ORIGINAL ARTICLE

Maternal serum copper and zinc levels and premature rupture of the foetal membranes

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Abstract

Objective: To examine the correlation of zinc and copper serum concentration level, body mass index, age and parity with premature rupture of the membranes.

Methods: The cross-sectional study was conducted between 2009 and 2010 at the fertility ward of Amiralmomenin Hospital of Semnan University of Medical Sciences, Iran. It comprised 100 full-term pregnant women with and without premature rupture of the membranes and 50 non-pregnant women as controls. The diagnosis of rupture of membranes was made on the basis of gross leakage of fluid within the vagina and a positive nitrazin test. A sample of 5mL blood was collected. The levels of zinc and copper were determined by an enzyme-linked immunosorbent assay method. Mean values among the three equal groups were compared using standard analysis of variance. Statistical significance was set at $p<0.05$.

Results: Pregnant women with ($p<0.027$) and without ($p<0·019$) premature rupture of the membranes had significantly lower serum zinc concentration than non-pregnant women. Inversely, the maternal serum copper concentration level was higher in both groups of pregnant women than in the controls ($p<0.001$). However, the results suggest that the decreased plasma zinc concentration and increased copper concentration in pregnant women were not the cause of premature rupture of the membranes at term.

Conclusion: Zinc and copper concentration levels in maternal serum had no effect on premature rupture of the membranes.

Keywords: PROM, Zinc, Copper, Pregnancy. (JPMA 64: 770; 2014)

Introduction

The membranes encircling the amniotic cavity[1] may be ruptured before the onset of labour[2,3] at any gestational age, even at 42 weeks gestation,[4] which is called premature rupture of the membranes(PROM). It occurs in approximately 2% to 18% of pregnancies.[2] Reduced collagen concentrations, altered collagen cross-link profiles,[1,5] increased concentrations of biomarkers of oxidative damage,[5] and extensive changes in collagen metabolism[6] have been described as risk factors for PROM.[1] A number of micronutrients are known to serve as antioxidants or essential cofactors for antioxidant enzymes.[7] One of this necessary micronutrient is zinc, which has a very important role in normal embryogenesis, intrauterine growth[8] and helps the mother during labour.[9] Previous studies have shown that the maternal zinc level in women with PROM and preterm labour is significantly lower than in women without such complications.[10,11] In addition, numerous animal experiments and observational studies suggest the potential role of zinc deficiency in labour and delivery-related complications such as PROM. Supplementation studies, however, do not confirm these associations.[12]

A number of studies have shown that serum copper increases during pregnancy. Also, the lower levels of serum copper during pregnancy are correlated with some pathological conditions.[13-15] Fu[15] and Artal et al.[15] have reported that the serum copper level is significantly lower in women with PROM than in controls. However, another study[16] found no such relationship. Bro et al. also reported no difference in the serum zinc and copper concentration levels in mothers with delivery complications and those with normal pregnancies and deliveries.[17] The conflicting results of the previous studies and the limited number of large-scale studies in this area underlined the need for additional research on impacts of zinc and copper serum concentration level on PROM. The current study was planned with that need in mind.

Patients and Methods

The cross-sectional study was conducted between 2009 and 2010 at the fertility ward of Amiralmomenin Hospital of Semnan University of Medical Sciences, Iran. Informed written consent was obtained from all the 150

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participants, and all procedures were approved by the institutional ethics board. Consecutive patients were recruited on the basis of convenient sampling during their visits to the hospital. The sample size was determined based on the data of Sikorski's et al.[11] study, which showed that the mean and standard deviation (SD) of serum zinc concentration for pregnant women with and without PROM was 4.33±1.18 and 5.97±1.39 respectively. With confidence interval (CI) of 99% and power of 95%, the sample size for each group was calculated to be 22 subjects in each group. But for obtaining exact results, the sample size was increased to 50. The subjects were divided into three equal groups of 50 each: 1) full-term pregnant women with PROM, 2) full-term pregnant women without PROM, and 3) non-pregnant women. Participants in the three groups were matched for body mass index (BMI), age and parity. The exclusion criteria comprised previous PROM, abortion, intercourse within the preceding two weeks, BMI less than 20, polyhydraminos, breech delivery, uterus anomalies, smoking, trauma, intra-uterine infections, Wilson's disease, and enteropathic dermatitis. The diagnosis of rupture of membranes was made on the basis of gross leakage of fluid within the vagina and a positive nitrazin test[18] and from positive microscopic ferning and pH tests performed during a speculum examination.[19] A personal interview was conducted with each participant. The purpose of the interview was to provide the participant with an opportunity to share their personal experience and any additional information that they found relevant to their medical condition. Following the interview, a sample of 5mL blood was taken from each participant at the central laboratory. The specimens were centrifuged and the plasma was removed and frozen at -20°. The frozen plasma was sent to immunological laboratory for further examination. The level of zinc and copper concentration was determined by enzyme-linked immunosorbent assay (ELISA) method (DRG. Italy) as described previously[20] using an atomic absorption spectrophotometer (Variant Spectra AA 220). Zinc and copper levels of 70-114ug/ml and 80-155ug/ml respectively were considered normal. Statistical analyses were performed using Kolmogorov-Smirnov, Chi-square and, Kruskal-Walis, One Way analysis of variance (ANOVA) and Tukey tests. The p-value less than 0.05 was considered statistically significant.

