SERUM FLUORIDE LEVELS AS A BIOMARKER IN PRE-ECLAMPTIC WOMEN: A CASE–CONTROL TERTIARY CARE HOSPITAL BASED STUDY

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ABSTRACT: The aim and objectives of the study were to determine (i) whether a high serum fluoride (F) level was an added risk factor for the development of pre-eclampsia in Kolar population, an area with endemic fluorosis, and (ii) whether the serum F levels could be used as a predictive diagnostic marker for pre-eclampsia. The study was designed as a case–control study in pregnant women after 20th week of gestation in the Department of Obstetrics and Gynecology of a tertiary care hospital. After obtaining ethics clearance and written informed consent from 300 pregnant women (150 cases and 150 controls), 5 mL of venous maternal blood was collected in a plain (red-capped) vacutainer and serum was separated for estimation of the serum F levels by using a fluoride ion-selective electrode. The serum F levels in the cases and controls were statistically analyzed by the Mann-Whitney U test. The mean±standard deviation in the cases and controls was 1.8±0.66 and 0.18±0.31 mg/L, respectively. The serum F levels were significantly higher in the pre-eclampsia cases with a z value of 14.4. We found that F is an added risk factor for pre-eclampsia and that a maternal serum F level of ≥1.8 mg/L can be considered as a diagnostic biomarker for predicting pre-eclampsia and the related pregnancy outcomes.

Keywords: Biomarker; Hypertension pregnancy outcomes; Pre-eclamptic women; Pregnancy; Serum fluoride.

INTRODUCTION

Pre-eclampsia (PE) is a pregnancy specific, multifactorial, and hypertensive disorder. It typically starts after the 20th week of pregnancy and is related to increased blood pressure (BP ≥140/90 mm Hg) and protein in the mother's urine (urinary albumin protein ≥300 mg/24 hr). The pathophysiology of PE is still not clear.[1,2] Rather than focusing on pathophysiology, finding risk factors may pave a way for a better understanding of PE. Fluoride (F) is present chiefly in water, soil, food, and air.[3] F intake at high levels may lead to toxicity in humans involving dental, skeletal, and non-skeletal fluorosis. PE may have genetic implications and is an environmental toxicity issue.[1] PE occupies a position between gestational hypertension and eclampsia, and is often complicated by factors like hypertension, proteinuria, and edema.[2,4] PE can give rise to serious complications in mother, fetus, and new born/infant. At present, there is no treatment for PE and it can only be resolved by removal of the placenta.[5]

Agrawal and Walia reported that while the overall incidence of PE during pregnancy in India was 28.7%, in Karnataka state in the Southern region of India, it is was 19.8%.[6] In Kolar, in Karnataka state, the incidence is 21%.[7] F, the ion of the element fluorine, is easily absorbed in the human body. The rural community mainly depends on untreated groundwater, accessed by wells and tube, for drinking and

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domestic use. Groundwater, may have a higher F content than surface water and this may contribute to the increasing incidence of F toxicity.[8]

As F is present in the environment and each person receives F from different sources in different quantities, the amount of absorption by the intestines varies from individual to individual. Therefore, it is challenging to assess the level of F intake by each individual. Some persons appear to be highly sensitive to F.[9] F accumulates in the body, especially in the calcium-rich hard tissues of teeth and bone. It is also present in skeletal muscle and other soft tissues. Approximately 40% of ingested F may be accumulated in the body tissues and the remainder may be excreted through urine, sweat, feces, etc. F consumption limits are determined by several factors.[10] It is evident that the analysis of F in body fluids is highly relevant and further the understanding of fluoride-induced toxicity. [11]

According to the Bureau of Indian Standards and the WHO, the permissible upper limits for F in the drinking water are 1.0–1.5 mg/ L.[12] In India, the drinking groundwater of 23 states out of 33 contains varying amounts of F.[13] In the southeastern belt, including Kolar, the F levels in drinking water range from 1.10 to 14.62 mg/L.[12] Fluorosis is prevalent in 20 of the 35 Indian States and Union Territories.[13,14] The incidence of fluorosis in Karnataka, especially in the Kolar district is high.[1] Besides Kolar, there are 15 more districts with fluorosis in Karnataka.[16]

The maternal serum F levels could be a predictive diagnostic marker for PE and high serum F levels could be an added risk factor for the increasing incidence of PE in F endemic areas. There are a few studies on the effect of F consumption on pregnancy[16,17] but none focused on F in PE, particularly in F endemic areas to examine the increasing incidence of PE and the associated pregnancy outcomes. Therefore, the present study was undertaken to examine the association between maternal serum F levels and PE.

