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Evaluation of catalase, myeloperoxidase and ferroxidase values in pregnant women with hyperemesis gravidarum

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ABSTRACT

Objectives: To investigate maternal serum catalase, myeloperoxidase and ferroxidase levels in pregnant women with Hyperemesis Gravidarum and to compare the results with healthy pregnancies.

Material and methods: In this study, 60 female patients admitted to the Health Sciences University, Gazi Yaşargil Training and Research Hospital, Gynecology and Obstetrics Department were evaluated. The patients were divided into two groups: Group 1 included 30 pregnant women with hyperemesis gravidarum; Group 2 included 30 healthy pregnant women. Pregnancies over 14 weeks were excluded from the study.

Results: The laboratory and laboratory characteristics of both groups are shown in Table 1. No significant differences were found between the groups in terms of the maternal age, gestational age, gravidity, parity, fasting glucose level, and BMI. The maternal blood CAT levels were significantly higher in the HG group (219.6 ± 111.3 kU/L) when compared to the control group (71.5 ± 52.5 kU/L) ($p < 0.001$). The maternal blood MPO levels were lower in the control group (121.5 ± 36.3 U/L) than in the study group (90.9 ± 56.4 U/L) ($p = 0.016$). However, the ferroxidase levels were similar between the two groups. The independent variables BMI, age, parity, gravidity and gestational week effects were adjusted according to the logistic regression method with groups. Significant differences were observed between the two groups in the levels of CAT (0.001), MPO (0.005) values.

Conclusions: This study suggests that antioxidants in response to oxidative stress gave different reactions with different mechanisms; Also, we believe that insufficient food intake suppresses the immune system and this has an important role on antioxidants.

Key words: catalase; myeloperoxidase; ferroxidase; hyperemesis gravidarum

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INTRODUCTION

Nausea and vomiting can be seen in 80% of pregnant women in the first months of pregnancy [1]. Nausea and vomiting is a severe form of hyperemesis gravidarum (HG) and occurs in 0.3–3% of all pregnancies. When severe nausea and vomiting occur more often than three times a day in a patient with ketonuria and a weight loss of more than 5%, the patient is diagnosed with HG [2–4].

The HG etiology has not yet been fully elucidated [5]; however, many oxidative stressors are known to play roles in HG [6], including an imbalance between the oxidants and antioxidants. Oxidative stress (OS) refers to an imbalance between the production of reactive oxygen species and the antioxidant defence system that buffers oxidative damage, resulting in cellular, molecular damage. It has been reported

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that antioxidant activity is significantly higher in women with healthy pregnancies when compared to women who are not pregnant [7].

The antioxidant defence system includes ferroxidase, which converts toxic ferrous iron to less toxic ferric iron, thereby reducing oxidative damage to the cellular and molecular [8, 9]. Catalase (CAT), an intracellular antioxidant, increases to compensate for redox reactions in OS [10]. Myeloperoxidase is another intracellular enzyme that acts as an antioxidant in neutrophils. A decrease in antioxidant activity or an increase in free radicals will lead to OS [11, 12].

In this study, the ferroxidase, CAT, and MPO serum levels in the blood of HG patients were determined, and the results were compared with those of healthy pregnant women.

MATERIAL AND METHODS

This prospective study included a total of 60 pregnant women admitted to the Gynecology and Obstetrics Department of the Health Science University Diyarbakır Gazi Yaşargil Training and Research Hospital in Diyarbakir, Turkey, between December 2017 and December 2018. The patients were divided into two groups: group 1 included 30 pregnant women with HG and group 2 included 30 healthy pregnant women. This research was conducted following the principles of the Helsinki Declaration, and informed consent was obtained from all of the participants.

The HG diagnosis inclusion criteria were as follows: severe and persistent nausea and vomiting more than three times per day during pregnancy, ketonuria, and greater than 5% weight loss [5]. Those patients with comorbid diseases (such as trophoblastic diseases, gestational diabetes, preeclampsia, thyroid diseases, infectious diseases, inflammatory diseases, renal diseases, hepatic diseases, and psychiatric disorders), smoking and alcohol habits, chronic medication use, and pregnancies over 14 weeks were excluded from the study. The following patient characteristics were recorded: maternal age, parity, gravidity, body mass index (BMI), and the gestational week at sampling.

