

Oxidative stress in recurrent pregnancy loss women

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ABSTRACT

الأهداف: التحقق من التغيرات الكيميائية المرتبطة بعملية أكسدة الدهون، وأكسيد النيتروجين، وفيتامين E عند النساء اللواتي فقدن حملهن بشكل متكرر مع النساء الحوامل السليمات وغير الحوامل.

الطريقة: أجريت دراسة الحالة في بغداد، مستشفى الكاظمية التعليمي خلال الفترة من سبتمبر 2008م حتى ديسمبر 2009م. اشتملت الدراسة على 96 امرأة، منهن 32 مريضة فقدن حملهن بشكل متكرر، بينما 32 امرأة حامل في الثلث الثالث و32 امرأة غير حوامل نماذج ضابطة. جمعت عينات الدم من المرضى أثناء فقدهن للطفل. تم استخدام مصل المرضى، والنماذج الضابطة لتقدير المالونالدهيد، وأكسيد النيتروجين، وفيتامين هاء.

النتائج: وجد أن هناك ارتفاعاً كبيراً وملحوظاً في مستوى المالونالدهيد بمصل المريضات اللواتي فقدن حملهن بشكل متكرر مقارنة مع النساء الحوامل في الثلث الثالث $p=0.002$ والغير حوامل $p=0.0001$. وقد أظهر مصل المرضى انخفاضاً كبيراً في مستوى كل من فيتامين E، وأكسيد النيتروجين عند مقارنتهم مع النساء الحوامل في الثلث الثالث، والغير حوامل. لم يلاحظ أي علاقة مهمة بين أكسيد النيتروجين والمالونالدهيد لمصل المرضى فيما ظهرت هناك علاقة مهمة بين المالونالدهيد، وفيتامين E، وأكسيد النيتروجين.

خاتمة: أن انخفاض مستويات أكسيد النيتروجين، وفيتامين E عند النساء اللواتي فقدن حملهن بشكل متكرر هو نتيجة لفقدان المتكرر وليس عاملاً مسبباً لفقدان الأطفال المتكرر.

Objectives: To investigate biochemical changes in lipid peroxidation, nitric oxide, and vitamin E in recurrent pregnancy loss women, and compare these with healthy pregnant, and non-pregnant women.

Methods: A case control study was conducted from September 2008 to December 2009 at Al-Khadimiya Teaching Hospital, Baghdad, Iraq. Ninety-six subjects were included in the study, 32 were patients with recurrent pregnancy loss (RPL), and 32 pregnant women in their third trimester, and another 32

non-pregnant women were used as controls. Blood samples were collected from each patient at the time of pregnancy loss. Serum from patients and controls was then used to estimate malondialdehyde (MDA), nitric oxide (NO), and vitamin E levels.

Results: There was a significant elevation in patient serum MDA compared with third trimester pregnant women ($p=0.002$) and non-pregnant women ($p=0.0001$). Both serum vitamin E and NO levels in RPL patients also showed a highly significant decrease compared with third trimester pregnant, and non-pregnant women. A highly significant difference was found in the MDA/vitamin E ratio between RPL and control groups, while no significance was found between RPL and control groups' NO/vitamin E ratio.

Conclusion: The decrease in NO production and vitamin E is a result of RPL and not a causative factor, as the RPL was without pathological cause, medication, or fibroid presence, and no significant difference was found between the NO/vitamin E ratio in RPL and controls group.

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Oxidative stress occurs when there is an elevated concentration of intracellular reactive oxygen species (ROS) in a steady state condition. However, when the balance between ROS and antioxidants is tipped towards overabundance of ROS, oxidative stress results.¹ This results from the imbalance of the oxidative-antioxidative system, when excessive free radical production occurs with low antioxidant defense, which leads to biomolecule chemical

