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Plasma free fatty acids in hyperemesis gravidarum pregnancy

Abstract: We evaluated the free fatty acids differences in plasma between hyperemesis gravidarum(HG) and healthy pregnant in first trimester pregnancy. Objective: We aimed to compare the plasma levels of DHA, AA and EPA, between HG patients and healthy pregnant women

Design: Fifty-two pregnants were involved in the study. Twenty-six pregnants of them were HG as study group, and twenty-six pregnants were enrolled as healthy pregnant women at the similar gestational age. The saturated fatty acids C14, C15, C16, C18, C20, C22, and C24; the omega-3 fatty acids eicosapentaenoic acid, (EPA) and docosahexaenoic acid, (DHA); the omega-6 fatty acids linoleic acid, arachidonic acid (AA), and homo-gamma-linolenic acid; and the omega-9 fatty acids oleic acid, erucic acid, and nervonic acid were analysed by gas chromatography.

Results: Statistically differences was not seen between the groups with maternal age, gestational age, or plasma levels of EPA, DHA, and AA. Statistically significant difference was seen between the groups with plasma levels of C20 and C22(p<0.05). C20 was declined but C22 was rised in the HG patients.

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Conclusion: EPA, DHA, or AA, which related to placental and fetal neural development are not changing from Hyperemesis gravidarum.

1 Introduction

Nausea and vomiting are common in pregnancy, occurring in 70–85% of all gravid women [1]. Hyperemesis gravidarum (HG) is a complication of pregnancy characterized by extreme nausea, vomiting, and dehydration in the first trimester [2,3]. HG causes uncontrolled vomiting requiring hospitalization, severe dehydration, muscle wasting, electrolyte imbalance, ketonuria, and loss of more than 5% of the body weight [4]. HG occurs in 0.3–2.0% of pregnancies [5], differs from the common nausea and vomiting experienced during pregnancy, and often requires hospitalization. Starvation is commonly seen in patients with HG [6].

Lipolysis, the removal of fatty acid chains from the glycerol to which they are bound in their storage form as triglycerides, is carried out by lipases. Fatty acids are then broken down to acetyl-CoA by means of beta oxidation inside the mitochondria. Under fasting conditions, adipose tissue initiates lipolysis and produces glycerol and free fatty acids (FFAs). The maternal liver converts these FFAs to glycerol and ketone to glucose, which can cross the placenta to maintain fetal metabolism [7]. FFAs are required as a source of energy for the development of the fetus and are used for maintaining the fluidity, permeability, and conformation of membranes; they are the precursors of prostacyclins, prostaglandins, thromboxanes, and leukotrienes [8]. Therefore, accurate measurement of plasma FFAs has important physiological and clinical implications. To understand and decrease the effects of HG-caused dehydration, muscle wasting, electrolyte

imbalance, ketonuria and weight loss, we wish to evaluate which FFAs are used as an energy source in pregnant women with HG.

Essential fatty acids (EFAs), such as α-linolenic acid (ALA), linoleic acid (LA), and their long-chain derivatives, can act as energy sources, but their main functions are structural and metabolic [8]. EFAs cannot be produced by mammalian tissues because of the need for desaturase enzymes and must be acquired through dietary intake [9]. Although the human body cannot produce *de novo* omega-3 and omega-6 long-chain polyunsaturated fatty acids (LC-PUFAs) [10], it can desaturate and elongate them by way of other enzymes, transforming LA to arachidonic acid (AA; 20:4n-6) and ALA to docosahexaenoic acid (DHA; 22:6n-3) and eicosapentaenoic acid (EPA; 20:5n-3) [11]. All the omega-3 and omega-6 fatty acids needed by the fetus must be supplied by the mother [12].

The most biologically important omega fatty acids are omega-3 (EPA and DHA) and omega-6 (AA) [13,14]. These mediate a number of key biological processes, including cell membrane physiology, signaling pathways, inflammation, gene regulation, and expression. They also have important functions in fetal and newborn neural development [15-17].