MATERIALS AND METHODS

Materials: The present study was designed as a case-control study in pregnant women after the 20th week of gestation in a tertiary care teaching hospital, the Department of Obstetrics and Gynecology at the Sri Devaraj Urs Medical College, Tamaka, Kolar district, Karnataka, India. The study commenced from August 2017 and sample collection was completed by October 2018. Approval for the study, was obtained from the Central Ethics Committee (Ref.No.SDUAHER/KLR/R&D/35/ 2017–2018).The diagnosis of PE in the Department of Obstetrics and Gynecology was based on the guidelines of the American College of Obstetricians and Gynecologists.[18] A total of 348 pregnant women (subjects) were involved in this study. Out of these, 48 dropped out, the 150 PE pregnant women were the cases and the remaining 150 normotensive pregnant women were the controls.

Exclusion and inclusion criteria for sample collection: A total of 348 women were involved in the study. The selection of samples was based on the following criteria:

THE INCLUSION CRITERIA FOR CASES: primigravida or multigravida ≥20th week of gestation in the age group of 18–45 years (reproductive age) in whom PE was diagnosed.

THE EXCLUSION CRITERIA FOR CASES: pregnant women with PE who have progressed to eclampsia, chronic hypertension, placenta previae, previous history of

diabetes mellitus, hypertension, gestational diabetes, gestational hypertension, ectopic pregnancy, no gestational sac, and < than 20 weeks of gestation.

Estimation of serum F: A written informed consent from 300 pregnant women was collected. Under aseptic precautions 5 mL of venous blood was collected in a plain (red-capped) vacutainer from both the PE (cases) and the normotensive pregnant women before delivery (controls). The vacutainer was left at room temperature for 1 hr and the blood was allowed to clot. The estimation of the serum F levels was done by using a F ion-selective electrode (Thermo Fisher's Scientific-ORION 96-09). Standardization of the instrument was done using different dilutions of standard solutions of 0.01 ppm, 0.1 ppm, 1 ppm, and 10 ppm and after every 20 samples, the calibration was verified.

Statistical analysis: The statistical analyses of the samples (cases and controls) were calculated by using the Statistical Packages for Social Sciences software SPSS (version 22.0; SPSS Inc, Chicago, IL, USA). The mean, median, range, and standard deviation (SD) of the maternal serum F levels in the cases and controls were calculated. The statistical significance of the maternal serum F values in the cases and controls were analyzed by the Mann-Whitney U test

RESULTS

In the study group of 150 cases and 150 controls, 143 cases and 144 controls were pregnant women from Karnataka. A few cases and controls were included from the neighboring states of Andhra Pradesh (4 cases and 3 controls) and Tamil Nadu, (3 cases and 3 controls) which are also endemic states for fluorosis (Table 1).

The maternal serum F levels in cases and controls from the three states are shown in Tables 1A–1C.

Table 1A. The serum fluoride (F) levels (mg/L) in pregnant women from various localities in the Indian states of Karnataka, Andhra Pradesh, and Tamil Nadu (No.=number of pregnant women, NA=not applicable, xU=upper quartile [75th percentile], xL=lower quartile [25th percentile], xU – xL=interquartile range [75th percentile – 25th percentile]), P/D=place or district

Locality	No.		Serum F levels		
			Median	xU—xL	xU–xL
State: Karnataka	143 144	Cases Controls	2.04 0.06	2—1 0.16—0.04	1 0.12
P/D: Kolar	62 70	Cases Controls	2.03 0.05	2—1 0.13—0.04	1 0.09
P/D: Mulbagal	28 20	Cases Controls	2.04 0.05	2—1 0.21—0.04	1 0.17
P/D: Bangarpet	17 16	Cases Controls	2.04 0.07	2.5—1 0.15—0.03	1.5 0.12
P/D: Malur	09 16	Cases Controls	2.03 0.08	2—1 0.16—0.04	1 0.12
P/D: KGF	6 1	Cases Controls	1.58 0.45	2—1 NA	1 NA

Table 1B. The serum fluoride (F) levels (mg/L) in pregnant women from various localities in the Indian states of Karnataka, Andhra Pradesh, and Tamil Nadu (No.=number of pregnant women, NA=not applicable, xU=upper quartile [75th percentile], xL=lower quartile [25th percentile], xU – xL=interquartile range [75th percentile – 25th percentile]), P/D=place or district

