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ORIGINAL ARTICLE

PEROXIREDOXIN 3 AND OXIDATIVE STRESS IN RECURRENT ABORTION PATIENTS

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Summary

The pathophysiology of recurrent spontaneous abortion (RSA) has been linked to oxidative stress (OS), which is defined as an imbalance between the formation of oxidants and the antioxidant defense system. The goal of this study was to assess the state of OS in recurrent spontaneous abortion by assessing some of its indicators in order to identify women who are at risk of abortion and enhance their reproductive health. Peroxiredoxin 3 (Prx3), progesterone (P4), glutathione (GSH), uric acid (UA), peroxynitrite ($ONOO^-$), and malondialdehyde (MDA) were measured in 40 healthy non-pregnant (HNP) women, 40 healthy pregnant (HP) women without abortion history, and 21 women with recurrent spontaneous abortion (Have at least ≥ 3 consecutive abortion). All subjects are of reproductive age, with the mother gestational age in the HP and RSA groups being ≤ 20 weeks. According to maternal gestational age, RSA and HP women were separated into two categories (1st & 2nd trimester). According to the findings of this study, Prx3 and GSH levels declined considerably in RSA patients compared to HP and HNP patients, but $ONOO^-$ and MDA levels increased statistically significantly in RSA patients compared to HP and HNP groups. However, P4 of RSA was found to be significantly lower in the HP group and much higher in the HNP group. The difference in uric acid levels between the RSA and HP groups was statistically significant, but the difference between the RSA and HNP groups was not.

Within the first and second trimesters of pregnancy, the difference between the RSA and HP groups showed statistically significant changes in oxidative stress-related biomarkers, with the exception of uric acid, which showed a non-significant difference between the two groups within the second trimester.

Finally, the effect of gestational age within RSA was revealed, with serum Prx3 and P4 showing a significant difference between the first and second stages of pregnancy, whereas other oxidative stress indicators were unaffected by pregnancy length within the RSA group.

Key words: Recurrent abortion; Oxidative stress; Antioxidants; Oxidants; Peroxiredoxin 3; Progesterone; Glutathione; Uric acid; Peroxynitrite; Malondialdehyde

Introduction

Early pregnancy loss, commonly known as miscarriage /or spontaneous abortion, is the most frequent complications of pregnancy and a serious public health concern for women throughout the world (1). Spontaneous

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abortion is the spontaneous termination of a pregnancy before the 20th week of pregnancy or before the fetal weight reaches 500 gm, and the fetus reaches viability. Approximately 12-24 percent of all pregnancies end in spontaneous abortion (2). Genetic, anatomical, endocrine, microbiological, behavioral, immunological and environmental variables all have a role in the pathogenesis of spontaneous abortion (3-5), Furthermore, there are a variety of causes for spontaneous abortion, one of which is oxidative stress (OS) is the main (6). The cause of 30 percent to 50 percent of abortions, however, is uncertain (7). Recurrent pregnancy loss (RPL) is one of the most common issues in reproductive medicine, and it may be an emotionally draining experience for couples who are impacted (8). Three or more consecutive spontaneous abortions before twenty weeks of pregnancy are referred to as recurrent spontaneous abortion (RSA), habitual abortion, or recurrent miscarriage (9). This medical disorder is thought to afflict 0.5% to 3% of women during their reproductive age (10). According to several studies, the reason of miscarriage is unclear in more than half of RPL instances (11). Abnormalities of Parental chromosomal, difficulties of structural uterine, propensity of maternal thrombotic, variables endocrine, infection causes in addition to environmental factors are all recognized etiologies of RPL (11, 12).

During early pregnancy, placental oxidative stress may cause difficulties such as recurrent abortion, congenital anomalies in diabetics, and preeclampsia (13, 14). OS may have a role in the etiopathogenesis of (RPL).

