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Ischemia Modified Albumin as a New Marker for Diagnosis of Early Pregnancy Losses

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Abstract

Research Article

The cause of a significant portion of pregnancy losses cannot be explained. Previous studies have suggested that trophoblast invasion and placental development occur in a relatively hypoxic environment during early pregnancy. In this study, we aimed to evaluate ischemic modified albumin (IMA) and thiol/disulfide ratios as an indicator of oxidative stress in early pregnancy losses. In this observational and prospective study, 30 healthy pregnant women, 30 pregnant women with early pregnancy loss in 6-14 weeks of pregnancy, and 30 healthy women with random sampling in reproductive age who applied to Alanya Alaaddin Keykubat University Education and Research Hospital Gynecology and Obstetrics Department were included. The patients' total disulphide parameters (Native Thiol [NT], Disulfide [D], and Total Thiol [TT]), disulfide/native thiol (index 1), disulfide/ total thiol (index 2), and native thiol/ total thiol (index 3) were studied. In statistical analyses, the level of disulfide was significantly lower in the early pregnancy losses (EPL) group compared to the control group. In the post-hoc analysis, the IMA level was significantly higher in the EPL and healthy pregnant group compared to the control group. In conclusion, the difference in IMA levels in our study and its compatibility with the literature suggests that this marker can be used in early pregnancy loss, however, in terms of thiol balance, there was no significant difference in native thiol and thiol in contrast to the literature, and in terms of disulfide, contradictory results are found. Thus, studies with larger populations are needed about the use of thiol and IMA in early pregnancy loss.

INTRODUCTION

Among the females at reproductive age, the incidence of first-trimester pregnancy loss is reported as 15-20% [1]. Early pregnancy losses are caused by factors based on genetics like in molar pregnancies, infection, immunology, and abnormal implantation, anatomy, and endocrinology. However, the group of inexplicable reasons is non-negligible. We may recognize whether trophoblasts abnormally multiply as early pregnancy losses are investigated, and oxidative stress, inflammation, and infection, vascular and chromosomal abnormalities might as well be detected [2]. In literature, there are several pieces of evidence that trophoblasts may invade with placental enlargement when the environment relatively exhibits hypoxic behavior in the term of early pregnancy [3,4]. Preeclampsia complication in pregnancies is observed at a higher level according to maternal IMA records, just as for diabetes mellitus and IUGR.[5] We have a lack of sufficient evidence for the practicality of IMA, and however, researchers need to further our topic in this study.

The detoxifying antioxidants for the radicals of hydroxyl, superoxide and hydrogen peroxide, and other reactive oxygen species (ROS) remain limited, and hence the oxidative balance is deteriorated, *called oxidative stress.* Mercaptain or thiols are classified as organic compounds containing hydrogen and sulfur atomic compounds bound to a carbon atom [5]. With others, albumin, protein, and low molecular weight thiols are included in the plasma thiol pool. The formation of disulfide or SS bonds results from the oxidation of thiols because of oxidants [6]. Caused by Cys residues from oxidative stress, these covalent bonds are defined as the disulfide bridge, which connects two groups of low molecular weight and protein thiols. The retro-reduction of a disulfide bond to the thiol groups can occur, leading to the dynamic balance of thiol-disulfide [7].

The structure of the albumin in plasma changes gradually with ischemia. When oxygen radicals are formed, the N-terminal end of the albumin is damaged, and this newly formed molecule loses its ability to bind to the metals and is

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called ischemia-modified albumin [8].

In this study, we aimed to evaluate IMA and thiol/ disulfide ratios as an indicator of oxidative stress in early pregnancy losses.

MATERIAL and METHODS

Ethical approval

Ethics approval was obtained on 04 March 2019 with number 4-4. All reported research involving "Human beings" conducted in accordance with the principles set forth in the Helsinki Declaration 2008.

Study design

In this observational and prospective study, 30 healthy pregnant women, 30 pregnant women with early pregnancy loss in 6-14 gestational weeks, and 30 non-pregnant healthy women in reproductive age were selected through a random sampling method. Alanya Alaaddin Keykubat University Education and Research Hospital Gynecology and Obstetrics Department joined the study following their signature of consent form during the period between March and June 2019. Patients with a history of two or more unexplained first-trimester miscarriages, a history of ischemic diseases, diabetes mellitus, heart disease, hypertension or any other known medical condition; multiple pregnancies, any major or minor fetal anomaly, absence of fetal pole on ultrasonographic examination or current smokers were excluded from the study. The patient information was retained, such as their demographic characteristics, BMI, age, gestational week, number of pregnancies, height, and weight obtained when they presented. These were physically examined, performing a complete blood count, and studying biochemical parameters, as documented.