alterations.³ Polyunsaturated fatty acids are oxidized in vivo by free radicals and other reactive species, and subsequent degradation of oxidized lipid molecules leads to the formation of several specific metabolites that include aldehydes of variable chain length, such as malondialdehyde (MDA).⁴ Lipid peroxides are disintegrated quickly and form reactive carbon compounds. Among these, MDA is an important reactive carbon compound, which is used commonly as an indicator of lipid peroxidation.⁵ In disease states, such as toxemia of pregnancy, an imbalance between lipid peroxidation and antioxidant mechanisms could impair normal endothelial function. Serum lipid peroxidation products are increased in pregnant women, and this increase is further augmented in toxemic patients with decreased antioxidant levels.² The antioxidants are substances that, when present at concentrations lower than an oxidizable substrate, will significantly delay or prevent oxidation of that substrate.⁶ These substances can exist in enzymatic and non-enzymatic forms. One of the non-enzymatic agents is the α -tocopherol (vitamin E).⁷ This agent is related to tocopherol compounds that have polar hydroxylated aromatic rings (chromanol rings) and non-polar isoprenoid side chains. Vitamin E is lipophilic and almost exclusively resides in cell membranes where the chromanol ring may be at the surface of the membrane, and the isoprenoid chain inserted into the non-polar bilayer.⁸ Since lipid peroxidation occurs on unsaturated fatty acid chains that reside within the lipid bilayer, and the chromanol ring is the active radical quenching part of the vitamin, the function of vitamin E as an anti-oxidant must involve considerable movement of the lipids and vitamin E to promote molecular interaction. Elevated plasma levels of lipid peroxides and glutathione, as well as lower levels of vitamin E and β -carotene, were reported in patients with recurrent abortion.⁸ Nitric oxide (NO) is a biological mediator synthesized from L-arginine by a family of NO synthases. It is an abundant reactive radical that acts as an important oxidative biological signal in a large variety of diverse physiological processes, including smooth muscle relaxation, neurotransmission, and immune regulation.⁹ Depending on cell type, NO is produced in an enzymatic reaction catalyzed by one of the 3 isoforms of NO synthase (NOS): neuronal NOS, endothelial NOS, and inducible NOS.¹⁰ As NO is highly labile, measurement of the relatively stable metabolites, nitrate and nitrite, is employed as an index of NO production and as a marker of NOS enzyme activity.¹¹

Recurrent pregnancy loss (RPL) is highly frustrating for both patients and physicians. Patients are considered with RPL when they lose 3 or more consecutive pregnancies before 20 weeks of gestation, and it affects 0.5-3% of reproductive women. Stress, either emotional

or psychological can be associated with RPL.⁷ Many causative factors may be associated with RPL, such as genetic abnormalities, uterine anomalies, autoimmune diseases, blood clotting disorders, infectious diseases, endocrinopathies, and polycystic ovary syndrome.¹² In approximately 50-60% of RPL, a causative factor cannot be identified and is therefore classified as idiopathic.¹³ The aim of this study is to investigate biochemical changes in lipid peroxidation, NO, and the non-enzymatic antioxidant vitamin E in unexplained RPL, and compare these with healthy pregnant, and non-pregnant women.

Methods. A case control study was conducted in Baghdad from September 2008 to December 2009. Study samples were collected from the Obstetric and Gynecological Department of Al-Khadimiya Teaching Hospital, Baghdad, Iraq, under supervision of a specialist. Only patients with a history of 3 or more unexplained RPL were included (n=32). Any patient with hormonal disturbances or diabetes mellitus, either during the current pregnancy or previously, uterine fibroids, on any medications (for example, aspirin) during the current pregnancy, or loss of pregnancy due to pathological causes were excluded from the study. Thirty-two healthy pregnant women at the end of their third trimester, and 32 healthy non-pregnant women were included as controls. Ethical approval was obtained from the local ethics committee, together with informed patient consent.

Ten milliliters of blood were taken from each patient at the time she was admitted to the hospital for pregnancy loss. The same amount of blood was aspirated from both the control groups. Serum was used to determine MDA,¹⁴ and NO using the spectrophotometric method. An NO enzymatic assay kit was used for NO (US Biological, Cat. #N2577-02, Swampscott, MA, USA) with an ELISA reader and washer (DiaMed Eurogen, Turnhout, Belgium). The complete reaction was read at 540 nm. High performance liquid chromatography (Shimadzu, Kyoto, Japan) was used to determine vitamin E levels.¹⁵ Patient and control samples were chromatographically analyzed with Octadecyl silanol column carbon-18 using a mobile phase 95% ethanol, 5% water, a flow rate of one ml/min and ultraviolet-visible detection at wavelength 230 nm.

Statistical data were expressed as mean \pm standard error (SE); statistical significance was determined when $p < 0.05$ by Student t-test using the Statistical Package for Social Sciences (SPSS Inc, Chicago, IL, USA) version 14.

Results. Patients in all groups were of a comparable age (Table 1). Significantly high MDA serum levels were

Table 1 - Comparison of levels of MDA, vit E, and NO between the patient and control groups (mean \pm standard error).

