ABSTRACT

Background: Despite prevalence of fluorosis in India, previous studies did not emphasize on the effect of maternal fluorosis on fetal outcomes. Objectives: To study the associations of higher maternal serum Fluoride (F) with low birth weight and poor APGAR count. Methods: One hundred and eight apparently healthy pregnant women aged 17-36 years were included in the study. Samples collected were maternal and cord blood, placenta and drinking and ground water. The samples were processed and analyzed for Fluoride. Fetal gestational age was measured on ultrasound scan and the birth weight of the baby on a digital scale. Fifth minute APGAR score was measured. Results: A significant negative correlation was found for maternal serum F vs Birth weight, Gestational Age and APGAR score. Significant negative correlations were also found for cord serum F vs Birth Weight, Gestational Age and APGAR score. Fairly negative correlations were also found for birth weight, APGAR score and Gestational age compared to Fluoride concentrations in maternal surface of placenta, fetal surface of placenta and marginal side of the placenta. However, significant positive correlations were observed when Gestational age was compared with Birth weight and APGAR score. When the maternal serum F was greater than 1 ppm, there was 10.58 times higher risk for low birth weight, 8.65 times higher risk for preterm delivery and 3.8 times higher risk for low APGAR score. When the cord serum F was greater than 0.22ppm, there was 2.76 times higher risk for low birth weight, 4.6 times higher risk for preterm delivery and 2.5 times higher risk for low APGAR score. Conclusion: With increased serum F in the mother, there is an inclination towards pre term delivery, low birth weight and poor APGAR count.

Key words: APGAR score, Placenta, Low Birth weight, Endemic fluorosis, Gestational age.

OBJECTIVES

To study the associations of higher maternal serum F with low birth weight and poor APGAR count in endemic fluorosis region.

MATERIALS AND METHODS

Selection of subjects: The study included one hundred and eight pregnant women at term aged 17 to 36 yrs residing in Nalgonda District, Andhra Pradesh, India. Reported pregnancy was confirmed on an ultra sound scan. Only those women who were pregnant for not more than 14 weeks at the time of scan were recruited. One hundred and twenty women were initially recruited in the study; but only 108 had delivery at our hospital. The women were under regular antenatal care of the Obstetricians in the Department of Obstetrics at Kamineni Institute of Medical Sciences, Nalgonda. All the women volunteered to participate in the study and gave their consent for the same. This study was
conducted with the approval of the institutional ethics committee during June 2009 to April 2010.

**Samples**

**Blood:** 3ml of maternal venous, and cord blood were drawn into disposable plain polystyrene tubes. Maternal blood was drawn during first hour of delivery and cord blood was collected at birth. Placenta was collected immediately after expulsion under sterile conditions and was carried to laboratory in an ice pack. Laboratory analyses were conducted in the Department of Biochemistry of Kamineni Institute of Medical Sciences. The samples were collected, handled and transported to the lab according to the guidelines given by clinical and laboratory standards institute/NCCLS (National Clinical Chemistry Laboratory Standards). The blood samples were centrifuged at 3000 rpm for 10 minutes and the serum was immediately analyzed for F.

**Placenta:** Placenta was collected immediately after expulsion under sterile conditions and was carried to lab in an ice pack. The placenta was divided into a central part and marginal part. The central part was further subdivided into maternal and fetal surfaces. 10 gm of tissue was cut from each part. These tissues were homogenized immediately using 10 mL normal saline at 100,000 rpm for 45 min in a pressure driven tissue homogenizer. Precautions were taken according to those given in the manual of the tissue homogenizer. Briefly, the speed of the homogenizer was not increased or decreased abruptly. It is done gradually; homogenization was always done in ice pack as it generates heat. The homogenization procedure developed was standardized and validated against other procedures. The tissue extract was processed immediately for F analysis. The homogenate was transferred into a centrifuge tube and centrifuged at 3000 rpm for 5 min. Now the total volume (Volume of buffer + volume of tissue fluid) was noted and the approximate dilution of the tissue fluid was calculated. The tissue extract was processed immediately for fluoride analysis and the results obtained were multiplied with appropriate dilution factor to calculate the actual values of fluoride.

**Water:** Drinking and ground water was brought by the family members of the subjects on request for F analysis since water was the major source of fluoride intake.