The placenta, which is responsible for the fetus's growth and development, must have a high metabolism throughout pregnancy. OS develops in response to increasing fetoplacental energy demands when there is an imbalance between the generation of oxidant substances and the ability of antioxidant systems to neutralize them, this lead to injury in trophoblastic tissue. Pregnancy losses may occurs in cases where it is an impossible to controls such injury (15).

PRXs (peroxiredoxins) are an antioxidant proteins family. In 1994, a family of thiolredoxin-dependent peroxidase reductase proteins was characterized for the first time (16). The PRXs family is the name given to this group of proteins.

The Members of PRXs family are antioxidant enzymes that perform peroxidase activity in addition to, the recovery and neutralization of hydrogen peroxide (H_2O_2), nitrogen peroxide as well as hydroperoxides (17).There are 6 members in this family. Prx3 belongs to the peroxiredoxin (PRX) family and is mostly found in mitochondria. It is thought to play a vital role in mitochondrial antioxidant defense and scavenging around (90%) of the mitochondrial hydrogen peroxide (18), making it essential for the mitochondrial homeostasis (19).

Progesterone (P4) is necessary for the start and continuation of a pregnancy (20). Because of progesterone's key function in early pregnancy, physicians and researchers have speculated that P4 shortage may be a cause of certain miscarriages; high levels of the hormone appear to decrease oxidative damage (21). Glutathione (GSH) is a master antioxidant that protects both the mother and her fetus from destructive effect of OS and preserves the growing and developing embryo's critical processes by managing differentiation of cell, death of cell, and many unique functions (22, 23). It protects cells from harm caused by lipid peroxides, reactive oxygen and nitrogen species (ROS, RNS) and xenobiotics by regulating cellular redox state (24). Uric acid (UA), it is a hydrophilic antioxidant produced during purine nucleotide metabolism that accounts for about (66%) of total oxygen scavenging action in blood sera (25). Peroxynitrite ($ONOO^-$) is a potent oxidant that causes lipid peroxidation (LPO), nitrosylation tyrosine residues of protein, DNA damaging , and other effects (26). Malondialdehyde (MDA) is a lipid peroxidation, and OS indicator (27, 28). MDA interacts with a variety of nitrogenous bases and proteins, resulting in several genetic alterations and illnesses (29).

As a result, pregnancy is a physiological condition marked by oxidative disruption, which contributes to the onset and progression of pregnancy-related problems (30). Prx3, P4, GSH, UA, $ONOO^-$, and MDA were identified as oxidative stress-related biomarkers in recurrent spontaneous abortion (RSA) in a recent study.

Materials and Methods

This research involved 101 female. They were separated into three essential groups: 40 healthy non-pregnant (HNP) women, 40 healthy pregnant (HP) without abortion history, and 21 recurrent spontaneous abortion patients (RSA) who had ≥ 3 consecutive miscarriages. The samples were gathered from the gynecological Teaching Hospitals

between January and July 2021. The HP and RSA women were further divided based on the maternal gestational age (1st & 2nd trimester groups). The ages of all participants are in reproductive age and the gestational age for HP and RSA women less than twenty weeks. All of the groups were given a complete history and clinical assessment. Each lady had her whole medical history collected, and ten mL of venous blood was extracted from each participant in this study. To complete blood serum separation, the blood samples were immediately transferred into plain tubes and put in a water bath at 37°C for 10 minutes before centrifugation at 3000 g for 15 minutes. The serum was isolated and used for tests of oxidative stress biochemical markers: Prx3 levels were evaluated using an Enzyme-Linked Immunosorbent Assay (ELISA) kit from Bioassay Technology Laboratory in China, while serum P4 levels were assessed using an ELISA kit from DiaPlus Inc. in Canada. The GSH levels were tested using the dithiobisnitro benzoic acid (DTNB) technique (31). Uricase is an enzymatic method for estimating serum uric acid (UA) that uses a kit (Biolabo/France) (32). Vanuffelene *et al*., 1998 developed a modified technique for measuring $ONOO^-$ (33). The thiobarbituric acid (TBA) technique was used to determine MDA levels (34).