Locality	No.		Serum F levels		
			Median	xU—xL	xU–xL
State: Karnataka	143	Cases	2.04	2—1	1
	144	Controls	0.06	0.16—0.04	0.12
P/D: Srinivasapura	3	Cases		NA	
	5	Controls		0.68—0.15	
P/D: Narsapura	0	Cases		NA	
	1	Controls		NA	
P/D: Domasandra	0	Cases		NA	
	1	Controls		NA	
P/D: Dinahalli	0	Cases		NA	
	1	Controls		NA	
P/D: Devanahalli	0	Cases	Cases 2.05 Controls 0.15	NA	Cases 1 Controls 0.53
	1	Controls		NA	
P/D: Gouribidanne	1	Cases		NA	
	0	Controls		NA	
P/D: Gaddur	1	Cases		NA	
	0	Controls		NA	
P/D: Siddalapet	0	Cases		NA	
	2	Controls		0.23	
P/D: Bangalore	1	Cases		NA	NA
	1	Controls		NA	NA
P/D: Hoskote	7	Cases		3—1	2
	8	Controls		0.28—0.05	0.23
P/D: Chintamani	7	Cases	Cases 2.05	2—1	1
	0	Controls	Controls 0.12	NA	NA
P/D: Chikballapur	1	Cases		NA	NA
	0	Controls		NA	NA
P/D: Muthyalapet	0	Cases		NA	NA
	1	Controls		NA	NA

Table 1C. The serum fluoride (F) levels (mg/L) in pregnant women from various localities in the Indian states of Karnataka, Andhra Pradesh, and Tamil Nadu (No.=number of pregnant women, NA=not applicable, xU=upper quartile [75th percentile], xL=lower quartile [25th percentile], xU – xL=interquartile range [75th percentile – 25th percentile]), P/D=place or district

Locality	No.		Serum F levels		
			Median	xU—xL	xU–xL
State: Andhra Pradesh	4	Cases	2.06	3.5—1.25	2.25
	3	Controls	0.032	NA	0.12
P/D: Chittor	2	Cases		NA	
	1	Controls		NA	
P/D: Mehaboobnagar	1	Cases		NA	
	0	Controls		NA	
P/D: Kadiri,	0	Cases		NA	
	1	Controls		NA	
P/D: Kuppam	0	Cases		NA	
	1	Controls		NA	
P/D: Madanapally	1	Cases		NA	
	0	Controls		NA	
State: Tamil Nadu	3	Cases	1.73	NA	2.5
	3	Controls	0.3	NA	0.12
P/D: Krishnagiri	3	Cases	1.73	NA	2.5
	3	Controls	0.3	NA	0.12

The maternal serum F levels were significantly higher (z value =14.4) in the PE cases compared to the controls (Table 2).

Table 2. Serum fluoride (F, mg/L) in the cases with pre-eclampsia and the controls (Values are mean±standard deviation [SD], n=number of subjects, p values calculated by the Mann-Whitney U test)

Serum fluoride (mean±SD, mg/L)		p value	z score
Cases (n= 150)	Controls (n=150)		
1.8±0.6	0.18±0.3	< 0.05	14.44

The incidence of the various clinical outcomes in the 150 cases with PE were: (i) intra-uterine growth retardation (IUGR) 46%, (ii) preterm delivery 19%, (iii) intra-

uterine deaths (IUD) 15%, (iv) anemia 15%, (v) hypothyroidism 8%, (vi) PE in their second pregnancy 6%, and (vii) twin pregnancies 3% (Table 3).

Table 3. Clinical outcomes due to pre-eclampsia (PE) (n=number of subjects, IUGR=intra-uterine growth retardation, IUD=intra-uterine death)

Clinical outcome due to PE	No. of cases (n=150)	Percentage
IUGR	69	46
Preterm delivery	28	19
IUD	22	15
Anemia with PE	22	15
Hypothyroidism with PE	12	8
First pregnancy PE, repeated in second pregnancy	9	6
Twin pregnancy	4	3

The mean±SD, median, and the interquartile range of IUGR, IUD, and preterm delivery are shown in Table 4.