### METHODS

1. **F Assay:** F was analyzed by ion selective procedure at pH 5.0 adjusted with TISAB (Total Ionic Strength Adjustment Buffer) III buffer using Eutech Epoxy Body Electrode. The instrument was calibrated and standardized using four solutions having F concentrations of 0.01 ppm, 0.1 ppm, 1 ppm and 10 ppm. The standards were run before analysis of each sample and the electrode was calibrated periodically. Other measures were followed according to those given in the instrument manual. Briefly, all solutions were analyzed in plastic ware and not glass ware; the electrode was calibrated every day, and samples were processed after checking the controls for each batch of ten samples.

2. **Birth Weight:** Following delivery, the baby was carefully weighed, following necessary precautions, on a digital scale (Seca 354/364) which has sensitivity of ±10 gm. The scale was calibrated periodically against standard weights and the calibration was rechecked. Babies weighing 2500 gm and above were considered normal while those weighing <2500 gm were considered low birth weight.

3. **APGAR Score:** The APGAR score is determined by evaluating the newborn baby on five simple criteria on a scale from zero to two, then summing up the five values thus obtained. The resulting APGAR score ranges from zero to 10. The five criteria (Appearance, Pulse, Grimace, Activity, Respiration) are used as a mnemonic learning aid. The test is generally done at one and five minutes after birth, and may be repeated later if the score is and remains low. Scores 3 and below are generally regarded as critically low. Scores 4 to 6 fairly low, and 7 to 10 generally normal. However, for the present study, fifth minute APGAR score is considered.

APGAR score is calculated from the score chart depicted in table 1.

4. **Gestational Age:** Gestational age was estimated by fetal biometry on an ultra sound scan. WHO guidelines were followed for the scan. The subject was given four to five glasses of water and instructed not to micturate till the scan was done. The bladder was not filled with a urethral

### Table 1: APGAR Score Sheet

<table>
<thead>
<tr>
<th>Score of 0</th>
<th>Score of 1</th>
<th>Score of 2</th>
<th>Score of the baby</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>blue or pale all over</td>
<td>blue at extremities body pink (acrocyanosis)</td>
<td>No cyanosis- pink body and extremities</td>
</tr>
<tr>
<td>Pulse Rate</td>
<td>Absent</td>
<td>&lt;100</td>
<td>≥100</td>
</tr>
<tr>
<td>Grimance (Reflex irritability)</td>
<td>no response to stimulation</td>
<td>grimmace/feebly cry when stimulated</td>
<td>cry or pull away when stimulated</td>
</tr>
<tr>
<td>Activity (muscle tone)</td>
<td>none</td>
<td>Some flexion</td>
<td>flexed arms and legs that resist extension</td>
</tr>
<tr>
<td>Respiration (Breathing)</td>
<td>absent</td>
<td>weak, irregular, gasping</td>
<td>strong, lusty cry</td>
</tr>
</tbody>
</table>
A significant negative correlation was found for maternal serum F vs Birth weight \( (r = -0.52; p < 0.0001) \), Gestational Age \( (r = -0.56; p < 0.0001) \) and APGAR score \( (r = -0.65; p < 0.0001) \). Significant negative correlations were also found for cord serum F vs Birth Weight \( (r = -0.41; p < 0.0001) \), Gestational Age \( (r = -0.35; p < 0.0001) \) and APGAR score \( (r = -0.41; p < 0.0001) \). Fairly negative correlations were also found for birth weight, APGAR score and Gestational age compared to Fluoride concentrations in maternal surface of placenta \( (r = -0.51, p < 0.0001); r = -0.53, p < 0.0001 \) and \( r = -0.46, p < 0.0001 \) respectively), fetal surface of placenta \( (r = -0.41, p < 0.0001); r = -0.47, p < 0.0001 \) and \( r = -0.34, p < 0.0001 \) respectively) and on the marginal side \( (r = -0.43, p < 0.0001); r = -0.47, p < 0.0001 \) and \( r = -0.38, p < 0.0001 \) respectively). However, significant positive correlations were observed when Gestational age was compared with Birth weight \( (r = +0.51; p < 0.0001) \) and APGAR score \( (r = +0.48; p < 0.0001) \). These findings suggest that with increased serum F in the mother, there is an inclination towards pre term delivery, low birth weight and poor APGAR count.

