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Research Article

The Consequences of Fasting During Pregnancy on the Thiole/ Disulfide Balance: An Observational Study

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Abstract

Aim: The objective of this research was to examine the impact of fasting on thiol-disulfide hemostasis in a population of healthy pregnant women.

Material and Methods: The study, conducted during Ramadan in 2021, included a control group of 53 pregnant women who were fasting and 57 pregnant women with similar demographics who were not fasting. The disulfide, native thiol, and total thiol concentrations in centrifuged blood plasma and venous blood plasma were all looked at. The ratios "disulphide/thiol 100," "disulphide/total thiol 100," and "thiol/total thiol 100" were all calculated.

Results: There were 32 pregnant women in the second trimester and 21 pregnant women in the third trimester among fasting participants, whereas there were 33 pregnant women in the second trimester and 24 pregnant women in the third trimester among non-fasting participants. Disulfide, disulfide/nativethiol*100, disulfide/totalthiol*100, and native/totalthiol*100 ratios were not significantly different between groups. A statistically significant difference was seen between the groups when comparing the mean values of native thiol and total thiol.

Conclusion: Our research is one of the first to examine homeostasis in pregnant women who were fasting, making it a remarkable advance in the field. The study demonstrated a discernible alteration in the thiol-disulfide balance, enhancing the process of oxidation. Fasting during pregnancy is associated with an increase in oxidative stress.

Keywords: Fasting, oxidative stress, pregnant women, ramadan, thiol-disulfide homeostasis

INTRODUCTION

Fasting is when you stop eating solid food, drinking drinks with calories, smoking, and using stimulants like coffee or tea on purpose for a certain amount of time. It typically involves some form of calorie restriction (1). During Ramadan, Muslims do a special kind of fasting, which means they can't eat, drink, smoke, or have sexual relations between dawn and dusk (1). The length of the fasting period is determined by the season in which Ramadan falls, and it lasts from about an hour before sunrise to sundown. For instance, fasting may last longer than 17 hours in several regions of the world during the summer (2,3).

Women are allowed to skip Ramadan fasts if they like, so long as they make up the days they miss after giving birth. However, most women would rather fast with their families throughout the month of Ramadan than make up the days later on their own. Hence, the practice of fasting throughout pregnancy occurs frequently among Muslim women (4,5).

The daytime rituals, lifestyle, and biorhythm of someone who is fasting change significantly throughout the month of Ramadan. The diurnal patterns of hormones, nutrient flow, and energy utilization may experience significant modifications during the period of fasting seen during Ramadan. These abnormalities might potentially lead to oxidative stress. "Oxidative stress" is described as a discrepancy between a cell's ability to produce reactive

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oxygen species (ROS) and its ability to do so while also protecting itself against oxidative damage (6).

There is a widely acknowledged consensus that pregnancy leads to an elevation in oxidative stress, which arises from a conventional systemic inflammatory response and heightened amounts of circulating (ROS). The placenta, which is the primary regulator of pregnancy, serves as the principal generator of ROS during this period (6-8). Accordingly, the elevated oxidative stress observed during pregnancy may result in possible tissue damage (6,8).

Numerous studies have looked at how Ramadan fasting affects inflammatory agents and oxidative damage using a variety of redox markers (9-16).

The cellular enzymatic and nonenzymatic processes that maintain redox equilibrium are essential for development and survival (17). However, conventional methods of measuring oxidative stress present a number of practical challenges. In the year 2014, Erel and Neselioglu introduced an innovative methodology that relies on evaluating the equilibrium between thiol and disulfide compounds in order to tackle this particular issue (18,19). Thiol sulfhydryl groups are essential for maintaining redox homeostasis, and thiols are responsible for the great majority of antioxidant capability (20). The oxidative balance is made up of the continuous and reversible exchange of thiols (the reduced state) and disulfides (the oxidized state). However, until recently, only the thiol side of the equilibrium could be identified, even using elaborate and costly technologies. But Erel and Neselioglu demonstrated that they could evaluate both factors rather well.

Thiol-disulfide hemostasis is not well studied in fasting pregnant women. There is only one study conducted by Ozturk et al., who studied oxidative stress in pregnant women during Ramadan (16). The number of participants in this research was small, and it was restricted to pregnant women in the 2nd trimester. The objective of this research was to investigate oxidative stress in fasting healthy pregnant. We used the serum concentrations of thiol and disulfide homeostasis species in the current investigation.

