Serum Lipid Profile, Oxidative Status, and Paraoxonase 1 Activity in Hyperemesis Gravidarum

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The aim of this study was to investigate lipid profile, paraoxonase 1 (PON1) activity, and oxidative stress status in the serum of hyperemesis gravidarum (HG) patients. Thirty-six HG cases and 36 normal pregnant were included in the study. Serum total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), apoproteins A1 (apo A1) and B (apo B), malondialdehyde (MDA), and total antioxidant activity (TAO) values and PON1 and arylesterase activities were determined. Although serum TC, TG, LDL-C, and apo B levels were not different among; the groups ($P > 0.05$), HDL-C ($P = 0.01$)

and apo A1 ($P = 0.007$) levels were lower in HG patients than in normal pregnant. HG group had significantly lower serum PON1 ($P = 0.03$) and arylesterase activities ($P = 0.03$) compared with the control group. Additionally, mean TAO values were lower ($P = 0.01$) and MDA levels were higher ($P = 0.02$) in HG group than in the healthy pregnant. A significant negative correlation between PON1 and MDA was found in HG group ($r = -0.33$, $P < 0.05$). The findings of this study have revealed that HG may be one of the conditions in which oxidant and antioxidant balance is impaired. *J. Clin. Lab. Anal.* 23:105–109, 2009. © 2009 Wiley-Liss, Inc.

Key words: hyperemesis gravidarum; PON1; serum lipids; MDA; TAO

INTRODUCTION

Although up to 80% of all pregnant women experience some form of nausea and vomiting during their pregnancy (1), the more severe cases in whom nausea and vomiting become augmented and continue throughout the day are termed as hyperemesis gravidarum (HG), which occurs in 0.5–2% of all pregnancies. Another definition of HG is vomiting severe enough to produce weight loss, dehydration, acid–base disturbances, ketonuria, and electrolyte imbalances, especially hypokalemia (2). Many hypotheses have been created to explain the etiology and pathophysiology of HG, including psychological factors, gastrointestinal tract dysfunction, endocrinological changes, infections, and immunological, metabolic, and anatomical factors. However, there is still no single theory sufficient to provide an adequate explanation for all the properties of HG (3,4).

Normal pregnancy is associated with a broad series of metabolic adaptations, which may also influence the metabolism of lipids and lipoproteins, being more prominent in the second and third trimesters (5).

Starvation itself induces profound metabolic changes in the body. A study, investigating serum lipid and lipoproteins in HG, showed that HG patients had lower levels of serum high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC), and apoprotein A1 (apo A1) compared with normal pregnancy (6).

Serum paraoxonase 1 (PON1), synthesized in the liver, is a HDL-associated enzyme that prevents oxidative modification of LDL and HDL. Serum PON1 is located on HDL and is responsible for the antioxidant activity of HDL. This protection is most probably related to PON1's hydrolyzing activity of lipid peroxide products (7). Serum PON1 activity was found to be reduced in a

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number of pathological conditions including myocardial infarction, diabetes, and hypercholesterolemia.

Increased lipid peroxidation and oxidative stress are observed during pregnancy owing to excess free-radical activity and impaired antioxidant defense system. In studies related to HG, low antioxidant enzyme activities (8) and increased oxidant stress (8,9) have been reported compared with normal pregnant.

The aim of this study was to evaluate PON1 (as an antioxidant enzyme) activity and total antioxidant activity (TAO) together with malondialdehyde (MDA) (as an oxidative stress marker) levels. We also investigated the possible correlations of MDA and TAO with PON in the healthy pregnant and HG patients.

MATERIALS AND METHOD

A total of 36 consecutive patients with HG were enrolled in this study. The diagnostic criteria for HG were as follows: severe vomiting (>3 times per day), weight loss of more than 5%, and at least one positive ketonuria. Routinely, blood glucose, thyroid-stimulating hormone, free T3, free T4, β human chorionic gonadotropin, and hepatic and renal function test measurements were done. Patients with multiple gestation, gestational trophoblastic disease, and other known possible vomiting causes such as thyroid diseases and psychological and gastrointestinal disorders were excluded. Thirty-six pregnant women without symptoms of nausea and vomiting were involved in the study as the control group. Gestational age was determined using the first date of last menstrual period and confirmed by ultrasonography. Written informed consent was obtained from all enrollees according to the criteria of the Ethical Committee of Medical Faculty, Ataturk University. Patient and control groups' ages and gestational ages were matched. Subjects of both groups were at between 11 and 15 weeks' gestation. Those who reported cigarette smoking, alcohol consumption, or antioxidant vitamin supplementation were excluded from the study.

