant.

Ethical issues & trial registeration

Present study protocol was approved by the regional committee of ethics in International branch of Shahid Beheshti University of Medical sciences as a thesis proposal for PhD degree in Nutrition Sciences. The approval has stressed that written informed consent should be taken from all the study subjects.

The Clinical trial was registered in the Iranian Registry of Clinical Trials under the number IRCT201405181597N3 accessible at this link:

http://en.search.irct.ir/search?query=IRCT201405181597N3

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Conflict of Interest

The authors declare that they have no conflict of interest.

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