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EFFECT OF ORAL ZINC SUPPLEMENTATION ON COPPER AND HAEMOGLOBIN LEVELS IN PREGNANT WOMEN

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Abstract: In the present study, pregnant women in different trimesters of pregnancy were randomly allocated to untreated control group (Gp A; n = 58), and zinc treated group (Gp B; n = 104). Both groups were administered ferrous sulphate 60 mg, and folic acid 5 mg, twice daily throughout the period of study. Gp B subjects were also administered 45 mg elemental zinc, in a single daily post lunch dose. Maternal blood and urine samples collected in each trimester, and at the time of delivery, and blood taken from the umbilical cord were tested for Cu levels. Maternal Hb was also estimated. In Gp A, mean serum Cu increased significantly from 117.15 ± 2.12 μg/dl in I trimester to 138.57 ± 0.92 μg/dl in III trimester ($P < 0.001$). In Gp B, serum Cu declined significantly from 115.64 ± 1.12 μg/dl in I trimester to 111.10 ± 0.99 μg/dl in III trimester ($P < 0.001$). Urinary Cu declined significantly from 47.24 ± 2.31 μg/24 hrs in I trimester to 37.43 ± 2.06 μg/24 hrs in III trimester ($P < 0.01$). Zn treatment did not alter differentially the serum Cu levels in anaemic and normohaemic subjects. Gp B cord blood serum Cu was significantly lower as compared to respective controls, significance being proportional to duration of zinc administration. Hb levels increased significantly in all subjects. Increase in Hb in Gp B was significantly higher in comparison to that in Gp A ($P < 0.05$). Elemental zinc when administered to pregnant women in a dose of 20-45 mg/day, causes improvement in Hb level, without leading to hypocupremia.

Key words: zinc copper anaemia pregnancy haemoglobin

INTRODUCTION

Serum zinc levels have been reported to fall below normal during pregnancy (1, 2). Zinc deficiency may cause maternal as well as foetal morbidity (3). There may be increased incidence of altered taste and smell, break-through bleeding, hypertension, and post partum haemorrhage in the pregnant women (4, 5). Zinc deficiency may also cause intrauterine growth retardation (I.U.G.R.), congenital malformations, low birth weight, low apgar score and prematurity in the baby at birth (6, 7). A supplementation of zinc during pregnancy has, therefore, been recommended by a number of workers (8, 9). The serum levels of zinc and copper are inversely correlated (10). Whereas zinc levels decrease during pregnancy, copper levels show an increase (2). However, following zinc supplementation, serum levels of copper decrease. Very high serum zinc levels may even lead to hypocupremia (11) which may lead to number of complications,

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mainly metabolic, as copper is a part of many important enzymes, not only in the periphery, but in CNS too. Keeping these points in view, a study was planned to observe the effect of oral zinc supplementation on copper levels in serum and urine of pregnant women. Copper was also estimated in umbilical cord serum, which reflected copper status of the baby.

The administration of zinc has been reported to be necessary during pregnancy, but it decreases absorption of dietary iron from the intestine.

Therefore, the effect of oral zinc supplementation on haemoglobin levels in pregnant women was also studied.

METHODS

The study was conducted at the Antenatal Clinic of J. N. Medical College, A.M.U., Aligarh. Pregnant women residing in Aligarh city and neighbouring villages were enrolled. Pregnant women reporting during I, II as well as III trimester of pregnancy were thoroughly examined and investigated as required. Those having systemic desease, or receiving steroids, zinc or copper supplementation were excluded from the study.

The pregnant subjects were divided into control (Gp A) and zinc treated group (Gp B). All pregnant subjects were administered ferrous sulphate 60 mg x BD, and folic acid 5 mg x BD throughout the study period. The afternoon dose was not administered in Gp B to avoid interaction with the post lunch dose of zinc. Gp B subjects were administered 45 mg elemental zinc as zinc sulphate, 200 mg tablet supplied by Yash Pharma Laboratories (Pvt) Ltd., Bombay in a single, daily, post lunch dose, from the day of reporting till delivery. Initially the number of subjects enrolled in both groups was same (i.e. 120 in each Gp), but subsequently, many subjects dropped out of study, and their data has not been recorded. The final number of subjects in control group was 58 and that in the zinc treated group was 104.

Five ml venous blood, and 24 hour urine samples were collected in each trimester since reporting, and also at the time of delivery. Blood sample was also collected immediately after delivery, from the umbilical cord. Immediately after clamping and cutting the umbilical cord, fresh jet of blood from the placental end was directly collected into vials. Haemoglobin levels were estimated from the maternal blood samples, just after collection. Blood was collected in sterilized plastic vials, which were kept at room temperature, till serum separated, which was then poured into fresh plastic vials. Hemolysed blood samples were discarded and were recollected at a later date. Urine samples were collected in sterilized glass bottles provided to the subjects. Urine volume was recorded; 5 ml urine was transferred to sterilized plastic vial and the rest discarded. Serum and urine samples were stored at 4°C and were analysed for zinc and copper within 72 hours, by G B C 902, double beam atomic absorption spectrophotometer.

