

Oxidative status and serum prolidase activity in tubal ectopic pregnancy

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Abstract

Objective: To determine the oxidative status and serum prolidase activity in tubal ectopic pregnancy and to see if there was any association between them.

Methods: The cross-sectional study was conducted during 2009 and 2010 at the Departments of Obstetrics and Gynaecology and Clinical Biochemistry under the Faculty of Medicine, Harran University, Turkey. It comprised 40 patients with tubal ectopic pregnancies and 42 women with healthy pregnancies. Serum prolidase activity was measured spectrophotometrically. Oxidative status was determined using total antioxidant capacity. SPSS 11.5 was used for statistical analysis.

Results: Total antioxidant capacity levels were lower in the ectopic pregnancy group than the healthy group ($p < 0.018$), whereas total oxidant status, oxidative stress index and prolidase activity were higher ($p < 0.05$).

Conclusion: Ectopic pregnancy may be associated with increased serum prolidase activity and oxidative stress, and this association may help to provide a better understanding about the pathogenesis of ectopic pregnancy.

Keywords: Ectopic pregnancy, Prolidase activity, Total antioxidant capacity, Total oxidant status, Oxidative stress index. (JPMA 63: 169; 2013)

Introduction

An ectopic pregnancy is a complication of pregnancy in which the fertilized ovum gets implanted outside the uterine cavity. About 2% of pregnancies are ectopic and of these, 98% occur in the fallopian tubes. There are a number of risk factors for ectopic pregnancies, but more than half of the identified ectopic pregnancies are in women without known risk factors.¹ Detection of ectopic pregnancy in early gestation has been achieved mainly due to enhanced diagnostic capability. Despite all the useful progress in diagnostic and detection techniques, ectopic pregnancy remains a source of serious maternal morbidity and mortality worldwide, especially in countries with poor prenatal care.² In a successful reproduction, fertilisation and early embryonic development begin in the Fallopian tubes which play a significant role in the physiological events. The embryo interacts with the female reproductive tract before implantation. Embryo-tubal transport is done with tubal ciliary beats and smooth muscle contractions. It has been hypothesised that tubal ectopic pregnancy is caused by a combination of retention of the embryo within the fallopian tube due to impaired embryo-tubal transport and alterations in the tubal environment allowing early implantation to occur.³ Tubal epithelial cells produce growth factors, cytokines, and other embryotrophic

factors of unknown identities to support embryo development in vitro. It has been suggested that an imbalance between the production of toxic compounds, such as oxygen-based free radicals and lipid peroxidase, and the detoxification and scavenging of these harmful molecules in vivo may affect pre-implantation embryo development⁴ and it has also been suggested that pathologic generation of nitric oxide (synthesised from L-arginine by three nitric oxide synthases in different tissues, including the fallopian tube) through increased nitric oxide synthases isoforms production may decrease tubal ciliary beats and smooth muscle contractions and, thus, affect embryo transport, which may consequently result in a tubal ectopic pregnancy.⁵

Prolidase is a manganese(Mn)-requiring homodimeric iminodipeptidase that releases carboxy-terminal proline or hydroxyproline from oligopeptides.⁶ It has been shown that an increase in prolidase enzyme activity is correlated with increased rates of collagen turnover.⁷ Increased oxidative stress might have a role in the changing of tubal environment⁸ and tubal epithelial cells might be replaced by collagen fibres. We hypothesised that there may be an association between serum prolidase activity and oxidative status in tubal ectopic pregnancy in patients without any known risk factors for tubal ectopic pregnancy. Thus, in the present study, we aimed at determining serum prolidase activity and oxidative stress markers, including total oxidative status (TOS), total antioxidant capacity (TAC) and oxidative stress index (OSI), and to see if there is any association between serum

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prolidase activity and the oxidative stress parameters in this complication of pregnancy.