Table 4. Mean±SD, median, upper and lower quartiles, and interquartile range of the serum fluoride (F) of the clinical outcomes of intra-uterine growth retardation, intra-uterine death, and preterm delivery in the cases with pre-eclampsia (PE) (IUGR=intra-uterine growth retardation, IUD=intra-uterine death, xU=upper quartile, xL=lower quartile, xU – xL=interquartile range)

Clinical outcome of PE	Mean±SD	Median	xU — xL	xU – xL
IUGR	1.9±0.8	2.0	2 — 1	1
IUD	1.8±0.8	1.6	2 — 1	1
Preterm delivery	2.0±1.1	2.1	3 — 1	2

DISCUSSION

The possible mechanisms for the pathophysiology of the harmful effects of F include enzyme inhibition, hormonal disruption, and neurotoxic interference. F toxicity in humans occurs with the consumption and absorption of excessive amounts of F, and can be affected by an individual's ethnicity, age, sex, hormonal status, and nutritional intake.[19] Fluorine in the form of fludrocortisone is used in medicine to increase blood pressure but has a variety of side-effects including high blood pressure, oedema, heart failure, low blood potassium, low immune system function, cataracts, muscle weakness, and mood changes.[20]

The adverse effects of F: High levels of F intake may lead to F toxicity affecting various organs and systems in the body. In pregnancy, the placenta permits F to enter

into the bloodstream of the fetus after the 20thweek of gestation.[21] After F crosses the placental membrane, its presence in the fetus is associated with perinatal risks such as premature rupture of membranes, prematurity, low birth weight, PE, and fetal death. These are mainly due to neurotoxic effects, interference with placental function, increased oxidative stress, inflammation, endothelial dysfunction, and the down regulation of nitric oxide.[22] A pilot study on fluorosis and hypertension in 125 individuals showed that hypertensive patients had high F levels with increased lipid peroxidation and oxidative stress.[23] Amini et al. reported a positive correlation between the mean F concentrations in groundwater resources and the prevalence of hypertension in males and females.[24] Oxidative stress has been associated with chronic diseases like hypertension. However, oxidative stress may not be the sole reason for increased blood pressure and other pro-hypertensive factors may also play a role, either directly or indirectly, e.g., salt intake, renin, angiotensin system, and sympathetic hyperactivity. The occurrence of oxidative stress in F toxicity will tend to increase blood pressure[25] and may even be one of the reasons for PE.

F and PE: With the notable exception of the work by Susheela et al. in treating anaemia in pregnant women with F toxicity to prevent the complication of low birth weight babies,[26-28] despite several investigations showing the effect of F consumption on various organs and systems, there is a dearth of procedures currently in place to prevent or treat F toxicity in pregnant women and children.

Studies show that F accumulates in the placenta and adversely affects fetal growth.[22] Chlubek at al. found that increased levels of F were present in the placenta of healthy pregnant women after the intake of F, at low concentrations, from water or air.[29] The human placenta has been found to be a biomarker of exposure to environmental F.[30] Gardiner et al. found, in 1952, that pregnant women in Newburgh, New York, USA, who drank artificially fluoridated water with 1.0–1.2 ppm (mg/L) of F had a placental F level of 2.09 ppm, which was an almost three-fold increase compared to the placental level of 0.74 ppm in pregnant women from Rochester, New York, USA, who drank non-fluoridated water with approximately 0.06 ppm.[31] The maternal blood F levels in the pregnant women in fluoridated Newburgh (mean 0.040 ppm) were approximately three times higher than the levels in the pregnant women in non-fluoridated Rochester (mean 0.014 ppm).[31]

MacArthur reported, in 2015, that from 1996 to 2004, the prevalence of PE was 19% higher (31.7 cases per 1000 deliveries) in the two most fluoridated regions of the USA (South and Northeast) than in the two least fluoridated regions (Midwest and West, 26.6 cases per 1000 deliveries).[22] The PE rate averaged 40% higher in the South (34.1 cases per 1000 deliveries) than in the West (24.3 cases per 1000 deliveries).[22] In 2004, the average fluoridation rate in the South's 16 states was 81%, compared with 46% in the West's 13 states.[22] An increase in the incidence of PE in fluoridated areas is evident and this is likely to be due to F toxicity.

The prevalence of PE is higher in populations in areas where calcium consumption is lower, and lower in populations given calcium supplementation during pregnancy.[32] The role of calcium in the pathogenesis of PE is unclear but numerous studies have specified that there was a reduced occurrence of PE where calcium supplementation was included in the diet during pregnancy.[33,34] Hypocalciuria and hypophosphaturia are significant in severe PE.[35] In rats a high intake of NaF was associated with increased abortions and intra-uterine death (IUD).[36] Chlubek et al.

