Effects of Prenatal Alcohol Exposure on the Developing Kidneys

Farahnak Assadi1*, MD

1. Pediatric Nephrologist, Rush University Medical Center, Chicago, Illinois, USA

Abstract

Objective: Clinical and experimental studies strongly suggest that prenatal alcohol exposure is associated with zinc deficiency and impaired renal tubular function. Whether maternal alcohol consumption during pregnancy causes renal tubular cell injury is unknown.

Material & Methods: Renal function was studied in 8 infants with fetal alcohol syndrome (FAS) and 8 healthy age‐matched infants. Renal function and structure were also examined in 11 offspring of rats exposed to alcohol during gestation.

Findings: Infants with FAS had limited ability to concentrate urine after water restriction (P<0.001) and impaired acidification after acute acid loading (P<0.001) compared to control group. Plasma zinc levels were lower (P<0.001) and urinary zinc excretion was higher (P<0.001) in infants with FAS compared to control infants. Scanning electron microscopic studies revealed cytoplasmic mitochondrial hypertrophy and vacuolar structures of the epithelial cells of the cortical collecting ducts in the rat kidney following fetal exposure to alcohol.

Conclusion: These findings suggest that offspring of rats exposed to alcohol during fetal life have renal functional and structural abnormalities that may be responsible in the genesis of renal functional abnormalities as described in infants with FAS.

Key Words: Renal tubular dysfunction; Structural renal abnormalities; Fetal alcohol syndrome; Infant

Introduction

Alcohol consumption during pregnancy is teratogenic and can give rise to a condition called fetal alcohol syndrome (FAS) in the offspring[1]. The principal features of children with FAS are characteristic craniofacial abnormalities, neurological and neuro‐behavioral abnormalities, growth retardation and skeletal deformities[2,3]. Other major congenital anomalies may include heart defects and renal structural anomalies[4,5].
Maternal alcohol consumption during pregnancy can also impair renal function in the offspring\(^6\-^8\). Experimental studies have also demonstrated that in utero exposure to alcohol in rats results in impaired renal acidification and defective urinary concentration similar to those described in infants with FAS\(^9\). The mechanism by which alcohol induces its effect is unknown. The similarity in the pattern of renal tubular dysfunction between the clinical and experimental studies suggests the possibility of alcohol-induced renal tubular cell injury as a causative factor in the development of renal dysfunction reported in infants with FAS.

This study was undertaken to investigate whether prenatal alcohol exposure is capable of inducing renal tubular cell injury and if so, to provide evidence of a causal relationship between renal morphology abnormalities and functional disturbances as documented in infants with FAS.

**Material & Methods**

Renal function was studied in 8 infants with FAS and 8 control subjects before and after fluid restriction and acute acid loading (ammonium chloride infusion 100 mg/kg over 4 h). Baseline urine and blood electrolytes, pH, osmolality, creatinine, \(\beta_2\)-microglobulin (\(\beta_2\)-M) and zinc, plasma renin activity (PRA) and plasma aldosterone (PA) levels were measured in all subjects. Eleven offspring of ethanol-exposed rats during gestation were randomly selected at 90 days of age and studied for renal morphology examinations.

Blood and urine levels of sodium, potassium, chloride, bicarbonate, urea and creatinine were determined with Astra analyzer (Beckman, Astra IV). Urine and plasma osmolality were measured by freezing point depression. Blood and urine pH were determined on an acid-base analyzer using direct-reading electrodes. Urine anion gap (mEq/L) was calculated as the sum of sodium and potassium minus chloride. PRA and PA level were measured by radioimmunoassay using the available kits (Squibb and Sons, Princeton, NJ, and Diagnostic Products, Los Angeles, CA; respectively). Creatinine clearance was calculated using Schwartz formula\(^9\). Blood and urine \(\beta_2\)-M were measured as described previously\(^{10,11}\).

