Study of Oxidant –Antioxidant Balance in Pregnant Women with Threatened Abortion

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Abstract

The aim of this study was to investigate the effects of threatened abortion on serum oxidant-antioxidants balance. Using the lipid peroxidation marker, malondialdehyde (MDA) and preventative antioxidants ceruloplasmin (Cp), uric acid (Ua) and albumin (Alb), in serum of patients with threatened abortion, as well as (50) healthy subjects as a control group. They divided into two groups as the following: Group (control): Included fifty healthy subjects aged (15-40 years). Group (patient): Included one hundred patients with threatened abortion (15-40 years). Results: The results show a presence of a significant increase in MDA and Cp, Ua and Alb in all groups of patients in comparison with control group.

Keywords: pregnancy, abortion , threatened abortion, oxidant-antioxidants balance.

Introduction

Pregnancy refers to the fertilization and development of one or more offspring, known as a fetus or embryo, in a woman's uterus. The term embryo is used to describe the developing offspring during the first 8 weeks following conception, and the term fetus is used from about 2 months of development until birth (1). In a pregnancy, there can be multiple gestations, as in the case of twins or triplets.

Childbirth usually occurs about 38 weeks after conception; in women who have a menstrual cycle length of four weeks, this is approximately 40 weeks from the last normal menstrual period. The World Health Organization (WHO) defines normal term for delivery as between 37 weeks and 42 weeks (2).

Pregnancy is typically divided into three periods, or trimesters, each of about three months. In medicine; pregnancy is also defined as beginning when the developing embryo becomes implanted in the endometrial lining of a woman’s uterus. The first 12 weeks of pregnancy are considered to make up the first trimester. According to the American Pregnancy Association, by the end of the first trimester, the fetus will be about 3 inches (76 mm) long and will weigh approximately 1 ounce (28 gm) (3). Weeks 13 to 28 of the gestation are called the second trimester. By the end of the second trimester, the expanding uterus has created a visible "baby bump". The third trimester of gestation spans from week 28 to the birth. The woman's belly will transform in shape as the belly drops due to the fetus turning in a downward position ready for birth. Pregnancy is associated with normal physiological changes that assist the nurturing and survival of the fetus. Biochemical parameters reflect these adaptive changes in most organ system and are clearly distinct from the non-pregnant state (4).

Abortion is common complication encountered during early pregnancy(5 -7). Threatened abortion, defined as vaginal bleeding before 24 weeks of gestation, is a common complication influenced about 20% of...
pregnancies. It has been shown to be associated with an increased risk of poor obstetric outcomes such as preterm labor, low birth weight, and premature rupture of membranes. Moreover, when pregnant women have bleeding, it may cause stress and anxiety for the mother-to-be about the outcome of pregnancy. So, it is necessary to be diagnosed and managed to prevent maternal or fetal mortalities and morbidities (8).

Lipid peroxidation (LPO) refers to the oxidative degradation of lipids by which free radicals steal electrons from the lipids (phospholipids) in the cell membranes, resulting in cell damage. LPO is to begin by hydroxyl or other radical. That extracts a hydrogen atom from a polyunsaturated lipids, thereby forming a lipid radical. The FRs chain reaction is propagated by reaction with O₂, forming the lipid peroxide. Rearrangement of the single electron result in degradation of lipid (9). The initiation of LPO of cell membrane begins when the OH radical effectively removes hydrogen from PUFA of the lipid molecule (RH) creating a lipid free Radical(R) (10).

To minimize the negative effects of ROS generated by any pro-oxidant, endogenous defensive mechanisms called antioxidant defense system, which utilizes enzymatic and non-enzymatic mechanisms. Antioxidants are naturally occurring substances that combat oxidative damage in biological entities. An antioxidant achieves this by slowing or prevention the oxidation process that can damage cells in the body. Antioxidants are thought to protect the body against the destructive effects of free radicals. Antioxidants neutralize free radicals by donating one of their own electrons, ending the electron-gain interaction. The antioxidant nutrients themselves don’t become free radicals by donating an electron because they are stable in either form. They act as scavengers, helping to prevent cell and tissue damage that could lead to cellular damage and illness (11, 12).