Venous blood samples were centrifuged at 4,000 rpm for 10 minutes, sera were separated and stored at –80°C until the MPO, ferroxidase, and CAT levels were analysed.

Activity of CAT was evaluated by Goth's method [13]. Sample (0.2 mL) was incubated in 1.0 mL substrate (65 µmol per in 60 hydrogen peroxide (H_2O_2) mmol/L sodium-potassium phosphate buffer, pH 7.4) at 37°C for 60 seconds. The enzymatic activity was stopped with 1.0 mL of 32.4 mM ammonium molybdate, and the yellow complex of molybdate and H_2O_2 was measured at 405 nm. CAT activity was presented kU/L.

Activity of MPO was evaluated by a modification of the o-dianisidine method [14], a kinetic measurement with a yellowish orange product ratio; MPO from the oxidation of O-dianiside in the presence of hydrogen peroxide was measured at 460 nm. MPO activity was presented in units per liter serum.

The ferroxidase level was evaluated using the Erel-specified method [15]. Although this method is calorimetric and automatic, enzymatically, it depends on the oxidation of the iron ion. The results were evaluated as the units per liter of serum.

The study protocol was approved by a regional committee (216).

Statistical analysis

For the comparative between-group analyses (case vs control), a chi-squared test was used for the categorical variables, and either the Student's t-test or the Mann-Whitney U test was used for the continuous variables. Independent variables were adjusted that reduced the BMI, age, parity, gravidity and gestational week effect with the independent logistic regression method. Differences were considered significant at $p < 0.05$. All statistical analyses explained R-software v.3.5.1 (R statistics software, Institute for statistics and mathematics, Vienna, Austria).

RESULTS

Clinical and laboratory results are shown in Table 1. No significant differences were found between the groups in terms of the maternal age, gestational week, gravidity, parity, fasting glucose level, and BMI. The maternal blood CAT levels were significantly higher in the HG group (219.6 ± 111.3 kU/L) when compared to the control group (71.5 ± 52.5 kU/L) ($p < 0.001$). The maternal blood MPO levels were lower in the control group (121.5 ± 36.3 U/l) than in the study group (90.9 ± 56.4 U/L) ($p = 0.016$). However, the ferroxidase levels were similar between the two groups. The independent variables BMI, age, parity, gravidity and gestational week effects were adjusted according to the logistic regression method with groups. Significant differences were observed between the two groups in the levels of CAT (0.001), MPO (0.005) values (Fig. 1–3).

DISCUSSION

In this study, which lasted less than 14 weeks, an imbalance was found between the serum OS markers and the antioxidant defence system markers when 30 pregnant women with HG and 30 normal pregnant women were compared. Low MPO values and statistically significantly higher CAT values were found in the serum samples of the HG patients when compared to the controls.

Previous studies have shown that the risks of preeclampsia, placental anomalies, and intrauterine growth retardation are increased in pregnant women with HG [16], and many events are known to cause OS in these women [6].

Table 1. Comparison between the hyperemesis gravidarum (HG) patients and the healthy controls

Variables	HG group Mean ± SD n = 30	Control group Mean ± SD n = 30	P value	P* value
MPO (U/L)	90.9 ± 56.4	121.5 ± 36.3	0.016	0.005
Ferroxidase (U/L)	517.1 ± 99.1	524.7 ± 126.7	0.796	0.917
Catalase (kU/L)	219.6 ± 111.3	71.5 ± 52.5	< 0.001	0.001
Maternal age (years)	25.0 ± 2.6	25.6 ± 2.9	0.39	
Gestational week	8.52 ± 2.27	7.79 ± 2.02	0.2	
Gravidity	2.29 ± 1.37	2.24 ± 1.27	0.88	
Parity	1.1 ± 1.16	1.14 ± 1.18	0.89	
BMI (kg/m^2)	23.1 ± 1.5	23.6 ± 1.1	0.11	
Fasting glucose (mg/dL)	87.7 ± 17.9	83.9 ± 9.5	0.31	