Patients and Methods

The cross sectional study was conducted at the Harran University Medical Faculty's Departments of Obstetrics and Gynaecology and Clinical Biochemistry during 2009 and 2010. The study group comprised women with ectopic pregnancies who were positively identified on transvaginal ultrasound scan and serum β -hCG levels. All women had been referred by their general practitioners or consultants because of suspected ectopic pregnancy. All women included in the study conceived spontaneously and were not taking exogenous progestogens. A full history was documented and clinical examination was carried out by the attending physician. An ultrasound scan was then performed using a high-frequency transvaginal probe and the patients had blood samples taken for the measurement of serum β -hCG. Informed consent for participation in this study was obtained from all the participants. During the study period, 40 of the patients who had tubal ectopic pregnancies and met the inclusion criteria, accepted to be part of the study. The study protocol conformed to the principles of the Helsinki Declaration and was approved by the Medical Ethics Committee of Harran University. Ectopic pregnancies that implanted outside of the fallopian tubes, patients who had symptoms of haemodynamic instability at the time of diagnosis, those who had additional diseases, history of alcohol abuse, smoking habit, intravenous drug abuse, antioxidant usage and patients who had known risk factors for ectopic pregnancy were excluded from the study. The study group included 40 ectopic pregnancies (5-8 weeks of gestation), and the control group comprised 42 healthy pregnant women with similar gestational ages. Among the 40 subjects, 23 had serum levels of β -hCG above 1500 IU/ml and ultrasound scan findings of them were the emptiness of uterine cavity or cervical canal and a mass with ultrasound appearances of an ectopic pregnancy was seen in either adnexa, separate from the ovary and corpus luteum. The other 17 in the group underwent serial β -hCG evaluation and ultrasound examinations. An increase over 48 hours of at least 66% was used as the cutoff point for viability⁹ and we also used the threshold of discrimination of intrauterine pregnancy to be around 1500 IU/ml of β -hCG.¹⁰ Patients with low β -hCG concentrations showed subnormal increase, and then underwent diagnostic dilatation and curettage (D&C) procedure. The biopsy materials were fixed with formalin 10% and examined at the Department of Pathology. Biopsy materials were stained with haematoxylin-eosin and assessed for the absence of chorionic villi and trophoblasts. After D&C, their subnormal increase of β -hCG levels

continued and diagnosis of ectopic pregnancies was confirmed. Patients who were diagnosed to have early pregnancy were included in the control group. The exclusion criteria for the subjects was also valid for the controls. Initially, there were 51 controls, but women who had any complication of pregnancy were also excluded from the study, and finally there were 42 patients in the control group.

The blood samples from the patients were taken before any medications and after overnight fasting. Blood samples were collected into empty tubes and immediately stored on ice at 4°C. The serum was then separated from the cells by centrifugation at 3000 rpm for 10 min. Serum samples for measurement of TOS and TAC levels and prolidase activity were stored at -80°C until they were used.

TAC was determined using the automated measurement method developed by Erel.¹¹ In this method, hydroxyl radical, which is the most potent biological radical, was produced. In the assay, ferrous ion solution, which was present in Reagent 1, was mixed with hydrogen peroxide, which was present in Reagent 2. The radicals thus 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, was measured. The assay has excellent precision values lower than 3%. The results are expressed as mmol Trolox Equiv./L.

TOS of serum was determined using the automated measurement method also developed by Erel.¹² Oxidants present in the sample oxidised the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction was enhanced by glycerol molecules, which were abundantly present in the reaction medium. The ferric ion made a coloured complex with xylenol orange in an acidic medium. The colour intensity, which can be measured spectrophotometrically, was related to the total amount of oxidant molecules present in the sample. The assay was calibrated with hydrogen peroxide and the results are expressed in terms of micromolar hydrogen peroxide equivalent per liter (μ mol H₂O₂ Equiv./L).

Percent ratio of TOS level to TAC level was accepted as the OSI. For calculation, the resulting unit of TAC was changed to mmol/L, and the OSI value was calculated according to the formula.¹³ OSI (Arbitrary Unit) = TOS (μ mol H₂O₂ Equiv./L)/TAC (mmol Trolox Equiv./L).

