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The assessment of maternal and umbilical cord homocysteine levels in obese pregnant women

B. Kaya[1], S. Kaya[1], H.L. Keskin[1], S. Aydoğmuş[1], G. Akçay Yegin[1], A. Ekiz[2], A.F. Avşar[3]

[1] *Ankara Atatürk Education and Research Hospital, Department of Obstetrics and Gynecology, Ankara*
[2] *İstanbul Kanuni Sultan Süleyman Education and Research Hospital, Department of Obstetrics and Gynecology, Istanbul*
[3] *Yıldırım Beyazıt University, School of Medicine, Department of Obstetrics and Gynecology, Ankara (Turkey)*

Summary

Purpose: The aim of this study was to compare the maternal plasma and umbilical cord blood homocysteine levels in obese and non-obese pregnant women. *Materials and Methods:* One hundred-ten term pregnant women, who completed their 37th gestational weeks and were not in active labor, were enrolled in the study. While 41 out of them were obese (BMI $\geq$ 30 kg/m^2), 69 were non-obese (BMI < 30 kg/m^2). The maternal plasma and umbilical cord homocysteine levels and umbilical cord pH values were compared between the groups. The statistical analyses were performed using *t*-test, Mann Whitney test, and Chi-square test. A $p < 0.05$ value was set as statistically significant. *Results:* The mean of age was higher in obese group in borderline significance (26.8 ±5.4 *vs.* 28.8 ±5.1, $p = 0.049$). The mean of gestational weeks, birthweight, the mode of delivery, and umbilical cord pH values were similar between the groups ($p > 0.05$). The maternal plasma homocysteine levels [median (interquartile range); 7.6 (4.1) *vs.* 7.1 (4.9)] and umbilical cord homocysteine values were not statistically different [8.6 (4.2) *vs.* 8.8 (4.5)] between the groups ($p > 0.05$). *Conclusion:* The maternal and umbilical cord blood homocysteine levels are not different in obese and non-obese pregnant women.

Key words: Pregnancy; Homocysteine; Obesity; Body mass index.

Introduction

Obesity is a chronic process that is common all around the world with an increasing prevalence and contributing to many chronic diseases and it is defined as excessive body fat accumulation [1-3]. It is a multifactorial situation developing under genetic, behavioral, environmental, physiological, and socio-cultural factors effecting the energy balance and fat storage [4]. Body mass index (BMI) over 30 kg/m^2 is evaluated as obesity [5]. While an increase in miscarriage, thromboembolic events, gestational diabetes, hypertensive disorders, cesarian section, and related maternal complications is observed, fetal complications such as fetal death, congenital abnormalities, and macrosomia are also shown to increase [6-8].

Homocysteine is a sulfur-containing amino acid and it is formed during the methionine metabolism. It takes charge in two important pathways containing methylation and sulfuration and it is not used in protein synthesis. Normal values of fasting plasma homocysteine is five to 15 µmol/L [9, 10]. Although homocysteine levels are found to be lower in normal pregnant women when compared to non-pregnant women, they are found to be even higher in pregnant women with preeclampsia, placental abruption, recurrent pregnancy loss, and intrauterine growth restriction when compared with normal pregnant women and it is indicated that it plays a role in the development of these aforementioned diseases [11-14]. Hyperhomocysteinemia has many mechanisms that cause atherogenic disease development such as blood vessel wall thickening, proliferation in the smooth muscle cells in the vessel intima layer, lipid accumulation on the blood vessel wall, platelet and leukocyte activation, increase in the low density lipoprotein (LDL) oxidation, thromboxane synthesis activation, and increased oxidative damage with radicals as a result of oxidation of homocysteine itself [15-18].

Although obesity and hyperhomocysteinemia have been separately associated with poor obstetric outcomes, there is limited information in the literature about the co-occurence of these two conditions at pregnancy. In the present study, the authors aimed to examine homocysteine levels in obese pregnant women and to ascertain if homocysteine levels contribute to the poor obstetric outcomes in obese pregnancies.