Ceruloplasmin is alpha-2-glycoprotein with a molecular weight of (132KDa), and it is encoded in humans by the (Cp) gene (13) also it is named with (Ceru-human), Cp-2) and ferroxidase Ceruloplasmin is an enzyme in the liver containing (six) atoms of copper in its structure. This enzyme to carry about (70%) of the total copper in human plasma while albumin carries (15%) (14). Uric acid is an end product of purine metabolism and is related to the purine bases of the nucleic acids in humans. Nearly 15 million years ago, one of our hominid ancestors acquired a mutation in the gene for uricase, the hepatic enzyme that degrades UA into allantoin. As a consequence, having lost the ability to express urate oxidase, humans are exposed to higher serum UA levels than other mammals (15, 16). Albumin (Alb) is a single polypeptide consisting of 585 amino acids with M.W. of approximately 66,248 Dalton, synthesized by the liver. It is most abundant in human plasma. Normally, it constitutes about 55-60% of all plasma proteins and has a serum half-life of about 20 days (17, 18).

The aim of this study is to investigate the oxidative stress by measuring the lipid peroxidation marker (MDA) in patients with threatened abortion, to evaluate serum antioxidant status in the mentioned disease by measurement (ceruloplasmin (Cp), uric acid (Ua) and albumin (Alb) compared with control group.

Patients and Method

Design of Study

One hundred in women with threatened abortion, and fifty women pregnant and six women non pregnant (control group) are included in this study.

Threatened abortion have attended consultative female unit clinic in maternity and children (Bent Al-Huda ) Hospital in Nassyrich, at the period between 10/9/2013 to 1/6/2014. It included (150) subjects, control (50) and patients (100).

Collection of blood sample

Disposable syringes and needles were used for blood with drawal (8ml) of blood was obtained from user women and control group by veinuncture at 10 a.m. The nest was allowed to clot at 37°C, and then centrifuged at 3000xg for 10 min. Sera were removed and stored at (-20°C) for later measurement biochemical parameters, unless used immediately.

Biochemical Parameters

Lipid peroxidation Marker (Serum MDA)

Determination of serum MDA level that consider as a lipid peroxidation marker were performed according to the method of (19). MDA concentrations were
calculated, using the molar extinction coefficient of MDA ($\varepsilon_{MDA}$) equal to 1.56 x10^5 mol⁻¹. Cm⁻¹(19). MDA formed from breakdown of polyunsaturated fatty acid, serves as a convenient index of peroxidation reaction.

**Serum Antioxidants**

Serum Cp concentration was measured by the method of (20) which using the extinction coefficient of Cp (ε Cp) equal to (0.68 ) to calculate it concentration. The bromocresol green (BCG) method, colorimetric method, is the simplest technique which have been developed to determine Alb concentration (21,22). Also, the Ua concentration was measured by colorimetric method (23,24).

**Statistical Analysis**

Statistical analysis was done using the software SPSS version 17.0; the results were expressed as mean ± standard error (mean ± SE). One way ANOVA-test was used to compare parameters in different studied groups. P-values (P ≤ 0.05) were considered statistically significant.

**Results and Discussion**

**Lipid Peroxidation Status (Malondialdehyde)**

Table (1) shows a significant increase in concentrations of serum MDA in patients group in comparison with control group (P ≤0.05). This result is similar to the result of (25, 26).

The increased MDA levels in threatened abortion is known to be due to increased generation of reactive oxygen species and increased oxygen demand along with reduction in activities of enzymes like superoxide dismutase, glutathione peroxidase and decrease in concentration of antioxidants like Vitamin C and Vitamin E (27).

Serum MDA levels were reported to be higher in abortion threatened women during the first trimester compared with normal pregnant controls (28, 29).

Oxidative stress is well known to initiate the caspase cascade leading to cell death in other systems. Concentrations of lipid peroxides increase in the decidua of women undergoing early pregnancy loss (30). Current evidence reveals that the architecture of the human first-trimester gestational sac limits fetal exposure to oxygen and tries to minimize the damage caused by oxygen free radicals. Failure of placentation is associated with an imbalance in ROS, which will further affect placental development and function and may subsequently have an influence on both the fetus and its mother (31).

**Table (1) Serum MDA concentration of study subjects**

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>MDA(nmol/ml) mean± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnants with T.A</td>
<td>100</td>
<td>22.61±1.20a</td>
</tr>
<tr>
<td>Normal pregnant s</td>
<td>50</td>
<td>6.44±0.67b</td>
</tr>
<tr>
<td>LSD</td>
<td></td>
<td>1.18</td>
</tr>
</tbody>
</table>

**Serum Antioxidants**

**Serum Ceruloplasmin Concentrations**

Table(2) show a significant increase in concentrations of serum Cp levels in both patient groups in comparison with control group (P ≤0.05). This result is similar to the result of (25,26).

