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Assessment of Some Biochemical Parameters in Iraqi Women with Recurrent Miscarriage

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Abstract

Miscarriage a is pregnancy loss that occurs within the first 23 weeks. The risk factors that increase miscarriage is multivariate. Spontaneous loss of a conception before the 20th week of pregnancy is known as a miscarriage. There are two types of miscarriage: spontaneous and recurrent, one or more sporadic miscarriages affects approximately 25 – 50 percent of all women. Recurrent miscarriage, on the other hand, affects about 1% of couples trying to conceive. There are a number of things that can cause a miscarriage, such as inflammatory and immune system diseases. This study aims to compare the levels of calcium, Zinc, lipid peroxidation (Malondialdehyde), and superoxide dismutase (SOD) in the serum of women who have experienced a miscarriage with those of women who are pregnant and healthy. The study included 140 blood samples of women who suffered from a miscarriage and a healthy pregnant group. Calcium and Zinc were evaluated in serum of miscarriage and healthy control group, serum Malondialdehyde MDA, an indicator of lipid peroxidation, serum SOD, antioxidant enzyme, and superoxide dismutase. The calcium concentration was low in aborted women compared with the healthy pregnant control group. Serum zinc levels were lower in women who had an abortion compared to those who were pregnant and healthy. The level of serum SOD and MDA in aborted women raveled differences significantly compared with the healthy pregnant group, a decrease in the mean level of SOD was noticed in aborted women in contrast to the healthy pregnant group. At the same time, MDA showed it enhanced among the aborted group compared with healthy delivered women.

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1. Introduction

Recurrent miscarriage RM is regarded as a multifactorial etiology that may be caused by inherited or acquired factors. Several studies and identifications of the molecular mechanism underlying the pathophysiology of RM have been conducted, but 50-60% of cases remain unidentified [1]. repeated pregnancy loss (RPL) is a significant reproductive health concern because it affects 2% – 5% of couples. Because of discrepancies in the definitions and criteria utilized, as well as the characteristics of the populations, the frequency of RPL varies substantially between reports [1].

Hypocalcemia in pregnancy is seldom, but it can occur in women with hypoparathyroidism and women with severe nutritional deficiencies. It can cause several complications for both mother and unborn child during pregnancy; it has been linked to hypertensive illnesses and has been shown to enhance the risk of preeclampsia and fetal development issues [2].

A connection between hypocalcemia and elevated blood pressure recently has been identified. In investigations on sheep, maternal hypocalcemia brought on by late-pregnancy fasting raised blood pressure and reduced uterine blood flow. The risk of pregnancy-related hypertension and dietary calcium consumption is negatively associated A healthy and balanced maternal diet is essential during pregnancy to meet the mother's daily nutritional requirements and those of the developing fetus, allowing the mother to preserve her stocks of nutrients in addition to those required for fetal health and foetal development. This is because total serum calcium levels decrease during pregnancy [3].

Pregnant women are particularly vulnerable to the adverse effects of zinc deficiency, intrauterine growth retardation (IUGR), low birth weight (LBW), and preeclampsia; early neurobehavioral development; immune system delays; preterm delivery; congenital abnormalities; spontaneous abortion;, and a rise in mortality rates,all adverse outcomes of low zinc levels during pregnancy are hazardous and permanent [4].

Several environmental factors have been associated with an increased risk of miscarriage: Obesity, defined as a body mass index >30 kg/m^2, smoking, excessive caffeine consumption, excessive alcohol intake, and cocaine. Mild-to-moderate alcohol intake is not associated with increased risk, a healthy lifestyle with minimal exposure to these factors should thus be encouraged in women with RPL [5]. A dangerous byproduct of polyunsaturated fatty acid (PUFA) autooxidation, malondialdehyde (MDA), is a vital sign of oxidative damage. The capacity of the membrane to let molecules in and out and its fluidity can be affected by lipid peroxidation. It may also prevent enzymes from functioning [6].

Several studies have reported a genetic predisposition to URPL, with an increased risk in siblings of patients with URPL. Various genetic association studies have been performed, and many candidate genes identified [5].

