

# In Twin Pregnancies, Zinc and Iron Decreased, while Copper Increased Minimally

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## Abstract

**Objective:** Along with the increase in the frequency of multiple pregnancies, an increase is observed in the frequency of fetomaternal negativities. In our study, we aimed to determine the iron, ferritin, hemoglobin, zinc, and copper levels in second-trimester multiparous twin pregnancies and compare them with the values in singleton pregnancies and healthy women with the same demographic characteristics.

**Methods:** Three groups were created in the study; control group, single pregnancy group, and twin pregnancy group. Fasting venous blood samples were taken from individuals. Iron, zinc, copper, and ferritin levels were measured.

**Results:** Compared with the control group, the ferritin (Fe), and zinc (Zn) values of the individuals in the single and twin pregnancy groups were statistically low while copper (Cu) levels were significantly high ( $p < 0.05$ ). Also, when compared with individuals in a single pregnancy group, a statistically significant decrease was found in Fe, and Zn levels in the twin pregnancy group ( $p < 0.05$ ). Although there was a minimal increase in Cu levels, this increase was not statistically significant in the twin pregnancy group.

**Conclusion:** Since changes in trace element levels can lead to fetomaternal adverse effects, we think that dietary habits should be monitored, and zinc, copper, ferritin, and iron levels should be followed in pregnant women.

**Key words:** Twin pregnancy, iron, zinc, copper, ferritin.

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## INTRODUCTION

The rate of twin pregnancies in all pregnancies is approximately 1-2% (1). In multiple pregnancies, an increase has been seen in recent years, and the reason for this is the development of reproductive techniques and advanced maternal age (2). The increase in the frequency of multiple pregnancies is accompanied by negativities related to maternal and fetus health. It has been reported that twin gestation is more likely to suffer pregnancy complications such as neonatal deaths, preterm birth, pregnancy-related hypertension, early membrane rupture, diabetes, preeclampsia, and intrauterine growth retardation, than those with a singleton gestation (3, 4). Many physiological changes occur during pregnancy. One of them is anemia which develops due to hemodilution. Despite a 30-50% increase in blood volume, a 17-30% increase in erythrocyte volume causes hemodilution and causes physiological anemia (5). According to the World Health Organization (WHO) data, anemia during pregnancy is observed in 51% of pregnant women. The majority of anemia cases are iron-deficiency anemia (6). According to the WHO, this condition, defined as gestational anemia, is when hemoglobin (Hb) levels are below 11 g / dl during pregnancy (7). Iron is involved in many metabolic processes in the body. In iron deficiency, pregnant women and fetuses are negatively affected. A positive correlation was found between iron deficiency in the mother and decreasing iron in the fetus. As a result of iron deficiency, myelination disorder in the fetus and brain development are disrupted, and cognitive development is affected by hypoxia occurring in the brain due to hemoglobin deficiency (8, 9). Iron deficiency anemia has been shown to cause low birth weight and prematurity (10,

11). Also, iron deficiency anemia can cause diseases such as hemorrhage, heart failure, and preeclampsia in pregnant women and increases the risk of death (12). In studies conducted, it has been found that iron anemia was observed in twin pregnancies more than single pregnancies (13). Serum ferritin levels are a non-invasive, safe indicator of total iron stores in the body (14). Ferritin level below 12 µg/l has been supporting the diagnosis of iron deficiency anemia. Trace elements are found in many reactions in the organism as a cofactor. Zinc and copper are essential trace elements. Zinc is the element in the structure of metalloenzymes such as DNA polymerase, RNA polymerase, superoxide dismutase, carbonic anhydrase, alkaline phosphatase, carboxypeptidase, and alcohol dehydrogenase (15). Also, studies are showing that zinc has a protective role against oxidative stress. Copper, another trace element, is found in the structure of enzymes such as superoxide dismutase (SOD), cytochrome oxidase, lysyl oxidase, tyrosinase, and regulates metabolic reactions (16).

In this study, we aimed to determine the levels of iron, ferritin, zinc, and copper in second-trimester multiparous-twin pregnant without any known risk factors and compare the values of women with singleton pregnancy with the same demographic characteristics.

