RESEARCH ARTICLE

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 superoxide 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.

DOI: https://doi.org/10.47391/JPMA.IQ-17

Introduction

The occurrence of two or more miscarriages is characterised as recurrent pregnancy loss.[1] 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.[2]

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.[3] 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.[3,4] It is considered the best index of human vitamin D status.[5]

According to an assessment of oxidative stress and antioxidant biomarkers, they are useful tools for estimating the risk factor of oxidative damage process and are closely associated with the occurrence of diseases causing foetal loss.[5]

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 (H_2O_2).[5]

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.[6] 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.[7]

College of the Health and Medical Technology, Middle Technical University, Baghdad, Iraq.

Corrospondience: Ruqaya Kahtan Abbas

email: ruqayaaljuboori60@gmail.com

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.[8]

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.[4]

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.[4]

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.[4]

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). SOD and MDA values for the placental tissues were also significantly different (p<0.05) (Table 4, Figure 2).

Table-1: Distribution of pregnant women in the miscarriage group (n=70).

Cases	First Trimester	Second Trimester	Third Trimester	Total
Aborted group	45 (64.3%)	21 (30%)	4 (5.7%)	70 (100%)

Table-2: Inter-group comparison of the serum concentration of calcium and vitamin D3.

Parameters	Study groups	n	Mean ± SE	*t*-test	*p*-value
Calcium (mg/dl)	Cases	70	7.87±0.11	11.50	>0.001
	Controls	70	9.44±0.07		HS
Vitamin D3 (ng/dl)	Cases	70	13.22±0.80	23.69	>0.001
	Control	70	43.47± 0.99	23.69	HS

SE: Standard error. HS: Highly significant.

Table-3: Inter-group comparison of the level of SOD and MDA.

Parameters	Groups	n	Mean ± SE	*t*-test	*p*-value
Serum. SOD (Unit/ml)	Cases	70	32.20±4.408	3.7	0.001
	Controls	70	60.17±6.21		(HS)
Serum MDA (Unit/ml)	Cases	70	10.41±1.55	3.63	0.001
	Controls	70	4.64±0.32		(HS)

SOD: Super oxide dismutase; MDA: Malondialdehyde, HS: Highly significant.

Table-4: Inter-group comparison of the level of SOD and MDA in placental tissues.

Parameters	Study group	n	Mean ± SE	*t*-test	*p*-value
Placenta. SOD	Cases	40	39.09±8.20	6.8	>0.001 (HS)
165-240 (Unit/ml)	Controls	30	182.82±19.16		
Placenta MDA	Cases	40	9.41±0.22	8.09	>0.001 (HS)
	Controls	30	6.72±0.24		

SOD: Super oxide dismutase; MDA: Malondialdehyde, HS: Highly significant.

Table-5: Correlation coefficient between the level of SOD and MDA in the serum samples of pregnant women in the miscarriage group.

Parameters	SOD		MDA	
	r-value	*p*-value	*r*-value	*p*-value
SOD	1	-	- 0.03	0.78
MDA			1	-

SOD: Super oxide dismutase; MDA: Malondialdehyde.

Table-6: Correlation coefficient between the level of SOD and MDA in placental tissue samples of pregnant women in the miscarriage group.

Parameters	SOD		MDA	
	r-value	*p*-value	*r*-value	*p*-value
SOD	1	-	- 0.4	0.001
MDA	-		1	-

SOD: Super oxide dismutase; MDA: Malondialdehyde.

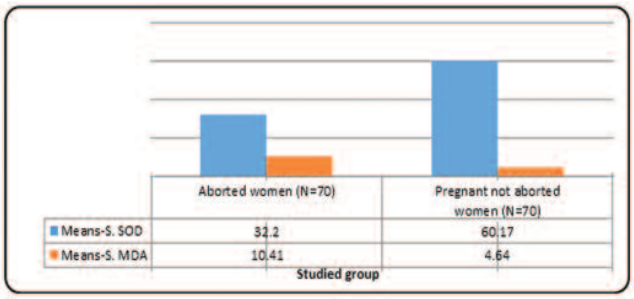

Figure 1: Inter-group comparison of mean and standard error of serum SOD and MDA.

