Study of the Relationship Between Antioxidant Enzymes in the Blood of Women Suffering From Miscarriage and The Effect of Heavy Metal Pollution on Them in Basra, Iraq

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ABSTRACT
In this study, environmental heavy metal pollution is associated with recurrent miscarriage. It is closely linked to oxidative stress and homeostasis imbalance due to reproductive toxicity resulting from (HM), as lead (Pb) and mercury (Hg) are considered among the most important biological factors that are hostile to human health and can reduce the possibility of a healthy pregnancy. This study aimed to investigate the effect of antioxidant enzymes as well as some heavy metals such as lead and mercury on recurrent miscarriage. Comparing them to healthy women with no history of miscarriage. Samples were collected from women who had miscarried, 50 samples, compared to 40 samples from healthy women who had not suffered a miscarriage previously. In Basra Women's and Children's Hospital in Basra, Iraq. During the reproductive age of patients and healthy people (18-40) years. The patients were divided into two groups according to place of residence (city center and countryside). And compare them with healthy women. He also highlighted oxidative stress because it is more affected by heavy metals and the appearance of women who have frequent miscarriages who live in rural areas, where oil and gas refineries are close to them, unlike the city. Due to direct exposed to pollution.

1. Introduction

A miscarriage is the spontaneous loss of a pregnancy before the twentieth week of pregnancy, Miscarriages are widespread Very, up to 1 in 5 Pregnancies ending before 12 weeks (1). Environmental pollution, such as the atmospheric air by waste from petroleum and natural gas, and the aquatic environment due to the throwing of industrial waste into rivers and bodies of water, and thus the accumulation of sediments in aquatic organisms for human use, has led to the emergence of multiple bad health consequences for humans, including frequent miscarriages in women (2). Therefore, pollution with heavy metals has been linked to the occurrence of harmful health phenomena, frequent miscarriages in women, and poor fertility, in addition to endocrine disorders (3). Therefore, heavy metal pollution has been linked to the occurrence of harmful health phenomena, recurrent miscarriage in women, poor fertility, in addition to disorders of the reproductive glands, pituitary gland, and hormonal balance (4,5). This imbalance leads to the disruption of various mechanisms, the exclusion of some essential elements, the cessation of the work of enzymes and the work of hormones, and the suppression of antioxidants, thus generating free radicals, the generation of peroxide of biomolecules, and oxidative stress. All of these processes may cause organ poisoning or fetal deformities and thus terminate the pregnancy through miscarriage (6,7,8). Given the above, the formation of reactive oxygen species (ROS) in the body, the body's vital systems work to balance the free radicals generated and enzymatic antioxidants, which act as the first line of defense to eliminate free radicals (9). For instance, iNOS catalyzes the synthesis of nitric oxide (NO.) from L-arginine, which in turn reacts with ROS to form several RNS (10, 11). Oxidants are defined as "any substance that may cause or enhance the release of other substances, generally through the release of oxygen." (12). Oxidants can be found in it has two forms, enzymatic and non-enzymatic. Common Enzymatic defenses include superoxide dismutase (SOD), catalase, and glutathione peroxidase Glutathione reductase. Non-enzymatic factors such as Ferritin, ceruloplasmin, transferrin, ascorbic acid (vitamin C), and-tocopherol (vitamin E) can also Acts as antioxidants. Some agents such as lyco- Coffee, metallothionein and bilirubin also contain Oxidation properties (13). As for (MDA), it is considered an indicator of oxidative stress resulting from the oxidation process, and this interacts with different groups of proteins and nitrogenous bases, and this interaction causes multiple changes and multiple diseases (14). From the above, pregnancy is considered a physiological condition affected by oxidative imbalance, and thus it causes the formation of diseases associated with

pregnancy (15) . This study aimed to investigate the effect of Mercury and Lead on aborted women and the extent of their effect on antioxidant enzymes and oxidative stress. And comparing them with healthy women who have no history of miscarriage. It has been shown that high levels of mercury and lead increase the risk of recurrent miscarriage and thus affect the imbalance between the levels of enzymatic antioxidants and the levels of free radicals. As a result of this, oxidative stress, represented by MDA values, increases, so its level increases in the blood of aborted women.

