

The Association of Serum Copper with Ceruloplasmin and Zinc with Folic Acid Levels in Recurrent Pregnancy Loss in Iraqi Women

Olaa Riad Kadim, Shatha M. J. Al-Khateeb, Esraa H. Humadi¹

Departments of Chemistry and Biochemistry and ¹Obstetrics and Gynaecology, College of Medicine, Al-Mustansiriyah University, Baghdad, Iraq

Abstract

Background and Objectives: Recurrent pregnancy loss (RPL) is the most common adverse pregnancy outcome. Micronutrients include minerals and vitamins are essential for normal function, growth, and development. Minerals have important effects on the health of the mother and the fetus. Micronutrient deficiency during pregnancy can lead to obstetric complications and even fetal death. **Materials and Methods:** A total of 90 pregnant women included in this study were between 7 and 20 weeks of gestation, their age was 18–40 years, and they were divided into three main groups. Patients: Group 1, pregnant women diagnosed with missed miscarriage no = 30; Controls: Group 2, ongoing pregnancy no = 30; and Group 3, nonpregnant women no = 30. Serum copper (Cu) and zinc (Zn) were determined using flame atomic absorption spectrometry. While the serum ceruloplasmin (CP) was measured by immunoturbidimetric assay and folic acid (FA) by enzyme-linked immune sorbent assay. **Results:** Serum Cu and CP were significantly higher in the women with RPL compared with healthy groups. Moreover, the levels of Zn were significantly lower in women with RPL compared with healthy control groups, and the result of serum FA was showed that there no significant variation in patients with RPL compared with control groups. **Conclusions:** The study showed that the alteration in the trace elements could participate with the RPL.

Keywords: Ceruloplasmin, copper, folic acid, recurrent pregnancy loss, zinc

INTRODUCTION

Recurrent pregnancy loss (RPL) defined as the spontaneous loss of a clinical established intrauterine pregnancy before the fetus has reached viability, thus it includes all pregnancy losses from the time of conception until 24 weeks of gestational.^[1] Micronutrients, minerals, and vitamins accessible from diet, they are essential for biological activity. Copper (Cu) is a trace element required for a wide variety of enzymatic reactions.^[2] Diet is the common source of Cu, and Cu can be found in the vegetarian diets and in the water by the Cu plumbing.^[3] Estrogen hormone stimulates the retention of Cu and this is the reason of Cu toxicity in women, and poor fertility rate can be increased during Cu toxicity.^[4]

Cu and ceruloplasmin (CP) levels increase significantly during pregnancy, and the accumulation of Cu mainly during the second and third trimester which stored in the liver primarily as metallothionein,^[5] thus the excess of these trace metal levels

may leads to increase the chances of miscarriage.^[6] CP, is an essential ferroxidase, it is synthesized in hepatocytes and secreted into the plasma, after the incorporation of 6 atoms of Cu.^[7] CP is an acute phase reactant, it carries about (70%) of the total Cu in plasma while about (15%) carried by albumin,^[8] so it could be as a sensitive indicators for assessment of the course of pregnancy and placental function.^[9]

Zinc (Zn) is an essential micronutrient that is important to the several biological processes including: enzyme activity, immune function, neurological function, and reproduction. It is important for the many aspects of metabolism through the

Address for correspondence: Ms. Olaa Riad Kadim,
Department of Chemistry and Biochemistry, College of Medicine,
Al-Mustansiriyah University, Baghdad, Iraq.
E-mail: lolaa_lolaa@yahoo.com

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incorporation in the antioxidant proteins (Cu/Zn superoxide dismutase), it is essential for the cellular division and differentiation. It is the vital nutrient that required for the normal embryogenesis.^[10]

There are many causes of decline in serum or plasma Zn during pregnancy such as first, low serum albumin, high estrogen levels; second, increased maternal blood volume has been suggested as factors lowering plasma Zn level consequently; and there are other reasons due to low Zn levels were prominent in women with dietary Zn deficiency.^[11]

Zn plays an important role in the absorption, synthesis, and biological activation of folate, therefore, and during pregnancy, Zn deficiency may lead to folate deficiency and as a conclusion lead to neural tube defects (NTDs) and many disorders in the fetal.^[12,13]

Folate (B_9) is a water soluble vitamin, it plays a cofactor in the reaction, which one-carbon can be transferred and acts as a central role in nucleic acid biosynthesis.^[14] Its involvement in the synthesis of DNA, RNA, and in the methylation of protein, and even DNA synthesis and further DNA repairing.^[15,16] Folate deficiency has a possible role factor in the RPL by raising the homocysteine levels in the body which leads to the damage of the endothelial vasculature all over the body, such as the placental vasculature.^[17] Folate can produce a lower risk of spontaneous abortion when taken pre-pregnancy and during pregnancy that showed by other study.^[18]

Aim of the study

The aim of the study is to test the role of trace element in the RPL.