## METHODS

### *Study design:*

This prospective study was performed after approval by the local ethics committee (approval number: KSU-08.01.2020/02). This study was conducted in patients who applied to the Obstetrics and Gynecology outpatient clinic of Necip Fazil City Hospital for routine pregnancy follow-up between February 2020 and January 2021. Three groups were

created in the study (n=26/per group). These groups are; Control group == Group I (= healthy, non-pregnant women), single pregnancy group = Group II (2nd trimester), and twin pregnancy group = Group III (2nd trimester). Control group women between the ages of  $25,2 \pm 1,9$  years, single pregnancy group women between the ages of  $26,2 \pm 2,8$  years, twin pregnancy group women between the ages of  $28,1 \pm 5,2$  years. All pregnant women were in the  $16,2 \pm 3,1$  th week of pregnancy. Pregnant women who did not have any known risk factors including high blood pressure, autoimmune disease, obesity, and did not smoke and use any preparations were accepted. The control group consisted of completely healthy women of similar age group. Fasting venous blood samples were taken from individuals. Iron, zinc, copper, and ferritin levels were measured.

#### ***Biochemical analyzes:***

##### ***Obtaining serum samples from study groups***

Fasting blood was collected of pregnant women who did not eat or drink anything other than water for at least eight hours during the night. Then, blood samples were centrifuged at 4500 rpm for 10 minutes to obtain serum samples, and were frozen in microcentrifuge tubes at  $-80^{\circ}\text{C}$  and stored until the study day.

***Estimation of Iron and Ferritin levels:*** Iron and ferritin levels were assayed according to the manufacturer's instructions by using the autoanalyzer (Cobas e 601 module, Roche Diagnostics, F.Hoffmann-La Roche Ltd., Kaiseraugst, Switzerland).

***Estimation of Zinc and Copper levels:*** For zinc measurement, samples were diluted with 5% glycerol for until  $\frac{1}{4}$  zinc solution was formed. For Cu measurement, samples were irrigated with 10%

glycerol until  $\frac{1}{2}$  copper solution was formed. The levels of both trace elements were determined by using atomic absorption spectrophotometer (Perkin Elmer A Analyst, model 800, USA).

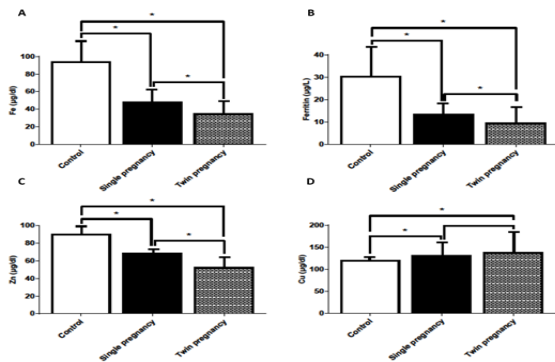
#### ***Statistical Analysis***

Statistical data comparisons were made using a standard software package (SPSS 20 for Windows; SPSS Inc., Chicago, IL, USA). Differences between groups were evaluated by one-way ANOVA, followed by the least significant differences (LSD) tests. All values were given as mean  $\pm$  SEM. P values  $<0.05$  were considered significant.

#### **RESULTS**

Zinc (Zn) levels ( $\mu\text{g/dl}$ ) were  $53.20 \pm 12.37$  in Group I,  $68.9 \pm 3.4$  in Group II,  $90.91 \pm 8.03$  in Group III, respectively. Copper (Cu) levels ( $\mu\text{g/dl}$ ) were observed to be  $139.31 \pm 33.97$  in Group I:  $132.42 \pm 28.10$  in Group II, and  $120.43 \pm 5.33$  in Group III, respectively. Iron levels ( $\mu\text{g/dl}$ ) were determined as  $35.62 \pm 12.20$  in Group I,  $48.61 \pm 15.38$  in Group II,  $94.53 \pm 23.61$  in Group III, respectively. Ferritin levels ( $\mu\text{g/L}$ ) were found to be  $9.71 \pm 6.48$  in Group I,  $13.62 \pm 6.42$  in Group II, and  $30.56 \pm 16.31$  in Group III, respectively. In light of these numerical data, there was no statistically significant difference between the ages of the groups. Compared with the control group, individuals in the single pregnancies group had statistically low Fe, Ferritin, and Zn levels, but Cu levels were significantly higher (figures 1A, B, C, D). The situation was the same in comparing the individuals in the twin-pregnancy group with the control group (figures 1A, B, C, D). Also, when compared with individuals in the single pregnancies group, a statistically significant decrease in Fe, Ferritin and Zn levels was found in the twin-pregnancy group (figures 1A, B, C). Although there

was a minimal increase in Cu levels in the twin pregnancy group compared to single pregnancies group, this increase was not statistically significant (figure 1D).