For the determination of prolidase activity, the serum was diluted 40-fold with 2.5 mmol/L Mn²⁺, 40 mmol/L Tris-HCl buffer (pH 8.0) and pre-incubated at 37°C for 2 h. The reaction mixture containing 30 mmol/L

gly-pro, 40 mmol/L trizma HCl buffer (pH 8.0) and 100 μ L of pre-incubation serum in 1 mL was incubated at 37°C for 30 min. Adding 0.5 mL of 20% trichloroacetic acid solution then stopped the incubation reaction. The supernatant was used for the measurement of proline by the method proposed by Myara^{14,15} which is a modification of Chinard's method.¹⁶ Intra-assay coefficients of variability (CV) of the assay was 3.8%.

Statistical analysis was done using SPSS 11.5. All data were expressed as mean \pm standard deviation (SD). The comparisons of non-parametric and parametric elements were performed with Mann-Whitney U test, and Independent samples T test respectively. A p value <0.05 was accepted as significant.

Results

The demographic and clinical data of the subjects and the controls were compared (Table-1). There were no statistically significant differences between the two groups with respect to maternal age, gestational age, parity, and body mass index (BMI) ($p > 0.05$).

Serum TAC levels were lower among the ectopic subjects

Table-1: Demographic and clinical characteristics of the study population.

	Controls (n = 42)	Subjects (n = 40)	p
Age, years	29.37 \pm 5.70	29.29 \pm 6.19	$p > 0.05$
BMI (Kg/m ²)	24.50 \pm 2.97	24.02 \pm 2.87	$p > 0.05$
Gestational age(weeks)	6.02 \pm 0.99	6.52 \pm 1.29	$p > 0.05$
Parity	4.07 \pm 3.16	4.14 \pm 1.92	$p > 0.05$

BMI: Body Mass Index.

Table-2: Prolidase activity, TOS, TAC and OSI in subjects and controls.

	Subjects (n = 40)	Controls (n = 42)	p
TAC (mmol Trolox equiv./l)	0.93 \pm 0.18	1.03 \pm 0.19	0.018
TOS (?mol H ₂ O ₂ Eqv./L)	16.91 \pm 4.97	14.39 \pm 3.30	0.008
OSI (arbitrary unit)	1.89 \pm 0.71	1.44 \pm 0.45	0.001
Prolidase (U/l)	688.46 \pm 37.98	630.43 \pm 54.47	< 0,001

TAC: Total antioxidant capacity. TOS: Total oxidative status. OSI: Oxidative stress index.

than the controls ($p < 0.018$), whereas TOS, OSI and prolidase activity were higher compared to the controls ($p < 0.008$, $p < 0.001$ and $p < 0.001$ respectively) (Table-2).

Discussion

Ectopic pregnancy is the most common life threatening emergency in early pregnancy. Although spontaneous resolution of ectopic pregnancy can occur, patients are at risk of tubal rupture and catastrophic haemorrhage. It has

been shown that oxidative stress plays a central role in the pathophysiology of many different disorders, including complications of pregnancy.¹⁷ Reactive oxygen species are produced in metabolic and physiological processes and our body is under constant oxidative attack from reactive oxygen species. A complex system of antioxidant defences generally hold this attack in balance. This balance can be perturbed, leading to oxidative stress. Oxidative stress is best defined in broad terms as an alteration in the pro-oxidant-antioxidant balance that leads to potential damage. Various tissues may control or prevent the damaging effects of the oxidant species by enzymic and non-enzymic antioxidant defence systems so the deleterious effects of the free radicals are kept in check by a delicate balance between the rate of their production and elimination by the different antioxidant systems. Any shift in this critical balance could result in an increase in peroxidative stress and may lead to cellular damage. Thus, the extent of oxidative damage in tissues depends on the balance of oxygen radical formation and the endogenous antioxidant capacity.¹⁸

