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Copper and Zinc in the Amniotic Fluid and Serum from High-Risk Pregnant Women (40557)[1]

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Copper is an essential nutrient for all mammals (1), where it is associated with proteins such as lysyl oxidase (collagen crosslinking), tyrosinase (pigmentation), ceruloplasmin (chief form of plasma copper), and ferroxidase (oxidation of Fe^{2+} to Fe^{3+}) (2). Although copper deficiency is relatively rare in man (2, 3), abnormalities in copper metabolism have been associated with reproductive problems in man. Hypocupremia in pregnant women has been suggested as an index of placental insufficiency in such cases as postmaturity, premature rupture of membranes, and in spontaneous abortions (4, 5). Along with other factors, low levels of copper in the drinking water of Wales have been correlated with a higher incidence of neural tube congenital malformations in human infants (6, 7). Copper deficiency in experimental animals produces reproductive failure and abnormal offspring (7, 8). Administration of copper-chelating agents, penicillamine, or WIN 18,446 produce teratologies in rat pups (7). Copper-chelating agents are widely used in the home, agriculture, food industry, and as plastic stabilizers where they could conceivably cause reproductive disorders by producing secondary copper deficiencies (7). Pregnancies occurring in untreated patients with Wilson's disease (abnormal tissue accumulation of copper) are prone to early abortions (9). Copper and zinc antagonize each other in animal tissues (7), and excess zinc may precipitate copper deficiency. Zinc deficiency has been associated with an increased incidence of gross congenital malformations in experimental animals and possibly man (10).

Analysis of amniotic fluid has been recently used to diagnose prenatal women for congenital malformations in the fetuses (11). Sodium, potassium, zinc, and some organic constituents have been measured in amniotic fluid (11, 12), but nothing is known about the concentration of copper or its interaction with zinc in amniotic fluid. It is important to know the levels of copper and zinc in amniotic fluid in light of the studies discussed above linking these two elements to fetal abnormalities. Thus, the purposes of the study presented below were: (i) To determine if the concentrations of copper in the amniotic fluid and serum of high-risk pregnant women are different from normal women and (ii) To determine if there is a statistical relationship between copper and zinc in these women.

Materials and methods. After signing informed consent forms, 147 pregnant patients donated amniotic fluid and/or blood at Crippled Childrens Division (UOHSC) Prenatal Diagnostic Clinic. Patients in the present study were placed into one of two general risk groups: at risk because of maternal age ($\geq$35 years, mean age = 38), or at risk because of positive history of genetic disorders, neural tube defects (NTD), or present genetic anomaly (mean age = 29). A control group consisted of pregnant women less than 35 years of age with no history of fetal anomalies (mean age = 34). The condition of the children to be born to the women in the groups is as yet unknown.

Samples were collected in plastic disposable syringes and stored in acid-washed (HNO_3) glass tubes. After separating the serum, the fluids were frozen for subsequent analysis by atomic absorption spectroscopy (Instrumentation Laboratories 151). Results are expressed as ppm wet tissue. Pearson's r value was calculated to determine associations between copper and zinc.

Results. The overall mean concentration of copper in the amniotic fluid in women 13–19 weeks pregnant was found to be 0.14 ± 0.004 (n = 137) (Fig. 1). Although early amniotic

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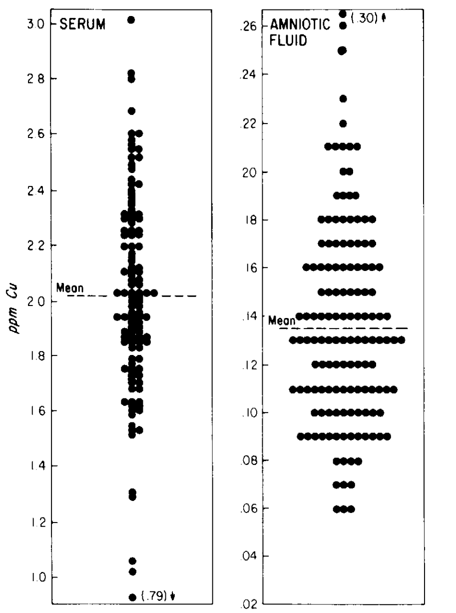

FIG. 1. Copper concentrations in the serum and amniotic fluid from pregnant women 13–19 weeks pregnant.

fluid has been described as compositionally similar in other substances to maternal serum (11), this was not found to be true for copper levels since overall maternal serum copper levels (2.02 ± 0.03, n = 119) were about 14-fold greater than levels of copper in amniotic fluid (Fig. 1).

In the present study, serum copper levels did not change appreciably during the 13–19 weeks of pregnancy (Fig. 2) as shown previously (12) and where serum and plasma copper levels during pregnancy are considered to be elevated above nonpregnant levels (5, 13, 14). Amniotic fluid copper values were fairly constant over the same gestational period (Fig. 2); although there was a tendency for copper levels in amniotic fluid to increase as gestational age increased.

Average copper concentrations in the serum and amniotic fluid in the group of patients with past history of or presently diagnosed fetal anomalies (by amniocentesis only), or in the group of older age patients were not significantly different from the group of normal patients in our study (Table I).

We found a statistically significant, positive correlation coefficient (r = 0.43) between copper levels in serum and copper levels in amniotic fluid (Table II). However, there were no significant correlations between copper and zinc in serum or amniotic fluid.

