

# Correlation between Maternal Copper Deficiency and Premature Rupture of Membranes: A Case–Control Study

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## Abstract

**Background:** The association between maternal serum copper levels and premature rupture of membranes (PROM) is debated. Aims: This study aims to study the relationship between maternal serum copper levels and the incidence of PROM and to determine risk factors of PROM in Aleppo University Hospital of Obstetrics and Gynecology. Settings and Design: Sixty pregnant women were subjected for this study. **Materials and Methods:** Pregnant women were subjected for analyzing serum copper concentrations. Maternal serum copper levels were measured by atomic absorption spectroscope. **Statistical Analysis:** By SPSS (version 22.0). **Results:** A significant correlation was found between maternal serum copper levels and the incidence of PROM. The relationship presents when copper level is considered as a categorical variable, as mothers with copper deficiency were significantly more vulnerable for PROM (Odds ratios = 0.15, confidence intervals: 0.04–0.61,  $P = 0.002$ ). Furthermore, the relationship presents when copper level is considered as a numerical variable, as mothers with lower serum copper levels were at significantly higher risk to develop PROM. Difference in means between the two groups was 40.87  $\mu\text{g/dL}$  with CI (14.98–66.75)  $\mu\text{g/dL}$  ( $P = 0.003$ ). **Conclusion:** Within its limitations, this study suggests a significant relationship between maternal serum copper levels and PROM.

**Keywords:** Case control, copper, premature rupture of membranes, Syria

## INTRODUCTION

Copper is a mineral crucially needed for maintaining a hemostatic oxidative state. Copper has an essential role in catalyzing metabolic reactions<sup>[1]</sup> and in synthesis of red blood cells in adults.<sup>[2]</sup> Biochemical studies indicate that copper is present in low concentrations in viable tissues, for its function, along with Vitamin C, in the synthesis of connective tissue fibers (i.e., collagen and elastin).<sup>[3]</sup>

Syncytiotrophoblast expresses a heavy metal-binding protein, metallothionein-1. This protein binds and sequesters a host of copper and other heavy metals, including zinc, cadmium, and lead.<sup>[4]</sup>

Copper is a coenzyme for Lysyl oxidase, an enzyme that initiates collagen cross-linking, in order to increase the tensile strength of fibrillar collagens, and then, the strength of amniotic membranes.<sup>[4,5]</sup> Copper also contributes to the formation of fetal organs such as differentiation of the central nervous system.<sup>[6]</sup>

Many studies have demonstrated a connection between maternal copper levels disturbances and the incidence of developmental abnormalities in fetal tissues. For example, increased levels of maternal copper are involved in preeclampsia<sup>[7]</sup> and neural defects.<sup>[8]</sup> On the other hand, decreased levels of maternal serum copper are associated with low birth weight<sup>[9]</sup> or intrauterine growth retardation.<sup>[10]</sup>

Premature rupture of membranes (PROM) is a rupture of amniotic membranes before the onset of labor. If PROM occurs before the 37<sup>th</sup> week of pregnancy, it is called preterm PROM. The prevalence of PROM is 2%–18% of all pregnancies.<sup>[11]</sup>

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PROM increases maternal and fetal infections and causes premature delivery. Nevertheless, there are many risk factors that could induce PROM such as PROM in previous pregnancies, uterine distension (e.g., polyhydramnios and multifetal pregnancy), lower genital tract infection, maternal trauma, and cigarette smoking.<sup>[4,12]</sup>

Decline in collagen concentrations and increased collagen catabolism, changed collagen structure, and increased oxidative stress have been suggested as the mechanisms of PROM.<sup>[5]</sup> Copper is known to serve as an anti-oxidant and a coenzyme for lysyl oxidase, which motivated researchers to investigate the relationship between copper levels and PROM, and the role of copper in the pathophysiology of PROM. The association between maternal serum copper levels and PROM is still debated.<sup>[13]</sup> In addition, the small sample size is a limitation of all related studies due to the low incidence rate of PROM.

This study aims to study the relationship between maternal serum copper levels and the incidence of PROM and to determine the risk factors of PROM in Syria. This study will be the first study to investigate the association of maternal serum copper levels with PROM and risk factors of PROM in Aleppo University Hospital of Obstetrics and Gynecology.

## MATERIALS AND METHODS

### Ethics

Before collecting the data, consent was obtained from the participants. All ethical considerations, including obtaining verbal consent, were approved by our institution.

### Study design

Sixty pregnant women in Aleppo University Hospital of Obstetrics and Gynecology were subjected for analyzing serum copper concentrations, using the simple random sampling. Research data were provided as supplementary file. The samples were divided into two groups. A case group, consisting of 27 women diagnosed with PROM, and a control group, consisting of 33 apparently healthy pregnant women with no problems or complications.

We collected the blood on ethylenediaminetetraacetic acid tubes. For the case group, we collected blood within 24–40 h from gestational membrane rupture. The blood was directly centrifuged and stored in -20°C until analysis.

