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ORIGINAL ARTICLE

PEROXIREDOXIN 3 WITH TOXIC METALS IN MISSED ABORTION PATIENTS

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Summary

The most vital factors that hostile health human are toxic heavy metals (THMs). Heavy metals are harmful environmental contaminants that can decrease the likelihood of a healthy pregnancy and afterwards impede a healthy birth. Both paternal and maternal toxic metal exposure could influence pregnancy, So the rates of pregnancy failure are constantly rising. The current study's goal is to explore the effect of Peroxiredoxin 3 antioxidant, as well as some toxic metals (TMs) such as arsenic, cadmium and mercury in missed abortion patients and compared with healthy pregnant and non-pregnant women without a history of abortion in order to evaluate the degree of this effect on this pathological situation. Additionally, it will demonstrate the connection between these biochemical variables and gestational age. Peroxiredoxin 3 (Prx3), Arsenic (As), Cadmium (Cd), and Mercury (Hg) as a (THMs) were estimated in 40 healthy non-pregnant (HNP) women, 40 healthy pregnants (HP) with no abortion history, and 20 women with missed abortion (MA). All woman participants are of reproductive age, with the maternal gestational age in the HP and MA groups being ≤ 20 weeks. Maternal gestational age was used to categorize MA and HP women into two groups (1st & 2nd trimester).

Regarding to the findings of recent research, Prx3 levels declined noticeably in MA patients compared to HP and HNP groups, on other hand the difference of toxic metals which represented in this study as: (As, Cd, and Hg) elevated statistically significantly in MA patients compared to HP and HNP groups. Within the first and second trimesters of pregnancy, the difference of Prx3 levels showed statistically significant reduction between the MA and HP groups. A statistical significance elevation was found between the two comparable gestational age of both groups in regard to blood serum (As, Cd, and Hg) levels. Lastly, the impact of gestational period within MA cases was revealed, serum (Cd) and (Hg) showing a significant variation between the first and second trimester of pregnancy, whereas Prx3 and (As) were unaffected by pregnancy advances within the MA group.

Key words: Missed abortion; Peroxiredoxin 3; Toxic metals; Arsenic; Cadmium; Mercury; Oxidative stress

Introduction

Miscarriage is a significant public health concern to heavy bodily, psychological, and economic burdens on persons, society and health system cares, (1). In order to prevent and treat pregnancy failure, it is vital to determine

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the risk factors for this illness. Several factors including endometrial notice and chromosomal disorders owing to their vital task in the incidence of miscarriage (2).

The definition of spontaneous abortion (SA), commonly referred to as miscarriage, is the loss of a pregnancy previous to viability (3). Around 80% of miscarriages happen before week twelve of pregnancy, which is referred to as an early SA (4).

The term "abortion" refers to the termination of a pregnancy before to 20 weeks of gestation. Threatened, inevitable, complete, incomplete, missed and septic abortion are types of SA (5). One of the most frequent types of early pregnancy loss (EPL) is MA, which affects 15% for all pregnancy women (7).

Missed abortion is a particular kind of miscarriage that occurs when the intrauterine products are not spontaneously expelled after the embryonic or fetal death. Women with MA may not exhibit any overt symptoms (8). Multiple etiologic variables have been discovered as contributing to MA at this time, including immunological factors, chromosomal abnormalities of parent, abnormalities of uterine, problems of endocrine, infections, inherited thrombophilia, as well as environmental factors (9), but the etiology is not well understood (7).

The class of protective and antioxidant proteins known as peroxiredoxins (Prxs or PRDXs). A family of thiol redoxin-dependent peroxidase reductase proteins (TSA) was originally identified in 1994 (10). These proteins are referred to as the PRXs family. The PRXs family members are antioxidant enzymes that quickly detoxify organic hydroperoxides (ROOH), peroxynitrite (OONO-), and hydrogen peroxide (H_2O_2) in addition to performing peroxidase activity. There are six isoforms in the Prxs family (11). Prx3 is one of the Prxs members family, which is mainly to be found in mitochondria. It is believed to be crucial for maintaining mitochondrial homeostasis since it scavenges (90%) of the mitochondrial H_2O_2 and protects them from free radicals (12, 13).

