Prospective study on biochemical markers causing recurrent miscarriages in Iraqi women of reproductive age

Ruqaya Kahtan Abbas, Amani Jasim, Qater Al Nada Ali Kanaem Al Ibady

Abstract

Objective: To compare the levels of elemental calcium, vitamin D3, malondialdehyde and super oxide dismutase enzymes in serum of women having recurrent miscarriages with normal healthy pregnant women.

Method: The prospective study was conducted at Al-Karkh Maternity Hospital, Baghdad, Iraq, and Al-Alawia Maternity Hospital, Baghdad, Iraq, from February to August 2022, and comprised adult pregnant women having recurrent miscarriages without being hypertensive or diabetic in group A and normal healthy pregnant women in control group B. Blood samples were obtained from all the subjects. Besides, placental tissues were obtained from some members from both the groups. Levels of elemental calcium, vitamin D3, malondialdehyde and super oxide dismutase enzymes in serum were evaluated and compared between the groups. Malondialdehyde and super oxide dismutase enzymes in the placental tissues were also compared. Data was analysed using SPSS 20.

Results: Of the 140 women, 70(50%) were in each of the 2 groups. The overall age range of the sample was 18-43 years. Placental tissues were obtained from 40(57.14%) in group A and 30(42.85%) in group B. In group A, miscarriage was reported in the first trimester in 45(62.3%) cases, 21(30%) in the second trimester and 4(5.7%) in the third trimester. Mean level of serum elemental calcium, vitamin D3, malondialdehyde and super oxide dismutase were significantly different between the groups (p<0.05), and the same was the case with the placental tissues (p<0.05).

Conclusion: The causes of recurrent miscarriage in women could be attributed to changes in the levels of elemental calcium, vitamin D3, super oxide dismutase and malondialdehyde in serum and placental tissues.

Keywords: Pregnancy, Abortion, Spontaneous, Malondialdehyde, Cholecalciferol, Placenta, Vitamins, Oxides.

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Introduction

The occurrence of two or more miscarriages is characterised as recurrent pregnancy loss. The risk is between 9% and 12% for women aged <35, but it rises to 50% for women aged >40. Different societies have employed various nomenclature systems.

Throughout gestation, essential vitamins and minerals, including vitamin D, support maternal health and foetal development via mechanisms that are integrated across placental, maternal and foetal compartments. However, in most cases, deficiencies or excesses of these trace elements are linked to human diseases. Because 25-hydroxyvitamin D (25-OH-D) is the most common circulating form of vitamin D in the human body, a small amount is metabolised in the kidney to other di-hydroxyl forms. It is considered the best index of human vitamin D status. Malondialdehyde (MDA) is one of the final products of polyunsaturated fatty acids peroxidation in the cells. An increase in free radicals causes overproduction of MDA. Malondialdehyde (MDA) is a marker of lipid peroxidation. The primary enzymes for the detoxification of superoxide anion are glutathione peroxidase (GPX) and superoxide dismutase (SOD), which are present in the cytoplasm of all aerobic cells that convert superoxide anion to hydrogen peroxide (H2O2).

Malondialdehyde (MDA) is one of the final products of polyunsaturated fatty acids peroxidation in the cells. An increase in free radicals causes overproduction of MDA. Malondialdehyde level is commonly known as a marker of oxidative stress and the antioxidant status in cancerous patients MDA, a major element, splits off from lipid peroxides in the pathogenesis of placental deficiency, which is the process of oxidative attack on essential cell components by reactive oxygen species (ROS) resulting from disturbed oxidant-antioxidant balance. Increased oxidative stress in the placental tissues during normal gestation, decrease in placental oxidative stress in the second and third trimesters appear to be a principle of physiological aspects of normal pregnancy.

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The current study was planned to compare the levels of elemental calcium, vitamin D3, malondialdehyde and super oxide dismutase enzymes in serum of women having recurrent miscarriages with normal healthy pregnant women.

