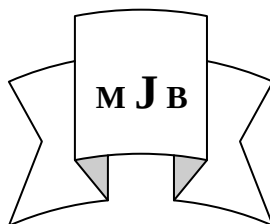


Relationship of Nitric Oxide and Malondialdehyde to Miscarriage

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Abstract

The present study was conducted to verify the oxidative stress status in miscarriage .To achieve this aim, 70 women with miscarriage (patient group) , and 25 healthy non pregnant (control group) were subjected to the study.

The statistical analysis (student's t-test) showed a significant ($p<0.001$) increase of nitric oxide (NO) and malondialdehyde (MDA) levels , in women with miscarriage when compared with those of the control group .

The linear regression analysis demonstrated a significant positive correlation for both MDA values($r=0.47$, $p<0.01$) and NO values($r=0.45$, $p<0.01$) with gestational ages .The same analysis indicated a significant positive correlation for MDA ($r=0.73$, $p<0.01$) and ($r=0.71$, $p<0.01$) for NO levels with the number of previous miscarriages was observed . The results indicate that oxidative stress may lead to miscarriage.

Key words: nitric oxide, malondialdehyde, miscarriage.

علاقة تركيز اوكسيد النتريك وتركيز المالوندايالدهايد مع الاجهاض

الخلاصة

تم تصميم الدراسة الحالية للتحقق من تغيرات الإجهاد التأكسدي عند مريضات الاجهاض التلقائي . شملت الدراسة ٧٠ مريضة و ٢٥ امرأة غير حامل سوية بوصفهم مجموعة سيطرة . تم تقدير مستويات اوكسيد النتريك و مالون ثنائي الألداهيد في أمصال المرضى ومجموعة السيطرة . وجد زيادة ملحوظة ($p<0.001$) في مستوى اوكسيد النتريك و مالون ثنائي الألداهيد لدى مريضات الاجهاض عند مقارنتها مع مثيلاتها في مجموعة السيطرة. اظهر تحليل الانحدار الخطي ارتباطاً معنوياً موجباً لكل من مستوى مالون ثنائي الألداهيد ($r=0.47$, $p<0.01$) ومستوى اوكسي النتريك ($r=0.45$, $p<0.01$) لدى المريضات نسبةً إلى التقدم في مدة الحمل . كما أظهرت النتائج وجود ارتباط معنوي موجب في مستوى كل من مالون ثنائي الألداهيد ($r=0.73$, $p<0.01$) ومستوى اوكسيد النتريك ($r=0.71$, $p<0.01$) مع عدد الاجهاضات السابقة . توضح النتائج ان الاجهاد التاكسدي يؤدي الى الاجهاض.

Introduction

The terms miscarriage and spontaneous abortion refer to pregnancy loss at less than 20 weeks gestation in absence of elective medical or surgical measures to terminate the pregnancy, this definition represent the more recent one [1].

Spontaneous abortion occurs in 10% to 15% of clinically recognizable pregnancies [2]. There are many causes of miscarriage including Chromosomal abnormality [3], Uterine abnormalities [4], Infection [5], Endocrinological abnormalities [6], Autoimmune diseases [7]. In addition to identifiable

causes of miscarriage, some other factors increasing risk of miscarriage have been reported such as maternal age [8], number of previous miscarriages [9], environmental factors [10].

Oxidative stress is best defined in broad terms as an alteration in pro-oxidant-antioxidant balance in favor of the former that leads to potential damage. Reactive oxygen species (ROS) are implicated in the course of oxidative stress which is now recognized to play a central role in the pathophysiology of many different disorder including complications of pregnancy [11].

Superoxide anion (O_2^-) is a reactive oxygen species that reacts quickly with nitric oxide (NO) in the vasculature. The reaction produces peroxynitrite. This is important because NO is a key mediator in many important vascular functions including regulation of smooth muscle tone and blood pressure, platelet activation, and vascular cell signaling [12].

Peroxynitrite itself is a highly reactive species which can directly react with various biological targets and components of the cell including lipids, thiols, amino acid residues, DNA bases, and low-molecular weight antioxidants [13]. Peroxynitrite is able to get across cell membranes to some extent through anion channels [14]. Lipid degradation occurs, forming products such as malondialdehyde (MDA) and ethane, that are commonly measured as end products of lipid peroxidation [15]. Lipid peroxidation is of particular significance in miscarriage. Studies demonstrated an elevated lipid peroxidation in miscarriage as measured by thio – barbituric acid reactive substance [16].