As industrialization progressed, it became possible for anyone to be exposed to hazardous metals, which eventually had an impact on people's reproductive systems and even embryos, causing early pregnancy loss (14-16). Most factors of environment, have recently come into focus as a potential cause of miscarriage and have become essential for identifying the unconventional and unique causes of spontaneous abortion (3). Heavy metals are typically described as those metals with a particular density of more than 5 gm/cm^3 that have a negative impact on the organisms live and environment (17). They can enter the body through a variety of routes, including as air, diet, water, and occasionally cutaneous exposure. Heavy metals are retained after absorption, where they build up in the body (18). Various organs of human are impacted by a different chronic & acute harmful effects of TMs. Examples of toxic effects of heavy metals include kidney & gastrointestinal dysfunction, neurological system diseases, vascular damaging, lesions of skin, malfunction of immune system, cancer and birth defects (19-21). The mechanisms for metals-induced toxicities to a biological system include indirect or direct ROS production, weaken antioxidant defense, reduced glutathione (GSH) diminution, inactivation of antioxidant defense enzymes, and oxidative stress (OS) (18, 22). However, some of (HMs) have the ability to bind only to certain macromolecules (18). Even at modest exposure levels, heavy metals including arsenic (As), cadmium (Cd), and mercury (Hg) can be harmful (23, 24).

Materials and Methods

This study was designed as a case-control, in which 100 women participated. The research population was consist of three main groups of wom¬en: 40 HNP women, 40 HP women with never abortion history, and 20 women with MA; The diagnosis of MA depend on the clinical history, physical examination, as well as obstetric ultrasound. Final diagnosis of MA based on the presences of the fetus in the uterus with negative fetal heart. Between January and July 2021, samples were collected from Gynecological Teaching Hospitals in Mosul City. The study's HP and HNP participants had no history of any reproductive illnesses. Using the mother's gestational age as an additional dividing factor, the HP and MA women which is subdivided into (1^{st} & 2^{nd} trimester groups). All of the participants are of reproductive age, and for HP & MA women, the gestational age is ≤ 20 weeks. A complete history and clinical evaluation for all groups are taken. In this investigation, ten milliliters of venous blood were taken from each woman, along with a complete medical history. To complete blood serum separation, the blood samples were immediately transferred into plain tubes and put in a water bath at 37°C approximately for 10 minutes subsequently centrifugation

at 3000 g for 15 minutes. The serum was separated and utilized for testing of Prx3 with some toxic metals; Prx3 levels were assessed via an Enzyme-Linked Immunosorbent Assay (ELISA) kit from the Bioassay Technology Laboratory in China; the toxic metals (TMs) tested in this study are: (As, Cd, and Hg) measured in blood serum using the atomic absorption (25).

Statistical Analysis

The t-test was used to statistically analyze the results for this study. As descriptive statistics, the Mean ± Standard deviation (SD) values were employed. P-values ≤ 0.05 was deemed as statistically significant (26).

Results

In current study, the comparison of the mean values of Prx3 and some TMs between HP and MA groups as shown in **Table (1)**, the results revealed that the blood serum of Prx3 level in patients with MA were significantly decline (31.23±3.55) compared to HP women (45.78±7.10). Whereas, a significant rise in the levels of (As, Cd, and Hg) were noticed in MA cases (6.218±0.40), (0.252±0.023) and (2.341±0.11) respectively as compared to HP group (4.035±0.37), (0.114±0.008) and (0.769±0.034) respectively.

Table 1. Prx_3 with toxic metals in MA patients as compared with HP group.

Biochemical parameters	HP group (n=40)		MA group (n=20)		P-value
	Mean	SD	Mean	SD	
Prx_3 (ng/ml)	45.78	7.10	31.23	3.55	0.0001***
As (µg/dL)	4.03	0.37	6.21	0.40	0.035*
Cd (µg/dL)	0.114	0.008	0.252	0.023	0.023*
Hg (µg/dL)	0.769	0.034	2.341	0.11	0.0001***

*Significant at (P≤ 0.05) , *** Significant at (P≤ 0.0001).

Table (2) shows the comparison of Prx3 and some TMs between HNP and MA women, a statistically significant reduction in the mean value of Prx3 was observed in MA patients (31.23±3.55) as compared with HNP group (55.97±8.37). While the current results showed a significant elevation of blood serum (As, Cd, and Hg) in MA group (6.218±0.40), (0.252±0.023) and (2.341±0.11) respectively in contrast with HNP group (3.746±0.55) (0.102±0.006) and (0.746±0.04) respectively.

