A CLINICAL INVESTIGATE STUDY FOR HOMOCYSTEINE AND SOME TRACE ELEMENTS LEVELS IN PREGNANT WITH PREECLAMPSIA AND PREMATURE BIRTH

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ABSTRACT:

Background: Homocysteine is a sulfhydryl-containing amino acid that is formed when methionine is demethylated and is needed for intravascular metabolism. Plasma homocysteine concentrations decrease normally in pregnancy, while the rise in homocysteine is associated with preeclampsia and is also responsible for the death of the fetus in the womb and repeated miscarriage, and intrauterine growth restriction have been associated with elevated homocysteine levels during pregnancy, clots of blood. These levels are linked to the influence of Hcy on blood vessels, endothelial activity, increased pro-oxidative, endothelial metabolic disorders.

Aims: Investigation of the relationship between elevation of homocysteine levels and levels of trace elements (selenium, copper, magnesium, and ferritin) in serum of pregnant women with preeclampsia and risk of preterm labor.

Patients and methods: The study extended from October (2020) to February (2021). The study included (130) women, were divided into Control group (60) women, divided into two groups, (30) women a healthy pregnancy, and (30) non-pregnant women, and Preeclampsia group Includes (35) women suffering from preeclampsia, preterm labor (35) women at risk of premature labor.

Results: The results show a significant increase in the level of homocysteine in the preeclampsia 13.27 ± 2.49 and preterm labor 13.03 ± 0.95 groups compared with control groups, a significant decrease serum magnesium (1.82 ± 0.31), copper (139.82 ± 33.63) and selenium (61.20 ± 19.09) in both groups of patients in preeclampsia and premature birth campare with control goups, while the results show a presence of a significant increase in Ferritin (21.27 ± 7.57) in both groups of patients campare with control goups.

Where it was found that there is a negative correlation between homocysteine and each of selenium, magnesium, and copper, and a positive correlation between homocysteine and ferritin.

Conclusion: Our study show that preeclampsia and premature birth are associated with higher ferritin concentration and lower magnesium, copper and selenium.

Keywords: Preeclampsia, premature birth, copper, selenium, magnesium, ferritin, pregnancy.

