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Original article

MaFOS-GDM trial: Maternal fish oil supplementation in women with gestational diabetes and cord blood DNA methylation at insulin like growth factor-1 (IGF-1) gene

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SUMMARY

Background: To evaluate the effects of maternal fish oil supplementation in women with gestational diabetes mellitus (GDM) on birthweight and DNA methylation at insulin like growth factor-1 (IGF-1) gene in their offspring.

Methods: Randomized controlled trial. A total of 120 women with GDM were randomized to one of the two groups between 24 and 28 weeks of the pregnancy: Group 1 (n = 52) received fish oil liquid softgel (Ocean plus®) and Group 2 (Placebo) (n = 68) sunflower oil liquid softgel. The birthweight and DNA methylation at IGF-1 gene of the offsprings were assessed.

Results: We observed a significant inverse association between fish oil use during pregnancy and birthweight (β = −0.18, s.e.:125, $P = .04$), corresponding to a 250 g lower birthweight among infants born to fish oil users. This association didn't persist in multivariate analysis. Cord blood IGF-1 was lower in fish oil group ($P = .001$). Cord blood DNA methylation percentages at CpG-1044 and CpG-611 sites of IGF-1 gene promoter 1 (P1) region were higher in fish oil group compared to placebo group ($P = .02$ and $P = .001$, respectively). However, CpG-1044 and CpG-611 methylations were not associated to birthweight ($\beta = 0.04$, s.e: 25.1, $P = .66$ and β = 0.04, s.e: 22.7, $P = 0.66$, respectively).

Conclusions: Maternal fish oil use has small effects on birthweight and DNA methylation when given to mothers with GDM at late pregnancy. Future studies are needed to show associations between maternal fish oil use and neonatal DNA methylations.

Clinical Trial Registration: "Fish Oil Supplementation in Women with Gestational Diabetes".
Identifier: NCT02371343.

1. Introduction

Gestational diabetes mellitus (GDM) is defined as glucose intolerance first detected during pregnancy [1]. GDM affects approximately 3–18% of pregnancies worldwide, with prominent variations in prevalence among ethnic groups [2]. The fetal programming hypothesis, that is Barker's hypothesis, suggests that high birthweight, high weight gain during pregnancy, maternal obesity, and GDM have been linked to future risk of adult disease in the offspring [3,4]. The association between fetal growth and later life cardio-metabolic events remains poorly understood. It was suggested that epigenetic mechanisms such as DNA methylation might be important contributors to fetal programming [5,6]. DNA methylation primarily occurs on cytosine residues in cytosine-phosphate-guanine (CpG) dinucleotides [7]. About half of human

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Abbreviations

BMI	body mass index
BW	birthweight
CpG	cytosine-phosphate-guanine
CS	cesarean section
DHA	docosahexaenoic acid
EPA	eicosapentaenoic acid
GA	gestational age
GDM	gestational diabetes mellitus
IGF	insulin-like growth factor
LC-PUFA	long-chain polyunsaturated fatty acids
NICU	neonatal intensive care unit
NS	nonsensitive
RCT	randomized controlled trial

genes contain CpG-rich regions termed CpG islands in their promoter regions. Many studies in humans revealed links between birthweight and DNA methylation [8,9].

It is well established that the insulin-like growth factor (IGF) system plays an important role in the regulation of feto-placental metabolism and growth [10,11]. IGF-1 promotes fetal growth by enhancing the placental efficiency to transfer nutrients, such as amino acids, to the fetus as well as the uptake and utilization of substrates by fetal tissues [12]. Desgagné et al. [13] showed that DNA methylation at the IGF genes might be involved in fetal growth and development.

Dietary omega-3 long chain polyunsaturated fatty acids (LC-PUFAs), particularly fish and marine mammal–derived eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are inversely associated with the risk of cardiovascular disease [14]. It was shown in animal studies that consumption of omega-3 LC-PUFA fatty acids increased the glucose utilization [15]. Epidemiological studies that examined the relationship between consumption of omega-3 LC-PUFA and GDM have given contradictory results [14–17]. Animal models suggested that LC-PUFAs might influence global DNA methylation patterns because of their role in one-carbon metabolism [18]. Lee et al. [19] reported that maternal supplementation with omega-3 PUFA during pregnancy might modulate global methylation levels and the Th1/Th2 balance in infants.