Materials and Methods

One hundred-ten term pregnant women, whose routine pregnancy follow-up was carried out at the obstetrics outpatient clinic of an educational and research hospital, were enrolled in the study. The study protocol was reviewed and approved by the local ethics committee. Informed consent was obtained from all subjects. The authors investigated 49 obese (BMI $\geq$ 30 kg/m^2) pregnant women for the study group and 61 non-obese (BMI < 30 kg/m^2) pregnant women for the control group at gestational week 37-41 of their singleton pregnancy, not engaged in active labor, with no medical

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disease or fetal anomaly and regularly followed antenatally were enrolled in both study and control groups. Gravida, parity, age, BMI in early pregnancy, gestational week at delivery, birth weight and delivery method were recorded for both groups. Maternal and umbilical cord homocysteine levels and umbilical cord pH values of the patients were measured. Pregnant women with chronic disease (diabetes mellitus, hypertension, renal disease, thyroid disfunction, cardiopulmonary disease, and autoimmune disease), pregnancy-related diabetes, hypertension and other pregnancy complications, history of poor obstetric outcome and fetal anomaly, smokers, multivitamin users, and those engaged in active labor were excluded from the study. The last menstruation date and the first trimester ultrasonographic measures of those who did not know their last menstruation date were used to determine the gestational age. Five cc peripheral venous blood samples were taken into ethylene diacetic acid (EDTA) containing tubes following eight to 12 hours of fasting in both groups. Right after the delivery, following the cordon clamping, venous blood samples were collected from umbilical cords and similarly taken into tubes with EDTA. Both maternal and fetal blood samples were placed in the refrigerator after the sampling and centrifuged at 3,000 cycles/ for ten minutes for one hour and its serums were separated. Serums were preserved at -25°C until being analyzed and the samples were studied on in 3 days at latest. Serum homocysteine levels were measured by use of chemiluminescence method [19]. Before the study was begun, a minimal sample size was calculated using G*Power ver. 3.1.3 software, and the minimum number of samples was determined as 98 subjects (49 cases for each group) to conform to the following statistical requirements: Type I (a) error = 0.05, Type II (b) error = 90%, and effect size (f) = 0.6 difference between group proportions of subjects. At the end of the study, data of 110 subjects (61 for non-obese and 49 for obese group) were evaluated; thus the actual power was carried out as 99%.

The data of the study were analyzed using SPSS Statistics 17.0 software. Before the statistical analysis was performed, the distribution of data was evaluated using the Shapiro-Wilk normality test.

It was observed that, whereas the data of variables body mass index, homocysteine levels in the maternal plasma and umbilical cord blood did not distribute normally ($p < 0.05$), the values of age, gestational age, umbilical cord pH, and birth weight were in conformity with the normal distribution ($p > 0.05$). Descriptive values of the parameters distributed normally were indicated as mean ± SD, and values of the variables that did not distribute normally as median (interquartile range - IQR).

In variables with continuous data, the parametric test (*t*-test) was used for comparison the normal distributed data among the groups, and non-parametric test (Mann-Whitney Test) was used in comparing data that did not distribute normally. The comparison of the data in categorical variables, gravida and parity, between the groups was performed using Chi-square test. As for the evaluation of the results, $p < 0.05$ was accepted as statistically significant.

Results

Whereas 49 (44.5%) of 110 cases enrolled in the study were obese, BMI in 61 cases was below 30. The mean age of all the cases was 27.6 ± 5.3 years and gestational age at birth was 39.0 ± 1.2 weeks. In all the cases, the average maternal plasma homocysteine level was 8.4 ± 3.9 µmol/L and the average umbilical cord homocysteine level was 9.9 ± 4.8 µmol/L. Whereas 70 (63.6%) of the cases delivered by vaginal delivery, 40 (36.4%) of the cases delivered by cesarean section. In eight (7.3%) of the cases, fetal distress findings were detected.

Table 1. — *Comparison of demographic data and homocysteine levels between the groups.*

	Normal weight (n=61)	Obese (n=49)	p
Age (years)*	26.8 ± 5.4	28.8 ± 5.1	0.049[a]
Gravida**	2.0 (1.0)	2.0 (1.0)	0.002[b]
Parity**	1.0 (1.0)	1.0 (1.75)	0.003[b]
Gestational age (weeks)*	38.9 ± 1.1	39.1 ± 1.3	0.487[a]
BMI (kg/m^2)**	27.0 (3.1)	31.3 (1.8)	< 0.001[c]
Maternal plasma homocysteine (µmol/L)**	7.6 (4.1)	7.1 (4.9)	0.947[c]
Umbilical cord blood homocysteine (µmol/L)**	8.6 (4.2)	8.8 (4.5)	0.675[c]
Umbilical cord pH*	7.29 ±0.06	7.30 ± 0.06	0.122[a]
Birth weight (grams)*	3293 ±403	3332 ± 399	0.621[a]

*Mean ± SD; ** Median (IQR); [a] *t*-Test; [b] Chi-square Test; [c] Mann-Whitney Test.