**Patients and Methods**

The prospective study was conducted at Al-Karkh Maternity Hospital, Baghdad, Iraq, and Al-Alawia Maternity Hospital, Baghdad, Iraq, from February to August 2022. The sample comprised adult pregnant women having recurrent miscarriages without being hypertensive or diabetic in group A and normal healthy pregnant women in control group B. Women with history of hypertension or diabetes, and those who were unable to comprehend the questions or not willing to participate were excluded.

After taking informed consent, 5ml serum blood samples were obtained from all the subjects and put in plastic tubes and left to clot. They were then, centrifuged at 3000 rpm for 10 min. sera, stored at -40 °C in small tubes with accurate labels with the name until the time of estimation. Levels of elemental calcium (Ca), vitamin D3, MDA and SOD in serum were evaluated and compared between the groups.

Besides, placental tissues were obtained from some members from both the groups. Placental tissues were weighed then homogenised in 10ml of 0.01M tris(hydroxymethyl) aminomethane (THAM) hydrochloride (Tris-HCl) buffer using tissue homogeniser. The obtained homogenates were centrifuged at 4,000g for 30min. Supernatants were stored at -80°C. Before analysis, the snap-frozen tissue was thawed and centrifuged at 14,500g for 15min for estimation of SOD and MDA in placental tissues.

Calcium was estimated by an enzymatic method using an automated spectrophotometer.

A competitive enzyme-linked immunosorbent assay (ELISA) was used to determine serum vitamin D, and vitamin D concentrations in all samples were spectrophotometrically evaluated at 450nm and analysed using an analysis kit to minimise analytical variation.

MDA and SOD levels in serum were measured using the calorimetric method. It was based on the reaction of MDA with thiobarbituric acid in an acidic medium at 95°C for 30min to form a thiobarbituric acid reactive product. The absorbance of the resulting product could be measured spectrophotometrically at 534nm.

The principle of the test depended on the capability of SOD to inhibit the phenazine methosulphate-mediated reduction of nitro-blue tetrazolium dye. The change in the absorbance over 5min was measured at 560nm for controls, and for the cases at 25°C.

Data was analysed using SPSS 20.0. Quantitative data was expressed as mean and standard deviation (SD), while qualitative data was expressed as frequencies and percentages. Pearson correlation coefficient were also employed where necessary. P<0.05 was considered significant.

**Results**

Of the 140 women, 70(50%) were in each of the 2 groups. The overall age range of the sample was 18-43 years. Placental tissues were obtained from 40(57.14%) in group A and 30(42.85%) in group B. In group A, miscarriage was reported in the first trimester in 45(62.3%) cases, 21(30%) in the second trimester and 4(5.7%) in the third trimester (Table 1).

The mean level of serum elemental Ca, vitamin D3 (Table 2) as well as MDA and SOD enzymes (Table 3, Figure 1) were significantly different between the groups (p<0.05).

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<th>Parameters</th>
<th>Study groups</th>
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<th>Mean ± SE</th>
<th>t-test</th>
<th>p-value</th>
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<tbody>
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<td>Calcium (mg/dl) Cases 70</td>
<td>7.87±0.11</td>
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<td>Vitamin D3 (ng/dl) Cases 70</td>
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<td>Serum. SOD (Unit/ml) Cases 70</td>
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<tr>
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<td>Serum MDA (Unit/ml) Cases 70</td>
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<tr>
<td>Control 70</td>
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<td>3.63</td>
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<td>Placenta MDA Cases 40</td>
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<tr>
<td>Control 30</td>
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<td>HS</td>
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</table>

SOD: Super oxide dismutase; MDA: Malondialdehyde, HS: Highly significant.
There was a negative correlation between SOD and MDA levels in the serum of group A (Table 5). Also, placenta’s MDA levels were higher in group A compared to group B, while placental SOD levels were lower in group A compared to group B (Table 6).

### Discussion

The occurrence of abortion in the first trimester was 64.3% in the current study, while it was 30% in the second trimester and 5.7% in the third trimester. An earlier study reported that risk of miscarriage was 1-3% in the first trimester and relative risk increased in line with the number of previous miscarriages.

