Peer reviewed article

Assessment of serum paraoxonase and arylesterase activities in early pregnancy failure

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Summary

Questions under study: Paraoxonase-1 (PON1) is an HDL-bound enzyme system which plays a key role in the protection of LDL and HDL from oxidation by hydrolysing activated phospholipids and lipid peroxide products**.** Oxidative stress has been involved in the pathogenesis of many complications of human pregnancy, including early pregnancy failure (EPF), preeclampsia and preterm labour. The purpose of this study was to determine serum paraoxonase/arylesterase activities and lipid hydroperoxide (LOOH) levels as an oxidative stress indicator in women with EPF.

Method: Paraoxonase/arylesterase activities and LOOH levels were assessed in women with EPF (n = 40) and healthy continuum pregnant women without EPF (n = 38) before 12 weeks' gestation. Serum basal and salt-stimulated paraoxonase/arylesterase activities were measured spectrophotometrically. LOOH levels were measured by ferrous oxidation with a xylenol orange assay. Student's t-test and the Pearson correlation were used for statistical analysis

Results: We found that basal and salt-stimulated paraoxonase/arylesterase activities were significantly lower in women with EPF than in women without EPF (all p <0.05), while LOOH levels were significantly higher (p = 0.009).

Conclusion: Our study showed that decreased paraoxonase/arylesterase activities and increased LOOH levels may play a role in ethiopathogenesis through increased susceptibility to lipid peroxidation in women with EPF.

Key words: paraoxonase; arylesterase; early pregnancy failure; lipid hydroperoxide

Introduction

Early pregnancy failure (EPF) is the most common complication of human reproduction, with an incidence ranging between 50 and 70% of all conceptions [1]. The aetiology and pathophysiology of EPF are not fully understood. There are many causes of early pregnancy failure, but it now appears that oxidative stress may play a role. An emerging confluence of opinion suggests that oxidative stress is one of the main underlying mechanisms in the pathogenesis of a continuum of disease processes such as spontaneous abortion, hydatidiform mole and preeclampsia [2]. Preeclampsia is an endothelial disease with major involvement of lipid-mediated oxidative damage [3]. Abnormalities of human placentation are associated with disorders that are unique to our species, such as preeclampsia and miscarriage [4]. The pathophysiological finding in the uteropla-

Abbreviations

BMI	Body mass index
Ca^{2+}	Calcium
CRL	Crown-rump length
EPF	Early pregnancy failure
HDL	High-density lipoprotein
HDL-C	HDL-cholesterol
LDL	Low-density lipoprotein
LDL-C	LDL-cholesterol
LOOH	Lipid hydroperoxide
oxLDL	Oxidised LDL
TChol	Total cholesterol
TG	Triglyceride
TVU	Transvaginal ultrasound
PON	Paraoxonase

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cental bed of patients with preeclampsia resembles atherosclerotic lesions, showing necrosis of the vessel wall and accumulation of lipid-laden foam cells with oxidised low-density lipoproteins (oxLDL) [5]. Underlying these changes is a common deficit of trophoblast invasion during the first and early second trimesters of pregnancy, and hence incomplete conversion of the endometrial spiral arteries [6].

Human serum paraoxonase-1 (PON1) is a high-density lipoprotein (HDL)-bound enzyme and its arylesterase/paraoxonase activities [7] are considered the major determinant of the antioxidant action of HDL [8]. Purified human PON1 inhibits LDL oxidation in vitro, and other studies have shown that PON1 prevents the formation of oxLDL, inactivates LDL-derived oxidised phospholipids and protects phospholipids in HDL from oxidation [9]. HDL and its associated PON quite possibly interrupt a process that would otherwise lead to oxidative damage [10]. Human PON1 has two genetic polymorphisms, one at position 55, which is a methionine (M)/leucine (L) substitution, and one at position 192, which is a glutamine (Q)/arginine (R) substitution. The polymorphisms affect the hydrolytic activity of PON1 isoenzymes with lipid-peroxides [11]. The PON1–192 R allele has been associated positively with coronary heart disease [12] and preterm delivery [13], thereby contributing to endothelial dysfunction [14]. Lipid hydroperoxide (LOOH) is a well-known marker of oxidative stress formed from unsaturated phospholipids, glycolipids and cholesterol by peroxidative reactions under oxidative stress. OxLDL is, besides membrane-bound cholesterol-derived hydroperoxides, the main form of LOOH responsible for the development of oxidative stress [15]. Concentrations of lipid peroxides have also been shown to increase in the villous and decidual tissues of women undergoing EPF [16]. Overall, the consequences are placental degeneration with complete loss of syncytiotrophoblast function and detachment of the placenta from the uterine wall. This mechanism is common to all miscarriages, the time at which it occurs in the first trimester depending on the aetiology [17].