Statistical analysis of data was done by Student's 't' test.

RESULTS

Serum copper level : In untreated control subjects, serum copper levels increased slowly and steadily with the advancement of pregnancy ($P < 0.001$) and were highest at term. In zinc treated subjects, however, the serum copper levels declined significantly ($P < 0.001$) within 15 days, as there was a gap of at least 15 days between collection of two consecutive samples. The serum copper levels in zinc treated subjects were lowest at term (Table I).

Urinary copper level : The 24 hour urinary copper levels in control subjects were limited to a narrow range, without any significant variation among subjects (P - NS). However, following zinc therapy even for one month (e.g. in subjects who reported during 8th month of gestation), the urinary copper levels decreased significantly as compared to levels measured at least 15 days before ($P < 0.01$; Table II).

TABLE I : Duration of therapy related effect of oral zinc sulphate administration 200 mg/day, from the day of reporting till term on serum copper levels (μg/dl) in pregnant women (data are $\bar{x} \pm$ SEM).

Group	*Duration of zinc therapy in months*						
	6-9 (Reported in I trimester)			*3-6 (Reported in II trimester)*		*Less than 3 (Reported in III trimester)*	
	Pre-treatment	*Post-treatment*	*Post-treatment*	*Pre-treatment*	*Post-treatment*	*Pre-treatment*	*Post-treatment*
	I Trimester	*II Trimester*	*III Trimester*	*II Trimester*	*III Trimester*	*III Trimester*	*III Trimester*
Control (n=58)	117.15 ± 2.12 (n=19)	128.05 ±1.33 (n=19)	138.57±0.92[b] (n=19)	129.07±1.43[b] (n = 28)	141.00±1.18[c] (n=28)	139.72 ± 1.12[c] (n=11)	143.00 ± 1.07[a] (n=11)
Zinc treated (n=104)	115.64± 1.12 (n=37)	113.13±1.07*[b] (n=37)	111.10 ± 0.99*[b] (n=37)	125.69 ± 0.93 (n=55)	112.65 ± 0.93*[b] (n=55)	137.08 ± 2.31[c] (n=12)	110.16 ± 1.39*[b] (n=12)

*P<0.001 vs control, [a]-P <0.005, [b]-P <0.001 vs pretreatment
[c] - P<0.001 vs pretreatment II trimester.

TABLE II : Duration of therapy related effect of oral zinc sulphate administration 200 mg/day from the day of reporting till term on urinary copper levels (μg/24 hrs) in pregnant women (data are $\bar{x} \pm$ SEM).

Group	*Duration of zinc therapy in months*						
	6-9 (Reported in I trimester)			*3-6 (reported in II trimester)*		*Less than 3 (reported in III trimester)*	
	Pre-treatment	*Post-treatment*	*Post-treatment*	*Pre-treatment*	*Post-treatment*	*Pre-treatment*	*Post-treatment*
	I Trimester	*II Trimester*	*III Trimester*	*II Trimester*	*III Trimester*	*III Trimester*	*III Trimester*
Control (n=58)	44.94 ± 3.74 (n=19)	43.36 ± 3.86 (n=19)	45.15 ± 3.53 (n=19)	46.14 ± 1.44 (n=20)	45.89 ± 2.36 (n=28)	43.09 ± 2.46 (n=11)	45.09 ± 2.42 (n=11)
Zinc treated (n=104)	47.24 ± 2.31 (n=37)	41.83 ± 2.20** (n=37)	37.43 ± 2.06* (n=37)	42.53 ± 1.34 (n=55)	36.81 ± 1.82* (n=55)	39.91 ± 3.21 (n=12)	34.16 ± 2.64* (n=12)

*P < 0.01 vs control;
**P < 0.02 vs pretreatment.

Umbilical cord blood serum copper level : The umbilical cord blood serum copper levels in control as well as zinc treated subjects were significantly low, as compared to maternal serum copper levels (P < 0.001). In zinc treated subjects the cord levels were significantly lower as compared to the cord levels in control subjects (P < 0.05 - 0.001). The significance of difference in cord levels between control and zinc treated subjects was directly proportional to the duration of zinc therapy. The cord copper levels in control as well as zinc treated subjects were not related to maternal serum copper levels (Table III).

Haemoglobin level : In zinc treated Gp, serum copper levels were not different in anaemic and normohaemic subjects (Table IV). There was a significant increase in haemoglobin levels in control as well as zinc treated subjects (P <0.05).

However, in zinc treated subjects, the increase in Hb levels was significantly higher than corresponding control (P < 0.05-00.01), when zinc therapy was given for more than 3 months (Table V).

TABLE III : Duration of therapy related effect of zinc sulphate administration (200 mg/day) from the day of reporting till term to pregnant women reporting in different trimesters of pregnancy, on the level of cord blood serum copper (μg/dl).

