Role Of Placenta To Combat Fluorosis (In Fetus) In Endemic Fluorosis Area

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Abstract : Fluoride (F) is known to cross the placenta from the maternal blood to the growing fetus. However, there are few studies on the role of placenta in conditions of high F intake in fluorosis endemic area. The present study is the first of its kind carried out in Nalgonda district, an endemic fluorosis area of Andhra Pradesh, India with an average ground and drinking water F of 10.94+2.09 ppm and 4.4+1.6 ppm respectively. The aim of the study is to determine the role of placenta in the transport of F from the maternal blood to the fetus in fluorosis endemic area. 200 healthy pregnant women aged 17-36 years were inducted in the study. All the women had normal and uneventful delivery. Samples collected were maternal blood, cord blood & placenta. The placenta was divided into three parts- peripheral, maternal and fetal surfaces. Placental extracts were obtained and stored at -20° C until processed. F concentrations of the newborn were well within the normal reference range despite high maternal F concentrations (1.62±0.78 ppm). Placental F concentration on the peripheral side (2.54±1.55 ppm) was two fold higher than the maternal serum F (1.62±0.78 ppm; p< 0.001) and six fold higher than the cord F (0.45±0.35 ppm; p< 0.001). Placental F on the maternal (1.62±0.78 ppm) and fetal surfaces (1.41±0.77 ppm) were three fold higher than that of cord blood (p<0.001). On the peripheral part of placenta F was found to be 1.5 times higher than maternal and fetal surfaces. Thus, it can be deduced that placenta does accumulate F especially in the peripheral part when women are exposed to relatively high F concentrations in water and food. The study also suggests that placenta can act as a backstop or guard for the passage of F to the fetus; thus protecting the developing fetus against neonatal fluoride complications.

Key-words: Cord fluoride; Endemic Fluorosis; Fluoride transport; Human placental fluoride; Serum Fluoride.

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INTRODUCTION : Fluorosis is a disease condition that manifests as dental or skeletal fluorosis or both, resulting from higher intake of fluoride (F) above 1.5 ppm per day[1, 2]. Results of studies comparing F levels in maternal and umbilical cord venous blood plasma [3]as well as intra uterine studies[4] suggest that F passes through placenta. Whether and to what extent the placenta can act as a filter and limit the transmission of F to the fetal circulation remains a matter of debate[5]. The aim of the study is to determine the role of placenta in the transport of F from the maternal blood to the fetus in a fluorosis endemic area.

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.

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 F.

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 [8,9].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. 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 everyday, and samples were processed after checking the controls for each batch of ten samples.

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.

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.

RESULTS : 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. The water sources suggest higher intake of F than the maximum, 1.5 ppm, recommended by WHO and Indian Standard Code for Drinking Water[1, 2]. The demographic detail of the subjects is presented in table 1.

Table1: Demographics of subjects **(n=200)**.

Parameters	**Range**	**Mean ± SD**
Age group (Years)	17-36	26.4±5.2
Weight (Kgs.)	40-60	48.0± 5.2
Gravida	1 to 4	
Parity	0 to 4	
Type of delivery		
1. Vaginal	n = 84	
2. Lower Segment Caesarean Section	n = 116	

F values in the samples are presented in Table2.

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Table 2: Fluoride levels in placental extracts, maternal and cord serum.

FLUORIDE CONCENTRATIONS (PPM)					
	Maternal Serum	Cord Serum	Maternal Surface of Placenta	Fetal Surface of Placenta	Marginal Part of Placenta
Mean	1.62	0.45	1.62	1.41	2.54
SD	0.78	0.35	0.78	0.77	1.55
Median	1.71	0.32	1.42	1.23	2.36
Max	3.60	1.80	5.60	3.60	7.80
Min	0.30	0.10	0.66	0.13	0.72

These values indicate that F is essentially concentrated on marginal part of placenta. A strong positive correlation, r= 0.914349($p<0.001$), was found between F concentrations in maternal plasma and marginal part of placenta. Placental F concentration in the periphery was two fold higher than the maternal serum F ($p<0.001$) and six fold higher than the cord blood F ($p<0.001$) indicating that placenta acts as a backstop or guard for the passage of F to the fetus.

However, we found cord serum F levels reached that of maternal serum in one case and exceeded in 8 cases. The F concentrations of placenta on the maternal and fetal surfaces were three fold higher than the cord blood F which was statistically significant ($p<0.001$). Interestingly, there was no significant difference between the F concentrations of placenta on the maternal and fetal surfaces. Nevertheless, the F concentration on the peripheral part of placenta was found to be 1.5 times higher than on maternal or fetal surfaces. This indicates that placenta withholds F, mostly in the peripheral part.

DISCUSSION : Few studies have reported on transplacental F transport in fluorosis endemic areas. This is the first study in Nalgonda district of Andhra Pradesh, India. In 1955, Feltman and Kossel observed much higher concentrations of F in peripheral regions (in comparison with central ones) of two examined placentas[11]. In a Chinese investigation of F exposure and intelligence in children the effect of F on cognitive development appears to occur at an early stage of development of the embryo[12]. Further, a higher concentration of F has been found in embryonic brain tissue obtained from termination of pregnancy in areas where fluorosis due to coal burning was prevalent[12]. In our study, major source of fluoride was water and food. These studies emphasize the fact that F transport to the fetus needs to be curtailed for healthy growth and development of the fetus.

In our study, Cord F concentration reached on an average 30% of that in maternal serum. The mean value reported by other authors ranges from 60% in the study of Gupta et al, [13] and Shimonovitz et al[5] to 75% in the study of Shen and Taves[14] and 87% in a Polish study[15]. Cord F levels ranges from 0.003 – 0.078 µg/mL (1 mg/L = 1 µg/ mL = 1 ppm) according to Shimonovitz et al[5] to 0.11 – 0.39 µg/mL in Malhotra's study[16] while we found cord F levels of 0.1 – 1.8 ppm. We also found higher maternal serum F levels (0.3 – 3.6 ppm) than Shimonovitz et al[5] (0.003 – 0.041 µg/mL) and Malhotra etal[16] (0.12 – 0.42 µg/mL). However, our findings are supported by maternal F concentrations in the study of Gupta etal[13] (0.1 – 2.4 ppm).

The high maternal F concentration could be attributed to high F content in drinking water (1.2 – 6.3 ppm) as compared to the Polish study[15](0.4 – 0.8 ppm) and additional exposure to F by consumption of locally grown crops where mean ground water F is 10.94 ppm. Thus, as the US National Academy of Sciences states, it is not possible to assess the exposure to F only on the basis of drinking water F[17] as is found in our study, therefore, there is a need for health care researchers to assess the F content of soil, ground water and food crops and statistically relate to mean serum F concentration of the regional population to determine the intensity of exposure to F. Further, there is a need to translate similar type of community research into community health practice for the benefit of the population at large.

The present study supports the view that the placenta has a protective role on fetus by preventing transfer of excess F to the growing fetus and the capacity of placenta as F filter is still a point of debate. When the drinking water and food has high F concentration, the F content of the placenta is significantly higher than that of the mother serum, while the cord blood has the least. Thus, these findings indicate that the placenta represents a natural barrier to the passage of larger quantities of F to the fetus probably by binding to calcium ions in the placenta. Further studies are essential to demonstrate and establish these observations.

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