Venous blood samples were collected into tubes without anticoagulant. Serum was separated by centrifugation after clotting and stored at -80°C until assayed.

Measurement of the TAO: TAO of serum was determined using a novel automated measurement method developed by Erel (10). In this method, hydroxyl radical is produced. In the assay, ferrous ion solution is mixed by hydrogen peroxide. The sequentially produced radicals such as brown-colored dianisidiny radical cation, produced by the hydroxyl radical, are also potent radicals. Using this method, antioxidative effect of the sample against the potent free-radical reactions, which is initiated by the produced hydroxyl radical, is measured. The results are expressed as mmol Trolox Equiv./L.

Measurement of lipid and lipoprotein profile: The levels of serum TC, triacylglycerol (TG), HDL-C, and LDL-C were determined by enzymatic colorimetric methods using an Olympus Au 2700 autoanalyzer (Tokyo, Japan). Very low-density lipoprotein was calculated as TG/5. Apolipoprotein measurements were performed by an immuno-turbidimetric assay.

Assay of PON1 and arylesterase activities: PON1 activity was measured using diethyl-*p*-nitrophenylphosphate as a substrate as previously described (11). Briefly, 40 μL of serum (1:2 diluted with water) was incubated with reaction mixture at 37°C for 5 min, and then the enzymatic reaction was started by the addition of a substrate solution. The basal assay mixture contained 1.2 mM paraoxon, 1.0 mM CaCl_2 , and 50 mM Tris-HCl (pH 6.8). The increase in A_{405} was recorded. The amount of *p*-nitrophenol formed per minute was calculated from the molar absorption coefficient at pH 6.8 ($7,480 \text{ M}^{-1}/\text{cm}$). A total of 1 U/mL of PON1 was defined as 1 nmol of *p*-nitrophenol formed per minute. PON1 arylesterase activity was determined as the rate of phenyl acetate hydrolysis catalyzed by serum in a cuvette with 4.4 mM phenyl acetate in 50 mM Tris/acetate (pH 7.8) containing 20 mM of CaCl_2 at 37°C . The hydrolysis of phenyl acetate was monitored at 270 nm with a molar absorption coefficient at 270 nm of $1,310 \text{ M}^{-1}/\text{cm}$.

MDA assay: Serum MDA levels were determined spectrophotometrically according to the method described by Ohkawa et al. (12). A mixture of 8.1% sodium dodecyl sulfate, 20% acetic acid, and 0.9% thiobarbituric acid was added to 0.2 mL of serum, and distilled water was added to the mixture to bring the total volume up to 4 mL. This mixture was incubated at 95°C for 1 hr. After incubation, the tubes were left to cool under cold water, and 1 mL distilled water plus 5 mL *n*-butanol/pyridine (15:1 v/v) was added, followed by mixing up. The samples were centrifuged at $4,000 \times g$ for 10 min. The supernatants were removed, and absorbances were measured with respect to a blank at 532 nm. 1,1,3,3-Tetraethoxypropane was used as the standard. Lipid peroxide levels were expressed as $\mu\text{mol/L}$.

Statistical analysis: Results were given as mean $\pm$ SD. According to Kolmogorov Smirnov test, because the distribution of all analytes is normal, unpaired *t*-test was used to assess the differences between groups. Correlation analyses were performed using Pearson's correlation test. A *P* value <0.05 was accepted as significant.

RESULTS

Table 1 gives the characteristics of the study population. Gestational age, age, and BMI of patients with HG were not different from those of healthy controls

TABLE 1. Characteristics of the Study Population

	Control (n = 36)	HG (n = 36)
Age (yr)	26.97 ± 4.89	28.44 ± 5.18
BMI (kg/m ²)	24.39 ± 3.77	23.40 ± 3.46
Gestational age at the study (week)	13.25 ± 1.42	12.86 ± 1.05

HG, hyperemesis gravidarum.

