The relationship between serum copper, zinc, and glutathione peroxidase with malondialdehyde in women with unexplained recurrent miscarriage.

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Abstract
The study was designed to evaluate the relationship between serum copper, serum zinc, also with malondialdehyde (MDA) in women with unexplained recurrent miscarriage. Also, the study evaluated the relationship between glutathione peroxidase with malondialdehyde (MDA) in women with unexplained recurrent miscarriage. Blood samples were take from obstetric and gynecology outpatient departments of a ministry of healthy hospitals. Patient women with, at least, three consecutive fetal losses. Seventy five women with unexplained recurrent miscarriages (study group) and eighty healthy controls (group control) with no antecedent fetal loss. We found that significantly increased lipid peroxidation, measured as MDA, was demonstrated in women with unexplained recurrent (p< 0.02). The mean serum copper level in in women with unexplained recurrent was significantly lower than the control group (p< 0.01). In addition, the mean serum zinc level in women with unexplained recurrent was significantly lower than the control group(p< 0.04), and the mean serum glutathione peroxidase level in patients with miscarriage was significantly lower than the control group (p< 0.024). Moreover, a negative correlation was also observed between glutathione peroxidase, copper, zinc with MDA levels in the patient group but not in the control group.

Introduction
Spontaneous abortion is a new issue in terms of its social and economic impact. Today more and more women decide to conceive in their thirties or forties, but are often thwarted by infertility. Many women are career-orientated during the age of maximum fecundity. Competition and the desire to earn more is associated with a delay in the age at which the first pregnancy is attempted and possibly with other factors (e.g. stress, pollutants, smoking) that later increase the risk of miscarriage(1). Placental-related disorders of pregnancy are almost unique to the human species. These disorders, which affect around a third of human pregnancies, primarily include miscarriage and preeclampsia. Independent of the cause of the miscarriage, the
excessive entry of maternal blood into the intervillous space has two effects: (i) a direct mechanical effect on the villous tissue which becomes progressively enmeshed inside large intervillous blood thrombi (ii) and a widespread and indirect $O_2^-$-mediated trophoblastic damage and increased apoptosis (2,3,4). Reaction oxygen species (ROS), such as superoxide anion radical ($O_2^-$) and hydrogen peroxide ($H_2O_2$), are constantly produced during metabolic processes in all living species. Under normal physiological condition, cellular ROS generation is counterbalanced by the action of antioxidant enzymes and other redox molecules. The balance between $O_2^-$ generation and elimination is important for maintaining proper cellular redox states. A moderate increase in ROS can stimulate cell growth and proliferation (5). However, excessive ROS accumulation will lead to cellular injury, such as damage to DNA, protein, and lipid membrane. Because of their potential harmful effect, excessive ROS must be promptly eliminated from the cells by a variety of antioxidant defense mechanisms, including important enzymes, such as superoxide dismutase (SOD), catalase, and various peroxidases. The cytosolic copper/zinc containing SOD (Cu,Zn-SOD, or SOD1) and the mitochondrial manganese-containing SOD (Mn-SOD, or SOD2) are essential enzymes responsible for catalyzing the conversion of $O_2^-$ to $H_2O_2$ (5,6). Other placental studies have shown the superoxide radical and its scavenging system play an important role in endothelial function but are not involved in parturition. The SOD activity of each cell type was largely copper- and zinc-containing SOD activity (7). Prime targets of reactive oxygen species are the polyunsaturated fatty acids in cell membranes causing lipid peroxidation, which may lead to damage of the cell structure and function (8). Additionally, decomposition of lipid hydroperoxides yields a wide variety of end-products, including malondialdehyde (MDA). Increased levels of serum malondialdehyde, a measure of lipid peroxide activity, have been found to be increased in women undergoing spontaneous abortion (9). A growing body of evidence has indicated that many trace elements play an important role in a number of biological processes by activating or inhibiting enzymatic reactions, by competing with other elements and metalloproteins for binding sites, by affecting the permeability of cell membranes or by other mechanisms (10). It is, therefore, reasonable to assume that these trace elements would exert action directly and indirectly, in normal and pathological pregnancy. Various studies have suggested that these trace element induce and spontaneous abortions (11). Divalent ions of transition metals can promote lipid peroxidation in vitro and much attention is currently focused on lipid peroxidation in the pathogenesis of metal toxicity (12). The mechanisms of pathogenesis could be mediated by direct effects of certain trace elements (e.g., Fe, Zn, and Cu) on the formation of hydroxyl free-radical from hydrogen peroxide and superoxide via the Fenton and Haber-Weiss reaction(5). More recently, other authors found the decrease glutathione peroxidase activity play an important role in the occurrence of spontaneous abortion (13). Also, GP catalyzes the reduction of hydrogen peroxide and organic hydroperoxides, thus preventing lipid peroxidation of cell membranes and acting as free radical scavenger. In recent years, using MAD as a marker of oxidative stress, there has been growing interest in studying the role played by lipid peroxidation in spontaneous abortion (14,15,16). Therefore, the present study was undertake to evaluated the association between MDA (a marker of oxidative stress) with glutathione peroxidase, serum cupper, and serum zinc in miscarriage.
Materials and methods:

Fifty women with unexplained recurrent miscarriage had at least three consecutive miscarriage in early pregnancy stage. All mean age of patients was 27.1±11.2, with a range of 18-42 years. There were 70 healthy volunteers (28.2±10.6, 19-41 years) used as control subjects. Control group that had delivered at least tow live children and had not suffered from spontaneous miscarriage, fetal death, or placental abruption. Women were excluded from the study, if they had any of the following abnormalities, uterine anomalies, fibroids, Ascherman’s syndrome, and genetic disorder. And endocrine factors including polycystic ovary disease, thyroid dysfunction, and diabetes. Reproductive tract infection such as rubella, and herpes viruses. Before sampling collection, if any gradients regarding medication taken by patients that would interfere with homocysteine test such as methotrexate, folic acid, or exposed hours ago to nitrous oxide were considered.

Fasting blood samples (10 mL) were collected from patients and controls. The blood samples were centrifuged at 3000 rpm for 10 min at 4°C. both the patients and controls sera were stored at 4°C in an ice chest for no longer than 24 h before freezing.

Statistical analysis

The results are expressed as mean ± SD (1SD). Statistical analysis was performed using student’s t-test; p values <0.05 were considered significant.

Materials

The entire chemical in this were imported from BDH Co and Sigma chemical co. Expected Kits from Giesse for copper and zinc.

Assessment of the lipid peroxidation activity:

The assessment of lipid peroxidation process is achieved via determination the byproduct; Malondialdehyde(17).

The level serum malondialdehyde was determined by a modified procedure described by Guidet B. and Shah S.V.(18). In brief; to 150 µl serum sample add the followings: 1 ml trichloroacetic acid 17.5 %, 1ml of 0.6% thiobarbituric acid, mixed well by vortex, incubate it in boiling water bath for 15 minutes, then allowed to cool.

Then add 1ml of 70% TCA, and let the mixture to stand at room temperature for 20 minutes, centrifuged at 2000 rpm for 15 minutes, and take out for scanning spectrophotometrically.

\[
\text{Absorbance at 532 nm} = \frac{L \times E_0}{D}
\]

\(L\): light bath (1cm)
\(E_0\): extinction coefficient 1.56 x 10^5 M\(^{-1}\) Cm\(^{-1}\).
\(D\): dilution factor

Determination of total glutathione peroxidase activity(GPx)

GPx activity was measured by the method described by Rotruck et al (19). Briefly, reaction mixture contained 0.2 ml of 0.4 M Tris-HCl (BDH) buffer pH 7.0,0.1 ml of 10 mM sodium azide (Fluka), 0.2 ml of serum, o.2 glutathione, 0.1 ml of 0.2 mM Cumene hydroperoxide (Fluka). The contents were incubated at 37 C for 10 min. The reaction was arrested by 0.4 ml of 10% TCA, and centrifuged. Supernatant was assayed for glutathione content by using Ellman’s reagent(19.8 mg of 5,5'-dihydrobiisnitro benzoic acid (DTNB) in 100 ml of 0.1% sodium nitrate).
Determinations of serum trace metals:

Assessment of serum copper colorimetrically:
Serum copper is measured by a colorimetric method using commercially available kit (Giesse).

Assessment of serum zinc colorimetrically:
Serum zinc is measured by a colorimetric method using commercially available kit (Giesse).