Statistical Analysis

The data was statistically examined using the t-test. The data is presented as means with standard deviations (SD). A significant variation was considered when *P*-values was ≤ 0.05 (35).

Results

In recent research, the comparison of the mean values of Prx3 and some oxidants and antioxidants markers between HP and RSA groups as shown in **Table (1)**, a highly significant reduction in the levels of Prx3, P4 and GSH was noticed in RSA (33.67±8.79), (15.99±2.50), and (3.55±0.21) compared with HP group (45.78±7.10), (45.40±4.98) and (5.00±0.46) respectively. While a significant elevation in levels of UA, $ONOO^-$ and MDA were noticed in RSA cases (4.83±0.82), (95.88±16.90) and (9.64±1.87) as compared with HP group (3.95±0.79), (84.01±13.32) and (7.48±1.06) respectively.

Table 1. Prx3, some antioxidants and oxidants levels in RSA patients as compared with HP group.

Biochemical parameters	HP group (n=40)		RSA group (n=21)		*P*-value
	Mean	SD	Mean	SD	
Prx3 (ng/mL)	45.78	7.10	33.67	8.79	0.0001***
P4 (ng/mL)	45.40	4.98	15.99	2.50	0.0001***
GSH (µmol/L)	5.00	0.46	3.55	0.21	0.0001***
UA (mg/dL)	3.95	0.79	4.83	0.82	0.013*
$ONOO^-$ (µmol/L)	84.01	13.32	95.88	16.90	0.040*
MDA (µmol/L)	7.48	1.06	9.64	1.87	0.0001***

*Significant at ($P \leq 0.05$), *** Significant at ($P \leq 0.0001$).

Table 2. Prx3, some antioxidants and oxidants levels in RSA patients as compared with HNP group.

Biochemical parameters	HNP group (n=40)		RSA group (n=21)		*P*-value
	Mean	SD	Mean	SD	
Prx3 (ng/mL)	55.97	8.37	33.67	8.79	0.0001***
P4 (ng/mL)	5.37	0.88	15.99	2.50	0.023*
GSH (µmol/L)	6.37	1.28	3.55	0.21	0.0001***
UA (mg/dL)	4.46	0.95	4.83	0.82	0.311
$ONOO^-$ (µmol/L)	75.51	19.25	95.88	16.90	0.013*
MDA (µmol/L)	4.30	0.84	9.64	1.87	0.0001***

*Significant at ($P \leq 0.05$), *** Significant at ($P \leq 0.0001$).

The differences in the mean values of studied Prx3 and some oxidative stress biomarkers between HNP and RSA women; as seen in **Table (2)**, a significant decline in the mean values of Prx3 and GSH was noted in RSA cases (33.67±8.79) and (3.55±0.21) as compared with HNP group (55.97±8.37) and (6.37±1.28) respectively. While the results showed a significant rises of blood serum P4, $ONOO^-$ and MDA in RSA group (15.99±2.50), (95.88±16.90) and (9.64±1.87) respectively in contrast with HNP group (5.37±0.88), (75.51±19.25) and (4.30±0.84) respectively, the elevation of uric acid in RSA group statistically not significant.

The gestational age effects on Prx3 and some oxidants and antioxidants studied in this research was shown in **Tables (3, 4)**.

The results shows a significant decrease of serum Prx3, P4 and GSH in 1st stage of RSA group (35.30±4.48), (8.38±0.98) and (3.83±0.69) respectively in contrast with a comparable stage of HP group (43.61±5.87), (26.55±1.91) and (5.54±0.95) respectively. However, UA, $ONOO^-$ and MDA reported a significant increase in RSA cases within 1st stage (4.95±0.68), (96.60±15.00) and (9.05±1.06) respectively in comparison with 1st stage of HP (3.80±0.50), (88.02±13.18) and (7.01±0.98) respectively; as seen in **Table (3)**.