This prospective case-control study examined women in the first trimester of pregnancy to compare plasma FFAs and plasma levels of DHA, AA, EPA, and other fatty acids in HG patients and healthy pregnant women.

2 Materials and methods

2.1 Subjects

Ethical permission for this study was obtained through the Gulhane Military Medical Academy Ethics Committee (GATA KAEK-14047). The patients were informed about the examination process, and their consent was obtained. The study was carried out over a 16-month period (January 2013–April 2014).

2.2 Clinical studies

Twenty-six women with normal pregnancies (control group) and 26 women with pregnancies complicated by HG, ketonuria, and weight loss of more than 5% of body weight were enrolled in the study. Gestational age was confirmed by obstetric ultrasonography to be between 7 and 12 weeks. The exclusion criteria were fetal malfor-

mation, maternal disease, diabetes, alcohol abuse, or infection. All were singleton pregnancies, and none of the women were smokers. Maternal venous blood and urine samples were taken from all patients in the morning.

2.3 Assessment of dietary fatty acid intake

Measuring dietary fat intake can be difficult with use of food-frequency questionnaires (FFQs). Biological markers are more objective than FFQs, because biological markers do not depend on food-intake composition databases or the appropriateness of FFQ items (18). Levels of erythrocytes and C15 (pentadecanoic acid) in plasma can be used as diagnostic markers of dietary fat intake [19].

2.4 Biochemical analysis

Biochemical analysis was performed as described by Folch et al. [20]. Blood samples were collected in ethylenediaminetetraacetic acid (EDTA) tubes and kept on ice. Plasma for fatty acid analysis was separated by centrifugation at 3,000 rpm for 10 min. at 4°C and then frozen at −80°C until analysis. Lipids were extracted from 0.20 mL of plasma in chloroformmethanol (2:1) containing 10 mg/L of butylated hydroxytoluene (BHT) [20]. Thin-layer chromatography (Hewlett-Packard 6890 gas chromatograph; Minnesota, USA) was used to separate and evaporate plasma phospholipids until dry under nitrogen gas. Fatty acids were classified against authentic lipid standards obtained from Nu-Chek Prep, Inc. (Elysian, MN, USA). We evaluated the saturated fatty acids (SFAs) C14 (myristic acid), C15 (pentadecanoic acid), C16 (palmitic acid), C18 (stearic acid), C20 (arachidic acid), C22 (behenic acid), and C24 (lignoceric acid); the omega-3 FFAs C22:5n3 (EPA) and C22:6n3 (DHA); the omega-6 FFAs C18:2n6 (LA), C20:4n6 (AA), and C20:3n6 (homo-gamma-linolenic acid); and the omega-9 FFAs C18:1n9 (oleic acid), C22:1n9 (erucic acid), and C24:1n9 (nervonic acid) (Table 1).

2.5 Statistical analysis

Data were presented as mean ± standard deviation and analyzed with SPSS version 15 (SPSS Inc., Chicago, IL, USA). The relationships between the study and control groups were assessed with Mann–Whitney U tests. A level of significance equal to or less than 0.05 was used to determine statistical significance.

Table 1: Demographic characteristics of the participants and measurements of plasma free fatty acids for the control and hyperemesis gravidarum groups

