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Zinc Status of Infants with Fetal Alcohol Syndrome

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ABSTRACT. Plasma and urinary zinc levels were examined in 6 infants with fetal alcohol syndrome to determine whether zinc deficiency, if present in fetal alcohol syndrome patients, is secondary to an increased urinary zinc excretion. Six infants born to nonalcoholic mothers served as controls. There was no significant difference in creatinine clearance, urine flow rate, or plasma albumin concentrations between the two groups. Plasma concentrations of zinc were significantly lower in fetal alcohol syndrome patients (62.5 ± 2.8 μg/dl) in comparison to controls (71 ± 1.8 μg/dl), ($p = 0.0001$). Urinary excretion of zinc in fetal alcohol syndrome patients averaged 646 ± 125 μg/24 h, significantly higher than in control subjects (76.6 ± 22 μg/24 h), ($p = 0.0001$). Thus (1) lower plasma zinc levels are present in infants with fetal alcohol syndrome and (2) increased urinary zinc excretion appears to be responsible for decreased plasma zinc concentrations. (*Pediatr Res* 20: 551–554, 1986)

Abbreviations

FAS, fetal alcohol syndrome
PZn, plasma zinc concentration

The teratogenicity of alcohol has been demonstrated in humans through clinical and epidemiological studies and in animals through controlled laboratory experiments (1). The effects on offspring range from decreased birth weight and functional deficits at lower levels of alcohol intake to FAS and early or late fetal death at higher doses (2, 3). The principal features of FAS include prenatal and postnatal growth retardation, facial dysmorphic features, and central nervous system involvement (4, 5). The mediation of these effects through the nutritional status of the mother with respect to zinc has been hypothesized (6). Hyperzincuria and tendency toward lower PZn concentrations have been reported in alcoholic patients (7, 8). Studies of zinc deficiency in human pregnancy have also shown a strong positive correlation with fetal dysmorphogenesis (9) in a manner similar to those reported in rat pups when the dams were restricted in zinc intake during gestation (10).

In view of the fact that excessive alcohol intake may deplete body stores of zinc and since zinc plays a vital role in DNA synthesis and cell division (11, 12), one may hypothesize that a relative zinc deficiency during gestation may be one of the mechanisms underlying alcohol-induced fetal dysmorphogenesis.

The present study was designed to evaluate zinc status in infants with FAS and to determine whether zinc deficiency, if present in the FAS, is secondary to an increased urinary zinc excretion.

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PATIENTS AND METHODS

Six alcoholic mothers and their offspring (age 6 to 12 months) were selected from a large group of inmates incarcerated at a Tehran Prostitutional Center in 1978. The mothers were spree drinkers and had had a history of alcoholism from 5 to 8 yr. None was addicted to any other drugs. All satisfied the criteria for alcoholism as defined by the criteria committee of the National Council of Alcoholism (13). The six selected infants of these women were recognized as having the pattern of altered growth and morphogenesis characteristic of the FAS (4, 5) (Table 1). All presented in early infancy with symptoms including poor feeding, vomiting, polyuria, dehydration, and failure to thrive. None had malabsorption syndromes, glucosuria, aminoaciduria, proteinuria, hyperphosphaturia (defined as tubular reabsorption of phosphate <85%), or hypercalciuria (urine calcium/urine creatinine >0.25). Normal blood concentrations of glucose (91.5 ± 5 mg/dl), calcium (8.1 ± 0.3 mg/dl), phosphate (4.0 ± 0.4 mg/dl), alkaline phosphatase (125 ± 11 U/liter), total proteins (6.8 ± 1.1 g/dl), and albumin (3.7 ± 0.3 g/dl) were obtained in all patients. Liver function tests were normal with SGOT 32 ± 4 U/liter, SGPT 21 ± 3 U/liter, and total bilirubin 0.5 ± 0.1 mg/dl. Tuberculin skin tests were negative and sweat tests were normal. Six infants born to nonalcoholic mothers with postnatal ages of 4 to 10 months (mean 6.9 months) and weights of 5.0 to 9.8 kg (mean 7.38 kg) served as controls. Although the control infants were younger than the FAS infants, group mean values for weight and height were not significantly different. Other characteristics of both the FAS and control infants have been reported previously (27). Dietary zinc intake by the FAS patients and the normal control infants appeared to be similar. All were fed cow's milk formula after birth. Solid foods were introduced by the end of the 4th month of life in the form of cereals, fruit juice, and pudding providing a caloric intake of 105 (range 100–110) kcal/kg/day. This diet contained 3 to 5 mg zinc by calculation. Strained cooked vegetables and meats were added into the diet by the end of the 6th month. Twenty-four-h specimens of urine were collected by means of bladder catheterization directly into sterilized and acidified plastic containers and immediately frozen for later analysis. At the midpoint of each urine collection 3 ml venous blood samples were drawn into acid-cleaned sterile plastic syringes through stainless-steel needles and immediately placed in plastic tubes containing heparin. Plasma was separated from erythrocytes within 1 h and then returned to plastic tubes. Urine and plasma specimens were frozen promptly at −20° C and analyzed later for creatinine (Beckman autoanalyzer methods) and zinc (atomic absorption spectrophotometry) (14). All specimens were analyzed fresh or within 24-h of storage. The intraassay coefficient of variation of the method for plasma