Based on these findings and according to the important role of the IGF system in metabolism and fetal growth regulation, the potential effects of maternal fish oil supplementation on cord blood IGF1 DNA methylation profiles in women with GDM were investigated.

2. Patients and methods

2.1. Design and setting

The MaFOS-GDM trial is a multicenter, prospective, randomized controlled trial (RCT) in which the supplementation with fish oil of pregnant women with GDM was compared with placebo. The trial was conducted from January 2015 and January 2017 at 3 tertiary maternity and children hospitals from different geographical regions of Turkey (Dr. Sami Ulus Maternity and Children Research and Training Hospital, Ankara; Memorial Dicle Hospital, Diyarbakır; Balıkesir Maternity and Children Hospital, Balıkesir).

Pregnant women with GDM who were 18–40 years old, in their 24–28 gestational week, residents of one of the study centers, planning to remain in the area for the next year, and who provided informed consent were eligible. Women were randomized by balanced blocks using sealed envelopes to receive either fish oil (Ocean Plus, 1200 mg, EPA 384 mg DHA 252 mg, Ocean®, Germany; 1 capsule/day) or placebo (sunflower oil; 1 capsule/day) until delivery. Placebo capsules were similar in appearance and taste to the fish oil capsules. Of the 140 women randomized, 120 completed the study with 52 women in control group and 68 women in treatment group (Fig. 1). Study participants and members of the study team were unaware of the randomization schedule of the study.

We calculated length of gestation in days by subtracting the date of the last menstrual period from the date of delivery. If gestational age according to the second-trimester ultrasound differed from that according to the last menstrual period by >10 days, we used ultrasound dating to determine gestational age.

Characteristics of participants were collected at baseline using standardized previously prepared forms and anthropometric measurements were obtained by trained health workers.

Prior to delivery, women were asked to complete a questionnaire and provide information about, height, weight prior to pregnancy, weight gain at the end of the pregnancy, vitamin supplementation during pregnancy, and smoking habits. Body mass index (BMI) prior to pregnancy was calculated as weight (kg) divided by height (m) squared. Medical records were abstracted at delivery to obtain information on birth outcomes. Maternal serum samples were obtained for glycosylated hemoglobin (HbA1c) measurements from all participants just after birth. Infant cord blood samples were also obtained to measure IGF-1 levels and genetic analyses.

Outcomes Primary outcome was IGF-1 DNA methylation percentages and secondary outcome was the birthweight of the offsprings.

2.2. Sample collection

Maternal serum HbA1c was measured with high-performance liquid chromatography (HPLC) just after birth. For genetic tests, cord blood specimens were collected in EDTA-containing tubes and stored at −80 °C until required. At the same time, cord blood serum was also obtained to measure IGF-I levels. Serum IGF-1 was measured with the chemiluminescence technique using the Immulite 2000 immunoassay system (Siemens Health Care, London, UK).

2.3. DNA methylation analysis

DNA was extracted by using QIAamp DNA Mini Kit, according to the manufacturer's protocol (Qiagen; Valencia, CA, USA), and quantity and quality assessed using a NanoDrop 1000 micro volume spectrophotometer (Thermo Scientific; Wilmington, DE, USA). Infant genomic DNA (0.5–1 μg) was modified by treatment with sodium bisulfite, using EpiTect Fast Bisulfite Kit (Qiagen). Bisulfite treatment of denatured DNA converts all unmethylated cytosines (C) to uracils but leaves methylated cytosines unchanged. Uracils then converted to thymidines (T) during PCR. Quantitative detection of cytosines and thymidines allows assessing DNA methylation status. Methylation status of CpG sites of interest were determined by next-gene DNA sequencing. Amplification of region of CpG sites from bisulfate converted DNA samples was carried out by PCR; by using site specific PCR primers which don't have CpG sites on them. After PCR, amplification efficiency was checked by using 2% agarose gel electrophoresis. Reactions with specific banding on agarose gel electrophoresis were purified by using Nucleo-Fast® 96 PCR purification plates (MACHEREY-NAGEL GmbH, Co., Germany) and quantified by using NanoDrop1000 spectrophotometer (Thermo

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