Results
Women with unexplained recurrent miscarriage had significantly higher MDA levels (p<0.02) than controls, which suggested the presence of increased oxidative stress are shown in Table (I). The serum Cu level tended to decrease in these patients, the mean value of Cu concentration in patients was (70.3 ± 14.62) mg/L while the mean value for control was (154.3 ± 15.10) mg/L. Cu level was found to be lower in patient group compared with controls with P value of less than 0.05 as shown in Table (II). The serum Zn level tended to decrease in these patients, the mean value of Zn concentration in patients was (61.2 ± 15.9) mg/L while the mean value for control was (97.6 ± 23.7) mg/L. Zn level was found to be lower in patient group compared with controls with P value of less than 0.05 as shown in Table (III). The serum glutathione peroxidase level tended to decrease in these patients, the mean value of glutathione peroxidase activity in patients was (130 ± 33.1) U/L while the mean value for control was (164 ± 24.5) U/L. Glutathione peroxidase level was found to be lower in patient group compared with controls with P value of less than 0.05 as shown in Table (IV). Variation of MDA, trace element concentration, and glutathione peroxidase level showing enhancement or depression in women with unexplained recurrent miscarriage as shown in figure (1). Both serum MDA and Cu levels were significantly lower than those of the controls (p<0.05). As shown in figure (2), a significant negative correlation was found between serum MDA levels and serum Cu levels (R²=0.56, p<0.05). Similarly, both serum MDA and Zn levels were significantly lower than those of the controls (p<0.05). As shown in figure (3), a significant negative correlation was found between serum MDA levels and serum Zn levels (R²= 0.54, p<0.05) and also shown in figure (4), significant negative correlation was found between serum MDA levels and serum glutathione peroxidase activity level (R²= 0.58, p<0.05).

Table I: Mean malondialdehyde concentration (nmol/mL) of controls group and patients group.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean value of MDA conc.</th>
<th>SD</th>
<th>P&lt;0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>39.5</td>
<td>17.2</td>
<td>0.02</td>
</tr>
<tr>
<td>Patients</td>
<td>60.2</td>
<td>15.2</td>
<td></td>
</tr>
</tbody>
</table>

Table II: Mean copper levels (µg/dL) of controls group and patients group.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean value of Cu levels</th>
<th>SD</th>
<th>P&lt;0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>154.3</td>
<td>15.1</td>
<td>0.05&lt;P</td>
</tr>
<tr>
<td>Patients</td>
<td>70.3</td>
<td>14.62</td>
<td></td>
</tr>
</tbody>
</table>

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Table III: Mean zinc levels (µg/dL) of controls group and patients group.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean value of Zn levels</th>
<th>SD</th>
<th>P &lt; 0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>97.6</td>
<td>23.7</td>
<td>0.04</td>
</tr>
<tr>
<td>Patients</td>
<td>61.2</td>
<td>15.9</td>
<td></td>
</tr>
</tbody>
</table>

Table IV: Mean glutathione peroxidase activity levels (U/L) of controls group and patients group.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean value of GPx levels</th>
<th>SD</th>
<th>P &lt; 0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>164</td>
<td>24.5</td>
<td>0.024</td>
</tr>
<tr>
<td>Patients</td>
<td>130</td>
<td>33.1</td>
<td></td>
</tr>
</tbody>
</table>

Figure (1). Malondialdehyde (MDA) and metal concentration, and glutathione peroxidase (GPx) in women with unexplained recurrent miscarriage and controls.

Figure (2). Correlation between serum Cu and MDA levels in women with unexplained recurrent miscarriage (n=50, R^2 = 0.56, p<0.05)
Figure (3). Correlation between serum Zn and MDA levels in women with unexplained recurrent miscarriage (n=50, $R^2=0.54$, p<

Figure (4)
Correlation between glutathione peroxidase and MDA levels in women with unexplained recurrent miscarriage (n=50, $R^2=0.58$, p<0.05).
**Discussion**
Placental-related disorders of pregnancy are almost unique to the human species. These disorders, which affect around a third of human pregnancies, primarily include miscarriage and pre-eclampsia. In other mammalian species, the incidence of both disorders is extremely low. (1).

Evolution for mammals living on dry land has been closely linked to adaptation to changes in O\(_2\) concentration in the environment (20).

Most of the O\(_2\) used during the oxidation of dietary organic molecules is converted into water via the combined action of the enzymes of the respiratory chain. Around 1–2\% of the O\(_2\) consumed escapes this process and is diverted into highly reactive O\(_2\) free radicals (OFRs) and other reactive O\(_2\) species (ROS) at a rate dependent on the prevailing O\(_2\) tension (16). When the production of OFRs exceeds the natural cellular protection, indiscriminate damage can occur to proteins, lipids and DNA.

The physiological hypoxia of the first trimester gestational sac may protect the developing fetus against the deleterious and teratogenic effects of OFRs. Recent evidence also indicates that the hypoxia is necessary to maintain stem cells in a fully pluripotent state (21), for at physiological levels free radicals regulate a wide variety of cell functions, in particular transcription factors (20).