Table 1. *Clinical features observed in infants with FAS*

Feature	Patient 1	2	3	4	5	6
Age (mo)/sex	12/F	10/F	10/M	8/F	7/F	6/M
Growth deficiency (≤10th percentile)						
Prenatal	+	+	+	+	+	+
Postnatal	+	+	+	+	+	+
Facial characteristics						
Short palpebral fissures	+	+	+	+	+	+
Hypoplastic philtrum	+	+	−	+	+	+
Hypoplastic maxilla	+	+	+	+	−	−
Microcephaly (≤3rd percentile)	+	−	+	+	+	+
Central nervous system						
Developmental delay	+	+	+	+	+	+
Hypotonia	−	−	+	−	+	−
Skeletal						
Joint anomalies*	−	−	−	+	−	−
Altered plamar crease pattern	−	+	−	−	−	−
Cardiac anomaly†	−	−	−	−	+	−

* Syndactyly of the second and third toes bilaterally and limitation of motion at elbows.

† Atrial septal defect; +, present; −, absent.

zinc was 0.011 and of the method for urine, 0.013. The corresponding interassay coefficients of variation were 0.019 and 0.03, respectively. Recovery of zinc determined by the method was 98%.

Creatinine clearance was estimated as urinary creatinine concentration × urinary flow rate/plasma creatinine concentration.

The protocol for this study was approved by the Institutional Review Board, The University of Medical Sciences of Iran. Informed written maternal consent was obtained for all patients prior to study. Statistical analysis of the results was done using the Student's *t* test for unpaired variables. Data are presented as the mean ± SD.

RESULTS

The results of the PZn determinations in FAS patients are presented and compared to the data in normal subjects (Fig. 1). The mean PZn in the FAS patients was 62.5 ± 2.8 μg/dl. In normal infants, the mean value was 71 ± 1.8 μg/dl ($p = 0.0001$). PZn levels in all FAS patients were lower than in all control subjects. No FAS patient had a value greater than 65 μg/dl.

Total urinary excretion of zinc is shown in Figure 2. The mean excretion in the patient group, 646 ± 125 μg/24 h, was significantly higher than that for the normal group, 76.6 ± 22 μg/24 h ($p = 0.0001$). Only one of the six normal infants had values greater than 100 μg/24 h. In contrast, zinc excretion of 391 μg/24 h or greater was found in all patients with FAS. Five of the six patients with FAS had values greater than 680 μg/24 h. There was no significant difference in creatinine clearance or urinary flow rate between the two groups (Table 2).

DISCUSSION

The present study suggests that infants exposed to intrauterine alcohol have lower plasma Zn levels and increased urinary Zn excretion as compared to control infants when examined 6 to 12 months after birth.