2. Methodology

Study Subject

The 90 participants in this study were divided into two groups: the case group, which included 50 patients. Samples were collected from Basra Women's and Children's Hospital in Basra, and in the Department of Chemistry, College of Science, University of Basra, and the control group included 40 patients who had no history of miscarriage. Patients with recurrent miscarriages were divided according to residence between the city center and the district. The ages of the study participants ranged from (18-40) years. childbearing age.

Sample Collection

Sample collection: A volume of 8 ml of blood samples was kept in gelatinous tubes and allowed to clot at 37°C for 30 minutes, after which it was centrifuged at 3000 rpm for 5 minutes in order to collect serum samples for biochemistry. The serums were stored at freezing temperature (-20°C) and were ready for use in measuring elements and biochemical factors.

Biochemical assessments

The heavy elements lead (Pb) and mercury (Hg) were measured in the blood of patients and the control group by preparing the samples for measurement by performing a digestion process on the samples in order to get rid of the protein present in the serum to facilitate the measurement of the elements lead and mercury. They were measured using a device ICP_OES, Thermo Fisher Scientific, Germany), and antioxidant enzymes were measured Including catalase (CAT), glutathione peroxidase (GPX), superoxide dismutase (SOD), and measuring oxidative stress by measuring malondialdehyde (MDA). The measurement was done using an ELISA device.(by sunlong Biotech Kit).

Statistical Analysis

SPSS for Windows (version 26: USA) was used to analyze the relationships between heavy metals, oxidative stress, and antioxidant enzymes in control groups and patients

3. Results and discussion

The levels of heavy metals among study participants are shown in Table(1) and figure(1) . There were differences in lead and mercury levels in women with recurrent and first miscarriages living in rural areas and city centers compared to the healthy group. The mercury values in cases of recurrent miscarriage in rural areas were higher than those in the first miscarriage in the same geographical area, and then lower than that in the case of the first and recurrent miscarriages in the city center, respectively (27.979 ±31.917, 11.303±5.879, 7.371±3.426, 7.647±2.499). And compared to the values of the control group for both the countryside and the city center, respectively (0.650±1.175, 0.712±1 .614). It obtained high statistical significance with (p values= 0.001)

The lead values for women with repeated miscarriages in the countryside were higher than the lead values for women with a recurrent and first miscarriage in the city center, and the lead values for women with a first miscarriage in the rural area were lower, respectively (508.862±383.43, 360.072±75.261, 304.926 ± 92.405, 288.988±73.590 ppb).

The control group had pb values for both the city center and the countryside, which were lower than

the lead values in aborted women, respectively (269.83±42.770, 244.512±20.163 ppb), therefore, it obtained high statistical significance with (p values= 0.000).

Table 1: Comparative the levels of Heavy metals according to Residency for cases and control

Heavy elements	**Residency**		**Groups**		**P-value**
			Case	Control	
			Mean ± S.D	Mean ± S.D	
Hg (ppb)	Center	Primary	7.647±2.499	0. 712±1 .614	0.001
		Recurrent	7.371±3.426	0.712 ±1 .614	0.001
	District	Primary	11.303±5.879	0.650±1 .175	0.001
		Recurrent	27.979±31.917	0. 650±1 .175	0.001
Pb (ppb)	Center	Primary	304.926±92.405	269.83±42.770	0.000
		Recurrent	360.072±75.261	269.83±42.770	0.000
	District	Primary	288.988±73.590	244.512±20.163	0.000
		Recurrent	508.862±383.43	244.512±20.163	0.000