Later the cases were divided into two as normal weight (n=61) and obese (n=49) and the two groups were compared. The age of obese cases was higher in borderline significance when compared to normal-weight cases (28.8 ± 5.1 *vs.* 26.8 ± 5.4, $p = 0.049$) (Table 1). Gestational week at delivery and birth weight were similar between two groups ($p > 0.05$) (Table 1). Gravida and parity were found to be higher in obese pregnant women ($p < 0.05$). Delivery methods were also similar in two groups. While the delivery rate by cesarean was 36.1% (22/61) in normal-weight cases, it was 36.7% (18/49) in obese group ($p = 0.827$). Whereas fetal distress developed in three (4.9%) of normal-weight cases, five (10.2%) of obese cases developed fetal distress ($p = 0.264$). Umbilical cord pH levels were similar in both groups (7.29 ± 0.06 *vs.* 7.30 ± 0.06). Maternal plasma homocysteine levels [7.6 (4.1) *vs.* 7.1 (4.9)] and umbilical cord homocysteine levels [8.6 (4.2) *vs.* 8.8 (4.5)] were similar in both groups (respectively, $p = 0.947$ and $p = 0.675$) (Table 1).

Discussion

Physiological and metabolic stress elevate due to the increasing metabolic needs in order to meet the needs of the growing fetus. Due to the fact that high homocysteine levels cause endothelial disfunction, it is asserted that pregnant women with hyperhomocysteine are predisposed to placental vasculitis and endothelial disfunction. High homocysteine concentration and low folate level may effect DNA synthesis and cell division that may cause deterioration of cell proliferation and normal fetal growth [20, 21].

It is known that obesity is a chronic oxidative stress creating situation [22]. In a study including 5,131 women, Kabiru *et al.* assessed the relationship between the increase in BMIs and the pregnancy outcomes and they detected significant increase in the gestational diabetes, induction failure, perineal laceration, delivery by cesarean section, and postpartum infection ratios in normal-weight pregnant women with increased BMI during pregnancy. They also detected increase in the preeclampsia and operative vaginal delivery ratios in over-weight pregnant women with increased BMI and increase in the chorioamnionitis, induction failure, and delivery by cesarean in obese pregnant women with increased BMI [23]. The present authors did not detect significant difference between obese and non-obese groups as for the parameters such as cesarean, fetal distress, gestational week at delivery, birth weight, and umbilical cord pH. The fact that they did not find such difference in the present study, whereas in the literature, significant difference is found between obese and non-obese pregnant women as for the parameters examining the pregnancy outcomes, may be due to the limited number of the patients.

Bukhari *et al.* found that serum homocysteine levels are significantly high in obese subjects when compared to normal-weight subjects. They commented that homocysteine is an independent risk factor in the development and progression of obesity [24]. Andersson *et al.* detected that serum homocysteine levels in pregnant women begin to decrease as of the early phases of the first trimester when compared to non-pregnant women. They stated that the decrease in the homocysteine levels become even more evident in the weeks 15-17 and remain relatively stabile afterwards and that the levels return to pre-pregnancy levels in the postpartum day 2-4 [25]. Similarly, Kang *et al.* noted that plasma homocysteine levels decrease by almost 50% around 16th gestational week [26]. As the responsible mechanism for this decrease, they supported the hypothesis of increased glomerular filtration rate, increased plasma volume and accordingly developed hemodilution, and increased homocysteine intake of the fetus [25, 27].

Hyanek *et al.* detected the homocysteine levels significantly low in normal pregnancies, when compared to women in reproductive and climacteric periods. They stated that plasma homocysteine levels were slightly higher in complicated pregnancies, when compared to normal pregnancies [28]. Açılmış *et al.* found the maternal and fetal serum homocysteine levels significantly higher in severe preeclamptic patients when compared to mild preeclamptic patients and the control group; they underlined no significant difference between the mild preeclamptic patients and the control group in terms of serum homocysteine levels [29]. Vollset *et al.* detected that in women with high plasma homocysteine levels, preeclampsia risk (32%), and prematurity risk (38%), where high and low birth weight risk was even higher (101%). While they found a significant relationship between neural tube defect, club foot anomaly, and plasma homocysteine levels, they could not detect any relationship between placental abruption risk and homocysteine levels. Consequently, they correlated high concentration homocysteine with poor pregnancy outcomes [30].

