Plasma copper and zinc levels in pregnant women in Gaziantep, Turkey

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ABSTRACT

Objective: Trace elements such as copper and zinc are important factors on the health of pregnant women, fetus and embryonic development. Since these elements have important influences on health of pregnant women and the growing fetus, this study was designed to evaluate the zinc and copper status of pregnant women in Gaziantep, Turkey.

Methods: This study was designed as a prevalence study. Twenty-two health station region were chosen in 13 health centers in the province of Gaziantep city in 1999, and copper and zinc levels were examined in 415 pregnant women living in these regions. Plasma copper and zinc concentrations were determined spectrophotometrically. Analysis of variance and "t" tests were used in the statistical analysis of means.

Results: In the examination, plasma copper and zinc levels were found as 220.67 mg/dl and 124.91mg/dl. While zinc concentrations were not altered significantly throughout gestation, copper levels of pregnant women increased significantly as duration of pregnancy progressed.

Conclusion: Plasma copper was higher in all trimesters of gestation when compared to normal range of non-pregnant women. The zinc levels of pregnant women were mostly within normal range. It decreased significantly in the pregnant women >35 years of age and in those whose education was literacy and less. Therefore, zinc supplementation in pregnant women is recommended in these groups.

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Pregnancy is a time of increased nutritional needs, both to support the rapidly growing fetus and to allow for the changes occurring in the pregnant woman's body. Throughout pregnancy, recommended intakes of many vitamins and minerals are higher than those recommended prior to pregnancy.[1,2] Trace elements have important influences on the health of pregnant women and growing fetus.[1-4] Zinc and copper are of particular importance during pregnancy and the role of these trace elements in fetal development and growth is well documented in the literature.[1-14] Copper is of particular importance, especially early in life for the development and maintenance of myelin;[1-5] zinc is essential for normal embryogenesis, fetal growth and protein synthesis.[1-3] Deficiency of trace elements during intrauterine existence is closely related to mortality and morbidity in the newborn.[2-4,12] Therefore, the aim of this study was to investigate the levels of plasma copper and zinc during pregnancy in this region. But some factors (such as age of women, number of pregnancies, time interval between previous deliveries) that may be related to levels of copper and zinc were also investigated.

Methods. The average number of births is approximately 20,000 per year in Gaziantep City center. The least estimated sample size was 96 by; supposing the frequency of zinc or copper deficiency to be 10% (considering d=0.06 and a=0.05); and sufficient sample size was estimated to be 288. All health centers in city center (except for one) were included in the study. Four

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centers were not included due to insufficient health personnel. The number of pregnant women included in the study from each health center was determined by the number of pregnant population of each health center. Twenty-two health stations and 22 midwives were chosen among 13 different health centers region in Gaziantep City. We preferred to choose the region of midwives who we thought that they would cooperate better with us. Pregnant women living in each health station region participated in our study until we had obtained the ideal number of the sample for each health station. Therefore, women participating in the study were representative of the general population. Especially pregnant women within the third trimester were included in the study. But also pregnant women within their first and second trimesters were enrolled in the study to evaluate the changes during pregnancy. At first, a form including data concerning pregnant women and their pregnancy (such as age, and education of women, weeks of gestation and total number of pregnancy) was applied to obtained from pregnant women by the midwives. After that, 10 ml of venous blood samples were obtained from each women using standard venipuncture technique between 9.30-11.00 am after 12 hour fasting. Blood specimens were collected in heparinized tube. Venous blood samples were brought to the laboratory in the morning hours. All blood samples were centrifuged (3000 rpm for 10 minutes) and stored at -20°C until analysis, which was performed in the same run. Hemolysed specimens were excluded. Glassware were cleared of surface trace metal contamination by soaking overnight in 50% nitric acid-hydrochloric acid (1:3 volume per volume) solution, followed by rinsing with deionized water. The water specifications were one water with ≥14mW/cm^2 resistance and iron, zinc, and copper content of less than 10mg/l.[15] Plasma copper and zinc concentrations were determined with Bathokuproine with deproteinization method (Boehringer, Mannheim, Germany) and 5-Bromo-adenosine 3'-phosphate-5' phosphosulfate (5-Br-PAPS) method (Elitech Diagnostics, France) according to the manufacturer's instruction (UV, visible spectrophotometer, Shimadzu). The plasma copper and zinc levels were also compared with normal level for non-pregnant women.[16] Plasma copper values between 80 and 155 mg/dl and plasma zinc values between 50 and 150 mg/dl were considered as normal range. All the data was entered into the computer, and statistical analyses were performed by using SPSS 6.0 statistical program. In the statistical analyses, "t test" (independent samples), analysis of variance and Kruskal-Wallis analysis of variance tests (if data were not normally distributed) were used to compare the mean values, and Chi-square test was used to compare the countable values.