Table 3. Prx3, some antioxidants and oxidants levels in 1st stage RSA patients compared with 1st stage HP group.

Biochemical parameters	1st stage HP group (n=20)		1st stage RSA group (n=12)		*P*-value
	Mean	SD	Mean	SD	
Prx3 (ng/mL)	43.61	5.87	35.30	4.48	0.047*
P4 (ng/mL)	26.55	1.91	8.38	0.98	0.0001***
GSH (µmol/L)	5.54	0.95	3.83	0.69	0.023*
UA (mg/dL)	3.80	0.50	4.95	0.68	0.033*
$ONOO^-$ (µmol/L)	88.02	13.18	96.60	15.00	0.048*
MDA (µmol/L)	7.01	0.98	9.05	1.06	0.010*

*Significant at ($P \leq 0.05$), *** Significant at ($P \leq 0.0001$).

Table (4) shows the comparison of Prx3 and some oxidative markers between RSA and HP groups within 2nd stage of gestation, a significant decline was noticed in the mean values of Prx3, P4 and GSH in 2nd stage of RSA group (31.51±3.76), (26.13±6.95) and (3.18±0.80) respectively in comparison with 2nd stage of HP (47.96±4.18), (64.24±5.30) and (4.45±0.92) respectively, while $ONOO^-$ and MDA showed a significant elevation in 2nd stage of RSA cases (94.92±14.79) and (10.43±1.28) respectively in contrast with a comparable stage of HP (80.01±13.34) and (7.96±0.94) respectively. Serum uric acid showed a non-significant change between the two groups within 2nd stage of pregnancy.

Table 4. Prx3, some antioxidants and oxidants levels in 2nd stage RSA patients compared with 2nd stage HP group.

Biochemical parameters	2nd stage HP group (n=20)		2nd stage RSA group (n=9)		*P*-value
	Mean	SD	Mean	SD	
Prx3 (ng/mL)	47.96	4.18	31.51	3.76	0.018*
P4 (ng/mL)	64.24	5.30	26.13	6.95	0.018*
GSH (µmol/L)	4.45	0.92	3.18	0.80	0.031*
UA (mg/dL)	4.10	1.00	4.68	1.07	0.949
$ONOO^-$ (µmol/L)	80.01	13.34	94.92	14.79	0.036*
MDA (µmol/L)	7.96	0.94	10.43	1.28	0.001**

*Significant at ($P \leq 0.05$), ** Significant at ($P \leq 0.0001$).

Finally, The variation between 1st & 2nd stage within RSA cases as seen in **Table (5)** reported that a significant decline of serum Prx3 in 2nd stage of RSA cases compared with 1st stage while P4 showed a significant increase in 2nd stage compared with the 1st stage of RSA group. Others oxidative stress-related biomarkers give a non-significant difference between the two gestational stages of RSA group.

Table 5. Compared of Prx3, some antioxidants and oxidants levels in 1st and 2nd stage RSA patients groups.

Biochemical parameters	1st stage RSA group (n=12)		2nd stage RSA group (n=9)		*P*-value
	Mean	SD	Mean	SD	
Prx3 (ng/mL)	35.30	4.48	31.51	3.76	0.045*
P4 (ng/mL)	8.38	0.98	26.13	6.95	0.006*
GSH (µmol/L)	3.83	0.69	3.18	0.80	0.180
UA (mg/dL)	4.95	0.68	4.68	1.07	0.824
$ONOO^-$ (µmol/L)	96.60	15.00	94.92	14.79	0.801
MDA (µmol/L)	9.05	1.06	10.43	1.28	0.147

*Significant at ($P \leq 0.05$).

Discussion

By assessing blood levels of several OS indicators, this study aimed to assess the state of OS in women with RSA and HP presently in the first and second trimesters, as well as compare them to HNP women.