Results

Primary characteristics of the subjects were noted at the outset (Table-1). BMI values revealed that overall 5(10%) women with PROM, 8(16%) without PROM, and 9(18%) non-pregnant women were obese (p<0.633). Overall, 54(36%) women were in the 25-29 years age group. Mean age of pregnant women with and without PROM and the non-pregnant women was 27.5±5.8, 27.9±4.6 and 28.4±6.9 years respectively. Age was not significantly different among the groups (p<0.764). Mean parity in the three groups were 1.78±0.84, 1.70±0.79 and 1.66±0.82. The difference was not statistically significant (p<0.744). Pregnant women with (p<0.027) and without (p<0.019) PROM had significantly lower serum zinc concentration than the non-pregnant controls (Table-2). Serum zinc concentration did not have any significant relationship with PROM in pregnant women. Maternal serum copper concentration was significantly higher in pregnant women (both with and without PROM) than in non-pregnant women (p<0.001). However, there was no significant difference in the maternal plasma copper

Table-1: Demographic and micronutrient levels in serum of pregnant women and non-pregnant women according to different variables.

Variables	Pregnant PROM		Non-pregnant	P-value
	Yes(n=50)	No(n=50)		
Body mass index				0.633
Healthy (20-24.9)	29 (58%)	21(62%)	30 (60%)	
Overweight(25-29.9)	16 (32%)	11(22%)	11 (22%)	
Obese (≥30)	5 (10%)	8 (16%)	9 (18%)	
Age(in years)				0.764
Less than 20	3 (6%)	3 (6%)	3 (6%)	
20-24	12 (24%)	7 (14%)	14 (28%)	
25-29	20 (40%)	22 (44%)	12 (24%)	
30-34	7 (14%)	14 (28%)	12 (24%)	
≥35	8 (16%)	4 (8%)	9 (18%)	
Parity				0.744
1	24 (48%)	25 (50%)	28 (56%)	
2	13 (26%)	15 (30%)	11(22%)	
3	13 (26%)	10 (20%)	11 (22%)	

PRPM: Premature rupture of the membranes.
Chi-square and, Kruskal-Walis, and One Way ANOVA tests were used to determine significance.

Table-2: Micronutrients levels in serum of pregnant women with and without PROM and non-pregnant women.

Micronutrients	Pregnant PROM		Non-pregnant	[a]p
	Yes(n=50)	No(n=50)	No(n=50)	
Zinc (ug/dl)	80.4±23.0	79.8±26.0	92.9±22.8	0.01
Copper (ug/dl)	142.9±28.8	144.9±28.2	110.6±25.0	0.001

[a]Note: a values represent mean and the associated standard deviation. p <0.05 is considered statically significant.Micronutrients levels in serum of pregnant women with PROM compared with without PROM and non pregnant women. Kruskal-Walis, One Way ANOVA and Tukey testswere used to determine significance.
PROM: Premature rupture of the membranes.

concentration between the two groups of pregnant women, meaning that maternal serum copper concentration had no significant effect on PROM.

Discussion

The cause of PROM is almost certainly multifactorial.[1] We measured serum zinc and copper concentration levels in two groups of pregnant women with and without PROM and a group of non-pregnant women, and examined the potential relationship between plasma zinc and copper concentration level and PROM. The relationship between women's BMI, age and parity with PROM was also examined. Our results show that the serum zinc concentration is lower in pregnant women with and without PROM than non-pregnant women. Inversely, maternal serum copper concentration is higher in pregnant women than in non-pregnant women. The results suggest that the decreased plasma zinc concentration and increased copper concentration in pregnant women are not the cause of PROM at term. Also, no significant differences were found between groups in regard to age, or parity at term in Iranian pregnant women.

Previous studies[19,21] have reported that BMI of less than 20 is a definite factor for PROM in some patients. Underweight pregnant women may suffer from complications including PROM, anaemia, low Apgar score, preterm delivery, low birth weight of the baby, increased rate of perinatal mortality[22-27] andhigher plasma zinc concentration.[28] Obese mothers, however, show a higher prevalence of other diseases and complications such as Caesarean section, diabetes, hypertension, and PROM,[29] and infants born to obese mothers have longer hospital stays.[29] Nevertheless our results do not support the effect of BMI on PROM, although women in our study and the other studies had the same range of BMI, and women with BMI of less than 20 were excluded from the study. This difference might be due to the smaller sample size within the same BMI range in our study.

There have been suggestions that the highest PROM rate is observed in pregnant women who are 26 to 35 years old and the risk of PROM decreases as the age of pregnancy increases beyond 35 years.[30-31] The prevalence of abnormal labour and high-risk pregnancy outcomes increase in older women[32] due to the higher rate of other accompanying maternal diseases.[33] Our results compliment the results of Gosselink et al.[22] that age has no effect on PROM.