Results. In order to evaluate copper and zinc status during pregnancy and its relationship with some conditions of pregnant women, 415 pregnant women were included in this study. Eighty-five were in the first trimester, 37 were in the second trimester and 293 were in the third trimester. Both plasma copper and zinc levels were measured in 415 pregnant women. The mean age of women according to gestational weeks are given in **Table 1**. There was no significant difference between the groups regarding the age of pregnant women ($p>0.05$). The ages of women were similar in all groups. The mean copper and zinc concentrations according to gestational weeks are given in **Table 2**. The mean of plasma copper level was 220.67mg/dl. With the progression of gestational weeks, the level of plasma copper increased, and it was the highest in pregnant women between 32-38 gestational weeks (**Figure 1**). There was a significant increase in copper level during pregnancy ($p<0.01$). While the mean plasma copper concentration was 189.3mg/dl in the pregnant between 4-11 weeks, it was 229.04 mg/dl in pregnant between 36-38 weeks. There was statistically significant difference between the first and the last 2 groups

Table 1 - Mean age of pregnant women according to duration of pregnancy.

Duration of pregnancy (week)	Age (year)	
	n	Mean + SD
4 -11	36	25.14 ± 4.99
12-15	49	25.28 ± 5.79
16-27	37	25.88 ± 5.03
28-31	26	25.80 ± 5.94
32-35	137	25.37 ± 5.28
36-38	130	26.02 ± 5.46
Total	**415**	**25.61 ± 5.39**
p>0.05 (analysis of variance test)		

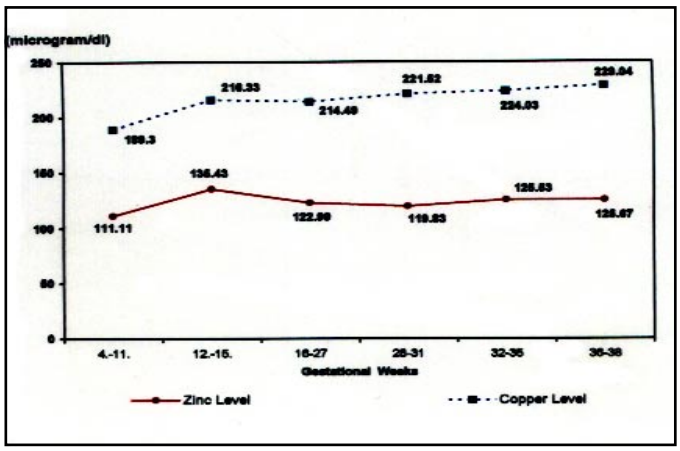

Figure 1 - Plasma copper and zinc levels of pregnant women.

Table 2 - Mean copper and zinc concentrations of pregnant women according to gestational weeks.

Gestational weeks		Copper		Zinc	
	n	Mean (mg/dl) ± SD	CI (95%)	Mean (mg/dl) ± SD	CI (95%)
4-11	36	189.30 ± 49.81*	172.44 - 206.15	111.12 ± 41.10	97.20 - 125.02
12-15	49	216.33 ± 47.46	202.70 - 229.97	135.43 ± 36.67	124.90 - 145.96
16-27	37	214.49 ± 52.60	196.95 - 232.03	122.99 ± 58.85	103.37 - 142.62
28-31	26	221.52 ± 69.74	193.35 - 249.69	119.83 ± 21.09	111.31 - 128.35
32-35	137	224.03 ± 61.51*	213.64 - 234.42	125.53 ± 33.63	119.85 - 131.22
36 -38	130	229.04 ± 63.94*	217.94 - 240.13	125.67 ± 34.35	119.71 - 131.63
Total	**415**	**220.67 ± 60.32**	**214.85 - 226.49**	**124.91 ± 37.39**	**121.30 - 128.52**
Results of analysis		p=0.003		p=0.094	

*There was a significant difference between the first and the last 2 groups (analysis of variance test).

Table 3 - Mean copper and zinc concentrations of pregnant women* according to some conditions.