A study reported that mother’s calcium metabolic stress, in addition to insufficient intake of calcium or vitamin D, had a negative effect on foetal growth. Calcium is the bone mineralisation substrate. If calcium intake is not sufficient, the skeletal mass cannot be built or maintained, as 99% of calcium is stored in the bones, while the remainder is placed in intracellular, extracellular compartments. Half of the calcium in plasma is free or ionized, and only the ionised calcium is metabolically active and influences the body functions. Of the other half, 40% is transported while being partly bound to plasma proteins, and the rest is bound to small anions, such as phosphate, carbonate, citrate, lactate and sulphate. A study in 2022 found a significant link between vitamin D deficiency and missed abortions, and serum 25-OH-D levels were also lower in mothers who miscarried compared to those who had a normal pregnancy. This was in line with the current findings. Numerous causes contribute to this deficiency’s high prevalence rate, including osteoporosis in mothers and new-borns.

The current findings related to SOD were in agreement with a study in Turkey, indicating that SOD activity may have a crucial role in the preservation of fertility and early pregnancy. Literature showed that antioxidant activity significantly increased through healthy gestation and in pregnancy-related diseases and increased total oxidant status might be involved in the pathophysiology of vaginal bleeding through early first trimester case of pregnancies.

MDA, a major breakdown product, can be employed to assess the degree of lipid peroxidation. ROS oxidative attacks on vital cell components as a result of an unbalanced oxidant-antioxidant system are acknowledged to have a role in the pathophysiology of placental insufficiency disorders, like pre-eclampsia and foetal growth limitation.

Placenta is the main source of oxidative stress during gestation because of the metabolic demand to support normal foetal development. Placental ROS plays a role in the regulation of cell signalling pathways’ response to stimuli and insults, including disruptions in the maternal blood supply to the placenta and inflammation. Oxidative stress develops in a number of maternal, placental and foetal diseases, leading to abortion when the placental generation of ROS overwhelms the endogenous antioxidant defences.

A study evaluated MDA and SOD to elucidate the role of oxidative stress in miscarriage, and significantly high MDA levels indicated systemic oxidative stress.

Further supporting the current findings are studies that...
reported significantly higher serum MDA levels and lower SOD activity in patients with a history of recurrent miscarriage than in women with a healthy pregnancy. An increase in MDA levels may reflect an overproduction of lipid peroxides and an impaired antioxidant defence mechanism, as well as an increasing concentration of lipid peroxides in the villous and decidua undergoing early pregnancy.²²

An elevation in MDA levels along with decrease in SOD levels and other antioxidant enzymes associated with the enhancement of lipid peroxidation may result in spontaneous miscarriage, and, therefore, serum SOD activity is important for corpus luteum activity, embryonic development and the maintenance of early pregnancy.²³

When oxidative stress develops early during pregnancy, it can impair placental development and enhance syncytiotrophoblast degeneration. Hemispheric sensitivity of syncytiotrophoblast to OS during the first trimester may contribute significantly to idiopathic recurrent pregnancy loss (RPL).²² One study documented an increase in oxidative stress in placental tissues during normal gestation. As the pregnancy progressed, placental antioxidant protection mechanisms increased and lipid peroxidation markers resulted in a diminution in oxidative stress, causing decrease in placental oxidative stress in the second and third trimesters.⁷

Rising of antioxidants has been documented in healthy pregnancies, while diminished levels are found in women with a history of miscarriages.⁷

The current findings are also in line with previous studies.⁴,²⁴,²⁵

The current study has limitations as the sample size was not calculated which could have reduced the power of the study. Besides, the sample size was small as there were not enough cases available.

Conclusions

Miscarriage in the first trimester was higher than in the second and third trimesters. Also, elemental Ca, vitamin D₃, MDA and SOD levels were significantly different among women with miscarriages compared to healthy pregnant women. The same was the case with SOD and MDA values for the placental tissues.

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Conflict of Interest: None.

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References