MATERIAL AND METHOD

This observational research was granted approval by the Trabzon Kanuni Training and Research Hospital's Ethics Committee for Clinical Research. Women who visited the Obstetrics and Gynecology Clinic at Trabzon Kanuni Training and Research Hospital between April 13 and May 12, 2021, were selected as the study's subjects (the month of Ramadan). Only pregnant women expecting a single child who were also fasting throughout Ramadan were considered eligible for this study. All pregnant individuals involved in the research provided informed consent.

The G*Power 3.1.9.4 statistical program's power analysis and the findings of previous studies were used to establish the necessary sample size. The research determined the critical values for Type I error (α) and Type II error (β) to be 0.05 and 0.95, respectively. In order to achieve a statistical power of 95% with a sample size of 96 participants, it was determined that a minimum of 52 patients were required in each group (16). A group of 60 participants was allocated to each experimental group, accounting for an anticipated attrition rate of 10% (Figure 1). Due to the increased oxygen pressure within the feto-maternal unit in the latter stages of the first trimester, this study did not include pregnant women.

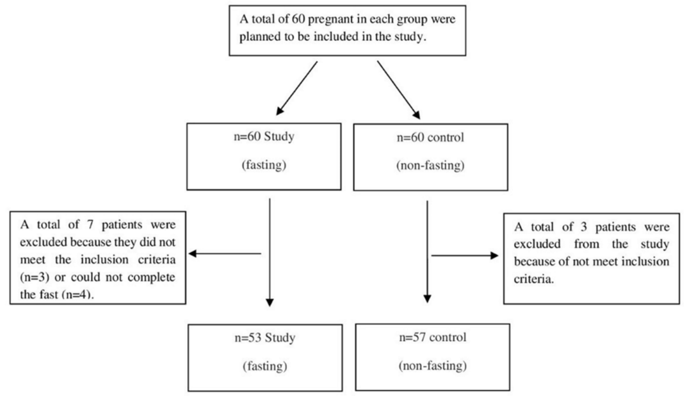

Figure 1. Participant flowchart

We excluded women who were in labor, had ruptured membranes, were carrying multiples, had fetal abnormalities, smoked or drank, or had significant medical disorders, including diabetes or inflammatory illnesses, and pregnant women who were COVID-19 positive during the study. All of the pregnant women in both the fasting and non-fasting groups took their prescribed medicines as usual, without any additional vitamin or mineral supplements. Oxidative stress values were examined in women who fasted for at least 15 days. Before and after at least 15 days of fasting, the native thiol and total thiol concentrations in the study were compared.

In the process, 10 cubic centimeters of blood from a vein are taken and put into tubes that have already been filled with ethylenediaminetetraacetic acid (EDTA). The cellular and plasma components were separated using centrifugation at a force of 1500 times the acceleration due to gravity for a duration of 10 minutes. The specimens were stored in a freezer maintained at a temperature of -80 degrees Celsius. Erel and Neselioglu used sodium borohydride (NaBH4) as a means to assess the levels of native thiol, total thiol, and disulphide in plasma (5). This was accomplished by converting the plasma's dynamic disulphide bonds into inert thiol groups. To inhibit further reduction of 5,50-dithiobis-2-nitrobenzoic acid (DTNB) and the newly formed disulfide bond resulting from the DTNB reaction, formaldehyde was used to eliminate the surplus NaBH4. Total thiol concentration was determined using a specialized Ellman's reagent. It was decided that mercaptoethanol solutions would be used for the calibrations. The concentration of disulfide was calculated as half of the difference between the total and the native thiol amount. Lastly, we determined the "disulfide/thiol 100," "disulfide/total thiol 100," and "thiol/total thiol 100" ratios.

Statistical analysis was conducted using SPSS version 23.0 (SPSS, Chicago, IL, USA). The data were provided in the form of the mean plus the standard deviation (SD). Kolmogorov-Smirnov testing showed that the dataset was normally distributed. The difference between the study and control groups was determined using an independent t-test. Within-group comparisons were made using a student t test. The cutoff for significance was set at a p value of 0.05.

RESULTS

Of the 60 patients included in the study group, 2 were excluded from the study due to COVID-19, 4 due to inability to complete fasting, and 1 due to premature birth. Of the 60 patients included in the control group, 2 were excluded because of COVID-19 and 1 due to gestational diabetes. Table 1 provides an overview of the demographic characteristics of each subgroup. Table 2 presents the mean values for native thiol, total thiol, disulfide, and the ratios disulfide/native thiol*100, disulfide/total thiol*100, and native/total thiol*100 for pregnant women who fasted and those who did not. In the fasting group, there were 32 second trimester and 21 third trimester pregnancies. In the non-fasting group, there were 33 second- and 24 third-trimester pregnant women. Disulfide and disulfide/nativethiol*100, disulfide/totalthiol*100, and native/totalthiol*100 ratios did not differ significantly across groups.

