Original article

Evaluation of Oxidative Stress in Ectopic Pregnancies

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Abstract. *Purpose:* The aim of the study is to show the relationship between oxidative stress and ectopic pregnancy. *Materials and Methods:* A total of 62 patients, 31 in the ectopic pregnancy group (study group) and 31 in the first-trimester pregnancy (control group) were included in the study. Patients between 18-45 years of age who had tubal ectopic pregnancy diagnosed by transvaginal ultrasonography and serum βHCG values were included in the study group. Serum thiol- disulfide hemostasis were measured from venous blood. *Results:* Between the control group and the ectopic pregnant group; there was no statistically significant difference in terms of age, total thiol, albumin, disulfide, index 1 (disulfide / total thiol), index 2 (disulfide / native thiol), and index 3 levels (p> 0.05). The area under the ROC curve for native thiol measurements was statistically significant in distinguishing the control group and the ectopic pregnant group [AUC = 0.657, 95% CI: 0.521-0.793, p = 0.034]. *Conclusion*: This study shows that ectopic pregnancies may be associated with the presence of high oxidative stress. Especially in early stage suspected patients, demonstrating the presence of oxidative stress together with serial β-HCG follow-up may be helpful in diagnosis. (www.actabiomedica.it)

Keywords: disulfide, ectopic pregnancy, oxidative stress, thiol

Introduction

Ectopic pregnancy is a complicated condition characterized by the embryo being located outside of the uterine cavity, which can lead to maternal death due to haemorrhage, especially with late diagnosis (1). The frequency of ectopic pregnancy among all pregnancies is around 2% and 98% of them are located at the tubes. Less frequently an ectopic pregnancy can be located at the ovaries, cervix, or it can be intraligamentary, cornual or abdominal (2-4). The diagnosis is usually made by a series of transvaginal ultrasonographic (TVUS) examinations and determinations of serum β-HCG levels. In the diagnosis of ectopic pregnancy, the sensitivity of transvaginal ultrasonography is 87-99% and its specificity is 94-99.9% (5-7).

Early diagnosis of an ectopic pregnancy enables the use of fertility preserving treatment modalities; which are expectant management or medical treatment. Especially in unruptured ectopic pregnancies treatment with methotrexate, feticide with intrathoracic potassium chloride injection and/or uterine artery embolization can be applied (3, 8). In the cases of late diagnosis with ruptured ectopic pregnancies and hemodynamic instability surgery might be the only choice of treatment (9).

In a healthy pregnancy fertilization and early embryonic development starts in the tubes. Ciliary move-

ment in the fallopian tubes transport the embryo to the uterine cavity where the implantation occurs. Tubal epithelial cells excrete many factors for the development of the embryo, such as growth factor, cytokines, embryotropic factors. Although the pathophysiology of tubal ectopic pregnancy is not fully understood, it is assumed that impaired transport of the embryo and/or environmental changes in the fallopian tubes lead to early implantation (10). The balance of oxidant-antioxidant status is important for the optimum physiological condition of an organism (11). With the effect of increased oxidative stress, the tubal environment may change and tubal epithelial cells can be replaced by collagen fibers adversely affecting the embryo transportation (12). Furthermore, it is believed that through an imbalance in the oxidant-antioxidant status leading to an accumulation of reactive oxygen species (ROS) embryonic development can be impaired before implantation (11). In addition, it has been suggested that the pathological production of nitric oxide synthase isoforms may reduce tubular ciliary activity and smooth muscle contractions, and thus embryo transfer may be affected, resulting in a tubal ectopic pregnancy (13).

Compounds containing thiol groups are organic substances that play an important role in defense against oxidative stress with their reducing characteristics. While oxidative products such as ROS formed in the organism are reduced by transferring their excess electrons to thiol-containing compounds thiol groups are oxidized (14). Oxidation of thiol groups causes the formation of disulfide bonds. This is a reversible reaction, and the disulfide bonds formed can be reduced back to thiol groups. Thus, dynamic thiol-disulfide homeostasis (TDH) is achieved. Dynamic thiol-disulfide homeostasis plays a critical role in antioxidant defense, detoxification, apoptosis, regulation of enzymatic activity, and cellular signal transduction (15, 16)

Ischemia-modified albumin (IMA) is a protein whose levels increase in plasma as a result of oxidative stress and can therefore be used as a marker of oxidative stress (17). In a previous study, increased IMA levels were observed in an ectopic pregnancy (18).

We based this study on previous data and the assumption that increased oxidative stress causes impaired tubal motility leading to early implantation, which is therefore associated with ectopic pregnancy. We aimed to evaluate whether dynamic TDH can be used as an oxidative stress marker in ectopic pregnancies by determining the serum native thiol, total thiol, and disulfide levels.