TABLE 2. Serum Lipid and Lipoprotein Profile of Subjects

Parameters	Controls	HG
TC (mg/dL)	171.83 ± 37.90	158.44 ± 40.11
TG (mg/dL)	118.47 ± 36.78	115.61 ± 64.29
HDL-C (mg/dL)	56.91 ± 14.03	48.80 ± 11.94*
LDL-C (mg/dL)	108.14 ± 24.82	101.85 ± 27.17
VLDL-C (mg/dL)	23.61 ± 7.32	23.16 ± 12.80
Apo A1 (mg/dL)	154.24 ± 29.83	133.32 ± 33.40**
Apo B (mg/dL)	73.89 ± 17.53	70.19 ± 18.93

Results were given as mean ± SD. * $P = 0.01$ and ** $P = 0.007$ compared with controls. HG, hyperemesis gravidarum; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; VLDL-C, very low-density lipoprotein cholesterol; apo A1, apoprotein A1; apo B, apoprotein B.

TABLE 3. Oxidant and Antioxidant Parameters in Controls and HG

Parameters	Controls	HG
PON1 (U/mL)	177.35 ± 76.61	141.22 ± 61.77*
Arylesterase (U/mL)	94.76 ± 32.90	78.52 ± 29.83*
TAO (mmol Trolox Equiv./L)	1.63 ± 0.18	1.50 ± 0.24**
MDA (μmol/L)	54.90 ± 9.29	60.13 ± 9.06***
PON1/apo A1	1.20 ± 0.56	1.45 ± 2.48
PON1/HDL	3.32 ± 1.67	3.03 ± 1.48

Results were given as mean ± SD. * $P = 0.03$, ** $P = 0.01$, and *** $P = 0.02$ compared with controls. HG, hyperemesis gravidarum; PON1, paraoxonase 1; TAO, total antioxidant activity; MDA, malondialdehyde; apo A1, apoprotein A1; HDL, high-density lipoprotein.

($P > 0.05$). The data including the lipid profile of patients with HG and healthy pregnant are given in Table 2. TC, TG, LDL-C, and apo B levels were not different between the groups ($P > 0.05$). HDL-C and apo A1 levels of the HG group were lower than those of the control group ($P = 0.01$, $P = 0.007$, respectively).

Mean TAO values were lower ($P = 0.01$) and MDA levels were higher ($P = 0.02$) in HG group than in the healthy pregnant. Patients with HG had significantly lower serum PON1 ($P = 0.03$) and arylesterase activities ($P = 0.03$) compared with the control subjects (Table 3). A significant negative correlation between PON1 and MDA was found in HG group ($r = -0.33$, $P < 0.05$). Additionally, TAO was significantly correlated with

PON1 ($r = 0.35$, $P < 0.05$) and MDA ($r = -0.37$, $P < 0.05$). In the control group, PON1 was not correlated with TAO or MDA.

To assess whether the increased PON1 activity was owing to elevated HDL and apo A1 levels, we standardized PON1 activity for HDL and apo A1 concentrations. The standardized values for HDL and apo A1 (PON1/HDL and PON1/apo A1 ratio) did not change significantly ($P > 0.05$ for both).

DISCUSSION

Although the exact pathophysiological mechanisms are not clear, it has been suggested that HG is associated with increased human chorionic gonadotropin levels, increased maternal serum estrogen, and *Helicobacter pylori* seropositivity. Social factors and ethnicity also play an important role in the development of HG (3). Whatever the cause is, HG is a condition of intractable vomiting, leading to fluid, electrolyte and acid-base imbalance, nutrition deficiency, and weight loss often severe enough to require hospital admission.

Previous reports (13) show higher levels of LDL-C, lower levels of HDL-C, and apolipoprotein A1 (6) in normal pregnancy than (6,13) in HG pregnancy. Our study indicates no significant differences between normal controls and HG subjects' TC, TG, LDL-C, and apo B levels, and HDL-C and apo A1 levels were significantly lower in HG patients. When looking into the above data, there seems to be a discrepancy between our finding and the previous studies in terms of LDL-C.

Oxidative stress is thought to be implicated in many pathologic processes of human disorders. Pregnancy is a physiological state associated with enhanced oxidative stress related to high metabolic turnover and elevated tissue oxygen requirements. Levels of peroxidation markers, such as lipid hydroperoxide and MDA, are higher in pregnant than in nonpregnant women (14). In addition to normal pregnancy, in studies related to HG, low antioxidant enzyme activities (8) and increased oxidant stress (8,9) have been reported compared with normal pregnant. In one study done by Fait et al. (15), it was found that plasma GSH levels were significantly lower in HG group than in pregnant controls. We also found an imbalance in the oxidant/antioxidant system characterized by increased oxidative stress and decreased antioxidant activity (decreased TAO and increased MDA).