Conditions	n	Copper/μg/dl Mean ± SD	Zinc μg/dl Mean ± SD
Age of women			
≤18	35	228.32 ± 57.70	123.80 ± 32.46
19-24	96	226.74 ± 66.95	126.66 ± 30.07
25-34	115	228.44 ± 57.18	128.49 ± 36.14
≥35	19	216.29 ± 79.81	102.24 ± 32.38
Result of analysis		p=0.88	p=0.016
Mother's education			110.76 ± 33.52
Non-literacy and literacy	60	221.73 ± 67.27	129.27 ± 31.88
Graduated a primary school	181	226.88 ± 60.45	134.32 ± 39.57
Graduated a secondary school or higher	26	234.55 ± 68.04	p=0.0004
Result of analysis		p=0.67	
Total number of pregnancies			
≤2	124	223.98 ± 61.69	130.26 ± 32.91
≥3	143	228.63 ± 63.59	121.56 ± 34.37
Result of analysis		p=0.54	p=0.036
Status of smoking			
Non-smoker	246	225.54 ± 61.60	126.88 ± 33.06
Smoker	20	240.83 ± 74,81	114.56 ± 37.30
Result of analysis		p=0.29	p=0.11
Time interval between the previous deliver			
<24 months	99	231.72 ± 60.48	126.99 ± 30.10
≥24 months	70	219.10 ± 64.55	125.45 ± 36.50
First pregnancy	61	229.78 ± 63.93	128.14 ± 36.75
Result of analysis		p=0.41	p=0.90
Abortion			
No	217	224.13 ± 63.48	125.01 ± 34.82
Yes	50	236.60 ± 58.37	128.18 ± 29.89
Result of analysis		p=0,20	p=0.55
Social insurance			
No	152	224.94 ± 61.72	125.77 ± 34.38
Yes	107	227.84 ± 64.08	127.22 ± 33.24
Result of analysis		p=0.71	p=0.73

* duration of pregnancy ≥ 32 weeks

(p<0.01). The mean of plasma zinc concentration was 124.91mg/dl. It was 111.12 mg/dl in the pregnant between 4-11 weeks, and 125.67mg/dl in the pregnant between 36-38 weeks. Zinc levels did not change significantly according to gestational weeks (p>0.05) (**Table 2**) (**Figure 1**). **Table 3** shows the mean of copper and zinc concentration by some factors (age of women, total number of pregnancy, time interval between the previous delivery and so forth). Only pregnant women with >32 weeks of gestation were enrolled in the evaluation to prevent the influence of gestational age. Plasma copper concentration did not significantly alter with these factors. But zinc concentration was significantly lower in pregnant women aged ≥35 years (p<0.05). Mean of zinc concentration was significantly lower in this age group than both 19-24 and 25-34 years groups. It was also significantly lower than the other groups of pregnant women whose education was literacy or less, (p<0.001), and lower in the pregnant women who had 3 or more pregnancy (p<0.05). Number of the pregnancies did not affect the zinc level. Both age and education of women independently affected the zinc level.

In our study, no relation could be found between copper and zinc levels. The copper and zinc levels were compared with normal level for non-pregnant women (**Table 4**). When the level of plasma copper was evaluated according to normal range of non-pregnant women, 87% of pregnant women were found to have high level of copper in the third trimester. This rate was 90.6% in the first trimester and 86.5% in the second trimester. There were not statistically significant differences between trimesters. The rate of copper deficiency was 10.2% in the third trimester of pregnancy. When the level of zinc was evaluated according to normal range of non-pregnant women, approximately 20-25% of pregnant women was found to have high level of zinc in all trimesters. But there were not statistically significant differences between the trimesters. The rate of zinc deficiency was 2% in the third trimester of pregnancy but it was 3.5% in the first trimester and 10.8% in the second trimester.

Table 4 - Evaluation of copper and zinc levels compared with normal range (for non-pregnant women) in all trimesters.