From the existing studies no reference could be obtained regarding the minimum level of maternal serum or cord serum F levels that have adverse outcome on the baby in endemic fluorosis area, an attempt was made by processing the data to obtain Odds Ratio and Pearson's Chi Square

### RESULTS

All the subjects in the study were resident of Nalgonda district (an endemic fluorosis area). These subjects use Government supplied Krishna River water for drinking and ground water for household activities like washing, cleaning etc, and also for cooking. Ground and drinking water samples were provided by all the participants \( (n = 108) \) and the F levels in the water samples were 10.64 ± 2.09 ppm and 4.4 ± 1.6 ppm respectively. These subjects depend on locally grown food crops and vegetables for their daily requirements. The water sources suggest higher intake of F than the maximum, 1.5 ppm, recommended by WHO and Indian Standard Code for Drinking Water. The demographic detail of the subjects is presented in table 2.

F content of the water sources and details of the neonates are presented in tables 3 and 4.

F values in the samples are presented in Table 5.
value and 1ppm for maternal serum Fluoride and 0.22 ppm for cord F was considered cut off in our study.

**Maternal F vs Fetal outcomes**
The gestational age ($\chi^2 = 16.8; p < 0.0001$), APGAR score ($\chi^2 = 4.37; p = 0.037$) and birth weight ($\chi^2 = 29.8; p < 0.0001$) of the baby were significantly associated with maternal serum F, where 1ppm was considered as cut off. It was also seen that when the maternal serum F was greater than 1 ppm, there was 10.58 times higher risk for low birth weight, 8.65 times higher risk for preterm delivery and 3.8 times higher risk for low APGAR score. These findings were summarised in table 6.

**Cord F vs Fetal outcomes**
Gestational age of the baby ($\chi^2 = 11.37; p < 0.001$) is significantly associated with Cord F while birth weight ($\chi^2 = 6.48; p = 0.11$) and APGAR score ($\chi^2 = 2.64; p = 0.1$) did not associate significantly. When the cord serum F was greater than 0.22ppm, there was 2.76 times higher risk for low birth weight, 4.6 times higher risk for preterm delivery and 2.5 times higher risk for low APGAR score. These findings were summarised in table 7.

**DISCUSSION**
There is wide variation with some correlation between fluoride concentration in maternal serum and cord blood, indicating that fluoride readily crosses the placenta. In general, average cord blood concentrations are approximately 60% of maternal serum concentrations, with proportionally lesser amounts present as higher maternal serum concentrations. Therefore, potential toxicity to the developing embryo and fetus in the setting of high maternal ingestion of fluoride has been a concern, evaluated in both animal and humans. Jendryczko et al. studied the associations of mineral components of human placenta with birth weight and infant head circumference and found that significant negative correlations were ascertained between the content of Pb, Cd, F, Hg and the birth weight. We also found fairly significant negative correlations between birth weight and F content in different regions of placenta. Makrozynski and Macho16 estimated F content of fetal bone as an indication of prenatal F exposure and found statistically significant negative correlations between the fetal age and F contents in the shaft and in the metaphyseal part of bones in sixty four fetuses with an intra uterine life ranging between 14-36 weeks.
However, we estimated the cord serum F as an indication of prenatal F exposure and found significant negative correlation with Gestational age of the fetus and also with other fetal outcomes like APGAR score and birth weight.

Apelberg et al.17 studied maternal and cord serum concentrations of Perfluoro compounds in people exposed to F in plastic industry in relation to weight and size at birth. Positive association was observed between Perfluoro compounds and Gestational age which was statistically not significant and which diminished with adjustment for key predictors of gestational age; after adjusting for gestational age, these chemicals were negatively associated with birth weight \( (p < 0.05) \). In a similar Japanese study18 done recently, similar findings were reported. Though the sources of F exposure in the present study differ from those cited afore, we also found a negative association of maternal and cord blood serum F with fetal outcomes.

Supporting evidence with a plausible hypothesis was given in a recent study done by AK Susheela19. They found in a study group \( (n = 205) \); on relatively F free food and water as compared to a control group \( (n = 115) \), the incidence of preterm deliveries was reduced by four times and low birth weight babies by two times. This study also reports that Iron and Folate supplementations to the pregnant women reduce the risk of low birth weight provided, they are accompanied by good nutrition and reduced F intake. Further, we found that when maternal serum F levels were above 1 ppm there was higher relative risk for adverse fetal outcomes. However, the relative risk was statistically significant only for Gestational age when cord serum F was above 0.22 ppm. This may be in part due to very low cord serum F levels which in turn may be due to sequestering of large amounts of F in placenta. However these cut offs may be established only after further studies that involve larger sample size.

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REFERENCES