Table 2. Prx_3 with toxic metals in MA patients as compared with HNP group.

Biochemical parameters	HP group (n=40)		MA group (n=20)		P-value
	Mean	SD	Mean	SD	
Prx_3 (ng/ml)	55.97	8.37	31.23	3.55	0.0001***
As (µg/dL)	3.74	0.55	6.21	0.40	0.029*
Cd (µg/dL)	0.102	0.006	0.252	0.023	0.010*
Hg (µg/dL)	0.746	0.04	2.341	0.11	0.0001***

*Significant at (P≤ 0.05) , *** Significant at (P≤ 0.0001).

Tables (3, 4) show the impact of gestational age on blood serum of Prx3 and some TMs studied in this study.

The results revealed a significant decline of blood serum Prx3 in MA group within 1st and 2nd trimester (32.26±2.03) and (30.55±4.23) respectively in contrast with a comparable trimesters of HP group (43.61±5.87) and (47.96±4.18)

respectively. Whereas, (As, Cd, and Hg) showed a significant elevation in MA group within 1st trimester (6.336±0.26), (0.165±0.03) and (2.999±0.16) respectively in comparison with 1st trimester of HP group (3.963±0.13), (0.111±0.008) and (0.761±0.032) respectively. Similar results of TMs noticed between MA and HP groups among 2nd trimester (6.139±0.72), (0.311±0.028) and (3.903±0.086) respectively in MA patients in contrast to women with normal pregnancy within 2nd trimester (4.108±0.63), (0.117±0.007) and (0.776±0.037) respectively; as seen in **Table (3, 4)**.

Table 3. Prx_3 with toxic metals in 1st trimester MA patients compared with 1st trimester HP group.

Biochemical parameters	1st trimester HP group (n=20)		1st trimester MA group (n=8)		P-value
	Mean	SD	Mean	SD	
Prx_3 (ng/ml)	43.61	5.87	32.26	2.03	0.018*
As (µg/dL)	3.96	0.13	6.33	0.26	0.0072*
Cd (µg/dL)	0.111	0.008	0.165	0.03	0.049*
Hg (µg/dL)	0.761	0.032	2.999	0.16	0.0001***

*Significant at (P≤ 0.05) , *** Significant at (P≤ 0.0001).

Table 4. Prx_3 with toxic metals in 2nd trimester MA patients compared with 2nd trimester HP group.

Biochemical parameters	2nd trimester HP group (n=20)		2nd trimester MA group (n=12)		P-value
	Mean	SD	Mean	SD	
Prx_3 (ng/ml)	47.96	4.18	30.55	4.23	0.001**
As (µg/dL)	4.10	0.63	6.13	0.72	0.028*
Cd (µg/dL)	0.117	0.007	0.311	0.028	0.039*
Hg (µg/dL)	0.776	0.037	3.903	0.086	0.005*

*Significant at (P≤ 0.05) , *** Significant at (P≤ 0.0001).

Finally, No statistical significance was found between 1st and 2nd trimester of MA patients in regard to blood serum of Prx3 and (As) levels, whereas a significant rises of serum (Cd) and (Hg) was noticed in MA cases within 2nd trimester (0.311±0.028) and (3.903±0.086) respectively as compared with MA cases within 1st trimester (0.165±0.03) and (2.999±0.16) respectively; as seen in **Table (5)**.

Table 5. Compared of Prx_3 with toxic metals in 1st and 2nd trimester MA patients groups.

Biochemical parameters	1st trimester MA group (n=8)		2nd trimester MA group (n=12)		P-value
	Mean	SD	Mean	SD	
Prx_3 (ng/ml)	32.26	2.03	30.55	4.23	0.672
As (µg/dL)	6.33	0.26	6.13	0.72	0.539
Cd (µg/dL)	0.165	0.03	0.311	0.028	0.022*
Hg (µg/dL)	2.999	0.16	3.903	0.086	0.025*

*Significant at (P≤ 0.05).

