Transplacental Transport of Fluoride, Calcium and Magnesium

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Abstracts: Background: In fluorosis endemic area, the amount of fluoride transferred to the fetus is significant in determining the early onset of fluorosis. The role played by placenta in transporting fluoride and other ions which are present in high concentrations in the local drinking water is studied. Aims & Objectives: The aim of the present study is to decipher the role of placenta in transport of fluoride to the fetus and to analyze the placental binding & distribution of fluoride, calcium and magnesium within the placenta. Materials & Methods: 200 healthy pregnant women aged between 17- 28 yrs were inducted in the study. All the women had a normal & uneventful delivery. The maternal blood, cord blood & placenta were collected immediately after delivery. The placenta was divided into 3 parts- the maternal side, fetal side & the peripheral. Separate tissue extracts were prepared from each site and analyzed. Fluoride, calcium and magnesium were quantified from the samples. Results: The average drinking water fluoride was 1.64±0.49ppm and ground water fluoride was 10.94±2.09ppm. Fluoride concentration of placenta on the maternal side was 1.62±0.787 ppm and in the periphery it was2.54±1.54 ppm ($p<0.001$) while that of the fetal side of placenta was1.41±0.776 ppm. Maternal and cord blood fluoride levels were1.21±0.79ppm and 0.45±0.304 ppm respectively ($p<0.001$). The concentrations of calcium in the maternal blood and cord blood were 9.67±1.53mg% and 9.89±1.89 mg% respectively ($P<0.5$). The levels of calcium in the placenta were 8.79±1.36 mg%, 9.68±1.69 mg%, 13.87±3.32 mg% respectively on the maternal surface, fetal surface and periphery ($p<0.001$). The magnesium concentration on the peripheral part of placenta was twofold higher than that of the maternal serum and cord serum ($p<0.001$) indicating a significant accumulation of magnesium on the peripheral parts of the placenta. However, the differences in the magnesium concentrations of the placenta on the maternal and fetal surfaces with that of the maternal and cord blood sera were found to be insignificant. Conclusion: The results show that, Fluoride, Calcium and Magnesium accumulate in placenta, with the highest concentration being in the marginal part. The placenta therefore acts as a barrier to the transport of fluoride ions and protects against early fluorosis. [Gurumurthy S M et al. NJIRM 2011; 2(3) : 51-55]

Key Words: placental transport, homogenization, fluorosis, calcium transfer.

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Introduction: The array of metabolic activities of placenta represents not only its metabolic requirements but also provides the means by which the placenta can regulate the quantity as well as the type of nutrients transferred to the fetus[1]. Placental transport provides a means of supplying nutrients to and removing metabolites from the fetus.

Many substances like glucose, amino acids and electrolytes, vitamins and ions are transported by carrier systems[2]. By the end of normal human pregnancy the fetus acquires approximately 28gm calcium and 0.7 gm magnesium mostly during the third trimester[3]. Trans placental passage of fluoride was studied and was proved that fluoride passes through placenta by as low as 30% in few studies to as high as 87% in others[4,5]. However there are no studies suggesting the amount of fluoride required for the fetus. The role of placenta as a fluoride filter and the placental transfer of fluoride, calcium and magnesium in fluorosis endemic region are studied.

Aims And Objectives: Present study was undertaken with aim to study whether babies born in endemic fluorosis area had higher serum fluoride levels at birth and to study whether the placenta plays a role in decreasing the transport of fluoride to the fetus so as to reduce the effect of fluorosis in the fetus. OBJECTIVES of the study are: To compare the maternal blood and cord blood and placental tissue levels of fluoride, calcium and magnesium. To study the accumulation of fluoride, calcium and magnesium in different regions of placenta i.e. maternal surface, fetal surface and

periphery and there by estimating whether there was a differential accumulation in the placenta.

Material and Methods: The study included 200 healthy pregnant women at term aged 17 to 36 yrs residing in Nalgonda District, AP, India. The women were under regular antenatal care of the Obstetricians in the Department of OBG 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 the 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) [6, 7]. The blood samples were centrifuged at 3000 rpm for 10 minutes and the serum was immediately analyzed for Fluoride, Calcium & Magnesium.