Elevated ROS levels can influence the oocytes and embryos in their environments for example

follicular or peritoneal fluid [17]. As implantation is a very well orchestrated process that involves complex interactions between the embryo and uterine environments, a burst of placental oxidative stress during establishment of maternal circulation may cause early pregnancy loss [18]. The oxidative stress affects both implantation and early embryo development by modifying the key of transcription and hence modifying the gene expression [17].

Recently oxidative stress appears to play a role in uterine contractions which may arise from free radicals induce stimulation of prostaglandin leading to uterine contraction which subsequently results in abortion [19].

Material and Methods

Seventy women with spontaneous abortion of age range 17-42 years were enrolled as patient group. Twenty five apparently healthy non -pregnant volunteers were included in this study. They were matched in their age with patients group.

Subjects suffered from any diseases such as diabetes mellitus, hypertension, cardiovascular disease, asthma, and liver disease which interferes with the data obtained, were excluded. Five milliliters (ml) blood samples were obtained from aborted women and control group by vein puncture. Samples were allowed to clot at room temperature, and then centrifuged. Sera were transferred carefully and stored at $-17\text{ }^{\circ}\text{C}$ until analysis time in suitable serum tube. In current study serum nitric oxide (NO), malondialdehyde (MDA) levels were measured.

Serum nitric oxide levels were measured by using nitric oxide kit. The kit uses the enzyme nitrate reductase to convert nitrate to nitrite. Nitrite is then

detected as a colored azo dye product of the Griess reaction that absorbs visible light at 540 nm by Bio ELISA reader [20].

The level of malondialdehyde was determined by modified procedure described by Guidet B. and Shah S.V. The test is based on the reaction of MDA with thiobarbituric acid (TBA); forming MDA-TBA₂ product that

absorbs strongly at 532 nm by using UV-VIS Spectrophotometer [21].

Results

The statistical analysis of data showed that there was a significant ($P < 0.001$) increase in MDA and NO levels in sera of women with miscarriage when compared with those of the control group as shown in table 1

Table 1 Serum nitric oxide (NO) ,malondialdehyde (MDA) levels in women with miscarriage and control group.

Parameter	Subject	No.	Mean $\pm$ SD	Range	P-value
NO (μ M)	Control	25	20.49 $\pm$ 2.02	15.22 – 23.76	0.001
	Patients	70	32.33 $\pm$ 12.69	18.85 – 60.88	
MDA (μ M)	Control	25	3.98 $\pm$ 1.22	1.76 – 6.98	0.001
	Patients	70	7.14 $\pm$ 3.98		

The impact of gestational age on the levels of serum NO, MDA in patients with miscarriage was verified by using the linear regression analysis. A

significant positive correlation ($r=0.47$, $p=0.01$) for MDA and ($r=0.45$, $p=0.01$) for NO levels in patients with the gestational age , see Fig1, 2.