Plasma Free Fatty Acids (µg/mL)	Hyperemesis Gravidarum n=26 Mean±Sd (Min-Max)-(Median)	Control Group n=26 Mean±Sd (Min-Max)-(Median)	p
Age	28±1 (22–35)	28±3 (22–36)	**0,876**
Gestatinoal age (week)	8±0,6	7,8±0,8	0,585
C15 (Pantadecanoic acid)	6.0 ± 6.1 (1.1–17.4)–(1.1)	8.8 ± 7.9 (1.1–34.6)–(9.3)	0,244
C20:4n6 (AA Arachidonic acid)	232.0 ± 21.6 (193–292)–(228)	237.4 ± 25.9 (19.6–279)–(234)	0,426
C22:5n3 (EPA Eicosapentaenoic acid)	47.9 ± 21.5 (0.4–68)–(53.9)	35.0 ± 30.4 (0.4–66.6)–(55.7)	0,566
C22:6n3 (DHA Docosahexaenoic acid)	79.0 ± 44.5 (0.7–118.5)–(101.2)	60 ± 49 (0.7–119)–(84.1)	0,082
C14 (Miristic acid)	102.5±79 (3.1–247)–(137.2)	112.7±78 (3.1–247)–(140)	0,297
C16 (Palmitic acid)	1115.9±264 (724–1762)–(1070)	1045.9±222 (706–1942)–(1038)	0,341
C18 (Stearic acid)	459.5±186 (35–752)–(482)	543.9±145 (69–775)–(549)	0,085
C18:1n9 (Oleic acid)	1026.9±157 (793–1362)–(1009)	1023.9±120 (709–1220)–(1034)	0,770
C18:2n6 (Linoleic acid)	1379.9±162 (1087–1714)–(1387)	1406.2±173 1027–1753)–(1410)	0,546
C20 (Arachidic acid)	18.5±7 (0.4–28.6)–(20.1)	22.1±3 (15.8–28.4)–(21.5)	0,039*
C22 (Behenic acid)	39.7±9.5 (0.8–53.3)–(40.2)	25.7±20 (0.8–66.5)–(33.5)	0,001*
C20:3n6 (Homogamma-linolenic acid)	67.5±46 (0.4–111.3)–(91.9)	80.3±48 (0.4–124.6)–(95.3)	0,361
C22:1n9 (Erucic asit)	5.6±4.3 (0.3–12.7)–(7.0)	3.0±4.3 (0.3–11.6)–(0.3)	0,066
C24 (Lignoceric asit)	28.8±14 (1–42.2)–(33.7)	26.8±15 (1.0–42.9)–(32.9)	0,538
C24:1 (Nervonic asit)	39.5±17 (0.6–55.1)–(46)	30.8±24 (0.6–59)–(45.2)	0,839

* Mann–Whitney U tests, "NS" not significant.

3 Results

The average gestational age of the HG group was 9.2 ± 1.2 weeks. In the control group, the average gestational age was 8.8 ± 1 weeks (p ˃ 0.05). The mean ages of the group of HG patients and the control group were 28 years and 26 years, respectively (p ˃ *0.05). No statistical difference* was observed between the groups as far as maternal or gestational age.

We found no significant difference between the groups in the levels of myristic acid, pentadecanoic acid, palmitic acid, stearic acid, lignoceric acid, EPA, DHA, LA, AA, homo-gamma-linolenic acid, oleic acid, erucic acid, or nervonic acid (p ˃ 0.05). In the HG and control groups, respectively, pentadecanoic acid was 6.5 ± 7.6 and 9.1 ± 6.0;

Table 2: Correlation analysis of groups (hyperemesis gravidarum [HG] and control group) with C20 and C22

	Correlation (HG and Control)	
	r	p
C20 (Arachidic acid)	0.290	0.037
C22 (Behenic acid)	−0.457	0.001

Spearman correlation test

AA was 236.68 ± 22.7 and 231.4 ± 25.8; EPA was 41.28 ± 26.0 and 41.9 ± 29.17; and DHA was 65.23 ± 50.6 and 76.8 ± 42.4.

Between the HG and control groups, we found a statistical difference in the levels of arachidic acid (18.5 ± 7

and 22.1 ± 3, p = 0.039) and behenic acid (39.7 ± 9.5 and 25.7 ± 20, p = 0.001) , respectively (Tables 1 and 2). The level of arachidic acid was lower in the HG group, while behenic acid was higher.

None of the patients in either group had any pregnancy complications.

4 Discussion

In some studies, improper maternal diet and weight loss have been seen to cause complications, such as low birth weight, antepartum hemorrhage, preterm birth, and fetal abnormality [21], while other studies have shown no difference in adverse fetal outcomes between women diagnosed with HG and normal pregnant women [22-24]. Fatty acid degradation, accompanied by ketosis, is dependent on the hunger level of pregnant women with HG. In all pregnancies, the fetus obtains EFAs from the mother by placental transfer [25]. In this study, we aimed to detect which FFAs in plasma undergo lipolysis to produce ketone bodies and, especially, to identify the omega-3 and omega-6 FFAs used for lipolysis.