We postulated that increased oxidative stress may be present in women with EPF, and in the present state of knowledge there has been no report involving the oxidative/antioxidative status of plasma in the EPF. We aimed to determine paraoxonase/arylesterase activities and LOOH levels as an oxidative stress indicator in women with EPF.

Materials and methods

The study was conducted in the Department of Obstetrics and Gynaecology, Harran University Hospital. This research was a descriptive study, and women with and without EPF and healthy continuum pregnant women were recruited consecutively. Informed consent for participation in the study was obtained from all the women. The study protocol was in conformity with the principles of the Helsinki Declaration and it was approved by the institutional ethics review board. In all, 78 pregnant women prior to 12 weeks' gestation were enrolled. Forty women with EPF were included. These pregnant women had had vaginal bleeding and/or lower abdominal pain for the first time in the previous few days (0–2 days). The diagnosis of EPF was based on the clinical history, clinical examination, and transvaginal ultrasound (TVU) results. In cases where pregnancy structures (a gestational sac without foetal heart rate) were identified by TVU, the final diagnosis of EPF was made. In 38 healthy continuum pregnant women without EPF before 12 weeks' gestation a gestational sac with foetal heart rate was identified by TVU. Ultrasound analysis was also used to detect embryonic remnants and to measure the crown-rump length (CRL). The time of foetal demise was estimated from the disparity in the foetal age as estimated from the date of the last menstrual period and based on the CRL. The women with EPF included in the study were examined at largely similar times after the foetal demise. They were compared for gestational age, gravidity, parity, abortus, maternal age, and body mass index (BMI) (table 1). Gestational age was calculated from the last menstrual period and confirmed by routine ultrasound test. Routine blood biochemistry, haematology, hormone levels, immunology and microbiological analysis were performed in all subjects.

Exclusion criteria were a gestational age later than 12 weeks (based on the first day of the last menstrual period and confirmed by a routine ultrasound), a history of recurrent pregnancy loss (3 or more consecutive pregnancy losses), chromosomal abnormalities, endocrine diseases, anatomical abnormalities of the genital tract, infections, immunologic diseases, trauma, internal diseases and intake of any chemical agent before the elective terminations. Exclusion criteria also included multiple pregnancies (twins or triplets), smoking, existence of diabetes mellitus, coronary artery disease, rheumatoid arthritis, systemic disease, connective tissue disease, coagulopathies, factor V Leiden, thrombophilia, systemic or local infection, hypertension, hyperlipidaemia, acute or chronic liver diseases (including hepatitis markers A, B and C positively), renal dysfunction, and anaemia.

Blood samples were obtained following an overnight fast. Blood samples of the study group were acquired just before dilatation and aspiration. Samples were drawn from the cubital vein into blood tubes, and the serum was

Table 1
Demographic parameters in women with and without EPF.