Prolidase (Enzyme Class 3.4.13.9) is a cytosolic Mn(II)-activated metalloproteinase that specifically hydrolyses imidodipeptides and imidotripeptides with C-terminal proline or hydroxyproline, and releases these two amino acids for collagen re-synthesis and cell growth. It is an ubiquitous enzyme which also plays an important role in intracellular protein catabolism, connective tissue metabolism and matrix remodeling,¹⁹ and its activity has been documented in erythrocytes, leukocytes, plasma, dermal fibroblasts, the kidney, brain, heart, thymus and uterus.²⁰ This enzyme has two forms such as prolidase I (Molecular Weight: 105,000) and prolidase II (Molecular Weight: 151,000), but only prolidase I has been found in human plasma.²¹ The collagen is the most abundant protein in the body and constituting more than a quarter of total body proteins.²² The last step of collagen degradation is mediated by prolidase (Enzyme Class 3.4.13.9) so the enzyme activity may be a step-limiting factor in the regulation of collagen biosynthesis.²³ It has been shown that an increase in prolidase enzyme activity is correlated with increased rates of collagen turnover and prolidase enzyme activity is affected by oxidative stress.²⁹ So oxidatively stressful events may cause tissue and cellular injury, including increased protein turnover such as collagen leading to abnormal remodelling of tuba uterina. Reactive oxygen species generating systems may attack tubal epithelium and collagen molecules, also degrade them and then may stimulate formation of fibrils by this collagen. So increased oxidative stress may increase prolidase activity and may have a role in tubal milieu changes and tubal epithelium may be replaced by collagen fibres and then tubal ectopic pregnancy may have been

seen in patients without any known risk factors for tubal ectopic pregnancy. The role of prolidase in the metabolism of collagen is shown by pathological conditions such as liver cirrhosis,¹⁴ and uterine leiomyoma.²⁵

The current study found increased serum prolidase activity in patients with tubal ectopic pregnancy which may be interpreted as an evidence of increased collagen re-synthesis. Tubal ectopic pregnancy may be associated with increased oxidative stress and increased serum prolidase activity. Increased serum prolidase activity and this association may help to provide a better understanding about the pathogenesis of tubal ectopic pregnancy.

The study hypothesised that oxidative stress and prolidase activity are related with tubal ectopic pregnancy. A search of MEDLINE for articles in the English language with the terms 'oxidative stress', 'prolidase activity' and 'ectopic pregnancy' revealed no entries. As such, the study is perhaps the first in the literature to evaluate a relationship between oxidative stress, prolidase activity and ectopic pregnancy. We found that TOS and OSI are increased whereas TAC is decreased in the ectopic pregnancy group, and prolidase activity is increased in ectopic pregnancies compared to healthy pregnancies. These findings provide support to the hypothesis.

Conclusion

Though there was evidence in support of the hypothesis, it is difficult to conclude whether these findings were the cause or the consequence of tubal ectopic pregnancy. The impact of prolidase activity on tubal ectopic pregnancy with regard to pathophysiological processes needs more comprehensive laboratory work and further clinical studies for clarifying the pathophysiological role of increased serum prolidase activity in tubal ectopic pregnancy.