Several mechanisms may lead to oxidative stress in patients with HG. The first mechanism may be lower intake of nutrient antioxidants. In many respects HG resembles fasting. There is, for instance, reduced food intake and hence limited availability of energy sources, fluids, vitamins, and other nutrients. In other words,

a combination of increased demands during pregnancy, the absence/insufficiency of nutritional intake, and malabsorption during HG are possible causes of these deficiencies. Additionally, oxidative stress can also be modified by certain environmental factors. For example, vitamins C and E are well-known environmental antioxidants. We confirmed this condition by showing a significant reduction in TAO levels and PON activity and elevated MDA levels in HG patients.

The second mechanism for increased oxidative stress may be *H. pylori* infection. During HG, Gram-negative bacterium that colonizes the gastric mucosa may increase the generation of reactive oxygen species. Some studies, performed on patients with HG, indicated a causative relationship between *H. pylori* infection and HG (16,17). However, more recently, it was found that there was not any relation between *H. pylori* and HG (8,18,19). Furthermore, Guney et al. (8) found that the decrease in antioxidant enzyme activities and the increase in MDA were not associated with *H. pylori* infection.

HDL protects against atherosclerosis by returning excess cholesterol from peripheral tissues back to the liver for reuse or excretion into the bile. Several reports suggest an antioxidative function for HDL, which may contribute to its antiatherogenic activity (20,21). Serum PON1 is located on HDL and is responsible for the antioxidant activity of HDL. PON1 activity can vary depending on different physiological conditions or pathological states. Significant reduction in serum PON activity during pregnancy in mice was demonstrated by Weitman et al. (22). We found a decrease in PON1 activity in HG compared with normal pregnant. Recently, Verit et al. also found that PON1 activity was significantly lower and lipid hydroperoxide levels were significantly higher in pregnant women with HG than in healthy pregnant women (23). As PON1 is an HDL-associated enzyme, reduced HDL levels could result in reduced serum PON1 activity in HG cases. In this study, the HDL-standardized enzyme activity (PON1/HDL ratio) was determined to assess whether the altered PON1 activity was owing to the HDL-C level reduction. We observed that the HDL-standardized PON1 activity was no longer different in patients with HG compared with controls, suggesting that the reduction in HDL-C could explain the reduced PON1 activity in HG patients. In addition, decreased serum arylesterase activity in HG patients could support this finding, as arylesterase activity was suggested to reflect the PON mass.

Serum PON1 and arylesterase activities are lowered as a result of an altered synthesis or secretion of HDL. In serum, because PON1 is entirely complexed to HDL, the reduction in PON1 activity may be owing to a reduction in HDL-C concentration in HG. As given in Table 2, HDL-C concentration decreased in HG patients. In addition, PON1 associated with circulating HDL

requires the presence of apo A1, which is also necessary for its stability and its optimal activity (1,2). Our results showed a reduction in apo A1 concentration in HG compared with normal pregnant.

The other explanation is that increased oxidative stress might lead to a low activity of PON1 in HG. Indeed, it was shown by Aviram et al. that PON1 is inactivated in the presence of lipid peroxides (24). There was a negative correlation between PON1 and MDA in our study. Therefore, increased oxidative stress in HG can be partly explained by their lower PON1 activities.

Several factors such as lipid peroxidation products and cytokines were also suggested to affect PON1 synthesis (25). The reduction in arylesterase activities, determined in this study in patient groups, could reflect the inhibition of PON1 synthesis. The significant inverse correlations between PON1 activity and MDA observed in our study allowed us to speculate that the oxidative stress could contribute to a reduction in PON1 activity.

As a conclusion, HG may be one of the conditions in which oxidant and antioxidant balance is impaired. HG reduces the PON1 activity and this decrease may be associated with reduced in HDL-C and apo A1 levels and increased oxidative stress. Further prospective and longitudinal studies are needed to investigate the association of PON activity with the development of atherogenesis in subjects with a history of previous HG.

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