Materials and Methods

This prospective study was conducted at Istanbul Kanuni Sultan Suleyman Training and Research Hospital Department of Obstetrics and Gynecology between March 2020 and September 2020. The study protocol was approved by the local Ethics Committee (2019/491). Written informed consent was obtained from all participants before their enrolment in the study.

31 patients between 18-45 years of age who were diagnosed with tubal ectopic pregnancy by TVUS and determination of serum β-HCG levels and who were at 5-8 weeks' gestation at the time of diagnosis were included in the study group. Patients with elevated β-HCG levels, which displayed an abnormal increase or stayed at the same level at 48 hours of two consecutive follow-ups, without any visible gestational sacs under TVUS, with a TVUS image of a possible gestational sac located at the fallopian tubes were included in the study. Patients with ectopic pregnancies located outside the fallopian tubes (cervical, ovarian, cornual, intraligamentary or abdominal), with hemodynamic instability at admission, patients with chronic diseases such as hypertension, diabetes mellitus, hypo-hyperthyroidism, renal or liver failure, patients who were smokers and/or alcohol abusers, patients using progesterone, and antioxidant drugs and with a history of endometriosis were excluded from the study.

31 patients with healthy singleton pregnancies in the first trimester (5-8 weeks of gestation) and with no additional chronic disease were included in the control group. The participants who met the inclusion criteria were enrolled in our study consecutively, as each tubal ectopic case was age-matched by a control case. All blood samples were obtained at admission before treatment begin 22 patients in the study group received methotrexate treatment and 9 received laparoscopic salpingectomy. In none of the patients' pathology reports, who received a dilatation and curettage, chorionic villus and trophoblasts were observed.

Biochemical assays

The blood samples were centrifuged at 2300×g for 10 min and stored at –80 °C until analysis. Serum TDH tests were measured by a recently described method using an automated clinical chemistry analyzer (Roche, Cobas 501, Mannheim, Germany) (15). Disulfide bonds were reduced to form free functional thiol groups with sodium borohydride. Unused reductant sodium borohydride was consumed and removed with formaldehyde to prevent the reduction of 5,5'-dithiobis-(2-nitrobenzoic) acid, and all of the thiol groups including reduced and native thiol groups were identified after the reaction with 5,5'-dithiobis-(2-nitrobenzoic) acid. Half of the difference between the total thiols and native thiols provides the dynamic disulfide levels. Index 1, 2 and 3 were calculated as follows; index 1= (disulfide/native thiol) x 100, index 2 = (disulfide/total thiol) x 100, index 3 = (native thiol/total thiol) x 100.

Statistical Analysis

Data analysis was performed by using IBM SPSS Statistics version 17.0 software (IBM Corporation, Armonk, NY, US). The distribution of continuous variables whether normal or not was determined by the Kolmogorov-Smirnov test. The assumption of homogeneity of variances was examined by Levene's test. Descriptive statistics for continuous variables were expressed as mean ± SD or median (25th – 75th) percentiles, where appropriate. The mean differences between cases and controls were compared by Student's t-test. Mann Whitney U test was applied for the continuous variables where the parametrical test assumptions were not met. Whether the laboratory measurements were statistically significant predictors on diagnosis or not was evaluated by receiver operating curve (ROC) analyses. Youden's index was applied for determining the optimal cut-off points for biochemical measurements in order to distinguish ectopic pregnancies from the control group. Sensitivity, specificity, positive and, negative predicted values, and diagnostic accuracy levels for native thiol and IMA in order to discriminate the patients with ectopic pregnancy from controls were also calculated. A p-value of less than 0.05 was considered statistically significant.

Results

Table 1 shows the comparison of age and laboratory parameters between the control and the study groups. No significant differences in terms of age, total thiol, albumin, disulfide, index 1, index 2, and index 3 levels were observed (p >0.05). The native thiol level in the study group was statistically lower (p = 0.033) and the IMA level was statistically higher than the control group (p = 0.043).

Table 2 shows ROC analysis results related to laboratory measurements in distinguishing the control and study groups. As a result of ROC analysis albumin, disulfide, index 1, index 2 and index 3 measure-

Table 1. Comparison of demographic and laboratory measurements between the control and the study group.