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[89]. The tissue extract was processed immediately for F analysis. The homogenate was transferred into a centrifuge tube and centrifuged at 3000rpm for 5min. Now the total volume (Volume of buffer + volume of tissue fluid) was noted and the approximate dilution of the tissue fluid is calculated. The tissue extract was processed immediately for fluoride, calcium and magnesium analysis and the results obtained were multiplied with appropriate dilution factor to calculate the actual values of fluoride, calcium and magnesium.

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: Fluoride 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.

Calcium assay was done by kit provided by coral diagnostics ltd., which is based on the principle of ortho-cresol-phthalein-complexone (OCPC) method[10,11]. The standards provided in the kit were run before processing each batch of ten samples.

Magnesium assay was done using Bioassay systems magnesium kit designed to measure magnesium directly in the biological samples without any pretreatment. The kit procedure is based on Dye Binding Method. The standards provided in the kit were run before processing each batch of ten samples.

Quality Control: Quality check was done for the above parameters. The results were evaluated by comparison with standards of known

concentration. Measures were taken by checking the kit to kit variability and the repeatability was checked by duplicate testing. The intra and inter assay coefficients of variation for all the parameters was maintained <5%.

Data Analysis: The data was processed in MS EXCEL and analysis was carried out using SPSS (17^{th} version). The results were statistically analyzed by the Student's t-test and by Pearson's correlation coefficient. A two tailed probability value of < 0.05 was taken as indicating significance.

Result: All the subjects in the study were resident of Nalgonda district (an endemic fluorosis area) since childhood. These subjects use Government supplied Krishna River water for drinking and ground water for house hold activities like washing, cleaning etc, and also for cooking. Ground and drinking water samples were provided by all the participants (n=200) 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.

All the babies born were normal and weighed between 2 to 3.2kgs. The gestational age of the babies was 282.19 ± 3.75days. All the babies born were examined by physicians from the department of pediatrics and clinical examination showed no abnormalities. The babies had a mean APGAR score of 8.25 ± 0.39. Concentrations of fluoride, magnesium and calcium in maternal serum, cord blood serum, marginal part of placenta, and central part of placenta are presented in Table 1.

Fluoride concentration of placenta on the maternal side was 1.62 ± 0.78ppm and in the periphery, it was 2.54±1.55ppm while that of the fetal side of the placenta is 1.41±0.776ppm. Maternal and cord blood fluoride levels are 1.62 ± 0.78ppm and 0.45±0.304ppm respectively. Serum fluoride levels in new born were well within the normal reference range and not in the fluorotic zone. Placental fluoride concentration on the periphery was twofold higher than the maternal serum fluoride ($p<0.001$) and six fold higher than cord blood fluoride ($p<0.001$) thereby indicating that placenta acts as a barrier for the passage of fluoride to the fetus. Similarly, the fluoride concentrations of placenta on the maternal and fetal surfaces were three fold higher than the cord blood fluoride ($p<0.001$). However, there was no significant difference between the fluoride concentrations of placenta on the maternal and fetal surfaces but the fluoride concentration on the peripheral part of placenta were found to be 1.5 times higher than on the maternal or fetal surfaces. This is indicates that placenta accumulates fluoride, mostly in the peripheral part, than in the central part i.e. on the maternal surface or fetal surface.

The concentration of calcium in the mother and cord sera was 9.67±1.53mg/dl and 9.89 ± 1.89 mg/dl respectively. On the peripheral part of placenta it was 13.87±3.32 mg/dl while on the maternal and fetal surfaces was 8.79±1.36mg/dl and 968±1.69mg/dl respectively. Calcium concentration on the peripheral part of placenta was about 1.5 fold higher than the maternal serum and the cord serum calcium concentrations (both $p<0.001$). The differences in the calcium concentrations of the placenta on the maternal surface and fetal surface with that of maternal serum and also cord serum are not significant.

The magnesium concentration on the peripheral part of placenta was twofold higher than that of the maternal serum and cord serum ($p<0.001$) indicating a significant accumulation of magnesium on the peripheral parts of the placenta.

Table 1: Distribution of minerals in different regions of placenta, maternal and cord blood sera.