Variable	RPL patients (n=32) Group A	Pregnant women (n=32) Group B	Non-pregnant women (n=32) Group C	T-test	P-value
Age (years)	27.53 \pm 8.84	28.72 \pm 8.18	27.91 \pm 7.36		
Serum MDA (μ mol/l)	0.76 \pm 5.9	0.56 \pm 1.2	0.34 \pm 2.5	A/B A/C B/C	0.002 0.0001 0.0001
Serum vit E (μ g/ml)	10.53 \pm 1.34	19.15 \pm 1.25	26.5 \pm 1.64	A/B A/C B/C	0.0001 0.0001 0.001
Serum NO (μ mol/l)	0.81 \pm 5.8	1.79 \pm 0.12	2.67 \pm 0.18	A/B A/C B/C	0.0001 0.0001 0.001

MDA - malondialdehyde, vit E - vitamin E, NO - nitric oxide, RPL - recurrent pregnancy loss

Table 2 - Comparison of serum MDA and NO to vit E ratios between the patient and control groups (mean \pm standard error).

Ratios	RPL patients (μ mol/mg) $\times 10^{-5}$ Group A	Pregnant women (μ mol/mg) $\times 10^{-5}$ Group B	Non-pregnant women (μ mol/mg) $\times 10^{-5}$ Group C	T-test	P-value
Serum MDA/vit E	4.79 \pm 0.54	3.28 \pm 0.2	2.15 \pm 0.55	A/B A/C	0.017 0.003
Serum NO/vit E	4.91 \pm 0.64	3.62 \pm 0.65	3.17 \pm 0.58	A/B A/C	0.194 0.078

MDA - malondialdehyde, vit E - vitamin E, NO - nitric oxide, RPL - recurrent pregnancy loss

found in RPL women compared with the control groups. There was also a significant higher level of MDA in pregnant women compared with non-pregnant woman. Both serum vitamin E and NO levels were significantly decreased in RPL women compared with the control groups (Table 1). There was also a significantly lower level of vitamin E and NO in pregnant women compared to non-pregnant woman. Using student t-test, the patient MDA/vitamin E ratio was highly significant when compared with both control groups. However, no significant difference was found between patients and controls for the NO/vitamin E ratio (Table 2).

Discussion. We examined oxidative stress represented by estimation of a lipid peroxidation marker (MDA), and studied its relation and/or association with one of the non-enzymatic antioxidants represented by vitamin E in addition to NO in unexplained RPL. Free radicals are difficult to measure directly due to their unstable and transient nature. They can, however, be measured indirectly as a marker of lipid peroxidation because of their tendency to cause it.¹⁶ Malondialdehyde was significantly elevated in the sera of RPL women compared with the sera of the controls, and is a result of increasing free radicals generation. An increase in MDA

levels during the progression of a healthy pregnancy is normal, as pregnancy is considered a stressful condition in which many physiological and metabolic functions are altered, generating free radicals that act on lipids to cause lipid peroxidation.^{17,18} Hence, why the MDA concentration is non-significantly increased in healthy pregnant women during their third trimester compared with concentrations in non-pregnant women. Regarding the elevation of MDA in serum of RPL women more than healthy pregnant women, and considering RPL as a stressful condition, Gupta et al⁷ explained that oxidative stress-induced damage has been hypothesized to play a role in spontaneous abortion and idiopathic RPL. While Safronova et al¹⁹ reported that pregnancy is characterized as an inflammatory state with the leukocytes showing changes similar to those found in sepsis, and increased generation of ROS was demonstrated in leukocytes by significantly higher levels of granulocyte spontaneous chemiluminescence in the recurrent abortion patients compared to a control group of healthy pregnant women.¹⁹ In 2006, Patil et al¹⁶ reported that cells have evolved a number of counteracting antioxidant defenses. These antioxidant defense mechanisms can be categorized under the headings of free radical scavenging and chain breaking antioxidants, and α -tocopherol

(vitamin E) is one of the nonenzymatic chain breaking antioxidants that limits the cellular concentration of free radicals and prevents excessive oxidative damage.¹⁶ Menevse et al³ studied the role of antioxidants and lipid peroxidation in RPL. They suggested that the significant decrease in vitamin E levels caused the significant increase in MDA level in RPL women. Their suggestion agrees with the current study findings when comparing MDA and vitamin E levels in pregnant women at the end the third trimester and non pregnant women (Table 1). Garba and Amodu²⁰ concluded that vitamin E is the most important chain breaking antioxidant, and it protects polyunsaturated fatty acids from peroxidative damage by donating hydrogen to the lipid peroxy radical. They also reported that because of the lipophilic property of the tocopherol molecule, vitamin E is the major free radical chain terminator in the lipophilic environment.²⁰