Serum IMA level measurement

The serum IMA level was measured based on the albumin cobalt binding test principle. Measurements were made in serum. After all the samples were collected in the cold chain, they were thawed and studied. For serum IMA measurement, 95 µl of patient serum was mixed with 5 µl of cobalt chloride and incubated for 5 minutes. The cobalt chloride concentration during incubation was 0.58 mmol/L. As a result of ischemia, very little of the cobalt is known to bind to albumin. After incubation, 25 µl of dithiothreitol (final concentration of 1.67 mmol/L) was added to the measuring cuvette to determine the cobalt that did not bind, therefore that the dithiothreitol that was not bound to the albumin would form a colored complex with cobalt. The resulting color complex was measured spectrophotometrically at a wavelength of 500 nm. After drawing a 5-point calibration curve in the range of 5-180 U/ mL, the absorbance values were evaluated in this calibration curve. Thus, IMA levels were calculated based on the calibration curve [9, 10].

Thiol balance measurement

Serum thiol-disulfide balance was studied spectrophotometrically with newly developed methods. The measurement method is a standard colorimetric method that has been previously described and has been applied many times in the literature. Briefly, reducible disulfide bonds were first reduced to form free functional thiol groups. The unused reducing sodium borohydride was consumed and removed with formaldehyde, and all thiol groups containing natural thiol groups were determined after reaction with DTNB (5, 5-dithiobis-2-nitrobenzoic acid). Half of the difference between total thiol and native thiol gives the amount of dynamic disulfide. After determining the native thiol and disulfide amounts, total thiol amount, native thiol/total thiol ratio, disulfide/total thiol ratio, and disulfide/native thiol ratio were calculated [11, 12].

Statistical analyses

The results were statistically analyzed using the Statistical Package for Social Sciences (SPSS) for Windows 20 (IBM SPSS Inc., Chicago, USA). The data distribution was tested using Kolmogorov- Smirnov. In a normal distribution, continuous variables were expressed as mean ± standard deviation. ANOVA was used to compare continuous variables. Tukey HSD and Bonferroni were used as post-hoc tests. The significance level was $p< 0.05$.

RESULTS

A total of 90 subjects (30 healthy pregnant women and 30 women with early pregnancy loss and 30 non-pregnant healthy women at the age of fertility) were included in the study. The groups of early pregnancy loss (EPL), healthy pregnant women (HPW) and non-pregnant women (NPW) are presented with their characteristics in Table 1.

Table 1: The characteristics of the patient and control groups

	Group 1	Group 2	Group 3
Age (years) (mean)	32,8214	28,2069	33,9615
BMI (kg/m^2) (mean)	24,3450	23,5765	24,9389
Smoking (years)	%6,7 (n=2)	%20 (n=6)	%13,3 (n=4)

In EPL, the mean native thiol value was 302,3400 ± 46,5206, while it was 330,0900 ± 35,9798 in HPW and 314,2733 ± 54,9279 in NPW. The mean total thiol value was 397,2700 ± 47,6392 in EPL, 426,9567 ± 40,2444 in HPW, and 421,6700 ± 57,9389 in NPW. The mean total thiol value was 47,4667 ± 7,85929 in EPL, 48,4367±7,70157 in HPW, and 53,7000±12,97 in NPW. In the intra-group comparison of the thiol levels, there was found a significant difference in terms of the total amount of thiol (p=0.049) and disulfide (p=0.035). Native thiol, total thiol, and disulfide levels of the study and control groups are given in Table 2.

The mean IMA was 0.7680 ± 0.09208 in EPL and 0.7690 ± 0.11031 in HPW and 0.6813 ± 0.17777 in NPW. There was found a significant difference in the intra-group comparison of the IMA levels (P = 0.009). The IMA levels of the study and control groups are also given in Table 2.

In post-hoc analyses, EPL subjects had significantly lower disulfide, and higher IMA levels and HPW subjects also had significantly higher IMA levels compared to NPW, the control group.

Table 2: IMA levels of early pregnancy loss, healthy pregnant and control group

		N	Mean	SD
IMA (g/L)	EPL	30	,7680	,09208
	HP	30	,7690	,11031
	Control	30	,6813	,15778
	Total	90	,7394	,12863
	Between Groups (p)			,009
Native Thiol (µmol/L)	EPL	30	302,3400	46,5206
	HP	30	330,0900	35,9798
	Control	30	314,2733	54,9279
	Total	90	315,5678	47,3363
	Between Groups (p)			,073
Total Thiol (µmol/L)	EPL	30	397,2700	47,6392
	HP	30	426,9567	40,2444
	Control	30	421,6700	57,9389
	Total	90	415,2989	50,3003
	Between Groups (p)			,049
Disulfide (µmol/L)	EPL	30	47,4667	7,85929
	HP	30	48,4367	7,70157
	Control	30	53,7000	12,978
	Total	90	49,8678	10,09579
	Between Groups (p)			,035
Disulfide/Native Thiol	EPL	30	16,1100	3,77079
	HP	30	14,8167	2,82868
	Control	30	17,7633	6,43495
	Total	90	16,2300	4,71208
	Between Groups (p)			,051
Disulfide/Total Thiol	EPL	30	12,0633	2,13000
	HP	30	11,3700	1,62187
	Control	30	12,8233	3,11345
	Total	90	12,0856	2,41873
	Between Groups (p)			,065
Native/Total Thiol	EPL	30	75,8800	4,28223
	HP	30	77,2700	3,24570
	Control	30	74,3500	6,19637
	Total	90	75,8333	4,83287
	Between Groups (p)			,063