The rat kidneys were removed under general anesthesia for morphologic examinations. The left kidney of each animal was cut longitudinally. Thin tissue sections (2 to 3 \(\mu\)m) were then cut and rinsed in 0.1 M sodium cacodylate buffer, fixed in 1.0% uranyl acetate and lead citrate, dehydrated in graded alcohol and embedded in 1:3 mixture of propylene oxide and epoxy resin. The tissue was then blocked in fresh resin using BEEM capsules and placed in an oven at 63°C for 24-36 h before sectioning. Thin sections were then cut with a diamond knife and viewed in a Cambridge Mark II electron microscope at 10 kV.

The protocol was approved by the Institutional Review Board on Human and Animal Care and Use Committees. Informed 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 mean (SD). A \(P\) value less than 0.05 was considered significant.

**Findings**

The clinical characteristics of infants with FAS are shown in Table 1. All presented in early infancy with symptoms of poor feeding, polyuria, failure to thrive and morphological characteristic of the FAS\(^1\-^3\). Baseline serum electrolytes, glomerular filtration rate (GFR), urinalysis, blood and urine anion gaps, \(\beta_2\)-M excretion, PRA, and PA levels did not differ between the two groups. Despite equivalent blood osmolality and GFR, the maximum urinary osmolality after 12 h of water deprivation in FAS patients was significantly lower compared to control infants [560 (107) vs. 965 (77) mOsm/kg; \(P<0.001\)], (Fig 1).
### Table 1- Clinical features observed in infants with FAS

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (month)/ gender</td>
<td>1 2 3 4 5 6 7 8</td>
</tr>
<tr>
<td>Weight/ Height &lt;10</td>
<td>+ + + + + + + +</td>
</tr>
<tr>
<td>Microcephaly</td>
<td>+ - + + + - -</td>
</tr>
<tr>
<td>Hypoplastic Philtrum</td>
<td>+ + + + + + +</td>
</tr>
<tr>
<td>Short Palpebral Fissure</td>
<td>+ + + + + + +</td>
</tr>
<tr>
<td>Hypotonia</td>
<td>- - + - - - -</td>
</tr>
<tr>
<td>Developmental delay</td>
<td>+ + + + + + +</td>
</tr>
<tr>
<td>Joint anomaly</td>
<td>- - - + + + +</td>
</tr>
<tr>
<td>Cardiac anomaly</td>
<td>- - - - + + +</td>
</tr>
<tr>
<td>Structural kidney anomaly</td>
<td>- - - - - - -</td>
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</tbody>
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F, female; M, male; (+), present; (-), absent; (°) syndactyly of the 2nd and 3rd toes bilaterally; (†) atrial septal defect

After ammonium chloride loading urine pH was significantly higher in FAS patients than in control infants [5.9 (0.97) vs. 5.10 (0.19); P<0.001], net acid excretion was lower and urine anion gap was higher in FAS patients than in control subjects [82 (11) vs. 131 (11.3) µEq/min and 28 (9) vs. -29 (12) mEq/l, respectively; P<0.001], (Fig 2). Scanning electron microscopic studies showed cytoplasmic mitochondrial hypertrophy and vacuolar structures of the epithelial cells of the cortical collecting ducts in the rat kidney following fetal exposure to alcohol (Fig 3).

### Discussion

The results of the present study demonstrate that offspring of alcohol-exposed rats during gestation develop renal cell injury in the distal nephron that may be responsible in the genesis of the impaired renal function in infants with FAS.

Since the distal nephron is primarily responsible for distal acidification[13] and maximal urine concentration[14], the impairment of this segment of the nephron is likely the cause of alcohol-induced renal...
dysfunction in infants with FAS.

It is known that alcohol has an adverse effect on zinc metabolism[15] and that prenatal zinc deficiency acts as a co-teratogen with alcohol in FAS.[16] The similarity in the pattern of impaired urine concentration and acidification[6,8] as well as hyperzincuria among infants with FAS[7] suggests the possibility of a transport defect in the distal nephron during gestation[9]. In the current study, the finding of cytoplasmic vacuoles and swelling mitochondrial of the epithelial cells of the distal nephron in the alcohol-exposed rat kidney is further evidence that the cellular damage in the distal nephron is responsible for the renal tubular dysfunction in FAS patients.