Decreased PZn levels can result from inadequate dietary intake (15, 16), impaired absorption (17), excessive urinary excretion (7, 8, 18), and inherited defects in zinc metabolism such as occurs in acrodermatitis enteropathica (19). Inadequate dietary zinc intake cannot account for the lowered PZn levels observed in our FAS patients since PZn deficiency did not occur in any of the six control subjects with comparable dietary intake. PZn levels are known to be dependent on plasma protein concentration since approximately 80 to 90% of zinc is bound to protein, mainly albumin (20). In this regard, plasma protein concentrations in FAS patients (6.8 ± 1.1 g/dl) were similar to those of controls (7.1 ± 1.4 g/dl) (p = NS). There was no evidence to suggest that any of the FAS patients had chronic diarrhea, malabsorption syndrome, or liver disease. Thus none of the usual disturbances in zinc absorption or metabolism was suspected in our patients.

The finding of marked hyperzincuria in virtually every FAS patient suggests that the increased urinary excretion of zinc may be the cause for the low PZn values. Each patient with FAS had a markedly increased urinary zinc excretion at the time of hypozincemia (Table 2). The mechanisms and factors determining the renal excretion of zinc are largely unknown. The albumin-bound zinc is in equilibrium with small molecular weight amino acids, primarily histidine and to a lesser extent cysteine (21). Since histidine-zinc ligands are of relatively small molecular weight, they cross the renal glomerulus and are readily excreted in the urine (21). Approximately 0.5 mg of zinc is excreted each

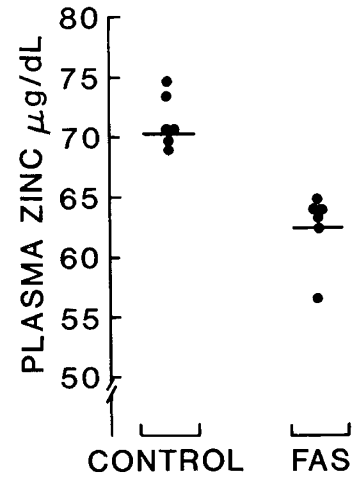

Fig. 1. Plasma zinc concentrations are compared in FAS infants and normal controls. Mean values are given by the *horizontal lines.*

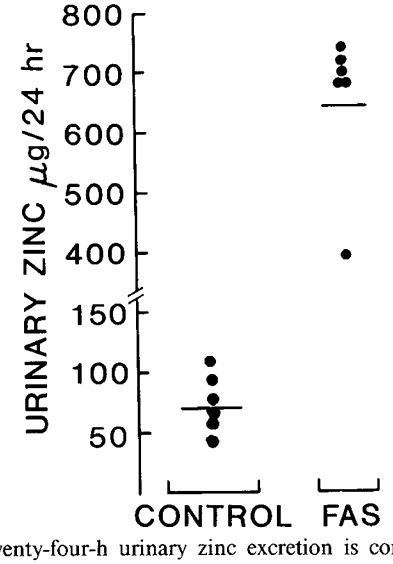

Fig. 2. Twenty-four-h urinary zinc excretion is compared in FAS infants and normal controls. Mean values are given by the *horizontal lines.*

Table 2. *Summary of data obtained in six infants with FAS**

Patient	Wt (kg)	C_{cr} (ml/min)	PZn (μg/dl)	V (ml/day)	UZn·V (μg/day)
1	9.1	65	57	374	715
2	8.3	60	64	302	696
3	8.2	63	65	331	686
4	7.4	58	62	302	391
5	5.9	51	65	273	684
6	5.3	47	63	316	704
Mean ± SD	7.2 ± 1.6	57 ± 7	62.5 ± 2.8†	316 ± 34	646 ± 125†
Controls ($n = 6$)					
Mean ± SD	7.4 ± 1.5	60 ± 9	71 ± 1.8	331 ± 29	77 ± 22

* C_{cr}, creatinine clearance; V, urine flow rate; UZn·V, urinary zinc excretion.
† $p = 0.0001$.

day in the urine of adults and a similar quanity is excreted in the sweat (21).

The alterations in renal physiology responsible for the increased zinc excretion in FAS patients are also obscure. Previous studies have shown that the congenital malformations that result from severe prenatal zinc deficiency are probably caused by a defect in DNA and protein synthesis (11, 22). In addition, several investigators have shown that zinc deficiency lowers the activity of alcohol dehydrogenase in the intestine, liver, kidneys, testes, and bones of rats in comparison to their pair-fed controls (11, 23). Reduced activity of alcohol dehydrogenase, a zinc metalloenzyme, has been reported in alcoholic patient (24). The participation of zinc in the dehydrogenation of ethanol (25) and glutamic acid (26), two substrates that are clearly implicated in the metabolic changes seen in alcoholic patients, supports this view (21). Although not proven, it seems possible that the observed hyperzincuria in FAS infants may be related to the enzyme inhibition.