Nomenclature			
ELISA	Enzyme-linked immunosorbent assay	SOD	Superoxide dismutase
HRP	Horseradish peroxide	PUFA	Polyunsaturated fatty acids
IUGR	Intrauterine growth retardation	ROS	Reactive oxygen species
LBW	Low birth weight	MDA	Glycation Hemoglobin

Reduced SOD activity may be responsible for ending spontaneous abortions by promoting prostaglandin production. Additionally, it has been shown that early in the first trimester, elevated plasma levels of SOD were linked to successful full-term pregnancies. SOD activity may thus play a crucial role in the preservation of fertility and early pregnancy. Interest in the antioxidant status of both healthy pregnancy and disorders associated with pregnancy has grown during the past several years. Studies have shown that antioxidant activity significantly increases during healthy pregnancy and in conditions associated to pregnancy [6].
Lipid peroxidation process denotes to the damage of polyunsaturated fatty acids that caused by oxidative stress and leads to irreversible damage of cell membrane. An increase in the generation and the manifestation of free radicals or reactive oxygen species (ROSs) and a loss in the biological system's ability to quickly detoxify the reactive middle or repair the damage that results are the two main contributors to oxidative stress [7]. SOD is one of the most important antioxidant enzymes, and its activity is considered the first line of defense in the fight against oxidative stress (OS). Cu/ZnSOD, also known as SOD1, Mn-SOD, or SOD2, and extracellular enzyme, or SOD3, are the three isoforms of SOD [7].

2. Materials and Methods

2.1. Study design

This study was done at two leading medical centers in Baghdad, Karkh Maternity and Al Alawia Maternity Hospitals, from February 2022 to August 2022. 140 women aged 18 to 43 were enrolled in this study; they were divided into the patient's group, 70 women and 70 healthy pregnant women.

2.2. Collection of samples

Quantifying the ml of venous blood from each patient by first allowing the blood to coagulate in plastic tubes. The tubes were then centrifuged at 3000 rpm for 10 minutes. After that, the sera were placed in small, labeled tubes and frozen at -40 degrees Celsius until they were needed.

2.3. Methods

Frozen serum and whole blood were brought to room temperature to defrost before being tested for inorganic elements. Zinc was tested using Flame absorption atomic spectrophotometry (FAAS), while inorganic elements were measured using graphite furnace atomic absorption spectrophotometry (GFAAS). The principle of FAAS is the dissociation of an element from its chemical bonds caused by the heat of a flame, resulting in the formation of unexcited or grounded stated atoms. These neutral atoms can absorb radiant energy and transform into excited states when exposed to light with a specific wavelength. The concentration of trace elements in the sample affects how much radiant energy is absorbed in the specific wavelength of each element. Typically, a calibration curve obtained using standards of known concentration is used to determine the analyte's concentration [8].

2.3.1. Assessment of Calcium in serum sample

Under alkaline conditions, this compound is formed when calcium ions combine with 5-nitro-5'-methyl-BAPTA (NM - BAPTA). The second stage involves a reaction between this complex and EDTA. The change in absorbance is measured photometrically at Wavelength (340 - 378) nm and is directly concentrations of superoxide dismutase, lipid peroxidation, and a few trace metals (Zn, Ca) (MDA). Serum was extracted from 5 proportional to the calcium concentration. Automatic sampling, reagent delivery, mixing, processing, and printing results were carried out. Working calibration solutions of zinc were prepared from stock solutions ranging from zero to at least three or more concentrations. Aspiration of blank (Standards of 0 concentration) has been made in order to prepare baseline to read 0 absorbances; this step was frequently repeated in order to correct baseline drift. Working standard was sequentially analyzed from the most diluted to the most concentrated ones [9].

2.3.2. Determination of zinc

For calibration, zinc concentrations (0.0, 50, 100, 150, and 200 g/dl) were prepared. 0.5 ml of serum was taken from the thawed sample and diluted to 5 ml with deionized water (1:10). After mixing, the diluted serum was aspirated into flame [10].

2.3.3. Determination of SOD in serum

This kit uses Competitive-ELISA. This kit includes a human SOD-coated micro-ELISA plate. Human SOD1 on the solid phase supporter competes for locations on the biotinylated Detection Ab. Incubating the microplate after adding Avidin conjugated to HRP. The plate's unbound sample, standard, and conjugates are removed. Each well receives a TMB substrate solution. Measure the color change at 450 nm after stopping the enzyme-substrate reaction. SOD is compared to a standard curve to assess human SOD concentration [11 - 14], Fig. 1.

2.3.4. Determination of MDA in serum

Assaying with enzyme-linked immunosorbent assay (ELISA). The plate was pre-coated with an anti-human-MDA antibody. The MDA from the sample is added to the wells with the coated antibodies, and a biotinylated human MDA antibody binds to the MDA from the sample. Streptavidin-HRP is then added to bind the Biotinylated MDA antibody. Following incubation, unbound Streptavidin-HRP has washed away.

Human MDA concentration is then connected to the additional substrate solution and the resulting color. The reaction is terminated by adding an acidic stop solution, and its completion is detected by measuring its absorbance at 450 nm [14], Fig. 2.