Table 1. The table shows the demographic data of the groups

	Control (n=57)	Study (n=53)	p
Age (year)	28.40±8.81	27.8±6.3	0.258
BMI (kg/m2)	24.80±3.12	24.3±4.10	0.213
Parity	2.68±1.61	2.51±1.75	0.196
Gestational age (week)	26.34±8.50	27.26±9.60	0.120
Oral intake (hour)	6.42±1.21	6.28±1.27	0.089
Fasting time (hour)	16.14±1.18	16.25±1.23	0.143

Data is presented mean±std

Native thiol and total thiol levels were much lower in the study group than in the control group, but there were no major changes in the pre- and post-oxidative stress indicators in the control group (Table 2). A statistically significant difference was seen between the groups upon analyzing the mean values of native thiol (Figure 2) and total thiol (Figure 3).

Table 2. The levels of oxidative stress indicators in the study and the control groups

		Study (n=53)	Control (n=57)	p*
Native thiol	**Before**	430.15±56.3	431.23±64.4	0.246
	After	396.20±66.3	429.40±69.8	**0.012**
	p**	**0.022**	0.311	
Total thiol	**Before**	473.30±69.20	475.65±69.2	0.255
	After	433.40±72.10	471.80±73.1	**0.007**
	p**	**0.006**	0.295	
Disulfide	**Before**	19.80±8.77	20.22±8.87	0.121
	After	18.60±9.75	21.18±9.61	0.166
	p**	0.280	0.219	
Disulfide/Native thiol*100	**Before**	4.60±1.12	4.68±1.21	0.433
	After	4.75±2.53	5.04±2.38	0.527
	p**	0.512	0.499	
Disulfide/Total thiol*100	**Before**	4.18±1.67	4.25±1.83	0.457
	After	4.24±2.07	4.50±1.92	0.499
	p**	0.501	0.518	
Native thiol/Total thiol*100	**Before**	90.90±5.71	90.66±4.13	0.411
	After	91.52±4.15	91.01±3.83	0.496
	p**	0.388	0.405	

Data is presented mean±std. *Independent t test. **Paired t test

Figure 2. The mean values of native thiol in the study and control groups are shown in the box plot graph

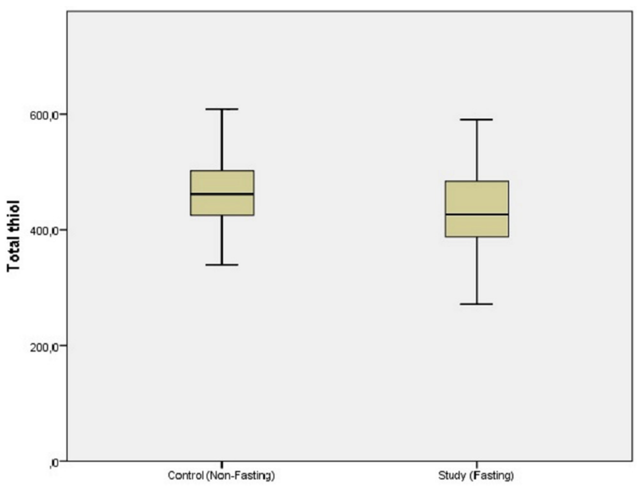

Figure 3. The mean values of total thiol in the study and control groups are shown in the box plot graph

DISCUSSION

The current investigation included an assessment of thiol disulfide hemostasis and, as a result, oxidative stress levels in pregnant women who fasted throughout the month of Ramadan. Based on the results obtained from this investigation, it was observed that the average concentrations of native thiol and total thiol in individuals who underwent fasting exhibited a statistically significant decrease compared to those who did not undergo fasting.

The prevailing consensus acknowledges that pregnancy is associated with an elevation in oxidative stress, which arises from a systemic inflammatory response and heightened levels of circulating (ROS). The placenta, which serves as the central regulator of pregnancy, is primarily responsible for generating (ROS) during this period (7). Accordingly, the elevated oxidative stress observed during pregnancy may result in possible tissue damage (8,21).

The biological significance of thiols and disulfides includes the static stability of the structures of proteins, the control of the activities of proteins and enzymes, receptors, transporters, Na-K channels, and transcription (19). The dynamic equilibrium state of thiol/disulfide plays crucial roles in several biological processes, including antioxidant defense, detoxification, apoptosis, control of enzyme activity, transcription, and cellular signal transmission systems (22). The plasma thiol pool is mostly composed of albumin (23).