MATERIALS AND METHODS

The study protocol was approved by the Ethical Committee of the Collage of Medicine/AL-Mustansiriya University/Department of Clinical Biochemistry. Our sample was collected from patients admitted to AL-Yarmouk Teaching Hospital in Bagdad between November 2017 to March 2018. The gestational age is calculated by the 1st day of reliable menstrual period and/or by early ultrasound scan. The study design is case-control. A total of 90 pregnant women included in this study were between 7 and 20 weeks of gestation, their age was ranged between 18 and 40 years, they were divided into three main groups. Group 1: Pregnant women diagnosed with missed miscarriage between 7 and 20 weeks of gestation and had history of at least three consecutive miscarriage no = 30; Group 2: Pregnant women between 7 and 20 weeks of gestation with single viable fetus without history of miscarriage no = 30; and Group 3: Nonpregnant women without history of miscarriage no = 30. Blood sample was collected before termination of missed miscarriage and then put the blood in the gel containing tubes, and the serum was then obtained and stored at -20°C until analysis.

Exclusion criteria

Women with Rh immunized, medical diseases such as diabetes mellitus and thyroid disease, congenital abnormality of uterus,

thrombophilia screen (+ve), antiphospholipid syndrome, twin pregnancy, and fetus with congenital abnormality. The determination of Zn and Cu levels: for estimation of Zn and Cu trace elements was conducted by flame atomic absorption spectrometry at wave length equal to 213.9 and 324.7 for Zn and Cu, respectively. The determination of ceruloplasmin by cobas c311 analyzer for quantitative immunological determination of human ceruloplasmin in serum and plasma. The determination of folic acid (FA): Determination of FA by “enzyme-linked immune sorbent assay” based on biotin double-antibody sandwich technology to assay Human FA.

Statistical analysis

SPSS 22.0.0 (Chicago, IL), MedCalc Statistical Software version 14.8.1 (MedCalc Software byba, Ostend, Belgium; 2014), Prism version 7.00 for Windows (GraphPad Software, La Jolla, California, USA), software package used to make the statistical analysis, p value considered when appropriate to be significant if less than 0.05 used for entering coding and statistical analysis of the data. The contentious variables were presented as mean and standard deviations (SDs). Pearson's Chi-square was used to assess the association between the categorical data of the included cases. The ANOVA test was used for comparing between more than two independent means. The level of significance in this study was $P < 0.005$.

RESULTS

The study include 30 patients, their age was ranged from 18-40 years with (Mean \pm SD) of serum copper in group 1 (RPL) (165.3 ± 13.5), group 2 (ongoing pregnancy) (136.1 ± 20.0), and group 3 (non-pregnant) was (119.8 ± 22.0), respectively. The mean serum Cu level in groups was statistically highly significant $P < 0.001$ [Figure 1].

CP in RPL group was significantly higher compared to nonpregnant women, no significant difference between ongoing pregnancy and nonpregnant women and between ongoing pregnancy and RPL women, as illustrated in Figure 2.

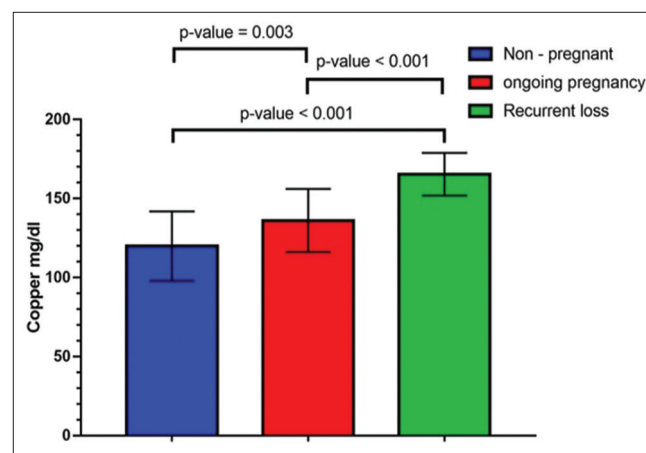


Figure 1: Difference in serum copper concentration in the recurrent pregnancy loss and healthy control

Zn in recurrent loss group was significantly lower compared to nonpregnant women and normal pregnancy, no significant difference between normal pregnancy and nonpregnant women, as illustrated in Figure 3.

The result was showed that there is no significant difference between the groups, in FA concentrations as illustrated in Figure 4.