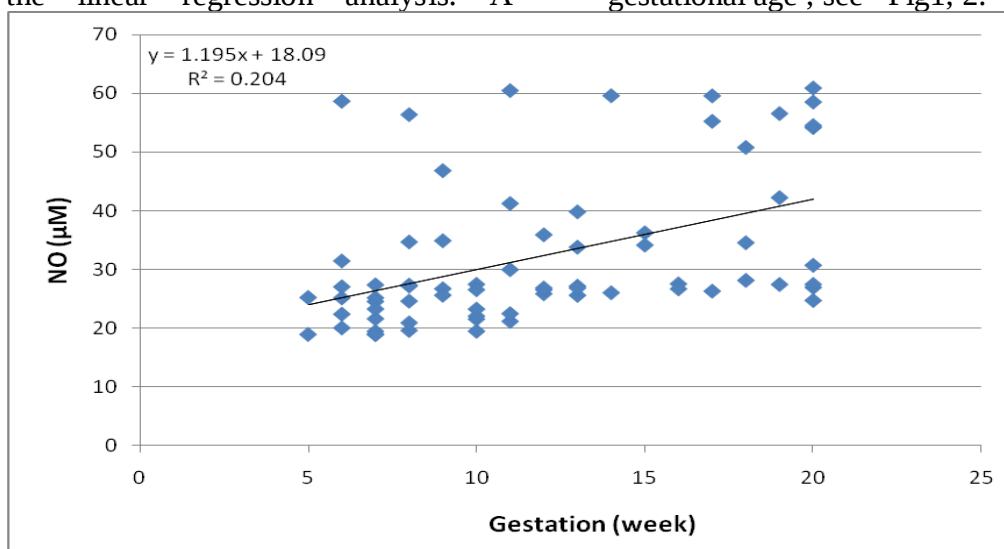


Fig. 1 Correlation of serum nitric oxide (NO) levels with gestation in women with miscarriage.

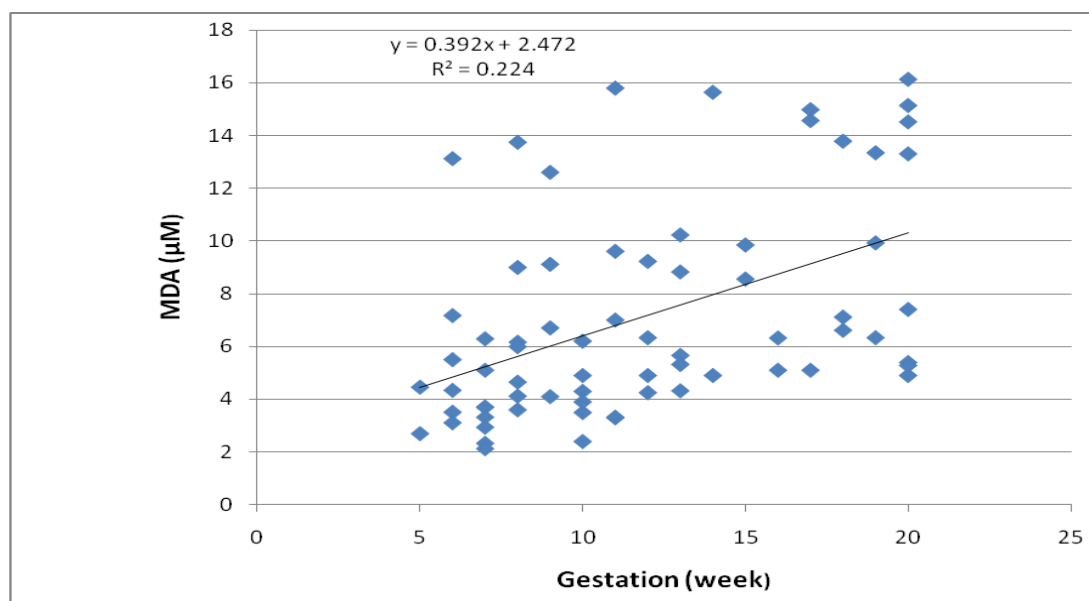


Fig.2 Correlation of serum malondialdehyde (MDA) levels with gestation in women with miscarriage.

The linear regression analysis was applied to evaluate the effect of number of previous miscarriages on the levels of NO, MDA in the patients. A significant positive correlation of

NO values ($r = 0.71$, $P < 0.01$), and MDA values ($r = 0.73$, $P < 0.01$) with number of previous miscarriages in miscarried women was observed as shown in Fig 3, 4