Oxidative stress-induced placental dysfunction may be a common cause of the multifactorial and polygenic etiologies of abortion, recurrent pregnancy loss, defective embryogenesis, hydatidiform mole, and drug-induced teratogenic effects. Placenta tissue may be a major source of lipid peroxidation products in pregnancy. Mihiailovic et al (22) showed that increased level of lipid peroxides in placental tissue during pregnancy.

In the study, we found MDA levels were elevated in the serum of pregnancy with abortion Concentrations of lipid peroxides have also been shown to increase in the villous and decidual tissues of women undergoing early pregnancy loss(23). Sane et al (24) concluded that oxidative stress may be associated with miscarriage. The products of lipid peroxidation reactions in the serum and tissues of failure pregnancy had been analyzed by Sigino et al (25). They found that serum MDA levels were elevated in pregnancy with abortion. In present study, our findings are in agreement with most of the earlier studies suggesting the abortion might be at risk from oxidative cell damage. Therefore, we suggested that the increase in the lipid peroxidation, through metabolic pathways, causes the increased production of MDA that leaks into the blood stream, consequently causing increased levels of MDA in failure pregnancy. Alteration in serum Cu and Zn levels occur in women with abortion. Copper is an essential element required for the formation of many enzymes with important roles in the human body. During pregnancy, the maternal serum copper concentration is increased due to the higher levels of ceruloplasmin that are the result of elevated oestrogen levels(26). Zinc is also essential component of genetic material and, a zinc deficiency can cause chromosome changes in both partners leading to an increase risk of miscarriage. Zinc is found in high concentration in sperm and adequate levels are needed to make the outer lager and tail. A correlation between miscarriage induction and the changes of trace elements such as copper and zinc, which facilitate the production of free radical, has been described (11). Previous results have also shown that women with miscarriage serious disturbances in the status of trace element, especially those involved in the antioxidant system i.e., Cu and Zn (11,12,13). Our findings show that a negative correlation was observed between serum Cu, the serum Zn, and MDA levels in women with abortion but not in the control group. It has been known that the antioxidants chosen comprised of two from peripheral blood-plasma thiol and ceruloplasma. Those changes were thought to offer
the cell protection from the damage caused by the increased oxidative stress associated with miscarriage (27). Ozgüneş H et al (28) found the positive correlation between the serum Cu level and ceruloplasmin activity in women with induced abortion. Other studies concluded that decrease serum Cu levels and serum Zn levels in women with either induced or spontaneous abortion compared with normal pregnancy (11). Bray et al (29) showed that essential biochemical function of zinc (Zn) is to serve as antioxidant. Zn has been shown to have an antioxidant role(s) in defined chemical system. Two mechanisms have been elucidated; the protection of sulfhydryl group, against oxidation and the inhibition of the production of reaction oxygens by transition metals. In the cytoplasm of eukaryotic cells, superoxide dismutase (Cu/Zn-SOD), one of the antioxidant enzymes, contains Zn in its active site (7,30).

GP is one of enzyme system that catalyze the breakdown of hydrogen peroxide. Spontaneous abortion have been associated with further decrease in (GPx). Our result are in agreement with those reported previously. Zachara et al (31) reported that the decreased blood glutathione peroxidase may play an important role in he aetiology of spontaneous abortion. Other studied that showed decrease blood glutathione peroxidase activity may be play an important role in occurrence of spontaneous(13). Biri et al (32) concluded reflected oxidative in placenta tissue of early pregnancy failure, as the oxidative processes seem to be counteracted by the physiological activation of antioxidant enzyme such as (GPx). From this point of view, our finding suggested that miscarriage progression may be due, at least in part, to the catalyzation of oxidative stress depend on Cu levels, Zn levels and glutathione peroxidase activity.

Conclusions

The presence of a relationship between oxidative stress and trace elements in women with unexplained recurrent miscarriage was observed in the present study. Also, the presence of a relationship between oxidative stress and glutathione peroxidase in women with unexplained recurrent miscarriage. Because only longitudinal studies could confirm causality by providing data on the MDA could be a result of the presence of unexplained recurrent miscarriage, but oxidative stress may not be implicated in unexplained recurrent miscarriage its development. We also suggest that increased oxidative stress present in may result from changes in the levels of certain trace elements. Additional studies, some of which we have already began, are required to explore the roles played by antioxidant (glutathione peroxidase) in women with unexplained recurrent miscarriage.

References

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