Primiparity, premature contractions, PROM in a previous pregnancy, and bleeding in the first trimester[34] are considered risk factors for PROM. We could not show that PROM occurs significantly among nulliparas than among multipara. This is in contrast with the results of the previous studies which have shown that PROM occur significantly more often among primipara than among multipara, and women with at least one PROM pregnancy in the past are at higher risk of premature rupture of membranes in their subsequent pregnancies.[30,35,36] Also, it has been reported that women with a history of PROM will have another PROM in their subsequent pregnancy in 13.5% cases, while for women with no such history PROM occurs only in 4.1% cases.[37] In addition, parity and cervical incompetence are risk factors for PROM among women who delivered small-for-gestational-age (SGA) neonates.[38] Also, in premature preterm rupture of membranes, when controlled for parity, age, marital status and race, the variables remain significant.[39] The fact that we included only mothers with equal number of gravidities, and also excluded the ones with history of PROM might have contributed to the differences in our findings in comparison with other studies. Further investigation is required to confirm such relationship.

Similar to the findings of the previous observations,[40,41] in our study serum zinc concentration in pregnant women was significantly (14%) lower than in non-pregnant women. Serum zinc concentration begins to decline in early pregnancy and continues to decline till term when the level of serum zinc concentration is almost 35% less than the level of concentration in non-pregnant women.[42,43] The decline in serum zinc concentration with pregnancy appears to be a normal physiologic adjustment and is not necessarily indicative of inadequate zinc nutriture.[44] The decline in the zinc level has been attributed to haemodilution, decrease in the level of zinc binding protein, hormonal changes during pregnancy,[45] and active transport of zinc from the mother to the foetus.[43] All of the aforementioned factors diminish the validity of using serum zinc level as an indicator of zinc nutriture during pregnancy.[46] Similar to previous reports,[17,46] we found no difference in serum zinc concentration between mothers with PROM and mothers with normal pregnancies and deliveries. Jameson[45] reported that women who give birth at the 37th week of pregnancy or earlier, or at the 43rd week of pregnancy or later have significantly lower serum zinc during early pregnancy compared to women who give birth at the 40th week of their pregnancy. Differences in characteristics of the participants may have contributed to the different findings. Subjects in our study were between 37th to 43th week of their pregnancy, but results do not correspond with the results of the studies that show a connection between reduced maternal serum zinc concentration during pregnancy or at delivery and a 3.5 to 7-fold increase in the risk of PROM.[11,47] In addition, research has shown that subnormal tissue zinc content,[10,11,48] and milder forms of zinc deficiency during pregnancy may

cause PROM at term[10,11,48-50] and preterm labour as well as inefficient uterine contraction.[12] The lower maternal serum zinc concentration may inhibit immunological competence in both mother and foetus and therefore increase the risk of amniotic infection and onset of PROM.[48] However, our results do not reveal a cause-and-effect relationship between the reduced concentration of serum zinc concentration during pregnancy and PROM.

Our results regarding the association between the level of serum copper and PROM is consistent with the results of a previous study by Bro et al[17] who showed that the level of serum copper concentration does not differ between mothers with and without PROM. Other studies have shown effects of serum copper level on PROM at term when comparing mothers with and without PROM. For example, Zhang et al,[16] found positive correlations between decrements of serum copper, amniotic copper, LOX, and collagen III and PROM. Therefore, the present study suggests that PROM is not related to the increased level of copper concentration during pregnancy.

Although we found a significant increase in serum copper concentration in pregnant women (with and without PROM) in comparison with non-pregnant women, but we found no correlation between the level of serum copper and PROM. A recent study[51] provides a possible explanation for such findings. Vukelicet al.[51] reported that maternal serum copper constantly increases during healthy, non-pathologic pregnancies. In our study, pregnant women (with and without PROM) had a significantly higher level of serum copper in comparison with non-pregnant women. Similar results have been reported by Masuda et al.[52] It has been suggested that high serum copper concentration during pregnancy might be due to an increased binding affinity with ceruloplasmin, increased ceruloplasmin production[17,52,53] as a result of elevated level of oestrogen[52,53] and passive transfer of ceruloplasmin across the placenta.[17,52,53] It is unlikely that a single pathophysiologic mechanism would cause all cases of PROM. Rather it is speculated that PROM is the result of a combination of processes. Various mechanisms, including mechanical and inflammatory processes, have been proposed as the cause of PROM.[54] Although the exact aetiology of PROM is not clear, but changes in interleukin 6(IL-6), granulocyte-colony stimulating factor (G-CSF), and C-reactive protein (CRP) concentration levels before and after delivery suggest that the aforementioned factors play a role in the aetiology of preterm delivery in women with PROM.[55] Abnormal collagen development caused by connective tissue growth factor (CTGF) and excessive apoptosis of membrane cells caused by Tumor necrosis factor-alpha are also related to the occurrence of preterm premature rupture of membranes.[56]

Conclusion

The decline in serum zinc concentration and increase in serum copper level at the end of pregnancy are normal physiological changes and do not contribute to PROM in Iranian pregnant women.

Acknowledgments

We are grateful to Semnan University of Medical Sciences and Health Services, Semnan, Iran, for financial support.

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