I. INTRODUCTION

Preeclampsia is a common pregnancy disease that affects 3-5% of all pregnancies. Preeclampsia onset is less common but has higher rates of maternal illness and perinatal mortality. The prominent pathological feature of early pre-eclampsia is an abnormal development of the placenta. The placenta in preeclampsia is usually characterized by the remodeling of the abnormal blood vessels that begins in the first trimester of pregnancy, where the flexible muscular wall cannot be replaced by fibrous material, as a result of this failure, the spiral arteries fail to transform into large (Boksla et al., 2016). Preterm labour is the leading cause of perinatal
morbidity and mortality in developing nations, with neonates with a gestational age of less than 32 weeks accounting for the bulk of deaths. Low birth weight (LBW) is also linked to an increased risk of death as well as short- and long-term health issues (Stylianou-Riga et al., 2018). Homocysteine is a sulfhydryl-containing amino acid that is formed when methionine is demethylated and is needed for intravascular metabolism. Homocysteine is thought to be a possible risk factor for the progression of atherosclerotic vascular disease processes that lead to cardiovascular disease (CVD) and death a stroke (Chrysant & Chrysant, 2018). Physiological levels of Hey are mainly determined by food intake and vitamin status. Usually, HHcy is characterized as >15 micromol/L levels (Moretti & Caruso, 2019). Plasma homocysteine concentrations decrease normally in pregnancy, as there is a presumed increase in the absorption of homocysteine from the fetus. The most well-known effect of homocysteine metabolism on pregnancy is its association with the problem of (NTD) neural tube defects. Folic acid, can reduce the risk of developing NTD, and for the first time a subsequent systematic review confirmed the benefit of folate before pregnancy and nutritional supplements in preventing NTD without any effect on miscarriage and ectopic pregnancy (Hague, 2003). Homocysteine levels during pregnancy decrease during the first three months of pregnancy, reaching a minimum during the second trimester, and then rise slightly during the third trimester. The rise in homocysteine is associated with preeclampsia and is also responsible for the death of the fetus in the womb and repeated miscarriage (Aubard et al., 2000). Trace elements refers to chemical elements present in very small quantities in a natural substance, with an average concentration of <100 μg / g. It is required for the growth and development of the organism, in the biological processes of trace elements of importance (Al-Fartusie & Mohssan, 2017). Every trace element contributes differently to each part in the body, the several major physiological and biochemical processes, the Interaction in biological processes between trace elements plays a role in The mediation of biological and chemical reactions that could be used for human health management (Rasdi et al., 2013). Selenium (Se) is an essential micronutrient for the proper functioning of all living organisms, it helps many enzymes such as glutathione peroxidase and is important in protecting against oxidative stress, and when supplementation is lacking, the risk of many diseases increases (Kieliszek, 2019). Selenium joins in several biological functions, which play an important role in body, such as The oxidative/antioxidant ratio, immune function control and inflammatory response moderation, thyroid hormone metabolism development and regulation, The regulation of oxidative stress (Bizerea et al., 2018). Serum and red blood cells are the main sources of magnesium (Mg). Magnesium is found in the human body in amounts of around 25 g. Magnesium is required for the action of over 300 enzymes in humans (DiNicolantonio et al., 2018), (Uwitonze & Razzaque, 2018). Copper is an trace essential element that is a cofactor for antioxidant enzymes, and thus is essential for health and for reducing the risk of disease. However, excessive copper may be harmful and could generate an oxidizing substance, which thus means an imbalance of antioxidant systems, (Song et al., 2017). Copper participates in the oxidative balance and immune processes and inhibits or activates many enzymes and is part of the enzyme Cu, Zn-DOS enzyme (superoxide dismutase), which is the enzyme of the first line of antioxidants. Copper's relationship to oxidative balance indicates a possible association with pregnancy-induced hypertension. Pregnancy-induced hypertension (PIH) occurs on average in 5–10% of pregnant women and increases maternal and fetal morbidity and mortality. This disease includes two main forms: gestational hypertension (GH) and preeclampsia (PE). (Lewandowska et al., 2019). Copper ions are needed for the health of bone tissue, according to numerous reports. Low serum copper levels are linked to lower BMD, while high serum copper levels are linked to deformities, hypoplasia, weak bones, and recurrent fractures. Copper may be used as a simple, radiation-free osteoporosis screening tool (Qu et al., 2018). Ferritin is a significant iron storage protein that retains a large iron center in its cavity and has ferroxidase activity. One of ferritin's major properties is its ability to attract iron ions and promote their mineralization by its ferroxidase activity (Arosio et al., 2017). Ferritin is a significant mediator of immune dysregulation, contributing to a cytokine storm by direct immunosuppressive and inflammatory-stimulating impact. The fatal outcome of COVID-19 has been linked to cytokine storm syndrome, COVID-19 intensity is thought to be influenced by ferritin levels. Individuals with moderate to severe COVID-19 saw a rise in ferritin levels in the blood, Ferritin levels are directly linked to the seriousness of COVID-19 (Vargas-Vargas & Cortés-Rojo, 2020) (Kowdley et al., 2012). Due to the expansion of red blood cell mass and the transition of the levels of iron to placental structures and the growing fetus, iron needs rise during pregnancy. Preterm delivery, small-for-gestational-age birth, and low birth weight are also linked to maternal anemia (Næs-Andresen et al., 2019).
II. PATIENTS AND METHODS

This study was conducted at department chemistry – faculty science, Alshatra general hospital, Maternity and children ( Bint Al- Huda ) Hospital and Al-Khalisa Health Dispensary in Thiqar - Iraq. The study extended from October (2020) to February (2021). The study included (140) women, 60 control subjects and 70 patients. controls and patients were divided into :

1- Control group : includes (60) women, divided into two groups, a group of (30) women enjoying a healthy pregnancy, and (30) non-pregnant women .

2- Preeclampsia group : includes (35) women suffering from preeclampsia.

3- Preterm labor: Includes (35) women at risk of premature labor.

The homocysteine was measured using the ELISA technique (BT LAB – China), Selenium level was measured using Atomic absorption spectrophotometer , And all of the magnesium, copper and ferritin were measured using Copas COBAS INTEGRA systems (Ferguson et al., 1964).

Collection of blood Samples:
Venous blood (5 ml) was collected from patients which recognized the clotting for 20 minutes at room temperature. After the blood clot, it was transferred to a centrifuge at (3000) in order to obtain the serum. The collected serum is used to estimate the variables in this study as it was stored in the freeze at (-80 °C) until use.

Statistical Analysis:
Statistical analysis was based on one way ANOVA (analysis of variations test with LSD (least significant difference)), differences were considered to be significant if p < 0.05. Statistical analysis was carried out using SPSS statistical version 23.0 SPSS Inc, Chicago, 111.