FFA levels in plasma and in the erythrocyte membrane (EM) have been studied previously [26]. The EM is a better source than plasma to measure fatty acids to gain information about long-term fat intake [27], in part because of the lower sensitivity and slower turnover rate of EM FFAs [28]. We evaluated the FFAs in plasma to look at shorter-term intake because our patients were in the first trimester of pregnancy and their problems had started only a few weeks before.

There is a positive correlation between birth weight and LC-PUFA concentrations in maternal plasma during the first trimester of pregnancy [29]. Heird et al. reported that AA is necessary for fetal growth [30], and Innis reported that DHA has an important function in retinal and brain development and function during the neonatal period [31]. Crawford et al. also reported that the dietary EFAs LA and α-linolenic acid (ALA) and their LC-PUFA products, mainly AA and DHA, are essential to brain and retinal development [32]. DHA is known to have an important function as a structural component in the retina and nervous system, because a deficiency of omega-3 fatty acids in the diet during the antenatal and postnatal period causes irreversible neuronal and visual deterioration [33]. DHA is found in the membranes of neuronal synapses and photoreceptor segments [34-36]. In early pregnancy, DHA is an important factor for fetal growth and development and also functions as a vascular remodeling and placen-

tal angiogenic factor [37]. Although many researchers have studied the concentrations of LC-PUFAs in maternal plasma, no studies have been carried out on HG patients to examine their levels of AA, DHA, and EPA fatty acids.

Adaptations in maternal metabolism result in the transport of maternal nutrients from the placenta to the fetus for fetal growth [38]. Our findings showed no statistical difference between the HG and control groups in maternal plasma levels of the SFAs myristic acid, pentadecanoic acid, palmitic acid, stearic acid, and lignoceric acid; the mono-unsaturated fatty acids oleic acid, erucic acid, and nervonic acid; the omega-3 fatty acids EPA and DHA; or the omega-6 fatty acids LA, AA, and homo-gamma-linolenic acid. Our study demonstrated that the fetus did not waste the necessary nutrients, especially AA, DHA, and EPA, even when maternal malnutrition and fatty acid degradation occurred in the first trimester of pregnancy.

Which fatty acids are used to produce ketone bodies by lipolysis in the hyperemetic pregnancy? We found that the long-chain SFA arachidic acid was present at lower levels in the HG group (p < 0.05), which indicates that it is used for producing ketone bodies; it would provide more energy than short- and medium-chain fatty acids. Although there were no comparable data found in the literature, our results provide evidence that arachidic acid is used to produce ketone bodies as an energy source in cases of maternal malnutrition caused by HG. The long-chain SFAs arachidic acid, behenic acid, and lignoceric acid are derived from dietary sources and may also be produced endogenously by the elongation of stearic acid (39). Long-chain SFAs exhibit distinct functions when compared to other long-chain SFAs; for example, they are known to influence liver homeostasis, retinal function, and anti-inflammatory functions [40].

Interestingly, we also found that the level of behenic acid was higher in the HG group than the control group. The reason for this is unclear. Behenic acid is a cholesterol-raising SFA in humans [41]. Although the mechanism requires further elucidation, Cater et al.'s findings indicate that dietary behenic acid is particularly potent in raising concentrations of total and LDL cholesterol [41]. Elevated levels of behenic acid may increase cholesterol synthesis in the fetus. The higher level of behenic acid in the HG group needs further investigation. In the future, it may also be useful to compare levels of arachidic acid and behenic acid with the severity of symptoms in HG patients.

The main limitation of our study was the small number of participants in the study group (n = 26) and the control group (n = 26).

5 Conclusion

Levels of plasma DHA, EPA, AA, and other FFAs were not affected by HG, but the level of the saturated long-chain FFA arachidic acid was reduced in HG patients compared with women having a normal pregnancy, although behenic acid was elevated.

Conflict of interests: No authors report any conflict of interest.

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