**Figure 1.** Compared serum Fe, Ferritin, Zn and Cu levels with the control group

Hematological parameters evaluations. A. Iron (Fe) levels, B. Ferritin levels, C. Zinc (Zn) levels, D. Copper (Cu) levels. The results are shown as mean  $\pm$  standart error mean (SEM) (n=26 / group). The symbol " \* " indicates that  $p < 0.05$ .

## DISCUSSION

Twin pregnancy rate has been increasing in recent years. In a study, it was observed that the rate of twin pregnancy in the United States in the last forty years increased by approximately 80%, and in 2016, one out of every 30 births was found to be twin births (17). Pregnancy due to advanced maternal age and assisted reproductive techniques were shown as the reason for this increase (2). The increase in the rate of twin pregnancy increases fetal and maternal risks. Maternally, pregnancy diabetes, hypertension, postpartum hemorrhage, anemia, uterine rupture, congenital losses; fatally, prematurity, and intrauterine growth retardation are seen more frequently in twin pregnancies than single pregnancies (18, 19). It is stated that some of these negative effects are caused by wrong and incomplete nutrition, trace element insufficiency, and wrong

physical exercise (20). In the case of twin pregnancy, the mother's body consumes approximately 10% more resting energy than single pregnancies and therefore requires 40% more calories (21, 22). In this case, the food which mother consumed is important in terms of consisting of trace elements. One of these trace elements, iron, is known as the cofactor of many enzymes in both fetus and pregnant. Its effects include DNA synthesis-repair, steroid hormone production, neurotransmitter synthesis, detoxification of foreign and harmful compounds, as well as synthesis and oxygen transport (23, 24). The prevalence of anemia during pregnancy is quite high. The majority of them are iron deficiency anemia, rate of anemia in all pregnant women has been determined as between 2% and 26% (25). A large number of fetal and maternal complications can occur due to anemia. Among them, maternally, sepsis, susceptibility to infection, increased postpartum hemorrhage; in fetus, motor-mental retardation, low birth weight, premature birth, premature rupture of membranes, and death are known (26-28). In our study, iron levels were lower in pregnant women compared to the control group. Moreover, in twin pregnancies, it was found to be lower iron levels than single pregnancies. Therefore, twin pregnancies carry more risks in terms of the complications mentioned above. It has been determined that ferritin is an indicator of body iron stores and its levels decrease in pregnant women (29). In our study, ferritin levels were lower in pregnant women compared to the control group. As with iron levels, ferritin levels were found to be lower in twin pregnancies compared to singleton pregnancies. Zinc, another trace element, is very important for both mother and baby. A positive correlation was found between serum zinc levels of mothers and infants.

Zinc has many different functions such as DNA, RNA and protein synthesis, stabilization of cell membranes, endothelial development, synthesis and use of some hormones, immunity development, antioxidant system development, and participation in the structure of enzymes (30). In studies, it has been shown that zinc deficiency in the mother causes adverse effects in the mother and baby, such as early membrane rupture, early and unexpected sudden abortions, prematurity, retardation of intrauterine growth, fetal neurological defects (31, 32). In our study, we found that zinc levels were significantly lower in pregnant women, especially in twin pregnancy, compared to other groups.

Copper is a trace element in the structure of enzymes such as SOD, cytochrome oxidase, lysyl oxidase, tyrosinase, which are involved in many metabolic events (33). Studies are showing that copper levels increase or decrease during pregnancy (34, 35). It has been reported that increased copper levels in some studies and decreased copper levels in others increase oxidative stress and damage DNA. It is thought to exert this adverse effect by decreasing the level of SOD, an antioxidant enzyme, or by increasing the formation of the hydroxyl radical (36, 37). In our study, we found that copper levels were higher in pregnant women than non-pregnant women. Also, although there was no statistically significant difference between twin pregnancies and single pregnancies, copper values were higher in twin pregnancies.

### CONCLUSIONS

In the light of these results, we think that changes in trace element levels may cause adverse maternal and fetal effects. Nutritional habits are important in pregnant women, especially in twin pregnancies, and

so zinc, copper, ferritin and iron levels should be followed during pregnancy period.

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**Ethics Committee Approval:** This study was performed after approval by the local ethics committee (approval number: KSU-08.01.2020/02).

**Peer-review:** Externally peer-reviewed.

**Author Contributions:** Concept: G.C, Design: G.C, A.T, Literature search: G.C, M.K, M.Y.G, Data Collection and Processing: G.C, A.K, K.H, Analysis or Interpretation G.C, K.H, M.Y.G, Writing: G.C, S.S, A.T.

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