III. RESULTS AND DISCUSSION:

Clinical and Characteristic Features of the Studied Groups:
There are a total of (130) subjects included in the current study, the group of patients (70) divided into two groups (preeclampsia and premature birth) were compared with the tow control group of healthy subjects (60). Characteristic data for all studied groups shown in Table 1:

Table 1 Characteristic data for studied groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>NO.</th>
<th>Age (Year)</th>
<th>BMI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non pregnant</td>
<td>30</td>
<td>32.35 ± 7.58</td>
<td>30.41 ± 4.70</td>
</tr>
<tr>
<td>Healthy preg.</td>
<td>30</td>
<td>26.25 ± 6.01</td>
<td>30.52 ± 4.09</td>
</tr>
<tr>
<td>Premature birth</td>
<td>30</td>
<td>31.05 ± 11.36</td>
<td>26.90 ± 3.35</td>
</tr>
<tr>
<td>Preeclampsia</td>
<td>30</td>
<td>30.85 ± 5.15</td>
<td>30.25 ± 5.23</td>
</tr>
<tr>
<td>L.S.D</td>
<td></td>
<td>4.15</td>
<td>1.87</td>
</tr>
</tbody>
</table>
Serum Homocysteine Concentration:

Table (2) shows a significant increase in the level of homocysteine in the preeclampsia and preterm labor groups compared to the healthy groups. The results of the current study are consistent with previous studies that found an increase in homocysteine in women exposed to preeclampsia and at risk of preterm labor (Dymara-Konopka & Laskowska, 2019). As a study showed Pisal et al., 2019, increase in homocysteine levels in the groups of preeclampsia and the risk of preterm labor correlated with B12 deficiency. Hyperhomocysteinemia can damage the vascular system that supports placental function during pregnancy, which can lead to miscarriage and other negative pregnancy outcomes. In women who have experienced repeated pregnancy loss, lowering homocysteine levels will increase pregnancy outcome (live birth) (Humadi, 2018).

Table (2): Serum homocysteine levels of control and patients groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>NO.</th>
<th>Homocysteine (nmol /ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean ±SD</td>
</tr>
<tr>
<td>Non pregnant</td>
<td>30</td>
<td>8.92&lt;sup&gt;b&lt;/sup&gt; ± 0.64</td>
<td></td>
</tr>
<tr>
<td>Healthy pregnancy</td>
<td>30</td>
<td>9.13&lt;sup&gt;b&lt;/sup&gt; ± 0.40</td>
<td></td>
</tr>
<tr>
<td>Premature birth</td>
<td>35</td>
<td>13.03&lt;sup&gt;a&lt;/sup&gt; ± 0.95</td>
<td></td>
</tr>
<tr>
<td>Preeclampsia</td>
<td>35</td>
<td>13.27&lt;sup&gt;a&lt;/sup&gt; ± 2.49</td>
<td></td>
</tr>
<tr>
<td>L.S.D</td>
<td></td>
<td>0.73</td>
<td></td>
</tr>
</tbody>
</table>

Legend as table (1)

Comparison of homocysteine levels with some trace elements:

There was statistically significant difference in the levels of trace elements selenium, copper, magnesium, and ferritin. Levels in preeclampsia and premature birth at (p>0.05) in the serum of preeclampsia and preterm birth groups compared with control groups Table (3) and figure (1).

Table (3) Homocysteine and (Se, Mg, Cu and ferritin) levels of control and patients groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Hcy</th>
<th>Se</th>
<th>Mg</th>
<th>Cu</th>
<th>Ferritin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non pregnant</td>
<td>Hcy</td>
<td>8.92&lt;sup&gt;b&lt;/sup&gt; ± 0.64</td>
<td>Se</td>
<td>81.26&lt;sup&gt;a&lt;/sup&gt; ± 7.21</td>
<td>Mg</td>
</tr>
</tbody>
</table>

No: Number of subjects.
SD: Standard deviation.
LSD: Least Significant Difference

(a, b, c) Means having different letters in the same column differed significantly (P<0.05).
Serum Selenium Concentration:

We note in Table (3) and figure (1) there is a decrease in selenium values for the groups of preeclampsia and risk of preterm labor compared to the two control groups, healthy and non-pregnant pregnancy (p = 0.002).

These results are consistent with several previous studies (Eze et al., 2020), (Vanderlelie & Perkins, 2011), (Mazloomi et al., 2021), (Yıldırım et al., 2019), (Duntas, 2020). Premature birth, miscarriage, preeclampsia, and intrauterine growth retardation are all linked to low blood Se levels during pregnancy, which is also followed by decreased GSH-Px activity. Reduced antioxidant enzyme activity during pregnancy results in oxidative stress within tissues, which contributes to premature birth, miscarriage, preeclampsia, and intrauterine growth retardation. Uncontrolled ROS formation is dangerous because placental production is highly dependent on oxygen levels (Zachara, 2018).

As noted in Figure (2) there is a negative association between homocysteine and selenium in the preeclampsia and premature birth risk groups with correlation coefficient (r = -0.504**, P.value = 0.002).