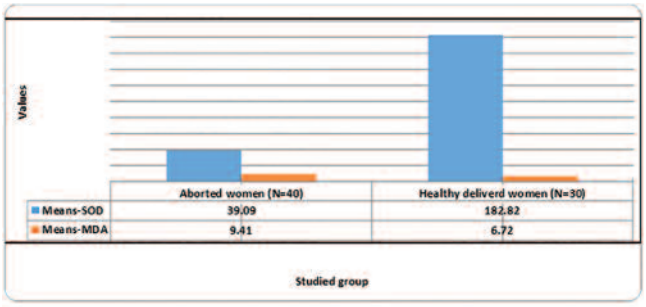

Figure 2: Inter-group comparison of mean and standard error of SOD and MDA in placental tissues.

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[9] 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.[10]

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.[11] 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.[12]

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.[12] 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.[13]

The current findings related to SOD were in agreement with a study in Turkey,[14] 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[15] and increased total oxidant status might be involved in the pathophysiology of vaginal bleeding through early first trimester case of pregnancies.[16]

MDA, a major breakdown product,[17] 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.[18]

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.[19] 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.[20]

A study evaluated MDA and SOD to elucidate the role of oxidative stress in miscarriage, and significantly high MDA levels indicated systemic oxidative stress.[21]

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.[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.

Acknowledgment: We are grateful to the Biochemistry Department and the Blood Collection Unit at Al-Karkh Maternity Hospital and Al-Alawia Maternity Hospital.

Disclaimer: None.

Conflict of Interest: None.

Source of Funding: None.

References

1. Zegers-Hochschild F, Adamson GD, de Mouzon J, Ishihara O, Mansour R, Nygren K, et al. International Committee for Monitoring Assisted Reproductive Technology; World Health Organization. International Committee for Monitoring Assisted Reproductive Technology (ICMART) and the World Health Organization (WHO) revised glossary of ART terminology, 2009. Fertil Steril 2009;92:1520-4. doi: 10.1016/j.fertnstert.2009.09.009.
2. Schreiber K, Sciascia S, de Groot PG, Devreese K, Jacobsen S, Ruiz-Irastorza G, et al. Antiphospholipid syndrome. Nat Rev Dis Primers 2018;4:17103. doi: 10.1038/nrdp.2017.103.
3. Kant S, Haldar P, Gupta A, Lohiya A. Serum calcium level among pregnant women and its association with pre-eclampsia and delivery outcomes: A cross-sectional study from North India. Nepal J Epidemiol 2019;9:795-803. doi: 10.3126/nje.v9i4.23150.
4. Cai Z, Zhang Q, Xia Z, Zheng S, Zeng L, Han L, et al. Determination of serum 25-hydroxyvitamin D status among population in southern China by a high accuracy LC-MS/MS method traced to reference measurement procedure. Nutr Metab (Lond) 2020;17:e8. doi: 10.1186/s12986-020-0427-7.
5. Kulkarni R, Deshpande A, Saxena R, Saxena K. A study of serum malondialdehyde and cytokine in tuberculosis patients. J Clin Diagn Res 2013;7:2140-2. doi: 10.7860/JCDR/2013/5736.3452.
6. Powe CE, Seely EW, Rana S, Bhan I, Ecker J, Karumanchi SA, et al. First trimester vitamin D, vitamin D binding protein, and subsequent preeclampsia. Hypertension 2010;56:758-63. doi: 10.1161/HYPERTENSIONAHA.110.158238.
7. Basu J, Bendek B, Agamasu E, Salafia CM, Mishra A, Benfield N, et al. Placental Oxidative Status throughout Normal Gestation in Women with Uncomplicated Pregnancies. Obstet Gynecol Int 2015;2015:276095. doi: 10.1155/2015/276095.
8. Dev K, Mir NA, Biswas A, Kannoujia J, Begum J, Kant R, et al. Dietary synbiotic supplementation improves the growth performance, body antioxidant pool, serum biochemistry, meat quality, and lipid oxidative stability in broiler chickens. Anim Nutr 2020;6:325-32. doi: 10.1016/j.aninu.2020.03.002.
9. Toth B, Würfel W, Bohlmann M, Zschocke J, Rudnik-Schöneborn S, Nawroth F, et al. Recurrent Miscarriage: Diagnostic and Therapeutic Procedures. Guideline of the DGGG, OEGGG and SGGG (S2k-Level, AWMF Registry Number 015/050). Geburtshilfe Frauenheilkd 2018;78:364-81. doi: 10.1055/a-0586-4568.
10. Almaghamsi A, Almalki MH, Buhary BM. Hypocalcemia in Pregnancy: A Clinical Review Update. Oman Med J 2018;33:453-62. doi: 10.5001/omj.2018.85.
11. Jafri L, Khan AH, Azeem S. Ionized calcium measurement in serum and plasma by ion selective electrodes: comparison of measured and calculated parameters. Indian J Clin Biochem 2014;29:327-32. doi: 10.1007/s12291-013-0360-x.
12. Fong J, Khan A. Hypocalcemia: updates in diagnosis and management for primary care. Can Fam Physician 2012;58:158-62.
13. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2011;96:1911-30. doi: 10.1210/jc.2011-0385.
14. Ozkaya O, Sezik M, Kaya H. Serum malondialdehyde, erythrocyte glutathione peroxidase, and erythrocyte superoxide dismutase levels in women with early spontaneous abortions accompanied by vaginal bleeding. Med Sci Monit 2008;14:CR47-51.
15. Deveer R, Deveer M, Engin-Üstün Y, Akbaba E, Uysal S, Sarikaya E, et al. Role of Oxidative Stress on Vaginal Bleeding during The First Trimester of Pregnant Women. Int J Fertil Steril 2014;7:271-4.