Han *et al.* noted that pre-pregnancy BMI effects the pregnancy outcomes. While correlating high CRP and low folate levels with preterm labor, they did not find any significant difference between obese and non-obese pregnant women in terms of the homocysteine level, which is another factor associated with preterm labor [31].

In the present study, the authors also did not find any significant relationship between maternal and umbilical cord homocysteine levels in obese and non-obese pregnant women. In the literature, generally there is a positive correlation between obesity and homocysteine levels, however such a correlation was not found in this study. It is possible that the authors could not detect the elevation that they expected in the homocysteine levels in obese women due to the fact that the present patients were obese pregnant women and pregnancy has a decreasing effect on the plasma homocysteine levels as was aforementioned (hemodilution, increased glomerular filtration, and fetus using the homocysteine).

Conclusion

Obesity and hyperhomocysteinemia are associated with both pregnancy complications and many medical problems in non-pregnant women, however there is conflicting information in the literature regarding the effect of the co-existence of the two during pregnancy. Whether hyperhomocysteinemia also contributes to poor obstetric outcomes of obesity is not clear yet, under the light of current information.

References

[1] Must A., Spadano J., Coakley E.H., Field A.E., Colditz G., Dietz W.H.: "The disease burden associated with overweight and obesity". *JAMA*, 1999, *282*, 1523.

[2] James P.T., Leach R., Kalamara E., Shayeghi M.: "The worldwide obesity epidemic". *Obes. Res.*, 2001, *9*, 228.

[3] Ogden C.L., Yanovski S.Z., Carroll M.D., Flegal K.M.: "The epidemiology of obesity". *Gastroenterology*, 2007, *132*, 2087.

[4] Racette S.B., Deusinger S.S., Deusinger R.H.: "Obesity: overview of prevalence, etiology, and treatment". *Phys. Ther.*, 2003, *83*, 276.

[5] Wells J.C., Fewtrell M.S.: "Measuring body composition". *Arch. Dis. Child.*, 2006, *91*, 612.

[6] Yu C.K., Teoh T.G., Robinson S.: "Obesity in pregnancy". *BJOG*, 2006, *113*, 1117.

[7] Yogev Y., Catalano O.M.: "Pregnancy and obesity". *Obstet. Gynecol. Clin. North Am.*, 2009, *36*, 285.

[8] Sirimi N., Goulis D.G.: "Obesity in pregnancy". *Hormones*, 2010, *9*, 299.

[9] Aubard Y., Darodes N., Cantaloube M.: "Hyperhomocysteinemia and pregnancy: review of your present understanding and therapeutic implications". *Eur. J. Obstet. Gynecol. Reprod. Biol.*, 2000, *93*, 157.

[10] Fattal-Valevski A., Bassan H., Korman S.H., Lerman-Sagie T., Gutman A., Harel S.: "Methylenetetrahydrofolate reductase deficiency: importance of early diagnosis". *J. Child. Neurol.*, 2000, *15*, 539.

[11] de Vries J.I., Dekker G.A., Huijgens P.C., Jakobs C., Blomberg B.M., van Geijn H.P.: "Hyperhomocysteinaemia and protein S deficiency

in complicated pregnancy". *Br. J. Obstet. Gynaecol.*, 1997, *104*, 1248.

[12] Rajkovic A., Mahomed K., Malinow M.R., Sorenson T.K., Woelk G.B., Williams M.A.: "Plasma homocysteine concentrations in eclamptic and preeclamptic African women postpartum". *Obstet. Gynecol.*, 1999, *94*, 355.

[13] Walker M.C., Smith G.N., Perkins S.L., Keely E.J., Garner P.R.: "Changes in homocysteine levels during normal pregnancy". *Am. J. Obstet. Gynecol.*, 1999, *180*, 660.

[14] Hague W.M.: "Homocysteine and pregnancy". *Best. Pract. Res. Clin. Obstet. Gynaecol.*, 2003, *17*, 459.