Parameters	Women without EPF (n = 38)	Women with EPF (n = 40)	p value
Maternal age (years)	28.8 ± 6.3	30.6 ± 4.4	0.143
Number of pregnancies	4.13 ± 2.7	5.3± 2.4	0.048
Number of deliveries	2.42 ± 2.2	2.95 ± 1.7	0.246
Number of abortions	0.71 ± 1.2	1.35 ± 1.3	0.026
Gestation age (weeks)	9.08 ± 2.2	8.48 ± 1.5	0.172
Body mass index (kg/m²)	26.6± 4.5	26.3± 4.4	0.774

immediately separated from the cells by centrifugation at 3000× g for 10 min, stored at –80 °C, and then analysed.

Paraoxonase and arylesterase activities were measured using commercially available kits (Relassay®, Gaziantep, Turkey). Paraoxonase activity measurements were performed in the absence (basal activity) and presence of NaCl (salt-stimulated activity). The rate of paraoxon hydrolysis (diethyl-p-nitrophenylphosphate) was measured by monitoring the increase of absorbance at 412 nm at 37 °C. The amount of generated p-nitrophenol was calculated from the molar absorptivity coefficient at a pH of 8.5, which was 18290 M^{-1} cm^{-1} [18]. Paraoxonase activity was expressed as U/L serum. Phenylacetate was used as a substrate to measure the arylesterase activity. Enzymatic activity was calculated from the molar absorptivity coefficient of the phenol produced, 1310M^{-1} cm^{-1}. One unit of arylesterase activity was defined as 1 µmol phenol generated/min under the above conditions and expressed as U/L serum [19]. Paraoxonase phenotype distribution was determined by a double substrate method, which calculates the ratio of salt-stimulated paraoxonase activity and arylesterase activity [18]. The coefficient of variation for measurement of serum PON activity was 3.3%. Serum LOOH levels were measured by the ferrous ion oxidation-xylenol orange (FOX-2) method as previously described [20]. The method is based on a known principle of the oxidation of Fe II to Fe III by LOOHs, under acidic conditions. The coefficient of variation for measurement of LOOH level was 5%. The levels of triglyceride (TG), total cholesterol (TChol), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C), and fasting glucose were determined using commercially available assay kits (Abbott®, Illinois, USA) with an Abbott Aeroset auto-analyser (Abbott®, Illinois, USA).

All statistical analyses were performed using SPSS 11.5 (SPSS for Windows 11.5, Chicago, IL). The comparisons of parameters were performed using Student's *t*-test and correlation analyses were performed using Pearson's correlation test, as usually used when data is distributed normally (according to One-Sample Kolmogorov-Smirnov test) and the study groups are large enough ($n \geq 30$). Continuous variables are expressed as mean ± S.D. The chi-square test was used to compare the demographic and clinical data of the subjects in patients and controls. Two-sided *p-values* of less than 0.05 were accepted as significant.

Results

Demographic and clinical data of the women with and without EPF are summarised in table 1. There were no significant differences between women with and without EPF in regard to age, gestational age, number of deliveries and BMI. However, there were significant differences between the groups in number of pregnancies and abortions ($p = 0.048$, $p = 0.026$ respectively).

Total cholesterol and triglyceride levels were higher in women with EPF than in those without EPF, although p values were not statistically significant ($p = 0.178$, $p = 0.065$ respectively). The LDL-C level was significantly higher in women with EPF than in women without EPF ($p = 0.015$). In contrast, the HDL-C level was significantly lower in women with EPF in comparison with those without ($p = 0.026$). Basal and salt-stimulated paraoxonase/ arylesterase activities were significantly lower in women with EPF than in women without EPF ($p = 0.017$, $p = 0.042$, $p = 0.002$, respectively), while LOOH levels were significantly higher ($p = 0.009$). In addition, the paraoxonase/HDL-C ratio did not differ significantly between women with and without EPF ($p = 0.095$) (table 2).

In women with EPF, basal paraoxonase and salt-stimulated paraoxonase/arylesterase activities correlated positively with HDL-C levels ($r = 0.398$, $p = 0.011$; $r = 0.350$, $p = 0.027$; $r = 0.334$, $p = 0.035$, respectively), while LDL-C levels were inversely correlated ($r = –0.490$, $p = 0.001$; $r = –0.471$, $p = 0.002$; $r = –0.290$, $p = 0.070$, respectively) (table 3). In addition, there was an inverse correlation between LOOH and basal paraoxonase and salt-stimulated paraoxonase/arylesterase activities ($r = –0.498$, $p = 0.001$; $r = –0.525$, $p = 0.001$; $r = –0.320$, $p = 0.044$, respectively) (table 3).