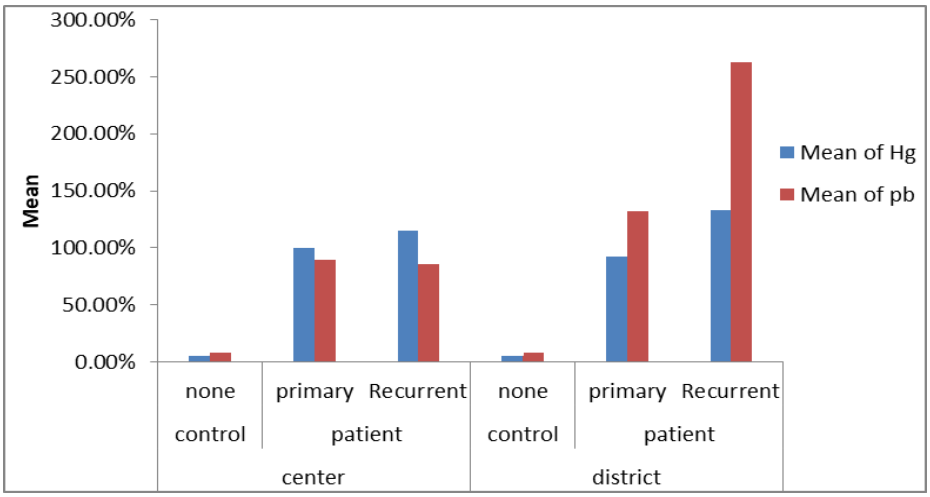

Figure (1): The level of (Hg) and (Pb) in In women with recurrent miscarriage and the control group.

The levels of antioxidant enzymes in the study are also presented in Table 2. The results of this study observed the levels of

Table 2: Comparative the levels of, SOD, CAT, GPX ,MAD according to Residincy for cases and control.

Antioxidant enzyme level	**Residency**	**Type of miscarriage**	**Groups**		**P-value**
			Case	Control	
			Mean ± S.D	Mean ± S.D	
SOD (u/l)	Center	Primary	15.385±8.305	18.050±12.740	0.000
		Recurrent	11.725±3.570	18.050±12.740	0.000
	District	Primary	9.033±1.287	19.658±10.547	0.000
		Recurrent	9.413±2.293	19.658±10.547	0.000
CAT (ku/l)	Center	Primary	0.398±0.440	6.202±11.603	0.000
		Recurrent	0.580±0.399	6.202±11.603	0.000
	District	Primary	0.458±0.379	3.651±1.097	0.000
		Recurrent	0.298±0.306	3.651±1.097	0.000
GPX (ng/ml)	Center	Primary	8.500±3.168	83.050±38.997	0.000
		Recurrent	6.175±2.270	83.050±38.997	0.000
	District	Primary	4.183 ±1.973	79.350±25.022	0.000
		Recurrent	2.609±1.345	79.350±25.022	0.000