Discussion

As demonstrated in the current study, Prx3 shows a clearly reduction in MA cases. The newly findings is in agreement with AL-Hamdani I.H. and Al-Helaly (2022), which found a markedly decline of serum Prx3 level

in recurrent spontaneous abortion patients (27), because of Prx3's antioxidant function in sustaining placentation, the autoantibodies versus this antioxidants may be lead to a miscarriage (28). Autoimmune antibodies can hurt the placenta and fetus through the pregnancy (29). Anti-Prx3 autoantibodies may develop in women who have repeatedly lost pregnancies (30). Downregulation of peroxiredoxin 3 in maternal placenta is a mechanism for early abortion, according to Liu *et al*., 2006 study (31).

There are relatively few studies examining the harmful consequences of toxic metal exposure on pregnancy outcomes and embryotoxicity (14). According to our research, women with MA had considerably higher blood TMs levels than those with HP and HNP. The new results are in line with those of Amadi *et al*., (2017), who found that the major toxic metals mercury, arsenic, and cadmium were linked to an increased prevalence of miscarriages in developing countries (32). These metals may cross the placenta barrier to fetus tissues at varying rates, increasing the risk of negative outcomes such low birth body weight, preeclampsia, preterm labor, fetal loss and stillbirth (33). Epidemiologic studies show that prenatal exposure to TMs (mercury, arsenic, and cadmium) might increase the likelihood of SA and stillbirths occurring when these metals are detected in the mother's blood or placenta (34-36). The arsenic may easily pass the placenta, especially during early pregnancy, according to human research on the correlation among arsenic-contaminated drinking H_2O and unfavorable pregnancy outcomes. This can result in spontaneous abortion SA, premature delivery, low birth body weight, and stillbirth (37). Otebhi and Osadolor's data from 2016 showed that women exposed to (As) in Nigeria had a 5.88% higher rate of miscarriages than those who weren't (38). The interruption of ATP synthesis at the levels of citric acid pathway lies at the center of the arsenic (As) toxicity mechanism. Additionally, there is an increase in the generation of H_2O_2, which might result in ROS and oxidative stress (OS) (39).

Elevated maternal blood (Cd) may also raise the chance of abortion up to two times more than in the control group (36, 40), that accumulate in some organs, cross the placenta barrier, lead to fetus teratogenic effects and may contribute to rise of embryonic demise (36, 41). The threatened abortion group's serum Cd levels were found to be significantly higher, according to Turan *et al.*, 2019 (42). Omeljaniuk *et al*., 2018, found a similar association in a sample of women who had been diagnosed with miscarriage, showing a greater level of blood Cd compared to pregnant women in the 1st trimester (36). Indirect reactive oxygen species (ROS) are a possible cause of cadmium's negative effects. The oxidative stress (OS) induced by cadmium are represented via the inhibition electron transport chain in mitochondria, the removal of redox active elements, reduction of glutathione's antioxidant capacity and deactivation of antioxidant enzymes (43). One of the possible causes for raised (Cd) levels in females may be due to increases considerably absorption of (Cd) with iron deficiency (44); iron (Fe) deficiency is frequently linked to pregnancy (45, 46); and it is important to note that the poisonous effects of Cadmium rise during pregnancy for the fetus & mother. Additionally, transcription of LDL receptor mRNA suppressed by Cadmium, which leads to a lower supply of cholesterol which essential for production of placental progesterone (47). Pregnant women in Nigeria with a history of pregnancy problems had a significantly higher mean blood mercury level than pregnant and non-pregnant ladies with no history of gestational problems ($q < 0.001$) (38).

According to a recent study, the apparent positive correlation between the (TMs) for MA and HP during the first and second trimesters of pregnancy in women may be explained by a decreased mechanism of detoxification as the pregnancy progresses in a pathological situation, while the apparent negative correlation between the Prx3 for MA and HP during these same trimesters may be explained by an increase in OS as the pregnancy advances.

Conclusion

The health of pregnant women may be adversely affected by toxic metals (TMs). Abortion may occur even if the blood level of TMs is below the dangerous dose limits. Therefore, the etiology, treatment, or prevention of abortion may depend on the increase of specific TMs levels in the blood.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Adherence to Ethical Standards

The study was approved by the Medical Research Ethics Committee at the University of Mosul. The study's approval code is UOM/COM/MREC/ 146 on January 04,2021.

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