Table 2

Lipid hydroperoxide level, PON/HDL ratio, basal/salt-stimulated paraoxonase/arylesterase activities and clinical parameters in women with and without EPF.

Parameters	Women without EPF (95% CI)	Women with EPF (95% CI)	p value
Basal paraoxonase (U/L)	268 ± 118 (229–306)	210 ± 87 (183–238)	0.017
Salt-stimulated paraoxonase (U/L)	787 ± 549 (606–967)	565 ± 391 (440–690)	0.042
Arylesterase (U/L)	186 ± 29 (177–196)	164 ± 32 (154–174)	0.002
Paraoxonase/HDL-C ratio	5.8 ± 2.7 (4.93–6.67)	4.9 ± 1.9 (4.33–5.52)	0.095
LOOH (µmol H_2O_2 Eqv./L)	6.2 ± 1.2 (5.80–6.62)	6.9 ± 1.1 (6.57–7.26)	0.009
HDL-C (mg/dl)	46.9 ± 8.0 (44.3–49.6)	42.9± 7.5 (40.6–45.4)	0.026
LDL-C (mg/dl)	72.2 ± 18.8 (66.0–78.4)	82.4 ± 17.3 (76.9–87.9)	0.015
Total cholesterol (mg/dl)	147 ± 28 (138–156)	155 ± 22 (148–162)	0.178
Triglyceride (mg/dl)	87.7 ± 25.6 (79.3–96.2)	98.5 ± 25.3 (90.5–107)	0.065

PON: paraoxonase, HDL-C: high-density lipoprotein-cholesterol, LDL-C: low-density lipoprotein-cholesterol,
LOOH: lipid hydroperoxide, EPF: early pregnancy failure, Values are reported as mean ± S.D, 95% CI; 95% confidence interval.

Table 3

The relationships among the paraoxonase activity, arylesterase activity, SS paraoxonase, LOOH, HDL-C, LDL-C, T chol and TG levels in women with EPF.

		TG	T Chol	LDL-C	HDL-C	LOOH	Arylesterase	SS paraoxonase
Paraoxonase	r	0.021	0.086	–0.490	0.398	–0.498	0.533	0.974
	p	0.899	0.596	0.001	0.011	0.001	<0.001	<0.001
SS paraoxonase	r	0.019	0.084	–0.471	0.350	–0.525	0.454	
	p	0.908	0.608	0.002	0.027	0.001	0.003	
Arylesterase	r	0.133	0.211	–0.290	0.334	–0.320		
	p	0.415	0.190	0.070	0.035	0.044		
LOOH	r	0.181	–0.168	0.147	–0.262			
	p	0.263	0.301	0.365	0.103			
HDL-C	r	–0.157	–0.051	–0.411				
	p	0.334	0.753	0.008				
LDL-C	r	0.116	0.286					
	p	0.477	0.074					
T Chol	r	0.219						
	p	0.174						

HDL-C: high-density lipoprotein-cholesterol, LDL-C: low-density Lipoprotein cholesterol, LOOH: lipid hydroperoxide, SS paraoxonase: Salt-stimulated paraoxonase, T chol: total cholesterol, TG: Triglyceride, EPF: early pregnancy failure