16. Sugino N, Nakata M, Kashida S, Karube A, Takiguchi S, Kato H. Decreased superoxide dismutase expression and increased concentrations of lipid peroxide and prostaglandin F(2alpha) in the decidua of failed pregnancy. Mol Hum Reprod 2000;6:642-7. doi: 10.1093/molehr/6.7.642.
17. Altuhafi A, Altun M, Hadwan MH. The Correlation between Selenium-Dependent Glutathione Peroxidase Activity and Oxidant/Antioxidant Balance in Sera of Diabetic Patients with Nephropathy. Rep Biochem Mol Biol 2021;10:164-72. doi: 10.52547/rbmb.10.2.164.
18. Aouache R, Biquard L, Vaiman D, Miralles F. Oxidative Stress in Preeclampsia and Placental Diseases. Int J Mol Sci 2018;19:1496. doi: 10.3390/ijms19051496.
19. Burton GJ, Jauniaux E. Oxidative stress. Best Pract Res Clin Obstet Gynaecol 2011;25:287-99. doi: 10.1016/j.bpobgyn.2010.10.016.
20. Toboła-Wróbel K, Pietryga M, Dydowicz P, Napierała M, Brązert J, Florek E. Association of Oxidative Stress on Pregnancy. Oxid Med Cell Longev 2020;2020:e6398520. doi: 10.1155/2020/6398520.
21. Banaei M, Torkzahrani S, Ozgoli G, Azad M, Mahmoudikohani F, Pormehr-Yabandeh A. Addressing the Sexual Function of Women During First Six Month After Delivery: Aquasi-Experimental Study. Mater Sociomed 2018;30:136-40. doi: 10.5455/msm.2018.30.136-140.
22. Al-Sheikh YA, Ghneim HK. 'The effect of micronutrients on superoxide dismutase in senescent fibroblasts'. Cell Biochem Funct 2011;29:384-93. doi: 10.1002/cbf.1761.
23. Ramandeep K, Kapil G, Harkiran K. Correlation of enhanced oxidative stress with altered thyroid profile: Probable role in spontaneous abortion. Int J Appl Basic Med Res 2017;7:20-5. doi: 10.4103/2229-516X.198514.
24. Bakacak M, Kılınç M, Serin S, Ercan Ö, Köstü B, Avcı F, et al. Changes in Copper, Zinc, and Malondialdehyde Levels and Superoxide Dismutase Activities in Pre-Eclamptic Pregnancies. Med Sci Monit 2015;21:2414-20. doi: 10.12659/MSM.895002.
25. El-Far M, El-Sayed IH, El-Motwally Ael-G, Hashem IA, Bakry N. Tumor necrosis factor-alpha and oxidant status are essential participating factors in unexplained recurrent spontaneous abortions. Clin Chem Lab Med 2007;45:879-83. doi: 10.1515/CCLM.2007.138.