found that the mean fluoride concentration in the maternal plasma of 30 healthy pregnant women at full term delivery was 4.27 μM/L while the levels in the marginal and central parts of the placenta were 42.1 μg/g of ash and 33.7 μg/g of ash, respectively.[29] They concluded that most placental fluoride was stored in the marginal part of the placenta, presumably as a result of the higher concentration of calcium found in that area.[29] Sastry et al. found, in a study of 200 healthy pregnant women, that the fluoride concentration on the peripheral side of the placenta (2.54±1.55 ppm) was two-fold higher than in the maternal serum (1.62±0.78 ppm) and six-fold higher than in the cord blood (0.45±0.35 ppm).[37] They deduced that the placenta accumulates fluoride, especially in the peripheral part, when women are exposed to relatively high fluoride concentrations in water and food.[37] The study also suggested that the placenta can act as a backstop or guard to stop the passage of fluoride to the fetus, thus protecting the developing fetus against neonatal fluoride-induced complications.[37]

Sastry et al.[38] found that increased serum F levels cause a high risk of preterm delivery and low birth weight babies. When the maternal serum F levels were >1 mg/L, there was a 10.58-fold increase in the risk of fetal complications and an 8.65-fold increase in the risk of preterm delivery. If the cord serum F levels were >0.22 mg/L there was a 2.76-fold increase in the risk of low birth weight and a 4.6-fold increase in the risk of preterm delivery. Sastry et al.[37,38] studied the F levels in maternal serum and cord blood. In the present study the maternal serum F levels were examined in pregnant women with PE and in normotensive pregnant women.

The values of maternal serum F levels can be used as a diagnostic biomarker for pregnancy complications like PE. If the mean±SD (standard deviation) of the maternal serum F levels are ≥1.8± 0.6 in F endemic areas the women will be prone to developing PE and related complications.

Opydo-Szymaczek and Borysewicz-Lewicka, in a study of 30 pregnant women at the time of giving birth, found that the mean concentration of F in maternal plasma, 3.54 μmol/L, (3.54 μM/L, 0.0673 mg/L) was significantly higher ($p<0.001$) than the level in venous cord blood 2.89 μmol/L, (2.89 μM/L, 0.0549 mg/L).[39] Similarly, Chlubek et al. found that the mean fluoride concentrations in maternal plasma of 30 healthy pregnant women at full term delivery was 4.27 μM/L (4.27 μmol/L, 0.0811 mg/L).[2] In the present study, while the mean serum F of 0.18 mg/L in the healthy women without PE was comparable to the values of 0.0673 and 0.811 mg/L found in healthy pregnant women at full-term by Opydo-Szymaczek and Borysewicz-Lewicka, and by Chlubek et al., respectively, the value found by us of 1.8 mg/L in the women with PE was approximately 10-fold higher than the value we found for the healthy pregnant women.[29,39]

While a few studies have estimated F levels in maternal plasma or serum, cord serum, and placentae,[22] none of the previous studies have examined the F levels in maternal serum in women with PE, especially in F endemic areas. Our study, in the Kolar district, an endemic area for fluorosis, is the first study of maternal serum F levels in PE. Based on all the relevant pieces of evidence, F appears to be a meaningful risk factor for PE**.** Our study results show that there is a strong association between the maternal serum F levels and PE ($p<0.05$). Based on our findings in the Kolar population, a maternal serum F level of ≥1.8±0.6 mg/L can be considered to be a diagnostic biomarker for predicting PE and the related pregnancy

outcomes of intra-uterine growth retardation, preterm delivery, intra-uterine deaths (IUD), anemia, hypothyroidism, PE in a second pregnancy, and twin pregnancies.

CONCLUSIONS

The finding, in pregnant women in the southeastern part of Karnataka, and especially in the F endemic areas like Kolar, of a strong association between the presence of a maternal serum F level of ≥1.8 mg/L and PE suggests that high maternal serum F levels may cause PE in pregnancy. We conclude that an increased serum F level is an added risk factor for development of PE and suggests that the estimation of the maternal serum F levels can be used as a diagnostic marker for predicting of development of PE. We recommend that the maternal serum F level may be introduced as a routine laboratory/clinical test in antenatal clinics in areas which are endemic for fluorosis or when there are indications that the fluoride intake may be high.

LIMITATIONS AND ASPECTS TO BE CONSIDERED IN FUTURE RESEARCH

In the present study the subjects were all from a fluoride endemic area and the serum fluoride levels were measured at ≥20 weeks. In future research, subjects from non-fluoride endemic areas might also be considered and the serum F measurement might be measured at more stages of gestation, e.g., early (< 34 weeks) and late (≥ 34 weeks)

AUTHORS' DECLARATION

The authors have no competing interests.

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