Discussion

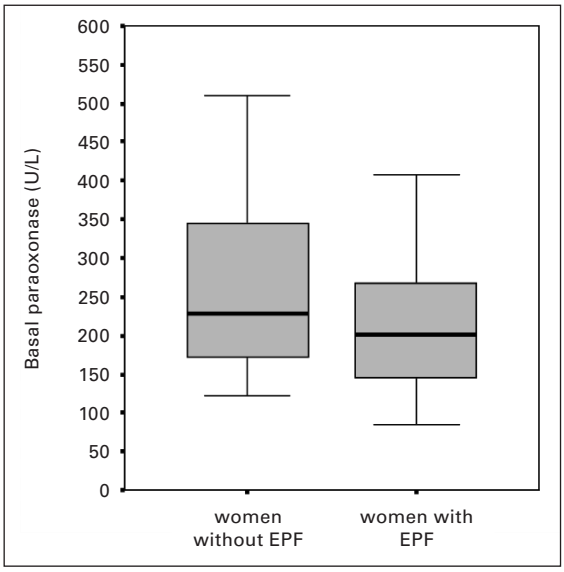

Figure 1

Reduced serum basal paraoxonase activity in women with EPF in comparison with women without EPF (p <0.05).

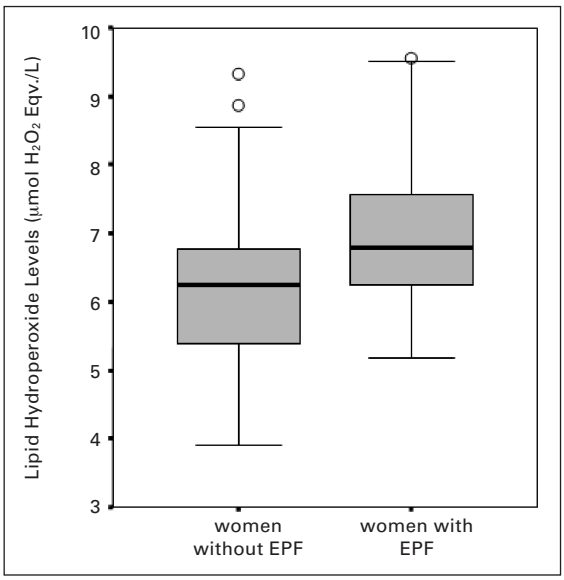

Figure 2

Increased serum LOOH levels in women with EPF in comparison with women without EPF (p <0.05).

In the present study we show that basal/salt-stimulated paraoxonase/arylesterase activities were significantly lower in women with EPF than in women without EPF, while LOOH levels were significantly higher (fig. 1, 2). To the best of our knowledge this is the first report of paraoxonase/arylesterase activities and oxidative status in women with EPF.

According to the data obtained from the current study, increased LOOH levels and decreased paraoxonase/arylesterase activities may be implicated in the presence of oxidative stress in EPF. In addition, it appears that decreased paraoxonase/arylesterase activities may play a role in the pathogenesis of oxidative stress in women with EPF, via increased susceptibility to lipid peroxidation. Lipid peroxides are directly involved in mediating maternal endothelial dysfunction, by increasing the production of thromboxane A_2 and the expression of cell adhesion molecules in the utero-placental vasculature, and also in the maternal peripheral vasculature [21]. Maternal plasma lipids are significantly elevated during pregnancy. Some studies have shown a preeclampsia-dyslipidaemia pattern of increased concentrations of TG, Chol, and LDL, and decreased concentrations of HDL [22]. Similar results were obtained in the present study from the pregnant women with EPF. Free fatty acids are particularly susceptible to oxidation, are increased in preeclampsia 15–20 weeks before the onset of the clinical disease [23], and may contribute to susceptibility to maternal oxidant stress. In contrast to miscarriage, where there is rapid and generalised placental tissue degeneration, in preeclampsia the placental damage is progressive and can be compensated for some time, depending on the sever-

ity of initial placental defect and intrinsic placental antioxidant capacity [17]. In the present study we observed that the LOOH level was increased in pregnant women with EPF, a finding which, with respect to increased oxidative stress, was consistent with previous studies.