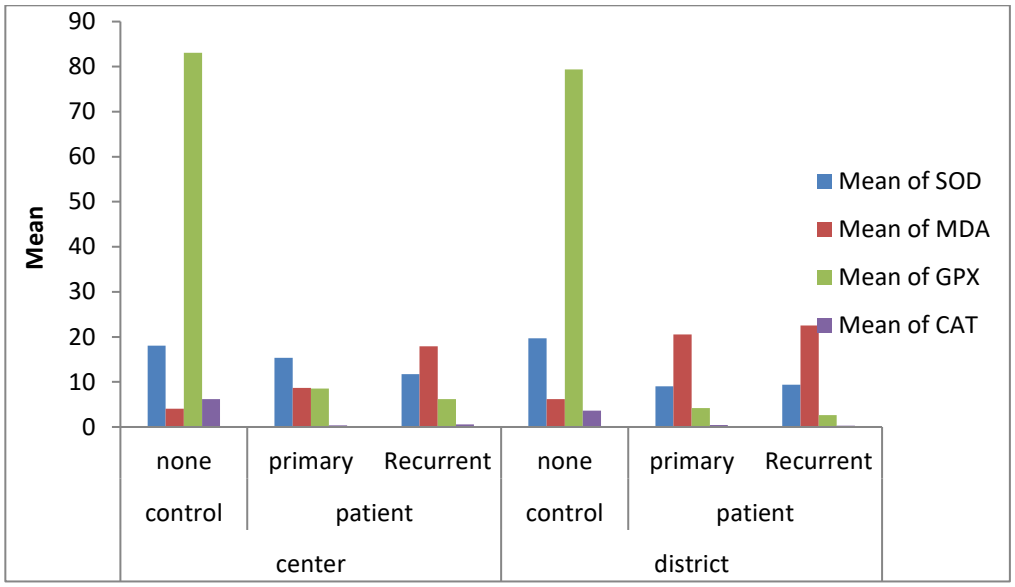

Figure (2): Levels of SOD, MDA, GPX, and CAT in the serum of Patient women and the control group.

The results of this study noted that SOD levels were the lowest in women with primary and recurrent miscarriages in the district, respectively (9.033±1.287, 9.413±2.293 U/L). Then, higher values were recorded in women who had miscarriages in the city center for the primary and recurrent miscarriages, respectively (15.385±8.305 ,11.725±3.570 U/L). Compared to the control group, which had high values. For both the city center and the district, respectively (18.050±12.740, 19.658±10.547 U/L). According to these differences occurring between the aborted women and the control group, there was a high statistical significance for the (P value = 0.000).

The study also showed CAT levels, which were the lowest values in women who had undergone recurrent and primary miscarriage in the district, then (0.298 ± 0.306, 0.458±0.379 KU/L). Slightly higher values were recorded in women who had a miscarriage in the city center for primary and recurrent miscarriages (0.398±0.440 , 0.580±0.399 KU/L). Compared to the control group, which had high values. For both city center and countryside respectively (6.202±11.603, 3.651±1.097 KU/L). According to these differences occurring between the aborted women and the control group, there was a high statistical significance of

(P value = 0.000).

Also, GPX levels showed a significant decrease in all women who had abortions, and the gradually decreased from primary and recurrent abortion in the city center primary and recurrent abortion in the district, respectively (8.500±3.168, 6.175±2.270, 4.183±1.973, 2.609±1.345, ng/ml). Compared to the control group, which showed high levels for both the city center and the countryside (83.050±38.997, 79.350±25.022), respectively.

It was highly statistically significant between patients with miscarriage and the control group, (P value = 0.000).

From Table (3) and figure (2) the results of oxidative stress MDA showed an increase in women who had recurrent and primary miscarriages in the district (19.245±6.352, 15.063±4.982µmol/l), while for women who had miscarriages in the city center for recurrent and primary miscarriages, they ranged from (13.387±3.172, 12.976±3.534 µmol/l), respectively. And compare. In the control group for the city center and the countryside, which amounted to (12.005±3.112, 14.245±3.414 µmol/l), respectively. It obtained high statistical significance for (p values = 0.000).

Table 3: the level of oxidative stress for both patients and the control group

Oxidative	Residency	Type of	Groups	P-value

stress study		**miscarriage**	Case	Control	
			Mean ± S.D	Mean ± S.D	
MDA (µmol/l)	Center	Primary	12.976±3.534	12.005±3.112	0.000
		Recurrent	13.387±3.172	12.005±3.112	0.000
	District	Primary	15.063±4.982	14.245±3.414	0.000
		Recurrent	19.245±6.352	14.245±3.414	0.000

Table 4: Correlation between the levels of antioxidant test with heavy metals Hg abd Pb levels