The significant decrease in NO levels in the serum of RPL women compared with the control groups is a result of an increase in the formation of free radicals especially in the form of superoxide, which is known to inactivate NO in chemical reactions forming the potent free radical peroxy nitrite anion (ONOO⁻), which is responsible for increase the production of MDA. Urban et al²¹ noted that NO, like homocysteine, is produced in blood vessel endothelium, and its deficiency may be one of the causes of baby loss.²¹ The current study results of significantly lower serum NO levels in pregnant women in their third trimester compared with non-pregnant women (Table 1) agrees with the results of Akturk et al²² and Kashiwagi et al,²³ where serum NO production increased in non-pregnant women compared with normal healthy pregnant women in their second and third trimester of pregnancy. However, the changes in NO production during normal pregnancy have varied in different studies. Schism et al²⁴ reported that maternal circulating nitrite levels decreased with advancing gestation, while, and Pasaoglu et al²⁵ and Choi et al²⁶ found that there were no changes in NO production during normal pregnancy compared to the nonpregnant state. A study by Zammiti et al²⁷ to evaluate vascular NO production in Tunisian women as a risk factor for RPL, concluded that there was no association between reduced vascular NO production and increasing risk of RPL, which is agreement with our study results where no significance was found between NO decreased levels among RPL women when compared with NO in both control groups as a ratio with vitamin E.

In conclusion, the decrease in NO production and vitamin E is a result of RPL and not a causative factor, since the RPL was without pathological cause, medication, or fibroid presence, and no significant

difference was found between the NO/Vit E ratio in RPL and control groups. A comparison between NO, MDA, and vitamins E and C in the placenta of recurrent pregnancy loss women at different trimesters with the placenta and cord blood of healthy pregnant women is recommended for further study.

References

1. Agarwal S, Gupta S. Role of reactive oxygen species in female reproduction. Part I. Oxidative stress: a general overview. *Agro Food Industry Hi-Tech* 2005; January/February: 21-25.
2. Mastalerz-Migas A, Steciwko A, Pokorski M, Pirogowicz I, Drobnik J, Bunio A, et al. What influences the level of oxidative stress as measured by 8-hydroxy-2'-deoxyguanosine in patients on hemodialysis? *J Physiol Pharmacol* 2006; 57 Suppl 4: 199-205.
3. Menevse E, Sivrikaya A, Karagozoglu E, Tiftik AM, Turk S. Study of elements, antioxidants, and lipid peroxidation in hemodialysis patients. *Turk J Med Sci* 2006; 36: 279-284.
4. Karatas F, Halifeoglu I, Karatepe M, Konar V, Canatan H, Colak R. Evaluation of Changes in Levels of Serum Selenium, MDA and Antioxidant Vitamins (A, E, C) in Diabetic Patients. *ARASTIRMA* 2006; 20: 391-395.
5. Rao G, Kamath U, Raghobama C, Sujatha P, Pragna R. Maternal and fetal indicators of oxidative stress in various obstetric complications. *Ind J Clin Biochem* 2008; 18: 80-86.
6. Mayne ST. Antioxidant nutrients and chronic disease: use of biomarkers of exposure and oxidative stress status in epidemiologic research. *J Nutr* 2003; 133: 933-940.
7. Gupta S, Agarwal A, Banerjee J, Alvarez JG. The role of oxidative stress in spontaneous abortion and recurrent pregnancy loss: a systematic review. *Obstet Gynecol Surv* 2007; 62: 335-347. Review.
8. Toy H, Camuzcuoglu H, Camuzcuoglu A, Celik H, Aksoy N. Decreased serum prolidase activity and increased oxidative stress in early pregnancy loss. *Gynecol Obstet Invest* 2010; 69: 122-127.
9. Choi JW, Im MW, Pai SH. Nitric oxide production increases during normal pregnancy and decreases in preeclampsia. *Ann Clin Lab Sci* 2002; 32: 257-263.
10. Maas R, Schwedhelm E, Kahl L, Li H, Benndorf R, Lüneburg N. Simultaneous assessment of endothelial function, nitric oxide synthase activity, nitric oxide-mediated signaling, and oxidative stress in individuals with and without hypercholesterolemia. *Clin Chem* 2008; 54: 292-300.
11. Hassan A, Gormley K, O'Sullivan M, Knight J, Sham P, Vallance P, et al. Endothelial nitric oxide gene haplotypes and risk of cerebral small-vessel disease. *Stroke* 2004; 35: 654-659.
12. Christiansen OB, Nielsen HS, Kolte A, Pedersen AT. Research methodology and epidemiology of relevance in recurrent pregnancy loss. *Semin Reprod Med* 2006; 24: 5-16.
13. Sugiura-Ogasawara M, Furukawa TA, Nakano Y, Hori S, Aoki K, Kitamura T. Depression as a potential causal factor in subsequent miscarriage in recurrent spontaneous aborters. *Hum Reprod* 2002; 17: 2580-2584.
14. Bateman RM, Jagger JE, Sharpe MD, Ellsworth ML, Mehta S, Ellis CG. Erythrocyte deformability is a nitric oxide mediated factor in decreased capillary density during sepsis. *Am J Physiol Heart Circ Physiol* 2001; 280: H2848-H2856.
15. Bompadre S, Tulipani S, Romandini S, Giorgetti R, Battino M. Improved HPLC column-switching determination of Coenzyme Q and Vitamin E in plasma. *Biofactors* 2008; 32: 257-262.