	Control group (n=31)	Study group (n=31)	p-value
Age (years)*	29.4±6.5	28.5±4.7	0.507†
Native thiol (μmol/L) *	427.4±81.9	379.4±90.8	**0.033**†
Total thiol (μmol/L) *	467.4±81.2	422.5±98.6	0.055†
IMA (ABSU) **	0.93 (0.84-0.97)	0.95 (0.89-1.08)	**0.043**‡
Albumin (g/dl) **	3.6 (2.8-3.9)	3.4 (3.0-3.7)	0.464‡
Disulfide (μmol/L) **	19.9 (15.2-24.1)	20.6 (14.7-23.9)	0.657‡
Index 1 *	4.9±2.0	5.8±2.5	0.107†
Index 2 *	4.4±1.6	5.1±1.8	0.100†
Index 3 *	91.2±3.2	89.7±3.7	0.100†

* Descriptive statistics were shown as mean ± SD, ** Data were expressed as median (25^{th} – 75^{th}) percentiles, † Student's t test, ‡ Mann Whitney U test.

Table 2. The results of ROC analyses

	AUC	95% CI	p-value
Native thiol	0.657	0.521-0.793	**0.034**
Total thiol	0.634	0.496-0.773	0.069
IMA	0.649	0.511-0.788	**0.043**
Albumin	0.554	0.404-0.704	0.464
Disulfide	0.533	0.386-0.679	0.657
Index 1	0.626	0.484-0.768	0.088
Index 2	0.626	0.484-0.768	0.087
Index 3	0.626	0.484-0.769	0.087

AUC: Area Under the Curve, CI: Confidence interval.

ments were not significant determinants (p >0.05), which can be used in distinguishing the study group from the control healthy subjects.

The area under the ROC curve for native thiol measurements was statistically significant in distinguishing the control group and the study group [AUC = 0.657, 95% CI: 0.521-0.793, p = 0.034] (Figure 1). Similarly, the area under the ROC curve for IMA measurements was also statistically significant in distinguishing the control group and the study group [AUC = 0.649, 95% CI: 0.511-0.788, p = 0.043] (Figure 2).

In Table 3, the best break-points and diagnostic performance indicators for native thiol and IMA measurements in determination of ectopic pregnancy can be seen. The best cut-off point for native thiol measurements in distinguishing ectopic pregnancy was 433.9. The sensitivity at this value was 74.2%, the specificity was 58.1%, the positive and negative predictive values were 63.9% and 69.2%, respectively, and the diagnostic accuracy rate was 66.1%. The best cut-off point for IMA measurements in detection of an ectopic pregnancy was 1.0235. The sensitivity at this value was calculated to be 35.5%, and the specificity 96.8%. Positive and negative predictive values were 91.7% and 60.0%, respectively and the diagnostic accuracy rate was 66.1%.

Discussion

In this study, we observed that the native thiol level was significantly lower and the IMA level was significantly higher in the study group. In addition, the area under the ROC curve for native thiol measurements and IMA was found to be statistically significant in distinguishing ectopic pregnancies. The best cut-off value for native thiol was calculated to be 433.9 and for IMA it was 1.0234. These results indicate that ectopic pregnancies may be associated with the presence of high oxidative stress. Especially in the early stages of a suspected ectopic pregnancy determination of elevated levels of oxidative stress along with serial βHCG follow-up can be helpful in early diagnosis.

Until recent years ectopic pregnancy was one of the important causes of maternal morbidity and mortality. With the development of diagnostic techniques conservative management has become possible. Although spontaneous resolution can be seen in ectopic pregnancies, patients have a risk of tubal rupture and hemorrhage. Therefore, an ectopic pregnancy is still a complicated condition that can cause maternal morbidity and mortality when diagnosed late. An accurate diagnosis can be made by transvaginal ultrasonography. However, invasive procedures may still be required. Its pathophysiology is not fully known, but it is proposed that the oxidant-antioxidant balance shifting in favor of oxidative stress might cause impaired tubal motility leading to implantation abnormalities, which is associated with ectopic pregnancy.

With the emergence of ROS, disruption of the oxidant-antioxidant balance causes the emergence of oxidative stress. In the presence of oxidative stress in the organism, compounds containing a thiol group undergo a reversible oxidation reaction forming disulfide bonds. They help maintain antioxidant balance and are then reduced back to thiol groups. Low total and native thiol levels have been shown to contribute to an increased risk of coronary artery disease in overweight adolescents with polycystic ovary syndrome (19). It is also known that oxidative stress increases in ectopic pregnancies (20). It has been shown in previous studies that tubal epithelial dysfunction may occur as a result of increased oxidative stress and the tubal environment may change (12) In addition, the high levels of IMA detected in ectopic pregnancies has shown that it can be used as a marker of oxidative stress, but there are no studies on the use of dynamic TDH in evaluation of ectopic pregnancies (18).