The observations presented above represent the first data on the concentrations of copper in amniotic fluid in women at risk for bearing children with birth defects. Our studies show that copper is a normal constituent of amniotic fluid. Furthermore, copper in amniotic fluid was found to be relatively constant in that copper levels were not influenced by gestational age during the early period of pregnancy (13–19 weeks) studied in this present report.

In another report (12), we presented data on the zinc content of amniotic fluid, which was found to contain zinc at about the same levels, 0.13 ± 0.01 ppm Zn, as copper. In the present experiment, we found no statistically significant relationship between copper and zinc in either amniotic fluid or serum in 147 pregnant women. This is in contrast to the negative association between copper and zinc noted in other animal tissues (8), and to the apparent "inverse relationship" between zinc and copper in serum reported during human pregnancy (15, 16). The latter conclusion was based upon the observation that serum zinc levels were decreased and serum copper levels were increased in pregnant women compared to levels 8 weeks after birth and to nonpregnant controls. However, these data represented only 20 pregnant women and the correlation coefficient between serum copper and zinc was only −0.25 (not statistically significant). While it is well established that serum copper does rise and zinc falls during pregnancy, we conclude that there is probably no consistent relationship between serum copper and zinc levels during the 13–19 weeks of pregnancy in the individual patient.

Previous work has indicated that abnormalities in copper metabolism may be asso-

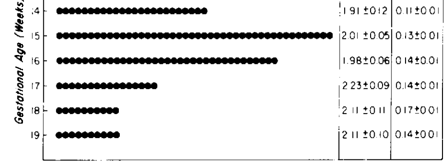

FIG. 2. Influence of gestational age in weeks on the concentrations of copper in the serum and amniotic fluid of pregnant women.

TABLE I. COPPER CONCENTRATIONS IN HIGH-RISK PREGNANT WOMEN[a]

Group	Mean age (years)	Cu (ppm) Amniotic fluid	Cu (ppm) Serum
Normal	34	0.14 ± 0.01 (18)[b]	1.98 ± 0.14 (15)
Older	38	0.14 ± 0.005 (81)	2.05 ± 0.04 (68)
Risk factor	29	0.13 ± 0.01 (39)	1.99 ± 0.04 (35)
Down's history[c]		0.14 ± 0.01 (12)	2.06 ± 0.07 (7)
An-hydrocephaly history		0.14 ± 0.02 (4)	1.92 ± 0.14 (4)
Trisomy 18-history		0.12 ± 0.02 (2)	2.11 ± 0.22 (2)
NTD and MM[d] history		0.12 ± 0.02 (5)	1.92 ± 0.12 (3)
Neonatal death history		0.16 (1)	1.68 (1)
Mental retardation history		0.17 (1)	1.55 (1)
Tay-Sach's history		0.12 ± 0.04 (2)	2.01 ± 0.49 (2)
Hurler's history		0.26 (1)	2.32 (1)
Muscular dystrophic brother		0.065 (1)	1.83 (1)
Hemophiliac brother		0.12 (1)	1.76 (1)
Unspecified congenital defect history		0.14 ± 0.03 (2)	2.41 ± 0.15 (2)
Miscarriages[e]		0.14 ± 0.02 (3)	2.15 ± 0.19 (4)
Present 14/21[f]		0.13 (1)	1.85 (1)
Present 1/15[g]		—	1.85 (1)
Present Down's[h]		0.12 ± 0.02 (2)	1.99 ± 0.09 (3)

[a] 13–19 weeks pregnant.
[b] Mean ± SE (number of patients).
[c] "History" includes previous children or in family.
[d] Myelomeningocele.
[e] Includes past and present miscarriages.
[f] Mother was a balanced carrier of a 14/21 translocation.
[g] Mother was a balanced carrier of a 1/15 translocation.
[h] Fetus diagnosed as trisomy 21 (Down's syndrome).

TABLE II. COPPER–ZINC INTERACTIONS IN PREGNANT WOMEN

Interaction	Pearson's r coefficient	Significance
Serum Cu vs amniotic Cu[a]	0.43	$P < 0.001$
Serum Zn vs amniotic Zn	0.02	NS[b]
Serum Cu vs serum Zn	0.05	NS
Amniotic Cu vs amniotic Zn	0.14	NS

[a] Mean values (ppm, mean ± SE): serum Cu = 2.02 ± 0.03 (118), serum Zn = 0.75 ± 0.01 (131), amniotic Cu = 0.14 ± 0.004 (138), amniotic Zn = 0.13 ± 0.04 (139).
[b] Not statistically significant ($P > 0.05$).

ciated with fetal anomalies in both man and experimental animals (6, 7). In our groups of high risk pregnant women we could find no obvious changes in the levels of serum or amniotic fluid at 13–19 weeks of pregnancy associated with past history of fetal anomalies, present genetic anomaly, or increased maternal age. Future studies should be directed towards correlating copper levels in the amniotic fluid and serum in these mothers in which the fetus is affected with a genetic disorder or birth defect.

Summary. Copper was found to be a normal constituent of the amniotic fluid in high-risk pregnant women at the level of 0.14 ± 0.004 ppm ($n = 138$). Copper in the amniotic fluid was positively correlated ($P < 0.001$) to maternal serum copper levels at $r = 0.43$. During the 13–19 weeks of early pregnancy, copper levels in the amniotic fluid and serum were not appreciably influenced by gestational age. There was no significant correlation between copper and zinc in serum or amniotic fluid. Copper levels in serum and amniotic fluid during this period were not altered in pregnant women having an increased risk of producing a child with a fetal anomaly.

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