ROS extensively damage cellular organelles including the mitochondria, nuclear and mitochondrial DNA, and cell membrane ultimately leading to cellular demise (32-34) ,cell function but also a physiologic one when present at very low levels. To prevent ROS-induced damage, cells have evolved antioxidant systems.
As a result, there is a delicate balance between ROS production and antioxidant activity that maintains a physiologic balance leading to cellular homeostasis. When this balance is perturbed by an excess of ROS production, a state of oxidative stress leading to cell damage and cell dysfunction (35,36).

Oxidative stress is manifested at the maternal–fetal interface from early pregnancy onwards. It plays a role in both the normal development of the placenta as well as in the pathophysiology of complications such as miscarriage, pre-eclampsia, intrauterine growth restriction (IUGR), and premature rupture of the membranes (37,38). Oxidative stress and the systemic inflammatory response are observed to a much greater degree in threatened abortion (39). There is irrefutable evidence of placental oxidative stress in cases of early onset threatened abortion, including increased concentrations of protein carbonyls, lipid peroxides, nitrotryosine residues and DNA oxidation (40, 41).

Exposure of explants to changes in oxygenation causes generation of ROS within the trophoblast and endothelial cells, placentas. Furthermore, labour, in which the placenta is exposed to repeated episodes of ischaemia–reperfusion, induces high levels of oxidative stress (42).

**Serum Uric Acid of concentration**

Table (2) shows the significant increase in uric acid level in all patient groups in comparison with their control groups (p ≤0.05), there is a significant increase in uric acid in patient group in comparison with control groups (43).

Uric acid is ubiquitous in body fluids and tissues, and its concentration in plasma is higher than that of most endogenous antioxidants, second only to albumin (44).

Plasma uric acid concentration is typically elevated in threatened abortion. It likely results from reduced uric acid clearance from diminished glomerular filtration, increased tubular reabsorption, and decreased secretion. Another possibility is from increased placental urate production compensatory to increased oxidative stress. Recently increased oxidative stress and formation of reactive oxygen species (ROS) have been proposed as another contributing source of Hyperuricemia noted in threatened abortion apart from renal dysfunction (45).

**Serum Albumin of Concentration**

Table (2) shows the significant increase in Albumin value in all patient groups in comparison with control groups (p ≤0.05). This result is similar to the result of (46).

Moreover, blood is at least comparable, if not more exposed to ROS than inside cells. Concentration of antioxidants is much lower in plasma than in cells (47). Several models of oxidation indicate that albumin plays key role in antioxidant functions challenging the historical idea that only the intracellular compartment needs strenuous defense against oxidants (48, 49).

Antioxidant activities of albumin result from its ligand-binding capacities. Albumin is well known for its ability to bind molecules, such as metals ions, fatty acids, drugs, and also hormones. The flexibility of the albumin structure adapts it readily to ligands, and its three domain design provides a variety of binding sites (50). Albumin has several important physiological and pharmacological functions. It transports metals fatty acids, cholesterol, bile pigments, and drugs. It is a key element in the regulation of osmotic pressure and distribution of fluid between different compartments. In normal conditions its half-life is about 20 days, and its plasma concentration represents equilibrium not only between its synthesis in the liver (51), and its catabolism, but also its transcapillary escape. In general, albumin represents the major and predominant antioxidant in plasma, a body compartment known to be exposed to continuous oxidative stress. A large proportion of total serum antioxidant properties can be attributed to albumin. Previous works have shown that more than 70% of the free radical-trapping activity of serum was due to human serum albumin (HSA) as assayed using the free radical-induced hemolysis test (52).
Table (2) Serum Antioxidant concentration (Cp, Ua, Alb) study subjects

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Cp (g/l)</th>
<th>Uric acid (mg/dl)</th>
<th>Alb (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnant s with T.A</td>
<td>100</td>
<td>6.15±0.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.76±0.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.79±0.06&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Normal pregnantn</td>
<td>50</td>
<td>3.75±0.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.41±0.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.22±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>LSD</td>
<td></td>
<td>0.66</td>
<td>0.17</td>
<td>0.14</td>
</tr>
</tbody>
</table>

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