It is also possible that the increased urinary zinc excretion is a result of impaired renal tubular function induced by intrauterine alcohol exposure. Additional support for this hypothesis was provided more recently in studies of renal acidification in FAS infants (27). These data show that infants with FAS have a defect in distal acidification and potassium excretion which cannot be attributed to abnormal aldosterone secretion. The high urinary excretion of zinc, whatever the cause, could deplete zinc stores of the body in FAS patients so that inadequate amounts of zinc would be available for cellular functions.

In a study of maternal zinc deficiency in the rat, Hurley (10) reported a high incidence of congenital malformations in the affected offspring. Subsequently Flynn *et al.* (9) examined the relationship between zinc status and FAS in a cohort of pregnant women and reported that alcoholic pregnant women have significantly lower zinc levels than comparable nonalcoholic pregnant women and that the low plasma zinc values were associated with an increased rate of fetal dysmorphogenesis. Furthermore studies on animal models of the FAS have demonstrated that the combination of zinc deficiency and alcohol consumption has more severe effects on the fetus than either alcohol or zinc deficiency alone (11, 12). In a more recent study Keppen *et al.* (28) examined the effects of alcohol on progeny of pregnant mice fed a zinc-deficient diet compared to those fed a diet with adequate zinc. The results of their findings are in accord with previous reports (11, 12), indicating that the teratogenic effects of alcohol and low dietary zinc in combination are much greater than the effects of either alone.

The findings in the present study that FAS infants, as did their mothers during gestation (9), have low plasma zinc levels suggest the possibility of preventing fetal dysmorphogenesis by supplementing alcoholic pregnant women with zinc. Additional support for this hypothesis has been provided in a recent study reported by Jameson (29). In this study 312 Swedish pregnant women were enrolled. The 64 women with low plasma zinc levels at 14-wk gestation who were then given zinc supplement (45 mg elemental zinc daily) had a higher percentage of normal deliveries and normal infants than the 69 women with low plasma zinc who were not supplemented (63 *versus* 22%), indicating that improvement of zinc nutriture during pregnancy can reduce the prevalence of birth defects. In this study none of the pregnant women was reported to be alcoholic. In an animal study of FAS, Keppen *et al.* (28) showed that fewer congenital malformations occurred in mice given the recommended amount of zinc compared to those given very low dietary zinc.

The ultimate question of the role of zinc in human FAS will require the demonstration that improvement of zinc nutriture in alcoholic women during pregnancy reduces the incidence or severity of birth defects in the offspring as compared to an untreated group. Further studies of zinc metabolism in alcoholic women and their neonates would seem justified.

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Announcements

European Society for Pediatric Research

The European Society for Pediatric Research will hold its next meeting in Groningen, The Netherlands, September 7–10, 1986. The following working groups will join the meeting: Paediatric Allergy and Clinical Immunology; Paediatric Pharmacology; Perinatology; and Paediatric Microcirculation. The main topics will be Nutrition and Metabolism, Hepatic Metabolism, Fetal and Neonatal Metabolism, Developmental Neurology, Genetics, Immunology, Pharmacology, Microcirculation, and Oncology.

Travel bursaries are available for young investigators, particularly from Eastern Europe. *For further information, contact* J. Fernandes, Department of Paediatrics, University Hospital, 59 Oostersingel, 9713 EZ Groningen, The Netherlands.

Australia Hosts 10th IUPHAR Congress

The 10th International Congress of Pharmacology will be held in Sydney, Australia, August 23–28, 1987. The organizers are planning a scientific program which will identify the key areas in which there is rapid growth. The format will include invited lectures, symposia, free communications, poster sessions, symposia and workshops.

The Congress will make full use of Sydney's famous attractions and facilities such as the Sydney Opera House and the harbor.

For further information, contact the Secretariat: 10th IUPHAR, GPO Box 2609, Sydney NSW 2001, Australia.