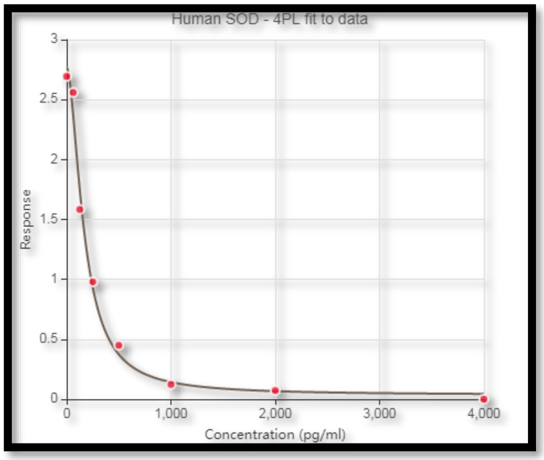

Fig 1. Standard curve for the concentration of human SOD

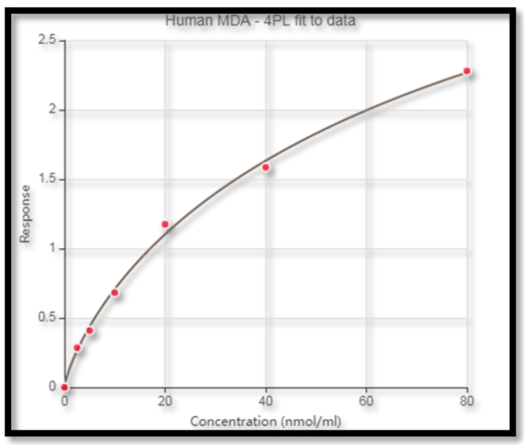

Fig 2. Standard curve for the concentration of human MDA

2.4. Statistical analysis

Data analysis was done using SPSS software package version 20.0. Quantitative data were reported as mean and standard error, while qualitative data were expressed as a number and percentage. When applicable, Student's t test and Pearson correlation coefficient were employed, and a p value of 0.01 and 0.001 was used to indicate if the results were significant.

3. Results

Table 1 showed the Mean and standard error of the aborted maternal age was (25.95± 0.92) while it was (25.51± 0.92) in the healthy, non-aborted group. No significant differences were documented between them.

Table 1 The Mean and SE of age of miscarriage women and healthy pregnant

Parameter	Groups	N	Mean±SE	T-test	*P-value*	C.S
Age (Years)	miscarriage women	70	25.95±1.00	0.32	0.7	N.S
	Healthy pregnant women	70	25.51±0.92			

In calcium level, the differences between the groups of pregnant women that are statistically significant who had healthy pregnancies and those who had abortions were found, as shown in Table 2 (9.44 ±0.07at a p-value of 0.001). When compared to the pregnant control group of healthy women.

Table 3 revealed significance differences in the level of zinc between aborted women 53.55± 1.81 and healthy 80.37±1.53 compared with aborted women at a P value >0.001.

Table 4 showed highly significant differences in the level of serum SOD and MDA in aborted women 32.20± 4.408, 10.41± 1.5, respectively, compared with the healthy Pregnant group (60.17± 6.21), (4.64± 0.32) decrease in the mean of level serum SOD was noticed in aborted women compared with the healthy pregnant group.

Table 2 Mean and standard error (SE) of the concentration of calcium level in serum of studied groups (aborted and healthy pregnant women)

Parameters	Groups	No.	Mean±SE	T-test	P-value	
Calcium (mg/dl)	Aborted women	70	7.87±0.11	11.50	>0.001	High significant
	Healthy pregnant women	70	9.44±0.07			

Table 3 Mean and Standard error (SE) of level of zinc concentration in aborted women and healthy pregnat groups

Parameter	Study Groups	N	Mean ±SE	T-test	P-value	C.S
Zinc (mg/dl)	Aborted women	70	53.55±1.81	11.28	>0.001	H.S
	Healthy pregnant women	70	80.37±1.53			

Table 4 Mean and Standard error (SE) of the levels of SOD and MDA in aborted women and healthy control groups

Parameter	Study groups	N	Mean±SE	T-test	P-value
Serum. SOD (Unit/ml)	Aborted women	70	32.20±4.408	3.7	0.001 (H.S)
	Pregnant not aborted women	70	60.17±6.21		
Serum MDA (Unit/ml	Aborted women	70	10.41±1.55	3.63	0.001 (H.S)
	Pregnant not aborted women	70	4.64±0.32		

4. Discussion

Our results are consistent with those of other studies that has found no statistically significant correlation between maternal age and the having full term baby. However, a study conducted by Fatema and Alkbay, 2016 found that the rate of abortion in the Kurdistan Region was 27.7% and that maternal age was significantly associated with the decision to have an abortion. No statistically significant variations in age at first pregnancy loss were found compared to a control group of healthy pregnant women.