References

- Dart RG, Kaplan B, Varaklis K. Predictive value of history and physical examination in patients with suspected ectopic pregnancy. *Ann Emerg Med* 1999; 33: 283-90.
- Paxton A, Maine D, Freedman L, Fry D, Lobis S: The evidence for emergency obstetric care. *Int J Gynecol Obstet* 2005; 88: 181-93.
- Shaw JL, Dey SK, Critchley HO, Horne AW. Current knowledge of the aetiology of human tubal ectopic pregnancy. *Hum Reprod Update* 2010; 16: 432-44.
- Lopes AS, Lane M, Thompson JG. Oxygen consumption and ROS production are increased at the time of fertilization and cell cleavage in bovine zygotes. *Hum Reprod* 2010; 25: 2762-73.
- Shao R, Zhang SX, Weijdegard B, Zou S, Egecioglu E, Norstrom A, et al. Nitric oxide synthases and tubal ectopic pregnancies induced by Chlamydia infection: basic and clinical insights. *Mol Hum Reprod* 2010; 16: 907-15.
- Lupi A, Rossi A, Vaghi P, Gallanti A, Cetta G, Forlino A. Nbenzyloxycarbonyl-L-proline: an in vitro and in vivo inhibitor of prolidase. *Biochim Biophys Acta* 2005; 1744: 157-63.
- Miltyk W, Surazynski A, Kasprzak KS, Fivash MJ Jr, Buzard GS, Phang JM. Inhibition of prolidase activity by nickel causes decreased growth of proline auxotrophic CHO cells. *J Cell Biochem* 2005; 94: 1210-7.
- Guerin P, Mouatassim S, Menezo Y. Oxidative stress and protection against reactive oxygen species in the pre-implantation embryo and its surroundings. *Hum Reprod Update* 2001; 7: 175-89.
- Dart RG, Mitterando J, Dart LM. Rate of change of serial betahuman chorionic gonadotropin values as a predictor of ectopic pregnancy in patients with indeterminate transvaginal ultrasound findings. *Ann Emerg Med* 1999; 34: 703-10.
- Morin L, Van den Hof MC; Diagnostic Imaging Committee, Society of Obstetricians and Gynaecology of Canada. Ultrasound evaluation of first trimester pregnancy complications. *J Obstet Gynecol Can* 2005; 27: 581-91.
- Erel O. A novel automated method to measure total antioxidant response against potent free radical reactions. *Clin Biochem* 2004; 37: 112-9.
- Erel O. A new automated colorimetric method for measuring total oxidant status. *Clin Biochem* 2005; 38: 1103-11.
- Harma M, Harma M, Erel O. Increased oxidative stress in patients with hydatidiform mole. *Swiss Med Wkly* 2003; 133: 563-6.
- Myara I, Myara A, Mangeot M, Fabre M, Charpentier C, Lemonnier A. Plasma prolidase activity: a possible index of collagen catabolism in chronic liver disease. *Clin Chem* 1984; 30: 211-5.
- Myara I, Charpentier C, Lemonnier A. Optimal conditions for prolidase assay by proline colorimetric determination: application to iminodipeptiduria. *Clin Chim Acta* 1982; 125: 193-205.
- Chinard FP. Photometric estimation of proline and ornithine. *J Biol Chem* 1952; 199: 91-5.
- Al-Gubory KH, Fowler PA, Garrel C. The roles of cellular reactive oxygen species, oxidative stress and antioxidants in pregnancy outcomes. *Int J Biochemist Cell Biol* 2010; 42: 1634-50.
- Burton GJ, Jauniaux E. Oxidative stress. *Best Pract Res Clin Obstet Gynaecol* 2011; 25: 287-99.
- Oono T, Yasutomi H, Ohhashi T, Kodama H, Arata J. Characterization of fibroblast-derived prolidase. The presence of two forms of prolidase. *J Dermatol Sci* 1990; 1: 319-24.
- Zanaboni G, Dyne KM, Rossi A, Monafò V, Cetta G. Prolidase deficiency: biochemical study of erythrocyte and skin fibroblast prolidase activity in Italian patients. *Haematologica* 1994; 79: 13-8.
- Cosson C, Myara I, Miech G, Moatti N, Lemonnier A. Only prolidase I activity is present in human plasma. *Int J Biochem* 1992; 24: 427-32.
- Di Lullo GA, Sweeney SM, Ko'rkko J, Ala-Kokko L, D. San Antonio JD. Mapping the ligand-binding sites and disease-associated mutations on the most abundant protein in the human, type I collagen. *J Biol Chem* 2002; 277: 4223-31.
- Lupi A, De Riso A, Torre SD, Rossi A, Campari E, Vilarinho L, et al. Characterization of a new PEPD allele causing prolidase deficiency in two unrelated patients: natural-occurrent mutations as a tool to investigate structure func. relationship. *J Hum Genet* 2004; 49: 500-6.
- Altindag O, Erel O, Aksoy N, Selek S, Celik H, Karaoglanoglu M. Increased oxidative stress and its relation with collagen metabolism in knee osteoarthritis. *Rheumatol Int* 2007; 27: 339-44.
- Wolanska M, Sobolewski K, Drozdewicz M. [Integrins and prolidase activity in uterine leiomyoma during tumor growth]. *Ginekol Pol* 2001; 72: 121-6.