Explanation: IMA: Ischemic modified albumin, HP: Healthy Pregnant, EPL: Early Pregnancy Loss

DISCUSSION

The placenta enlargement during pregnancy is associated with oxygen concentration. In a low-oxygen environment of the first trimester, the embryo grows and continues to grow in the second trimester with high oxygen capacity required. The growing fetus is in a protective environment from the harmful and teratogenic effects of free oxygen radicals, thanks to this physiological hypoxia of the early gestational sac[13]. Pathological oxidative stress may potentially lead to the loss of function and cell death, as reported that it has a part in early pregnancy loss, eclampsia, and fetal growth retardation[14, 15].

In a study of IMA and placenta percreata patients, IMA levels were found high in the Percreata group, and the increased oxidative stress was assessed as a contribution to the pathogenesis[16]. Previously, Özdemir et al.[17] and Cengiz et al.[18] found that IMA levels were higher in the groups of pregnancy loss. In the present study, we observed a significant difference between the IMA levels of EPL, HPW, and NPW, and in a post -hoc analysis, this difference was found to be significantly higher for the EPL subjects compared to the NPW. ($P = 0.009$). This study is the only one in the literature with a comparative analysis of pregnant and non-pregnant healthy women and patients with early pregnancy loss.

There are studies in the literature about thiol, another indicator of oxidative stress. For antioxidants, the contribution of thiols can be listed as defending against reactive oxygen species, regulating the programmed cell death, detoxifying, protecting from antioxidants, and realizing the cellular enzymatic activity. Çetin et al. made a comparison of the maternal serum thiol/disulfide ratios for healthy pregnancies and others with idiopathic intrauterine growth retardation and concluded with an impaired rate in pregnancies with IUGR[19].

Similarly, in the studies conducted on recurrent early pregnancy loss in the literature, the relationship between idiopathic recurrent pregnancy losses and thiol balance has been reported that native thiol decreased as disulfide was increased[20]. However, no significant difference has been observed in the thiol ratio in the studies regarding preterm membrane rupture[19]. In contrast to the literature, there was no change in native thiol, and disulfide increased in our study.

The significant decrease in total thiol levels in early pregnancy loss compared to the control group indicates a significant decrease in antioxidant capacity and deterioration of the balance in the direction of oxidative stress. In living organisms that breathe with oxygen, oxygen taken from outside turns into water as a result of many metabolic processes and reactions. During this transformation, the energy required for the organism is synthesized at the same time. An amount between 2 and 3% of the oxygen used in respiration does not turn into water and creates oxygen-borne radicals. As a result of the reduction of oxygen by taking an electron, the superoxide radical is reduced by taking two electrons, resulting in hydrogen peroxide. Adding the third electron creates the hydroxyl radical, and with the addition of the fourth electron, water is formed. Although superoxide and hydrogen peroxide radicals are not very toxic, they can easily turn into an extremely reactive hydroxyl radical with the catalytic effect of iron. Therefore, superoxide and hydrogen peroxide must be metabolized immediately by enzymes before they become more harmful. Due to their reactive nature, free radicals damage the lipid, protein and nucleic acids and disrupt the cell structure. It is vital that all oxidative damage is balanced by the antioxidant system. Acting as an antioxidant system component in the organism; There are also enzymes such as Superoxide dismutase (SOD), Glutathione Peroxidase (GSH-Px), Glutathione Transferase (GST), Catalase (CAT), Glutathione Reductase, vitamins and many proteins that bind metal ions. For these reasons, it is very important to have enough antioxidant capacity during pregnancy. Healthy nutrition and avoiding all factors that cause oxidative stress will provide a more positive pregnancy process[21-32].

In our study, we analyzed the data and examination results of the randomly sampled subjects based on the literature review that we conducted in order to observe the difference in IMA levels. As a result, we concluded that this marker is useful for the detection of early pregnancy loss, and in terms of thiol balance, there was, however, no significant difference in thiols and native thiols in contrast to the previous studies, furthermore contradictory results are found for disulfide. Further studies in a larger population or sample should be attempted to investigate the use of thiol and IMA for the issue of early pregnancy loss.

CONCLUSION

The reducible disulfide bonds are first reduced to form free functional thiol groups. The unused reducing sodium borohydride will be consumed and removed with formaldehyde

and after reaction with DTNB (5, 5-dithiobis-2-nitrobenzoic acid) all thiol groups containing reduced and natural thiol groups are determined. Significant decrease in total thiol levels in early pregnancy losses means relative increase in oxidative stress and decrease in antioxidant capacity. For this reason, a suitable and healthy lifestyle should be adopted to keep the antioxidant capacity high during pregnancy. In conclusion, if our research is supported by new studies, thiol balance may be an important laboratory marker in determining early pregnancy losses.

Conflict of interest

The authors declare that they have no conflict of interest.

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