To make disulfide bond structures that can be broken and put back together again, the thiol groups in cysteine residues, low molecular weight compounds, proteins, and other thiol groups are oxidized by interacting with oxidant molecules in the environment. The ability of disulfide bond structures to be broken down into thiol groups, which helps keep the thiol disulfide equilibrium, is important (24).

Since 1979, the measurement of just one aspect of this bilateral balance has been conducted. However, thanks to the invention of the novel method by Erel and Neselioglu, it is now possible to measure and evaluate both variable levels separately and collectively, allowing for a thorough evaluation (19). Thiol-Disulfide Homeostasis in fasting pregnant women has never been investigated until now, and our study is the first report in this area. In this study, we used an innovative methodology that allows the accurate quantification of cumulative thiol and disulfide levels. Additionally, we were able to quantify the specific amounts of native-total thiol (representing the reductive state) and disulfide (indicating the oxidative condition). The study revealed that the balance between thiol and disulfide was modified in response to variations in thiol levels among pregnant women who were fasting.

The research done by Ozturk et al. aimed to examine the impact of fasting during Ramadan on oxidative stress levels in pregnant women (16). The research revealed that there was no statistically significant disparity between the groups assigned to the study and the control conditions. Throughout their investigation, the researchers used total antioxidant status, total oxidant status, and the oxidative stress index as indicators. In the present investigation, a distinction was made from the previous research by including pregnancies in the third trimester. In the present work, an examination was conducted on the equilibrium of thiol-disulfide, a novel marker for oxidative stress. It was observed that there were substantial differences in the mean values of native and total thiols between the fasting and non-fasting groups. Furthermore, the individuals included in our research were subjected to a comparison both before and after a period of fasting. The study group exhibited a notable reduction in levels of native thiol and total thiol, while no discernible disparity was noted in the control group.

In the study conducted by Ibrahim et al., the impact of fasting during Ramadan on oxidative stress parameters and biochemical markers of cellular damage in healthy individuals was investigated. The findings revealed that, apart from a minor reduction in lipid peroxidative damage in erythrocytes, fasting during Ramadan did not result in any significant alterations in oxidative stress parameters or biochemical markers of cellular damage in the healthy subjects (14). In their study, Ibrahim et al. conducted an analysis of many biomarkers, including malondialdehyde

(MDA), glutathione, glutathione peroxidase, and catalase, in order to assess their potential as indicators of oxidative stress. In the present study, we conducted an assessment of thiol-disulfide levels, which diverged from the methodologies used in previous investigations. The cohort under scrutiny consisted only of pregnant females.

Numerous studies in the literature have documented a shift towards an oxidant direction in thiol-disulfide hemostasis in various obstetric complications, including preeclampsia, fetal distress, isolated oligohydramnios, gestational diabetes mellitus, and fetal growth restriction. These investigations specifically focused on examining thiol-disulfide dynamics in pregnant women. Based on the findings of previous research and the outcomes of our present investigation, it can be stated that the practice of fasting among pregnant women leads to a change in thiol-disulfide hemostasis towards an oxidant state (25-32). It can be said that pregnant women should not fast in order not to be exposed to oxidative stress during Ramadan and postpone it until after birth and even to the end of the breastfeeding period.

There is evidence indicating that, in pregnancies affected by COVID-19, there is a change in thiol-disulfide homeostasis towards an oxidant state. This observation suggests a potential involvement of ischemic processes in the etiopathogenesis of this emerging disease (33). It can be said that the COVID-19 pandemic period will also contribute to the oxidative stress caused by fasting.

The prospective design of this study was one of its strongest aspects. One of the limitations of our research is that we did not assess the incidence of obstetric problems such as preeclampsia, fetal distress, isolated oligohydramnios, prenatal diabetes mellitus, and fetal growth restriction. However, it was assumed that they would not provide statistically significant findings because of their low prevalence in otherwise healthy pregnancies. Obstetric complications can be evaluated in large scale studies. During the period of investigation, the dropout rate observed in the research exhibited fluctuations as a consequence of the global influence exerted by the COVID-19 pandemic.

CONCLUSION

In conclusion, this study is the first one in the scientific literature to look at the oxidative status of pregnant people who fasted during Ramadan, as well as the thiol-disulfide equilibrium of the mother's blood. The findings of the investigation indicated that there had been a change toward an oxidant direction in the thiol-disulfide balance. Antioxidant use may be recommended for fasting pregnant women.

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Conflict of Interest: *The authors declare that they have no competing interest.*

Ethical approval: *Ethics committee approval was received for this study from the ethics committee of Trabzon Kanuni Training and Research Hospital. Decision No: 2021/52 Date: 04.04.2021.*

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