Figure (1) Homocysteine and Trace elements levels of control and patients groups
Serum Magnesium Concentration:

As we note in Table (3) and figure (1) there is a significant decrease in the magnesium values in the groups of preeclampsia and the risk of preterm labor (p = 0.001). Mg deficiency may be a contributing factor in the development of preeclampsia (Kharb et al., 2018). These results are consistent with several previous studies (Darkwa et al., 2017), (Sukonpan & Phupong, 2005), (Saputri et al., 2020), (Rossokha et al., 2020). Results of some studies show that there is low levels of magnesium and calcium in women with preeclampsia. Low magnesium has consequences as there is a decrease in cerebral blood flow, an increase in the bursting of nerve cells, and a spasm of blood vessels. Magnesium has a protective effect on vessels. Clinical implications of low magnesium may be a constriction of blood vessels and a decrease in cerebral blood flow. Thus the tendency to preeclampsia. Doctors now agree that the drug for preeclampsia is magnesium sulfate. Its effects include increased blood flow, vasodilation, which prevents by selectively expanding cerebral blood vessels and relieving cerebral spasm associated with preeclampsia. It will prevent recurrent seizures in patient, and it is beneficial in reducing mortality and morbidity in both the mother and the fetus magnesium is capable shortening the hypertensive episode in preeclampsia (Kreepala et al., 2018).

As noted in Figure (3) there is a negative correlation between homocysteine and magnesium in the preeclampsia and premature birth risk groups with correlation coefficient (r=0.491, P value=0.001)
Serum copper Concentration:
As noted in Table (3) and figure (1) there is a significant decrease in the copper values in the groups of preeclampsia and preterm labor compared to the two non-pregnant and healthy pregnancy groups (p = 0.00 6) .Low maternal copper levels are linked to preeclampsia and can play a role in the disease's development (Kanagal et al., 2014) .These results are consistent with several previous studies (Keshavarz et al., 2017) , (Al-Jameil et al., 2014) , (Vukelić et al., 2012) , (Keshavarz et al., 2017) . Development restriction has been linked to oxidative stress. As cofactors of enzymes, micronutrients including copper, zinc, magnesium, manganese, and selenium play a role in antioxidant protection. Superoxide dismutase is an antioxidant enzyme that contain trace elements zinc and copper. Copper is a trace mineral that is essential for the production of enzymes. Many enzymes play a critical function in the human body. It plays a crucial role in pregnancy. Within the developing fetus, a wide range of enzymatic and other processes are developed (Mohamed et al., 2019) .

We notice in the figure (4) there is a negative correlation between homocysteine and copper in the preeclampsia and premature birth risk groups with correlation coefficient (r=-0.210 , P .value= 0.06 ).
Figure (4): Correlation between serum Hcy and Copper in preeclampsia and premature birth groups

**Serum Ferritin Concentration:**
As shown in the table (3) and figure (1) there is a significant increase in the level of ferritin in the basic groups compared to the control groups (p=0.002).

that serum iron and serum ferritin levels are significantly higher in pregnant women with pre eclampsia. Excess iron may be a cause of oxidative stress, which may play a role in the pathogenesis of preeclampsia (Maitra et al., 2019). These results are consistent with several previous studies (Shaji Geetha et al., 2020), (ElShahat et al., n.d.), (Ahn et al., 2021), (Abdel-Malek et al., 2018).

Ferritin is a cytosolic protein found in most tissues. It plays a significant role in the preservation of intracellular iron and has been the subject of several recent reviews. Ferritin is a 24-subunit protein that is divided into two types: H and L subunits (Wang et al., 2010), pre-eclamptic women have a higher level of serum iron and that does not necessarily indicate an abundance of iron. Elevated serum ferritin occurs in a number of clinical conditions involving non-utilization of iron and tissue destruction, such as hemolytic anemia, hepatic damage, or suppression of erythropoiesis leading to storage iron accumulation. He discovered that a high ferritin level was linked to an increased risk of cancer. Lower ferritin levels were linked to a lower risk of pre-eclampsia and pre-labor rupture of membranes, whereas higher ferritin levels were linked to a higher risk of preterm delivery and neonatal asphyxia (Maitra et al., 2019).

Figure (5) shows the positive correlation between homocysteine and ferritin in the preeclampsia and premature birth risk groups with correlation coefficient (r=0.447, P. value =0.002).

![Figure 5: Correlation between serum Hcy and Ferritin in preeclampsia and premature birth groups](image)

**IV. CONCLUSIONS:**
From the data in this study, it was conclude Increased homocysteine in the groups of preeclampsia and risk of preterm labor, Increased ferritin levels in women at risk of preterm labor and women with preeclampsia.

Significant decrease in the values of the trace elements copper, selenium and magnesium.

**REFERENCES:**