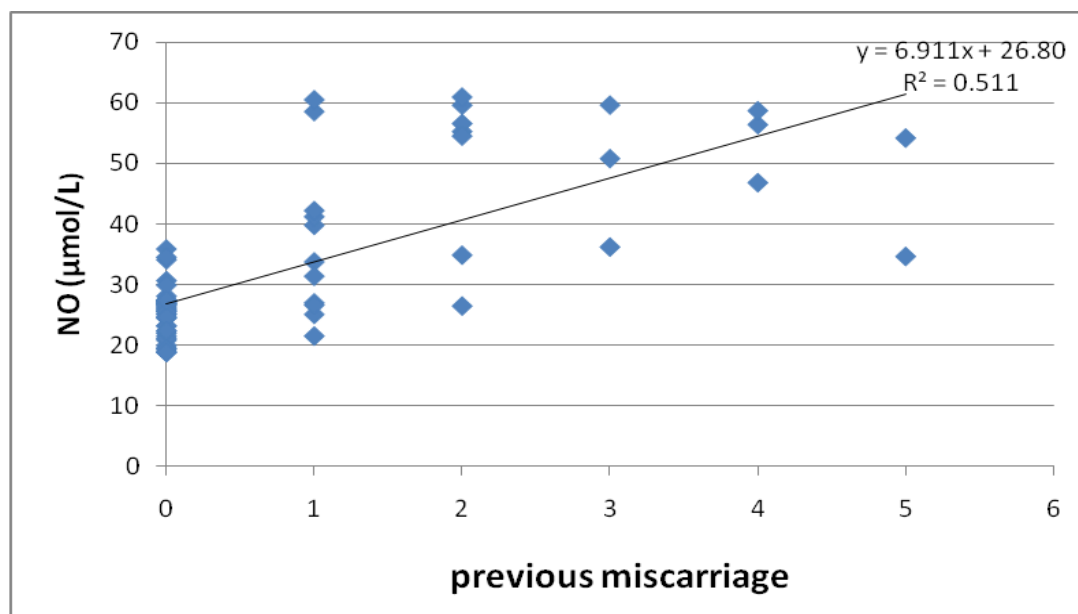


Fig. 3 Correlation of serum nitric oxide (NO) level with number of previous miscarriage in patient group.

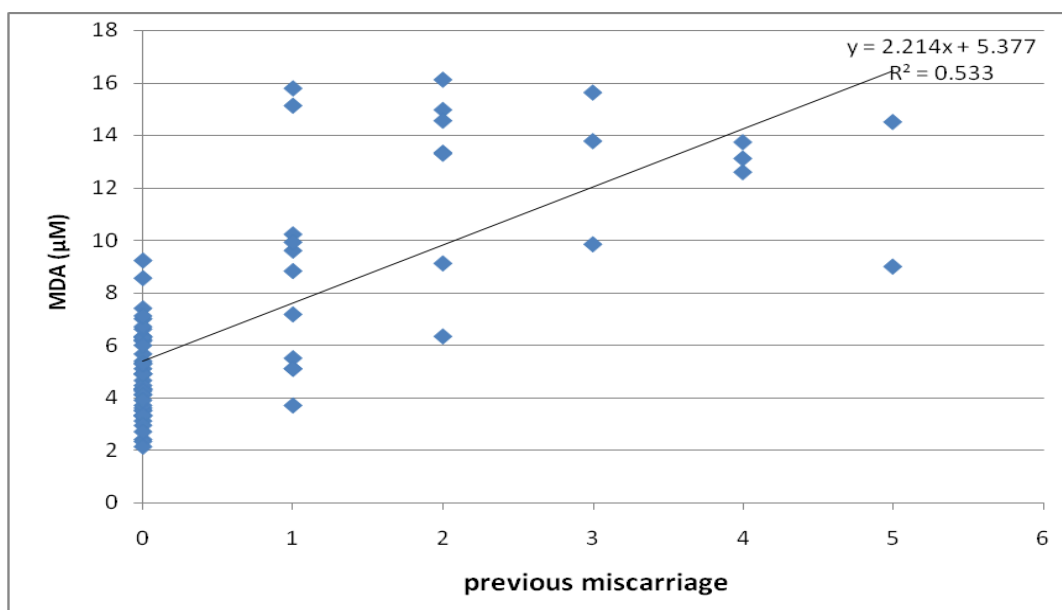


Fig. 4 Correlation of serum malondialdehyde (MDA) levels with number of previous miscarriage in patient group.

Discussion

In present study, NO levels were increased in patients comparing with control group. Activation of polymorphonuclear leucocytes and macrophages leads to increased production of ROS [22]. Increase in number and activity of macrophages is accompanied by release of more cytokines and other immune mediators, such as NO. This was initially implicated in low-grade inflammation, while elevated peritoneal NO is consistent with the increased number and activity of macrophages [23]. High levels of NO, such as those produced by macrophages, can negatively influence fertility and can cause pregnancy complications such as abortion. Macrophages stimulate endothelial NO synthase to release NO [24-26]. These abnormal immune responses might eventually stimulate macrophages and/or endometrial cells to persistently produce a large amount of NO and raise its levels [27].