Uzun et al. [24] suggested that elevated oxidative stress and oxLDL, dyslipidaemia and decreased PON1 activity may cause damage to the vascular endothelium. Aviram [10] reported that PON 1 may protect vascular tissue from oxidative damage, and purified PON1 exhibits the expected protection against oxidative damage from LDL. Oxidative stress has also been implicated as an important cause of recurrent pregnancy failure. Loss of antioxidant defences has been shown to be associated with pregnancy failure [25]. Biochemical markers of reactive oxygen species-induced membrane damage, such as lipid peroxidation products, reach high levels immediately before abortion [26]. It has been suggested that an oxidant/antioxidant imbalance is associated with pregnancy failure [27]. The lower antioxidant levels may aggravate pro-oxidant injury on endothelial cells, altering the prostacyclin-thromboxane balance and culminating in preeclampsia or abortion [28]. Increased oxidative stress in the placenta of women with preeclampsia is well documented [29]. Increased oxidative stress may alter placental vasculature, leading to early failure [2]. Hence mechanisms that prevent the oxidation of LDL have received increasing attention in recent years. One such mechanism is the prevention of LDL oxidation by PON1. It has been shown that PON1 lowers the sensitivity of LDL to lipid peroxidation [30]. Cellular stress and damage, including lipid peroxidation, were increased in tissues obtained from women with missed miscarriages compared with controls. Lipid peroxide formation, a marker of oxidative stress, is increased during pregnancy and EPF. OxLDL induces endothelial injury and inhibits apoptosis, platelet aggregation and endothelial nitric oxide synthase activity, all of which contribute to the atherosclerotic process and preeclampsia [31]. Maternal endothelial dysfunction may impair invasion of the spiral arteries by extravillous trophoblasts, which is necessary to create the high-flow, low-resistance uteroplacental vascular system that provides adequate blood supply for foetal growth [32]. We postulated that the hypothetical mechanisms in preeclampsia may also be responsible for EPF. In both diseases the placenta is a key source of factors which lead to similar metabolic changes [5]. In the present study we found that PON1 was decreased and the LOOH level increased in women with EPF who, as expected, are exposed to increased oxidative stress. Previous studies have shown that serum PON1 activity is associated with modulation of endothelial functions [8], regulation of coronary vasomotor tone and the presence and extent of coronary artery disease [33]. Germain et al. [34] reported that abnormal endothelial function in women with recurrent abortion, as well as the previously recognised association of growth restriction, preterm birth and abortion with an excess of cardiovascular disease in later life supports the concept that whatever increases the risk of cardiovascular disease in later life also increases the risk of implantation abnormalities.

In humans PON1 activity positively correlated with the quantity of vitamins C and E in the diet [26, 35]. Previous studies showed that consumption of dietary polyphenolic antioxidants, such as pomegranate juice hydrolysable tannins (punicalagin), or red wine flavonoids such as quercetin or cathechin, by E-deficient (E^0) mice [36–38] preserved serum PON1 activity by reducing oxidative stress, thereby contributing to PON1 hydrolytic activity on lipid peroxides in oxidised lipoproteins, macrophages and atherosclerotic lesions [39]. Vlachos et al. [40] reported that reduced total antioxidant status levels were found postdelivery in women with prolonged and/or obstructive labour followed by caesarean section. The activity of the PON1 enzyme correlates positively with the participants' total antioxidant status. Because preeclamptic conditions are closely related to lipid peroxidation [21], evaluation of PON 1 activity may be used as an indicator of HDL antioxidant capacity during the labour process.

In conclusion, we found significantly lower levels of paraoxonase/arylesterase activity and significantly higher LOOH levels in women with EPF. This evidence suggests that a decrease in the activities of paraoxonase/arylesterase may play an important role in the pathogenesis of EPF through increased susceptibility to lipid peroxidation. Since antioxidants increase the activity of PON1, it may be possible to conclude that addition of antioxidant vitamins such as vitamins C and E may be useful in preventing EPF as a supplementation therapy for the pregnant women whose PON1 activity is at low levels at the begining of pregnancy. Further studies are needed to clarify the possible mechanisms of paraoxonase/arylesterase activities and lipid peroxidation levels in the pathogenesis of EPF.

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