The mechanism by which this protective effect occurs is unknown. Alcohol-induced renal tubular cell injury could arise from the direct effect of alcohol on tissues or the metabolites of alcohol.

**Fig 2**- Comparison of urine pH, net acid excretion (NAE) and urine anion gap (AG) in FAS (○) and control (*) groups.

**Fig 3**- Scanning electron micrograph (original magnification x 12,800) of the cortical collecting tubule of rat following fetal exposure to alcohol. The mitochondria in lining cells are swollen and occasional vacuolar structures are seen in the basal cytoplasm of the epithelial cell.
Alcohol crosses the placenta and rapidly reaches the fetus. Extensive studies have demonstrated equivalent fetal and maternal alcohol concentrations, suggesting an unimpeded bidirectional movement of alcohol between the two compartments[17,18]. The fetus appears to depend on maternal hepatic detoxification because the activity of ADH in the fetal liver is less than 10% of that observed in the adult liver. Furthermore, the amniotic fluid acts as a reservoir for alcohol, prolonging fetal exposure[17,18]. Classes I and II ADH are primarily responsible for the initial degradation of alcohol to acetaldehyde and are shown to be protective against FAS.

Reduced activity of ADH, a zinc metaloenzyme, has been reported in alcoholic patients[19]. Alcohol and its toxic metabolites can alter fetal development by disrupting cellular differentiation and growth, disrupting DNA and protein synthesis and inhibiting cell migration[20]. Both alcohol and acetaldehyde modify the intermediary metabolism of carbohydrates, proteins, and fats. Both also decrease the transfer of amino acids, glucose, folic acid, zinc, and other nutrients across the placental barrier, indirectly affecting fetal growth due to intrauterine nutrient deprivation[19, 20]. Genetic susceptibility in FAS was suggested in some studies, with alcohol dehydrogenase (ADH) polymorphisms as a risk factor[21].

The medical care of the child with FAS is treatment for associated birth defects and intervention for potential cognitive and behavioral abnormalities. Some children with FAS present with clinically significant acidosis, fluid and electrolyte disorders, failure to thrive, impulsivity, hyperactivity, oppositional behavior, and/or sleep disorders[22]. Medication may assist with these symptoms.

The estimated rate of FAS in industrialized countries is 3 out of every 1,000 live births[23]. The incidence is probably much higher if infants with the milder form of the syndrome were included. In the United States, FAS has been regarded as the third most common recognizable cause of mental retardation with an estimated incidence of 1-2 cases per 1000 live births[24,25]. However, in some extremely high-risk areas, such as selected communities in South Africa where binge drinking in pregnancy is relatively common, careful in-school assessments have shown that rates of FAS may be as high as 4-5% of all children in the normal first grade in school[26].

Universal prevention attempts to promote the health and well-being of all individuals in a society or particular community by educating women about the risks of alcohol for the developing fetus and about the importance of avoiding alcohol consumption during pregnancy. This type of prevention can be accomplished with public education and primary care. Selective prevention and intervention is targeted to individuals in the population who are at increased risk, i.e., women of reproductive age who drink alcohol and who have the potential to become pregnant. This step can be accomplished with effective screening for alcohol use and with brief interventions.

**Conclusion**

In summary, renal evaluations of children born to alcoholic mother seem warranted for early diagnosis and correction of abnormalities that might contribute to growth and developmental failure in FAS. Since the syndrome is entirely preventable, it is important to educate the public on the deleterious effect of alcohol on the fetus. Further studies of zinc metabolism in alcoholic women would seem justified to demonstrate if improvement of zinc deficiency during pregnancy can reduce the prevalence of birth defects in their offspring.

**Acknowledgment**

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References