[15] Clarke R., Daly L., Robinson K., Naughten E., Cahalane S., Fowler B., Graham I.: "Hyperhomocysteinemia: an independent risk factor for vascular disease". *N. Engl. J. Med.*, 1991, *324*, 1149.

[16] Reis R.P., Azinheira J., Reis H.P., Bordalo A., Santos L., Adao M., *et al.*: "Homocysteinaemia after methionine overload as a coranary artery disease risk factor; importance of age and homocysteine levels". *Coron. Artery Dis.*, 1995, *6*, 851.

[17] McDowell I.F., Lang D.: "Homocysteine and endothelial dysfonction: a link with cardiovascular disease". J. Nutr., 2000, *130*, 369.

[18] Dikmen M.: "Homocysteine metabolism and association with diseases: A review". *Turkiye Klinikleri J. Med. Sci.*, 2004, *24*, 645.

[19] Ubbink J.B.: "Assay methods for the measurement of total homocyst(e)ine in plasma". *Semin. Thromb. Hemost.*, 2000, *26*, 233.

[20] Tang L., Mamotte C.D., Van Bockxmeer F.M., Taylor R.R.: "The effect of homocysteine on DNA synthesis in cultured human vascular smooth muscle". *Atherosclerosis*, 1998, *136*, 169.

[21] Scholl T.O., Johnson W.G.: "Folic acid: influence on the outcome of pregnancy". *Am. J. Clin. Nutr.*, 2000, *71*, 1295.

[22] Higdon J.V., Frei B.: "Obesity and oxidative stres: a direct link to CVD?" *Arterioscler. Thromb. Vasc. Biol.*, 2003, *23*, 365.

[23] Kabiru W., Raynor B.D.: "Obstetric outcomes associated with increased in BMI category during pregnancy". *Am. J. Obstet. Gynecol.*, 2004, *191*, 928.

[24] Bukhari S.A., Rajoka M.I., Nagra S.A., Rehman Z.U.: "Plasma homocysteine and DNA damage profiles in normal and obes subjects in the Pakistani population". *Mol. Biol. Rep.*, 2010, *37*, 289.

[25] Andersson A., Hultberg B., Brattström L., Isaksson A.: "Decreased serum homocysteine in pregnancy". *Eur. J. Clin. Chem. Clin. Biochem.*, 1992, *30*, 377.

[26] Kang S.S., Wong P.W., Zhou J.M., Cook H.Y.: "Total homocysteine in plasma and amniotic fluid of pregnant women". *Metabolism.,* 1986, *35*, 889.

[27] Malinow M.R., Rajkovic A., Duell P.B., Hess D.L., Upson B.M.: "The relationship between maternal and neonatal umblical cord plasma homocysteine suggests a potential role for maternal homocysteine in fetal metabolism". *Am. J. Obstet. Gynecol.*, 1998, *178*, 228.

[28] Hyanek J., Zivny J., Dolezal A., Calda P., Krofta L., Vinglerova H., *et al.*: "Homocysteine- its significance in gynecology and obstetrics". *Ceska. Gynekol.*, 2000, *65*, 406.

[29] Acilmis Y.G., Dikensoy E., Kutlar A.I., Balat O., Cebesoy F.B., Ozturk E., *et al.*: "Homocysteine, folic acid and vitamin B12 levels in maternal and umblical cord plasma and homocysteine levels in placenta in pregnant women with pre-eclampsia". *J. Obstet. Gynaecol. Res.*, 2011, *37*, 45.

[30] Vollset S.E., Refsum H., Irgens L.M., Emblem B.M., Tverdal A., Gjessing H.K., *et al.*: "Plasma total homocysteine, pregnancy complications and adverse pregnancy outcomes: the Hordaland Homocysteine study". *Am. J. Clin. Nutr.,* 2000, *71*, 962.

[31] Han Y.S., Ha E.H., Park H.S., Kim Y.J., Lee S.S.: "Relationships between pregnancy outcomes, biochemical markers and pre-pregnancy body mass index". *Int. J. Obes.*, 2011, *35*, 570.

Address reprint requests to:
B. KAYA, M.D.
Ankara Atatürk Education and Research Hospital
Department of Obstetrics and Gynecology
Ekin Street no. 21/8 Yesilyurt Bakirkoy
Istanbul 34149 (Turkey)
e-mail: kayabasak84@gmail.com