(data are mean ± SEM)

Group	*Duration of treatment in months*		
	6-9 (Reported in I trimester)	*3.6 (Reported in II trimester)*	*Less than 3 (Reported in III trimester)*
Control (n=58)	76.48 ± 1.98 (n=19)	73.51 ± 2.233 (n=28)	75.36 ± 3.40 (n=11)
Zinc treated (n=104)	60.35 ± 1.79*** (n=37)	62.83 ± 1.68** (n=55)	65.66 ± 2.57* (n=12)

*P<0.05, **P<0.01, ***P < 0.001 vs control.

DISCUSSION

In this study, serum copper levels tended to increase with the period of gestation and were at peak levels at terms. Increase in serum copper concentration during pregnancy is a part of physiological changes occurring during pregnancy. This increase in serum copper is attributed to increase in ceruloplasmin as result of elevated levels of estrogens (12).

When zinc was administered to pregnant women, there was a significant fall in serum copper levels in all trimesters of pregnancy. This was because of the inverse correlation between serum zinc and copper levels. When zinc levels increased beyond normal physiological limits due to continued administration,

TABLE IV : Duration of therapy related effect of oral zinc sulphate administration 200 mg/day, from the day of reporting till term on serum copper (μg/dl) in anaemic and normohaemic pregnant women.

(Data are $\bar{x}$ ± SEM)

Group	*Duration of zinc therapy in months*			
	6-9 (I Trimester)		*3.6 (II Trimester)*	*Less than 3 (III Trimester)*
Normohaemic (n = 29)	114.24 ± 2.38 (n = 29)	113.52 ± 2.69 (n = 29)	110.16± 1.27 (n = 45)	111.47 ± 4.81 (n=7)
Anaemic (n=23)	112.75 ± 4.21 (n=8)	110.47 ± 4.70 (n=8)	108.28 ± 3.95 (n=9)	108.50 ± 5.89 (n=6)

TABLE V : Duration of therapy related effect of oral zinc sulphate administration 200 mg/day from the day of reporting till term on haemoglobin level (g/dl) in pregnant women (data are $\bar{x}$ ± SEM).

Group	*Duration of zinc therapy in months*						
	6-9 (Reported in I trimester)			*3-6 (Reported in II trimester)*		*Less than 3 (Reported in III trimester)*	
	Pre-treatment	*Post-treatment*	*Post-treatment*	*Pre-treatment*	*Post-treatment*	*Pre-treatment*	*Post-treatment*
	I Trimester	*II Trimester*	*III Trimester*	*II Trimester*	*III Trimester*	*III Trimester*	*III Trimester*
Control (n=58)	9.14 ± 0.34 (n=19)	9.85 ± 0.28 (n=19)	10.48 ± 0.26[a] (n=19)	8.82 ± 0.21 (n=28)	9.60 ± 0.17[a] (n=28)	3.05 ± 0.48 (n=11)	10.18 ± 0.56[b] (n=11)
Zinc treated (n=104)	9.00±0.18 (n=37)	10.26 ± 0.19[b] (n=37)	11.22 ± 0.15*[c] (n=37)	8.64 ± 0.16 (n=55)	10.22 ± 0.13**[c] (n=55)	8.08 ± 0.41 (n=12)	10.28 ± 0.54[b] (n=12)

[a] - P < 0.05, [b] - P < 0.01, [c] - P < 0.001 vs pretreatment;
* - P < 0.05, ** - P < 0.01 vs control./

the copper levels declined proportionately. But the copper levels did not decrease beyond normal physiological limits. With continuous administration of zinc, there is a potential risk of hypocupremia (11). In this study, elemental zinc was administered in a single dose of 45 mg/day. This dosage is sufficient to take care of zinc deficiency, and daily zinc requirement in pregnant women, while the risk of inducing hypocupremia is minimised. The zinc - sufficient subjects, especially in developed countries require 20 mg/day elemental zinc during pregnancy to avoid hypozincemia (13). However, the Indian subjects are usually zinc deficient during pregnancy, and a dose of 20 mg of zinc per day is not sufficient to fulfil the zinc demand and also to avoid complications of zinc deficiency in mother as well as fetus/baby. For Indian women, therefore, 45 mg/day of elemental zinc is the proper dose to start with, which should be decreased to 20 mg/day, when the serum zinc levels indicate that the body zinc stores have been replenished. Thus the risk of iatrogenic hypocupremia is eliminated.

In the present study, oral zinc supplementation caused a further improvement in haemoglobin levels of pregnant subjects, in addition to that caused by iron and folate supplementation. Zinc and other cations complete with iron for absorption in small intestine, and vice versa (14). Therefore zinc and iron should not be administered together. There should be a time-gap of at least 3-5 hours between the administration of these two. This implies that the iron zinc combination preparations should not be prescribed to subjects. In order to check for probability of hypocupremia in zinc supplemented subjects, frequent serum level estimations are helpful. Blood can be collected at the same time, when samples for other routine investigations are being collected. If facilities for centrifugation are available, even 1 ml of blood sample is sufficient to obtain the required amount of serum.

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