The case manager obstetrician filled a survey to collect the information about the demographic characteristics, medical and obstetrical history, and suspected risk factors. This survey was filled by interviewing the cases and searching the records. Maternal serum copper levels were measured by standard atomic absorption spectroscope in Bioanalysis Department in Faculty of Pharmacy. Copper serum concentration of 165–222 µg/dL in the second trimester of pregnancy and 130–240 µg/dL in the third trimester was considered normal.<sup>[4]</sup> A participant with hemoglobin concentration of <11 mg/dl was considered anemic.

### Statistical analysis

We conducted the statistical analysis using the SPSS (Version 22.0; SPSS Inc.: Chicago, IL, USA). Categorical variables were presented as frequencies and percentages, whereas continuous data were summarized as mean ± standard deviation. We calculated odds ratios (OR) with confidence intervals (CI) using binary logistic regression analysis. Whichever indicated, we used Chi-square, Fisher's exact test, and Student *t*-test tests to calculate the *P* values. The *P* < 0.05 was considered statistically significant.

## RESULTS

The study included 60 pregnant women, 27 of them were in the case group, and 33 were controls. In the case group, the mean age was 31.21 ± 7.72 years, 26 cases were in their third trimester of gestation, and one in her 2<sup>nd</sup> trimester. In the control group, the mean age was 35.8 ± 2.75 years, 31 controls were in their third trimester, and two in their 2<sup>nd</sup> trimester. No significant differences in maternal or gestational age were found between the two groups. The characteristics of both groups are presented in Table 1.

Seventeen (67%) cases and 8 (24%) controls had copper deficiency. The relationship between copper deficiency and incidence of PROM was significant (OR = 0.15, CI: 0.04–0.61, *P* = 0.002) [Table 2].

The mean maternal serum copper level was (125.6 ± 47.4) µg/dL in the case group and (165.3 ± 51.4) µg/dL in the control group. Difference in means between the two groups was 40.87 µg/dL with CI (14.98–66.75) µg/dL. The difference in means between the two groups was statistically significant (*P* = 0.003).

There was no significant association between the incidence of PROM and PROM history, anemia, history of preterm

**Table 1: Mean, standard deviation and *P* value for the variants (mother age, gestational age and serum copper concentrations for both groups**

| Variants                            | Mean ± SD    |              | <i>P</i> |
|-------------------------------------|--------------|--------------|----------|
|                                     | Cases        | Controls     |          |
| Mother age (year)                   | 30.96±7.76   | 28.09±7.70   | >0.05    |
| Gestational age (weeks)             | 36.04±2.81   | 35.82±3.60   | >0.05    |
| Serum copper concentrations (µg/dl) | 124.41±47.88 | 165.27±51.36 | 0.003    |

**Table 2: Correlation between copper levels and premature rupture of membrane cases with *P* value, odd ratios, and confidence intervals**

| Maternal             | PROM |    | <i>P</i> | OR   | CI        |
|----------------------|------|----|----------|------|-----------|
|                      | Yes  | No |          |      |           |
| Copper deficiency    | 17   | 8  | 0.002    | 0.15 | 0.04-0.61 |
| Normal copper levels | 10   | 25 |          |      |           |

PROM: Premature rupture of membrane, OR: Odds ratio, CI: Confidence interval

**Table 3: Risk factors and its incidence in case and control groups with statistical values (odds ratio, confidence interval, and P value)**

|               | Risk factors |                      |           |               |                 |                                      |                   |                   |
|---------------|--------------|----------------------|-----------|---------------|-----------------|--------------------------------------|-------------------|-------------------|
|               | PROM history | Multiple pregnancies | Anemia    | Preterm labor | Polyhydramenios | High chronic intraabdominal pressure | Mechanical trauma | Vaginal infection |
| Control group | 1            | 3                    | 13        | 3             | 1               | 3                                    | 1                 | 1                 |
| Case group    | 4            | 1                    | 6         | 2             | 1               | 2                                    | 5                 | 9                 |
| OR            | 5.57         | 0.39                 | 0.44      | 0.80          | 1.23            | 0.80                                 | 9.14              | 4.21              |
| CI            | 0.58-53.11   | 0.38-3.39            | 0.14-1.38 | 0.12-5.17     | 0.07-20.64      | 0.12-5.17                            | 1.03-81.48        | 0.99-17.88        |
| P             | >0.05        | >0.05                | >0.05     | >0.05         | >0.05           | >0.05                                | 0.039             | >0.05             |

OR: Odds ratio, CI: Confidence interval

labor, polyhydramnios, high chronic intra-abdominal pressure, and vaginal infection. Mechanical trauma was the only studied factor that was significantly associated with PROM (OR = 9.14, CI: 1.03–81.48,  $P = 0.039$ ) [Table 3].