Sr. No	Parameters	Samples				
		Maternal Serum	Placenta			Cord Serum
			Maternal surface	Fetal Surface	Periphery	
01	Fluoride (ppm)	1.62±0.78*	1.62±0.78	1.41±0.77	2.54±1.55*	0.45±0.35*
02	Calcium (mg/dl)	9.67±1.53	8.79±1.36	9.68±1.69	13.87±3.32*	9.89±1.89*
03	Magnesium (mg/dl)	2.26±0.35	2.22±1.03	2.64±1.07	4.75±1.95*	2.21±0.45*

*p<0.001

However, the differences in the magnesium concentrations of the placenta on the maternal and fetal surfaces with that of the maternal and cord blood sera were found to be insignificant.

Based on these findings it can be deciphered that fluoride accumulates in the placenta with the highest concentration being on the peripheral part. The high fluoride levels in peripheral part as compared to the central part can be attributed to higher concentrations of calcium and magnesium in that region. Fluoride is therefore being sequestered by the placenta in order to prevent excess fluoride from reaching the fetus.

Discussion: The present study is conducted in Nalgonda District, Andhra Pradesh, India which is an endemic fluorosis area. The ground water fluoride is 10.94± 2.09ppm and the drinking water fluoride is 1.64± 0.49ppm. All the women inducted in the study were living in this region for more than 10 years. Many children in this area commonly present with features of fluorosis like mottling of teeth, delayed tooth eruption, deformities in limbs, frequently attacked by diarrhea and low I.Q. In this study it was found that the new born babies had fluoride levels well within the normal reference range.

In 1955, Feltman and Kossel observed much higher concentrations of fluoride in peripheral regions (in comparison with central ones) of two examined placentas [12]. These authors suggested that this differentiation was closely related to the calcium content of these parts of tissue. These findings were also discussed in studies by Shen and Taves [13] who showed that fluoride accumulation in placenta may be connected with local focuses of calcification. The present study also supports the view that higher concentrations of fluoride on peripheral part of placenta may be due to higher levels of calcium and magnesium in that region. M.E. Bruns, V. Wallshein and D.E. Bruns [14] reported the presence of a calcium binding protein in mouse placenta and studied its regulation. Their study suggest that the fetal levels of calcium were unchanged with higher ingestion of calcium but the placental calcium concentrations and calcium binding protein levels were significantly increased. Thus the higher calcium concentrations in placenta may be attributed to the presence of calcium binding protein which indirectly may be responsible for the higher fluoride concentrations in placenta when compared with the maternal and cord sera. A similar magnesium transporter was also reported which transports magnesium across placenta to the fetus[15]; thus contributing to the higher magnesium concentrations in placenta. In all animal species studied, calcium and magnesium were higher on the fetal compared to the maternal side of the placenta[16]. We also observed similar findings in our study. Further we found that the concentrations of these ions are highest on the marginal part of the placenta.

In recent Chinese investigations of fluoride exposure and intelligence in children the effect of fluoride appears to occur at an early stage of development of the embryo when the differentiation of brain nerve cells is taking place and development are most rapid [17]. Furthermore, a higher concentration of fluoride has been found in embryonic brain tissue obtained from termination of pregnancy in areas where fluorosis due to coal burning was prevalent. These observations reflect the view that the placenta protects the fetus from fluoride.

The present study also supports the aforesaid view that placenta has a protective role on fetus by preventing excess fluoride from passing to the growing fetus. When the drinking water and food has high fluoride concentration the fluoride content of the placenta is significantly higher than that of the mother serum, while the cord blood has the least. This result is supported by the work done by J Opydo and M. Borysewicz[18] on transplacental passage of fluoride in pregnant women in Poland assessed by maternal and cord blood plasma fluoride.

Conclusion: Based on the results, it is concluded that placenta can accumulate fluoride and Calcium in healthy women who are exposed in pregnancy to relatively high fluoride concentrations in water and food, the greatest amount of placental fluoride was found in the marginal part of the organ presumably as a result of the higher concentration of calcium and magnesium in that area. The findings from the present study indicate that the

fetus actively takes only that quantity of fluoride it needs or that the placenta represents a barrier to the passage of larger quantities of fluoride to the fetus.

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