Table 3. Diagnostic performance indicators for native thiol and IMA in terms for detection of ectopic pregnancy

	Definitions	Native thiol	IMA
The best cut-off point		<433.9	>1.0235
Sensitivity	TP/(TP+FN)	23/31 (74.2%)	11/31 (35.5%)
Specificity	TN/(TN+FP)	18/31 (58.1%)	30/31 (96.8%)
PPV	TP/(TP+FP)	23/36 (63.9%)	11/12 (91.7%)
NPV	TN/(FN+TN)	18/26 (69.2%)	30/50 (60.0%)
Accuracy	(TP+TN)/(N)	41/62 (66.1%)	41/62 (66.1%)

PPV: Positive predictive value, NPV: Negative predictive value, TP: True positive, FN: False negative, TN: True negative, FP: False positive, N: No. of total cases.

In a meta-analysis evaluating ectopic pregnancies and endometriosis, it has been shown that ectopic pregnancies are common in endometriosis patients (21). Endometriosis is a complex, chronic, estrogen-dependent disease and similar to ectopic pregnancy its pathophysiology is still not clear (22, 23). However, a proinflammatory environment in endometriosis triggers mechanisms such as proliferation and angiogenesis (23, 24). The association between endometriosis and oxidative stress is also known. Nevertheless, it is not possible to conclude if the increase in ectopic pregnancies among endometriosis patients is a result of this increase in oxidative stress.

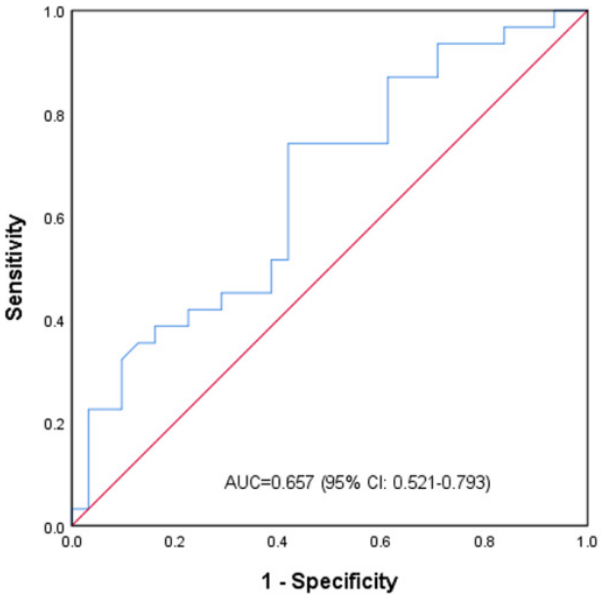

Figure 1: Graphical representation of the area under the curve for native thiol levels in the study group

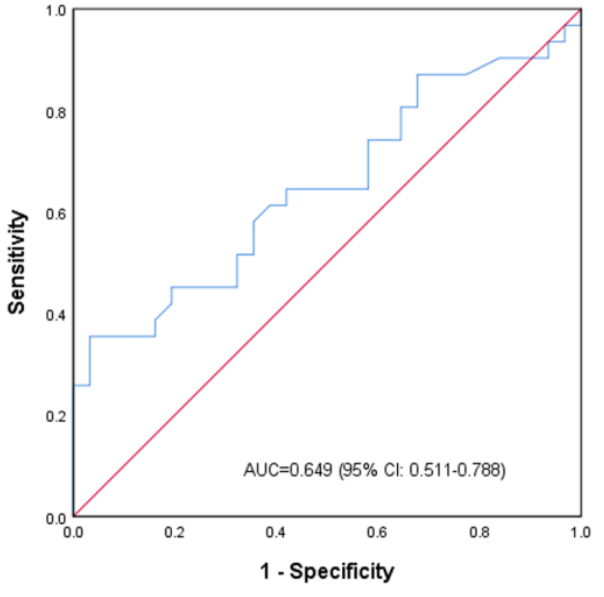

Figure 2: Graphical representation of the area under the curve for IMA levels in the study group

One of the limitations of this study is the small cohort of the study population. However, the prospective design and the significant results despite the small recruitment number are among its strenghts. This can be considered as a pilot study when designing larger multi-centered studies evaluating oxidative stress with dynamic TDH levels in ectopic pregnancies.

In this study, it was shown that native thiol levels decreased and IMA levels increased in patients with ectopic pregnancy. According to this study, since the pathophysiology of ectopic pregnancy is not fully known, determination of native thiol and IMA levels may be helpful in the diagnosis of patients with suspected ectopic pregnancies. However, routine investigation of these parameters in every patient may not be effective and/or practical. Although there are studies showing that oxidative stress increases in ectopic pregnancies, more studies are needed to conclude whether there is an association between oxidative stress and occurance of ectopic pregnancy.

Conflict of Interest: Each author declares that he or she has no commercial associations that might pose a conflict of interest in connection with the submitted article.

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