Correlations between the levels of Antioxidant test with heavy elements (Hg and pb) levels					
		SOD	MDA	CAT	GPX
Hg	Pearson Correlation	-0.087**	0.652	-0.113*	-0.272
	P-value	0.050	0.001	0.036	0.05
	N	50	50	50	50
pb	Pearson Correlation	-0.178*	0.803	-0.004**	-0.315*
	P-value	0.016	0.000	0.009	0.026
	N	50	50	50	50
****. Correlation is significant at the 0.01 level (2-tailed).**					
***. Correlation is significant at the 0.05 level (2-tailed).**					

As shown in Table 4 the results of this study show that there is an inverse relationship between (SOD, CAT, GPX) with (Hg) (-0.087, -0.113, -0.272), respectively, as well as the relationship between (SOD, CAT, GPX) and (pb) It is an inverse relationship (-0.178, -0.004, -0.315) respectively. From these results, it was shown that as the level of heavy metals (lead and mercury) increases, enzymatic antioxidants decrease.

While the relationship of (Hg, pb) with (MAD) is a direct relationship (0.652, 0.803).

Discussion

Many environmental pollutants are present in the air, water, and chemicals used in the home, which may be a cause of the risk of harmful pregnancy outcomes (16). Since pollution is everywhere, pregnant women may inhale, swallow, or absorb it through the skin, and this may directly affect pregnancy outcomes, including recurrent miscarriage (17,18).

Heavy metals are considered one of the most important sources of toxicity in the environment, including lead and mercury. Lead is considered to have a toxic effect on reproduction (19). This toxic effect affects cell functions by stopping the activity of many proteins, as well as the calcium manufacturing processes (20). Because of this toxic effect of lead, it will affect the activity of oxidative enzymes due to the oxidative stress to which the body is exposed (21), and residential areas may affect pregnancy outcomes due to economic, social, environmental, and work conditions (22). Since the areas of Basra Governorate are more vulnerable to pollution due to oil refining, natural gas, and petrochemical factories that were established near the areas on the district of Basra, pollution may be more frequent in these areas than in the city center. This study has shown that the percentage The increase in recurrent abortions in the district and sub-district areas is due to the highest percentage of contamination with heavy metals through the high levels of lead and mercury in the blood of aborted women who live in these areas. Because of this environmental pollution resulting from heavy metals, which are considered a major factor in the occurrence of miscarriage, as previous studies have shown, the process of producing free radicals will increase and this affects oxidative stress. Oxygen peroxide is produced, especially within the mitochondria, in order to fight the developing cells. Consequently, physical activity decreases due to Increased formation of reactive oxygen species (23,24). This study showed lower levels of CAT, SOD, and GPX L in women with recurrent miscarriages compared to women with no history of miscarriage. These are antioxidants that help get rid of free radicals, so their levels in the blood will decrease in women with recurrent miscarriages, so they are considered oxidative stress. Cause of pregnancy loss (25). As a result of

these oxidation processes, the level of MDA increases, as shown by this study with statistical significance (P = 0.000), although there are small differences between the aborted women and the control (26). As shown by Ozkaya et al., MDA levels in the blood of aborted women were higher than those in the control group (27). From this it is concluded that environmental pollution resulting from toxic heavy metals causes an increase in the production of oxygen peroxide, which causes an imbalance in the oxidative stress resulting from the conversion of oxygen peroxide into the O radical, (28) H_2O, and this will reduce the antioxidant enzymes that reduce free radicals and thus increase the level of MDA (29).

4. Conclusion and future scope

The study aimed to understand the relationship between some heavy metals resulting from environmental pollution and oxidative stress and its effect on recurrent and primary pregnancy loss. Heavy metals play a role in early pregnancy failure, as they have an effect on the oxidative system in the pregnant woman's body. Treating women with a history of recurrent pregnancy loss is still difficult. This study is considered a continuation of previous research efforts, which aim to reduce the burden of this condition. This is a difficult problem that has exhausted affected women for a long time, with its psychological and physical effects. It may contribute to effective treatment strategies.

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