SD — standard deviation, BMI — body mass index; MPO — myeloperoxidase
*The independent variables BMI, age, parity, gravidity and gestational week effects were adjusted according to the logistic regression method with groups

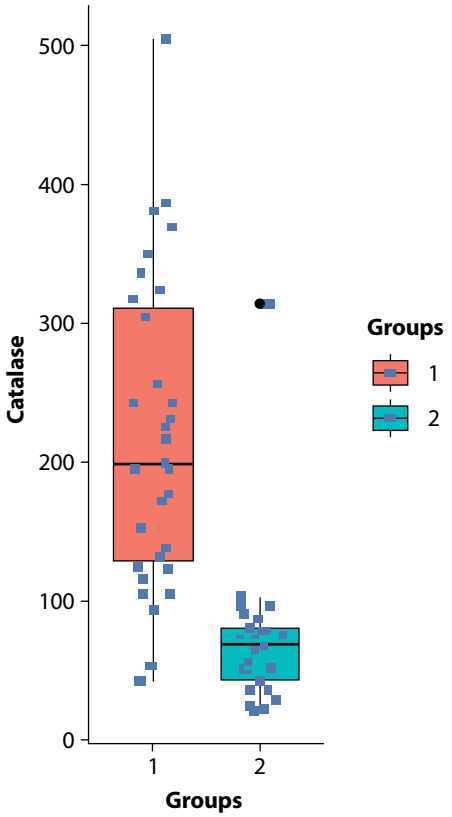

Figure 1. Compared Catalase values for the groups (Boxplot and scatter plot relationship between groups)

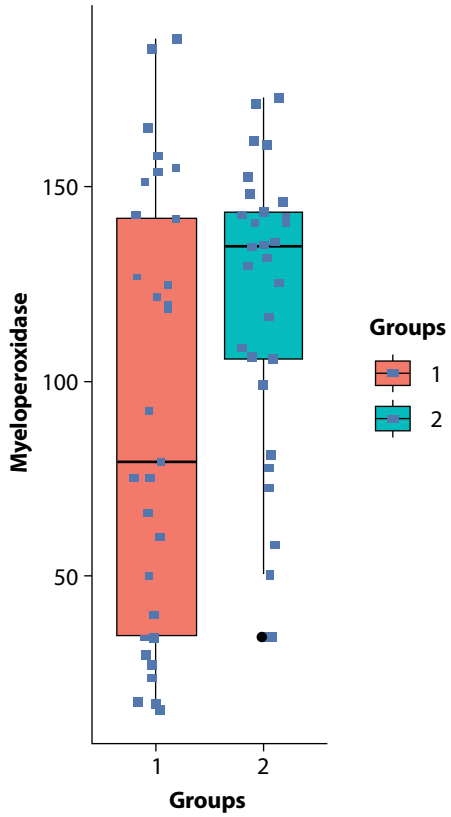

Figure 2. Compared Myeloperoxidase values for the groups (Boxplot and scatter plot relationship between groups)

The construction of reactive oxygen species in the blood is known as a normal process, and both enzymatic and non-enzymatic mechanisms are involved in counteracting the OS caused by increased reactive oxygen species levels. CAT, MPO, ferroxidase are some of the antioxidants that play this critical role [17].