In recent study, Prx3 shows a markedly reduction in RSA patients, because of their antioxidant action of Prx3 for supporting placentation, the autoantibodies against this antioxidants proteins may be caused miscarriage (36). Autoimmune antibodies can harm the placenta and the baby during pregnancy (37). Women who have had recurrent pregnancy loss may create anti-Prx3 autoantibodies (38). Liu *et al*. (39) noted that downregulation of Prx3 in the human placenta is a mechanism for early miscarriage. Furthermore, progesterone levels fall as a result of oxidative stressors damaging effects on steroidogenesis of luteal cell (40–42). ROS can also limit progesterone production by inhibiting cytochrome P450, LH receptors degradation and mitochondrial intracellular cholesterol transport (43). As a co-substrate for glutathione peroxide (GPx), glutathione (GSH) participates in the cellular defense mechanism against OS by scavenging reactive oxygen intermediates and free radicals, which explains reduced GSH concentration with increasing OS (44). As a result of the increased cellular demand for NADPH, the intracellular concentration of GSH is likely to drop. Because the quantity of NADPH available to replenish GSSG in GSH is much reduced, the non-enzymatic antioxidant defense mechanism (GSH) is severely reduced (45). Another measure looked at in this investigation was serum uric acid. In recurrent abortion cases, the level of UA was tested and found to be high signifying inverted OS. UA is responsible for over sixty% of free radicals scavenging activity in humans (46). As a consequence, UA might be utilized to identify OS tissues damage dysfunction. The levels of UA are low in uncomplicated pregnancies; concentrations drop by around (25% - 35%) in the first trimester, then grow throughout the pregnancy until they reach the level of non-pregnant at the end (47-49). The increased production of nitric oxide (NO) in recurrent abortion cases may be attributed to an increase in inducible nitric oxide synthase (iNOS) expression in miscarriage, as well as in recurrent spontaneous miscarriage, which in turn is responsible for the high $ONOO^-$ production by reacting superoxide anion radical ($O_2.^-$) with nitric oxide (NO) (50). It's a potent oxidant with a variety of tissue-damaging effects, including lipid peroxidation, tyrosine residues nitrosylation of protein and DNA damaging. It has been proven that nitrotyrosine is only generated by $ONOO^-$ action (26). The current findings are in line with those of Issa *et al*., who assessed the OS status in women who had spontaneous abortion. Their research found that women with abortion had greater serum MDA levels than non-pregnant healthy women (51). In the first trimester of pregnancy, Torkzahrani *et al*. found that serum MDA levels rose considerably in women who had spontaneous abortions compared to healthy pregnant women (52). In numerous research including pregnancy problems, MDA has been recognized as one of the most effective indicators of OS. Increased formation of lipid peroxidation, which is often begun by highly reactive free radicals, was one of the reasons

of MDA levels in abortus. Toxic stress was induced in cells by reactive electrophile species. High blood MDA levels showed an oxidation process in the membrane of the cell that caused endothelial cell injury either directly or indirectly through lipid peroxidation products activating other mediators. The endothelium membrane would leak, and molecules as small as an enzyme may escape through the destructive membrane, caused damaging DNA. If DNA damage occurred that could not be repaired by DNA repair mechanisms, the cell entered the apoptotic pathway and died, which at the fetal stage triggered the body's response to eject the products of conception, resulting in an abortus (53).

In the recent study, the appearance of positive correlation between the oxidative stress biomarker for RSA and HP within maternal gestational age (1st & 2nd trimester) may be attributed to increased OS as progress of gestation.

Conclusion

Finally, there is a link between oxidative stress indicators, the likelihood of recurrent abortion, and pregnancy advancement.

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Conflict of Interest

The authors declared absence of any conflict of interest.

Adherence to Ethical Standards

The study was approved by the Medical Research Ethics Committee at the University of Mosul. The study approval number and date UOM/COM/MREC/ 146 on 04/01/2021.

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