DISCUSSION

RPL is a surprisingly common occurrence. Whereas approximately 15% of all clinically recognized pregnancies result in spontaneous loss, there are many more pregnancies

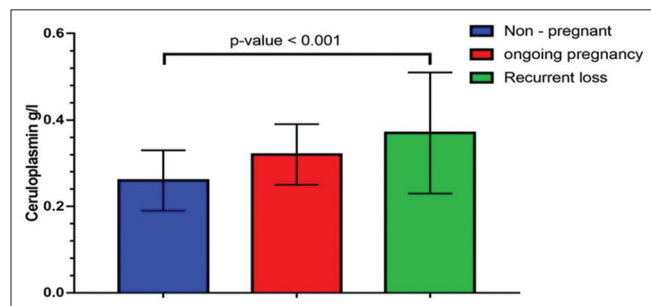


Figure 2: Difference in serum ceruloplasmin level in the recurrent pregnancy loss and healthy control

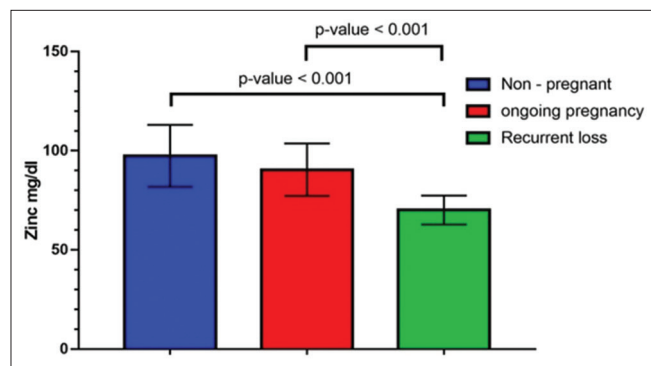


Figure 3: Difference in serum zinc level in the recurrent pregnancy loss and healthy control

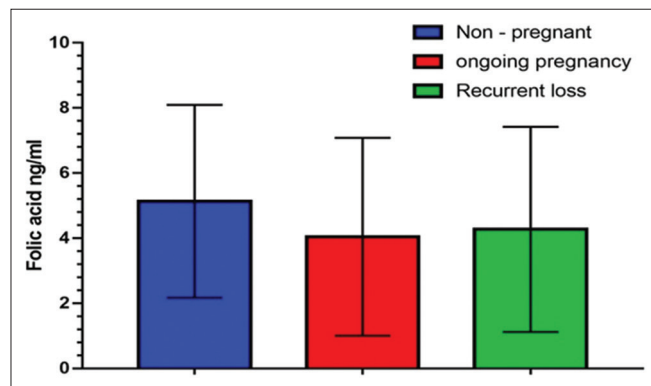


Figure 4: Difference in serum folic acid level in the recurrent pregnancy loss and healthy control

that fail prior to being clinically recognized. In the present study, there was a significant difference in Cu levels in Group 1, Group 2, and Group 3. The levels of serum Cu were also significantly higher in mothers with (RPL) in between all the three comparisons, which is agreement with Izquierdo Alvarez *et al.*^[9] in 2007 who showed that, high Cu levels in material serum are also considered detrimental being association with cerebral disorders in the fetus and increases the risk of spontaneous abortions.

The results of the current study showed that the levels of serum Cu and CP were significantly higher in women with (RPL) in between all three comparisons, that agreement with Prasad *et al.*^[19] in 2014, and serum Cu levels may be increase in pregnant women due to increase the binding affinity with CP, and this increase of CP production can be due to the passive transfer across the placenta, and increase Cu levels during pregnancy may be due to estrogen stimulated CP synthesis. Hence, the elevated level of hormone during pregnancy leads to rise of CP synthesis by making Cu more available through the mobilization from maternal tissues, especially liver.^[19]

In the present study, maternal serum Zn shows decrease levels in Group 1 (RPL) when compared with Group 2 (ongoing pregnant) and Group 3 (nonpregnant), similar findings by Hambidge^[20] in 2000 and Ahmed *et al.*^[21] in 2007. The deficiency of Zn may leads to loss of appetite in pregnant women and affects nutrients intake, which leads to poor fetal development.^[22] Zn deficiency has been suggested to influence the embryonic and fetal development by many mechanisms; first by decrease protein synthesis, second by reduced cell proliferation, and third by increases the rate of cellular oxidative damage.^[23]

Singla *et al.*^[24] conclude that Zn is necessary for the absorption, synthesis, and biological activity of folate, and Zn deficiency may be lead to folate deficiency in the embryo, that lead to a finally result in a NDTs.

In agreement with our study, Vila-Nova *et al.*^[25] study reported that there are no statistically significant changes between the three groups, also provided evidence that preconceptional FA use did not alter miscarriage rate. A study by Green *et al.*^[26] in 2003 proved that there is no significant effects of FA on plasma Zn concentrations.

CONCLUSIONS

It can be concluded that high levels of CP, Cu, and low level of Zn increase the risk of spontaneous pregnancy loss in Iraqi women. Thus, this alteration in the trace elements could be one of the causative factors with the RPL. Therefore, trace element is an important indicator of maternal nutritional status during pregnancy. Because trace elements play an important role during pregnancy and any reduction or elevation may lead to physiological abnormalities and disease, which may increase the incidence of adverse pregnancy outcomes. Therefore, pregnant women should maintain nutritional balance and have regular medical examinations.

Recommendation

I recommend measurement of serum trace element during the first trimester which may play role in reduce adverse pregnancy outcome and further study is recommended to test the predictive value of the trace element during pregnancy for any pregnant complication.

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Conflicts of interest

There are no conflicts of interest.

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