HG is characterized by cell-mediated immunity [18]. MPO, which is a member of the peroxidase superfamily, is found in the azurophilic granules in neutrophils and monocytes. MPO is released by the leukocytes in inflammatory conditions, and it catalyzes the formation of various reactive species, including HOCl; therefore, it plays a role in the body's defence against microorganisms [19, 20]. Another antioxidant that responds in inflammatory conditions is adenosine deaminase (ADA), which is an enzyme necessary for the differentiation of lymphoid cells, and it contributes

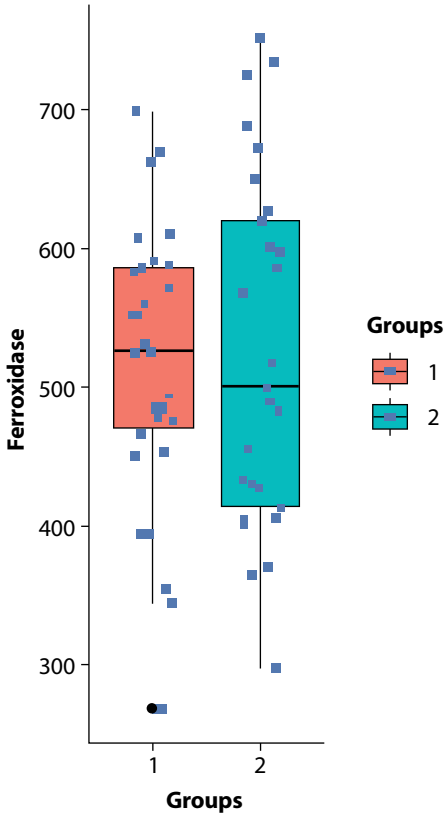

Figure 3. Compared Ferroxidase values for the groups (Boxplot and scatter plot relationship between groups)

to the production of cytokines. Although the exact mechanism is not known, lymphocytes or monocytes are thought to play an essential role in serum ADA activity [21, 22]. In the study by Biberoğlu et al. [23], although the serum ADA and CAT levels were higher in the HG group, the differences were not significant. There is no previous study of MPO in HG patients, this will be the first study. We found that the MPO levels were lower in our HG group than in the control group. We believe that the immune system is suppressed due to malnutrition, which lowers the MPO values. In addition, because HG related fasting normally leads to immune function suppression, it remains controversial whether the immune response is the cause of or a reaction to HG.

The production of CAT, which is an intracellular antioxidant enzyme, increases during OS to balance the redox reactions [10]. Güney et al. [24] found that CAT was significantly lower in HG and attributed to the deficiency of antioxidants taken with nutrients. However, Biberoğlu et al. [23] found that the CAT level was high, although this was not significant. We found that CAT levels were significantly higher in our study. In order to prevent the increase in oxidative radicals during the OS, we believe that the CAT level increases.

The copper metabolism is very complexly linked to iron metabolism. Two copper-containing enzymes ferroxidase I and ferroxidase II have the capability to oxidize ferrous (Fe2+) to ferric (Fe3+) form of iron. Ferric form is used for transport of iron. Ceruloplasmin (having ferroxidase I), is the predominant copper protein in plasma having antioxidant activity. Abnormalities in ceruloplasmin activity produce cellular iron storage that supports ferroxidase activity. The increase in copper in iron-deficient anemic mothers could be an offsetting mechanism to counteract anemia, and this is complemented by a surge in ceruloplasmin synthesis, which is having ferroxidase activity [25, 26]. Onaran et al. [27] found that the ceruloplasmin (ferroxidase 1) levels were similar between the HG group and the control group. Similarly, we did not find a significant difference between the ferroxidase levels between the groups. We believe that the levels of ferroxidase 1 do not change in patients with HG and that the nutrients and iron and copper inadequately keep each other in balance.

This study had some limitations. Initially, in the case of OS, antioxidant levels other than CAT, MPO and ferroxidase should be investigated. Many previous studies of these antioxidants have revealed different results; therefore, more work is needed. In addition, we did not analyze the antioxidant status of the patients before their pregnancies, which may have affected the OS outcome because we could not exclude the possibility of this occurring before the pregnancy. Finally, our sample size was small; therefore, future studies should include larger sample sizes.

CONCLUSIONS

In this study, the different antioxidant levels in the two groups showed that antioxidants, in response to OS, react differently with different mechanisms. Also, we believe that insufficient food intake suppresses the immune system in HG patients, and this plays an important role in the antioxidant levels.

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