MDA levels were found to be increased in women with miscarriage in relative to control group. MDA is a byproduct of lipid peroxidation, therefore, an elevation in MDA levels

may reflects an overproduction of lipid peroxides and/or impaired antioxidant defense mechanism. These lipid peroxides are produced mainly in the placenta due to membrane disruption by ROS [28].

Lipid hydroperoxides inhibit prostaglandin I_2 (PGI_2) synthase enzyme activity [29], whereas thromboxane $A_2(TXA_2)$ synthase activity is unchanged or even stimulated by lipid hydroperoxides [30]. The altered prostaglandins status might provoke vasospasm with platelets aggregation and exacerbation of placental ischemia, increased cell damage. This may lead to abnormal placentation and increased lipid peroxidation (amplification of oxidative stress) [31].

The present investigation pointed out a significant positive correlation of MDA and NO levels with the gestational age. This may indicate that the raised oxidative stress with consequent enhanced lipid peroxidation as the gestation was advanced.

To perceive the involvement of the elevated oxidative stress in miscarriage, we have to grasp the

changes of placental oxygen supply during pregnancy. In vivo O_2 data demonstrated that values for the intraplacental partial pressure of O_2 (PO_2) are two to three times at 8-10 weeks than after 12 weeks [32].

The first trimester uterine O_2 gradient exerts a regulatory effect on placental tissue development and function [33]. The physiological hypoxia of the first trimester gestational sac may protect the developing fetus against the deleterious and a teratogenic effects of free radicals. At the end of the first trimester, a burst of oxidative stress is evidenced in the periphery of the early placenta [18]. Focal trophoblastic oxidative damage and progressive villous degeneration trigger the formation of the fetal membrane [34].

Any factor causing abnormally high or rapidly fluctuating concentrations of O_2 will have a harmful and rapid effect on the early villous tissue. O_2 effects include chromosomal abnormalities which are found in at least 50% of miscarriages and are often associated with abnormal trophoblastic invasion of the uterine decidue [35].

Systemic NO production increases with advancing gestation during normal pregnancy. Elevated NO concentrations during pregnancy return to normal within 12 wk after delivery [36]. These human data and those from animal and in vitro studies [37].

Among the current enrolled patients, 62% miscarried in the first trimester, 60% miscarried in 8-12 week of gestation. Thus, it is rationally to suggest that the oxidative stress derangement of the miscarried patients in the first trimester in particular during 8-12 weeks is the motional factor. Many endothelial changes of potential relevance to habitual abortion, i.e there is a history of abortions, can be induced by lipid

peroxidation [30]. Abnormal placentation leads to placental oxidative stress and syncytiotrophoblast dysfunction, and it has been proposed as a cause of early abortion [38]. Enhanced lipid peroxidation has also been hypothesized to be associated with the pathophysiology of recurrent pregnancy loss [39].

In present study, the levels of MDA and NO were found to be elevated with number of previous miscarriages these observation compatible with suggestion that pregnancy is a physiological state associated with enhanced oxidative stress related to high metabolic turnover and elevated tissue oxygen requirements [40].

Free radical induced tissue damage of cell components and biomolecules (lipids, protein, and proteins) has been associated with the etiology of pregnancy-related conditions including miscarriage [41,42]. Many endothelial changes including increase the level of NO of potential relevance to habitual abortion, i.e there is a history of abortions, can be induced by lipid peroxidation [30]. Successful pregnancy requires the development of an adequate uteroplacental circulation. Abnormal placentation leads to placental oxidative stress and syncytiotrophoblast dysfunction, and it has been proposed as a cause of early abortion [38]. Enhanced lipid peroxidation has also been hypothesized to be associated with the pathophysiology of recurrent pregnancy loss due to antiphospholipid syndrome [39].

In present study, the levels of MDA and NO were found to be elevated with number of previous miscarriages these observation compatible with suggestion that the morphological and immunohistochemical markers of

cellular stress and damage including lipid peroxidation marker (MDA) was increased in tissues obtained from miscarriages compared with controls [43].

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