Level of copper and zinc	First trimester n	%	Second Trimester n	(%)	Third trimester n	(%)
Copper Level						
Low	1	(1.2)	-	(0.0)	3	(10.2)
Normal	7	(8.2)	5	(13.5)	35	(11.9)
High	77	(90.6)	32	(86.5)	255	(87)
Total	**85**	**(100)**	**37**	**(100)**	**293**	**(100)**
Zinc level						
Low	3	(3.5)	4	(10.8)	6	(2)
Normal	60	(70.6)	24	(64.9)	226	(77.1)
High	22	(25.9)	9	(24.3)	61	(20.8)
Total	**85**	**(100)**	**37**	**(100)**	**293**	**(100)**

x^2 test (p>0.05)

Discussion. Trace elements have important influences on the health of pregnant women and the growing fetus.[1] Trace minerals such as copper, zinc, selenium play a major role in health, since even minute portions of them can powerfully affect health.[1-4] Copper is necessary for the absorption and utilization of iron (helps activation of ferro-oxidase and oxidation of Vitamin C) and important for the development and maintenance of myelin.[2,3] The level of plasma copper increased with the increase of progesterone and estrogen.[15] During the pregnancy, the levels of plasma estrogens and progesterone exceed the normal with the effect of placental hormones.[17] Accompanying the rise in those hormones, an increase in plasma copper is expected. This increase can account for hormonal increase and gastrointestinal absorption which increases due to maturation and placental functions, especially in the last months of pregnancy.

In this study, the mean plasma copper concentration was 222.67 mg/dl. It was 189.30 mg/dl in the first 2 months of pregnancy. With the progress of pregnancy, the level of plasma copper increased and it was high in all trimesters. These findings were found to be similar to the other studies.[8,10,11,18,19] Furthermore, copper enters the structure of ceruloplazmin, which plays a role in the absorption of iron.[15] In those cases when the need of iron increases, absorption of copper also increases. Pregnancy is a case of deficiency of iron and its absorption. In consequence of this, it is expected that level of copper will increase in the pregnant who had anemia. It was reported that anemia prevalence is more than 40% in pregnant women in Gaziantep.[20] In this study we found that mean copper concentration was higher than in the previously reported studies.[9-13] This difference might be closely related to high rates of anemia prevalence in pregnant, as well as some differences of methodology.[20] It is also reported that anemia prevalence is increased in the third trimester.[20] This knowledge supports our results. In our study the level of plasma copper reached its highest level in the third trimester. When we evaluated plasma copper level according to some characteristics of pregnant women (such as age, and education level, and total number of pregnancy) copper level did not significantly alter with these factors. Zinc is an element, which is necessary for growth and development. Importantly deficiency of zinc during pregnancy can result in a greater incidence of fetal malformations.[1-3,6,9,12] During the pregnancy, the requirements for zinc increase.[2,6] In this study, the mean of zinc level was found as 124.91 mg/dl. Plasma zinc values between 50-150 mg/dl were considered as normal range for non-pregnant women.[16] According to these

criteria approximately 3% of pregnant women were found to be suffering from zinc deficiency. Approximately 77% of pregnant women within the third trimester were in normal range, 21% of them were in the high range. While zinc levels in our study were mostly normal, low zinc concentrations were reported in some other studies.[8,10,14] Zinc concentrations did not significantly differ between the gestational weeks. Similar findings were also reported in some other studies.[9,11] When we evaluated plasma zinc level according to some characteristics of the pregnant women; there was a significant decrease in the zinc level in the pregnant women aged ≥35 years. There are many documents concerning relationship between the age of pregnant women and congenital abnormalities in newborn.[21-23] There is an increased risk of congenital abnormalities in newborn whose mother are >35 years.[21-23] In this study a significant decrease in zinc level was observed at this age group. It is known that deficiency of zinc during pregnancy can result in a greater incidence of fetal malformations.[1-3,6,12] It is thought that an increase in congenital abnormalities at this age group may be related to a decrease in zinc level at the same age group. Also statistically significant decreases were found in pregnant women who had 3 or more pregnancies and in pregnant women whose education was literacy and less. The mean zinc concentration did not change significantly according to number of pregnancy. It was thought that the age of women independently impact on the zinc level but pregnancy number of women does not impact independently. It was also observed that the education of women independently impact on zinc level. It was thought that the education of women could have an effect on both economical status of the family and the knowledge of nutrition. While the relationship between zinc level and the time interval between the previous deliveries was reported in some studies,[6] in our study, there was no association between the zinc and the time interval between the latest 2 deliveries. In general, zinc status of pregnant women in Gaziantep appears to be adequate. The results of the present study enforce the need for zinc supplementation during pregnancy especially if the mother age is >35 years. Copper level was mostly above the higher limit of the reported references intervals and increased by the gestational period of the pregnant women living in Gaziantep city center. Copper deficiency is infrequent in our region.

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