The women who had abortions had lower Ca concentrations. According to this study, maternal calcium metabolic stress, rather than insufficient calcium intake or vitamin D deficiency, has a deleterious impact on fetal development [14]. Calcium levels are the substrate for the mineralization of bones. Insufficient calcium intake or excessive calcium losses prevent the development or maintenance of skeletal mass. In humans, hydroxyapatite accounts for more than 99 percent of the calcium, whereas the remaining 5–6 g are split between intracellular and extracellular compartments, with just 1.3 g outside of cells [15]. Only the ionized calcium, which makes up half of the plasma, is metabolically active and impacts the body's functions. 60% of the remaining plasma calcium is partially attached to minor anions such as phosphate, carbonate, citrate, lactate, and sulfate, and 40% is delivered partially linked to plasma proteins [16].

The American College of Obstetricians and Gynecologists has advised that women who are 19 years of age and older who are pregnant or breastfeeding should consume 1,000 mg of calcium daily, while teenage mothers need a little more. A large amount of calcium in their bodies keeps their bones healthy, and the rest is stored in their bodies to support the baby's growth [17]. Several observational studies suggest that diets low in essential vitamins and minerals can pose a significant reproductive risk.According to researchers, several decreases in the mean level of serum SOD were noticed in aborted women compared with the healthy pregnant group; optimal trace metal concentrations are also necessary for a good pregnancy and pregnancy outcome [18-19].

The metal zinc is an omnipotent metal that has amphoteric nature. Hence, it is ionized either in acidic or alkaline forms. Content of zinc is 2-3 ng the average body content of zinc is 2-3 g in an average adult [20].

Thaker et al., 2019, [21] were incompatible with the findings of the current investigation. They reported that compared to full-term groups, maternal serum Zn and calcium levels were lower in preterm subjects, and Premature membrane rupture is substantially less common in pregnant women. MDA showed it enhanced among aborted women health pregnant group. The results were in agreement with Numerous studies that discovered decreased total SOD activity in spontaneous abortions with vaginal bleeding and speculated that oxidative stress, as evidenced by reduced SOD activity, may be involved in the termination of spontaneous abortions by promoting prostaglandin synthesis.

Additionally, it has been noted that early in the first trimester, elevated plasma levels of SOD were linked to successful full-term pregnancies. SOD activity may therefore play a crucial role in preserving fertility and early pregnancy. Interest in the antioxidant status of healthy pregnancy and diseases associated with pregnancy has grown over the past few years. Studies have shown that antioxidant activity significantly increases during healthy pregnancy and in pregnancy-related conditions [21].

Malondialdehyde (MDA) is a significant byproduct of cellular breakdown (Altuhafi, Altun, and Hadwan 2021). To gauge the severity of lipid peroxidations, superoxide from lipid peroxides can be used. In addition to converting the Superoxide anion to hydrogen peroxide (H_2O_2), glutathione peroxidase (G-Px) plays a crucial role in detoxifying H2O2 into water and nonreactive oxygen molecules.

Reactive oxygen species' oxidative attack on vital cell components due to an unbalanced oxidant-antioxidant system is acknowledged to impact the development of placental insufficiency disorders like preeclampsia and fetal growth restriction. Early pregnancy failure may also be linked to the placenta's overall oxidative stress, which causes apoptosis and a decrease in type IV collagen [22], increases in oxidative stress18 and loss of antioxidant defences19 are associated with early pregnancy loss this have been demonstrated to be significantly correlated in women with early pregnancy loss [23].

Duhig et al. 2012 also reported that elevation in plasma levels of MDA along with decreased levels of SOD and other antioxidant enzymes was associated with enhanced lipid peroxidation, which may end in spontaneous abortion.[23] Serum SOD activity is reported to be important for corpus luteum activity, embryonic development, and maintenance of early pregnancy. In the gestational corpus luteum, theca interna cells stain heavily for SOD activity [24].

5. Conclusion

In this study, many solid findings are found. Firstly, the frequency in the occurrence of abortion in the first trimester was higher than what is observed in the second and third trimester. Secondly, the concentration of Ca was much lower in aborted women compared to the healthy pregnant control group. Additionally, the level of zinc in the blood of women who had an abortion was lower than that of healthy pregnant women. Finally, in women who had abortions, there was a decrease in the mean level of SOD. In contrast to healthy delivered women, MDA revealed that it improved in the group of women who had abortions. These findings shed the light on many aspects that are related to abortion and we recommend more studies to find out the actual mechanism/s that might lead to abortion.

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Declarations

Ethics approval and consent to participate: the study protocol was approved by the ethics committee of Republic epublicof Heath The purpose of the study was explained, and an informed written consent was taken before taking any data or doing any investigations. The participants were informed that their participation was voluntary and that they could withdraw from the study at any time without consequences.

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