16. Patil SB, Kodliwadmath MV, Kodliwadmath SM. Lipid peroxidation and nonenzymatic antioxidants in normal pregnancy. *J Obstet Gynecol India* 2006; 56: 399-401.
17. Patil SB, Kodliwadmath MV, Kodliwadmath SM. Study of oxidative stress and enzyme antioxidants in normal pregnancy. *Indian Journal of Clinical Biochemistry* 2007; 22: 135-137.
18. Soyöz M, Özçelik N, Kihnç I, Altuntas I. The effects of ochratoxin A on lipid peroxidation and antioxidant enzymes: a protective role of melatonin. *Cell Biol Toxicol* 2004; 20: 213-219.
19. Safronova VG, Matveeva NK, Avkhacheva NV, Sidel'nikova VM, Van'ko LV, Sukhikh GT. Changes in regulation of oxidase activity of peripheral blood granulocytes in women with habitual abortions. *Bull Exp Biol Med* 2003; 136: 257-260.
20. Garba IH, Amodu BO. Total serum vitamin C concentration in pregnant women: implications for a healthy pregnancy. *Rev Bras Saude Matern Infant* 2006; 6: 293-296.
21. Urban J, Jarocki S, Bielecki D, Urban R. Serum homocysteine and nitric oxide levels in pregnancy complicated with intrauterine fetal growth restriction. *Archives Perinatal Medicine* 2007; 13: 27-29.
22. Akturk A, Onal EE, Atalay Y, Yurekli M, Erbas D, Okumus N, et al. Maternal and umbilical venous adrenomedullin and nitric oxide levels in intrauterine growth restriction. *J Maternal Fetal Neonatal Med* 2007; 20: 521-525.
23. Kashiwagi M, Zimmermann R, Beinder E. Pathophysiology of pre-eclampsia: update on the role of nitric oxide. *Curr Hypertens Rep* 2003; 5: 493-497.
24. Schiessl B, Strasburger C, Bidlingmaier M, Mylonas I, Jeschke U, Kainer F, et al. Plasma- and urine concentrations of nitrite/nitrate and cyclic Guanosine monophosphate in intrauterine growth restricted and preeclamptic pregnancies. *Arch Gynecol Obstet* 2006; 274: 150-154.
25. Pasaoglu H, Bulduk G, Ogüs E, Pasaoglu A, Onalan G. Nitric oxide, lipid peroxides, and uric acid levels in pre-eclampsia and eclampsia. *Toboku J Exp Med* 2004; 202: 87-92.
26. Choi JW, Im MW, Pai SH. Nitric oxide production increases during normal pregnancy and decreases in preeclampsia. *Ann Clin Lab Sci* 2002; 32: 257-263.
27. Zammiti W, Mtiraoui N, Mahjoub T. Lack of consistent association between endothelial nitric oxide synthase gene polymorphisms, homocysteine levels and recurrent pregnancy loss in tunisian women. *Am J Reprod Immunol* 2008; 59: 139-145.

Related topics

Manizheh SM, Mandana S, Hassan A, Amir GH, Mahlisa KS, Morteza G. Comparison study on the effect of prenatal administration of high dose and low dose folic acid. *Saudi Med J* 2009; 30: 88-97.

Baran OP, Kervancioglu P, Akkus M, Nergiz Y. Ultrastructural investigation of the protective role of folic acid and vitamin E against toxic effects of valproic acid on maternal liver tissue during period of gestation. *Saudi Med J* 2006; 27: 407-409.

Aydin S, Ozeren M, Yenilmez E, Yulug E, Cobanoglu U, Arvas H. Morphologic alterations and immunohistochemical analysis of alpha-fetoprotein and CD34 in chorionic villi of anembryonic pregnancy. *Saudi Med J* 2006; 27: 154-60.

Sobki SH, Al-Senaidy AM, Al-Shammari TA, Inam SS, Al-Gwiser AA, Bukhari SA. Impact of gestational diabetes on lipid profiling and indices of oxidative stress in maternal and cord plasma. *Saudi Med J* 2004; 25: 876-880.