## DISCUSSION

Copper plays an important role in reproduction cycle and copper deficiency may result in some metabolic disorders like PROM; therefore, this study aims to determine this correlation. In this study, a significant correlation was found between maternal serum copper levels and in cadence of PROM. The relationship presents when copper level is considered as a categorical variable, as mothers with copper deficiency were significantly more vulnerable for PROM. Furthermore, the relationship presents when copper level is considered as a numerical variable, as mothers with lower serum copper levels were at significantly higher risk to develop PROM. Among the studied suspected risk factors, trauma was the only one to have a significant relationship with PROM.

This study has a major limitation which is the small sample size. This limitation presents in all related studies, as PROM has a low incidence rate,<sup>[5]</sup> and copper levels measurement is expensive, technically challenging, and these cases do not admit to our hospitals very frequently. The negative effect of the small sample size is clearly reflected by the wide CIs of the risk factors ORs. For the previous considerations, the results of our study as all related studies should be interpreted and generalized with caution.

The association between copper levels and PROM has always been debated. Artal *et al.*,<sup>[14]</sup> Fu *et al.*,<sup>[15]</sup> and Kiilholma *et al.*<sup>[16]</sup> found a significant association between copper levels and the incidence of PROM and the results in this research is consistent with those previous studies, while Seddighi Looye *et al.*,<sup>[17]</sup> Kucukaydin *et al.*,<sup>[18]</sup> Bakhshandeh Nosrat *et al.*,<sup>[19]</sup> Rahmanian *et al.*,<sup>[13]</sup> Zhang *et al.*,<sup>[7]</sup> and Shen *et al.*<sup>[20]</sup> found no significant relationship between copper levels and incidence of PROM.

There are multiple factors that lead to the contradiction between studies. First, the small sample sizes which causes less precision. Second, the difference in copper measurement techniques. Third, the difference in the case and control groups. Some studies studied PROM in term pregnancies

or regardless of the gestational age,<sup>[7,13-15,17,19,20]</sup> but other studies studied preterm PROM.<sup>[16,18,19]</sup> Furthermore, some studies used healthy term pregnancies without PROM as controls,<sup>[7,13-17,19,20]</sup> and some used preterm pregnancies without PROM as controls.<sup>[16,19,18]</sup>

## CONCLUSION

Within its limitations, this study suggests a significant relationship between maternal serum copper levels and PROM. There is still a debate about this association in the medical literature, and this debate has multiple causes related to the small sample size, to study design and to technical issues. Further studies are recommended to investigate this association with larger sample sizes, and more controlled design, and to thoroughly review and metaanalyze the available literature. This will help the next step, which is to study the effectiveness of copper supplementation to prevent pregnancy adverse events.

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Nil.

## Conflicts of interest

There are no conflicts of interest.

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## ملخص المقال باللغة العربية

العلاقة بين نقص النحاس وتمزق الأغشية المبكر في الأمهات السوريات: دراسة مراقبة الحالة

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الخلفية: تمت مناقشة العلاقة بين مستويات النحاس في مصل الدم وبين تمزق الأغشية المبكر في كثير من الدراسات.

الأهداف: تهدف هذه الدراسة إلى دراسة العلاقة بين مستويات النحاس في مصل الأم ونسبة حدوث تمزق الأغشية الباكر وتحديد عوامل الخطورة في مستشفى الولادة وأمراض النساء بجامعة حلب.

الإعدادات والتصميم: خضعت ستون امرأة حامل لهذه الدراسة.

المواد والطرق: تم إخضاع النساء الحوامل لتحليل تراكيز النحاس في الدم. تم قياس مستويات النحاس في مصل الأم بواسطة مطياف الامتصاص الذري. وتم التحليل الإحصائي للنتائج باستخدامنا SPSS (الإصدار 22.0).

النتائج: تم العثور على علاقة معنوية بين مستويات النحاس في مصل الأمهات ووقوع تمزق الأغشية الباكر. تظهر العلاقة عندما يعتبر مستوى النحاس متغيراً فنوياً، حيث كانت الأمهات المصابات بنقص النحاس أكثر عرضة بشكل ملحوظ لـ تمزق الأغشية الباكر (نسب الأرجحية = 0.15، فترات الثقة [0.04-0.61، CI]،  $P=0.002$ ). علاوة على ذلك، تظهر العلاقة عندما يعتبر مستوى النحاس متغيراً عددياً، حيث كانت الأمهات اللواتي لديهن مستويات منخفضة من النحاس في الدم أكثر عرضة للإصابة بتمزق الأغشية الباكر. كان الاختلاف في المتوسط بين المجموعتين 40.87 ميكروغرام/ديسيلتر ( $P=0.003$ ).

الخلاصة: ضمن حدودها، تشير هذه الدراسة إلى وجود علاقة مهمة بين مستويات النحاس في مصل الأم وتمزق الأغشية الباكر.

الكلمات المفتاحية: مراقبة الحالة، نحاس، تمزق الأغشية المبكر، سوريا.