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ARTICLE

Minerals, trace elements, Vit. D and bone health

The association of fluoride in drinking water with serum calcium, vitamin D and parathyroid hormone in pregnant women and newborn infants

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Abstract

Background Chronic exposure to fluoride in drinking water causes an increase in plasma fluoride levels that is related to a reduction in calcium transport across the renal tubule endoplasmic reticulum and plasma membrane. In the present study, it was hypothesised that varying levels of fluoride present in drinking water are associated with serum levels of calcium and the related hormones vitamin D and parathyroid hormone in pregnant women and newborn infants.
Methods This cross-sectional study included two groups based on the fluoride concentration in drinking water. One group was considered low/optimum in which the fluoride concentration in drinking water was <1 ppm, and the other group was considered a high fluoride group with ≥1 ppm fluoride in drinking water. In each group, 90 pregnant women were recruited at the hospital during delivery. The participants were given a questionnaire regarding their medical history, sunshine exposure duration, and supplement use and a food frequency questionnaire (FFQ). Fluoride was measured in drinking water, urine, maternal serum and cord blood. Serum calcium, vitamin D, and parathyroid hormone were measured in a fully automated analyser.
Results In pregnant women, drinking water that contained fluoride was significantly positively correlated with urine and blood serum. Low mean concentrations of vitamin D and deficient (<10 ng/ml) vitamin D were more prevalent among the high fluoride group irrespective of diet, sunshine exposure and supplementation. Serum calcium and parathyroid hormone (PTH) levels were significantly lower in the high fluoride group than in the low/optimum fluoride group in both pregnant mothers' blood and cord blood.
Conclusions Drinking water with high fluoride levels was significantly associated with calcium and the related hormones vitamin D and parathyroid hormone.

Introduction

Fluorine is the most electronegative element and has a strong tendency to acquire negative charges in solutions, forming fluorides. If fluoride is consumed optimally through drinking water and other sources, it is beneficial for the teeth and bones, but excessive exposure may lead to many adverse effects, such as tooth decay, osteoporosis and kidney, bone, nerve and muscle damage [1–3]. According to the American Academy of Pediatrics, daily intake should be 0.05–0.07 mg/kg body weight for optimal dental health benefits [4].

When the quantity of fluoride in drinking water and other sources increases, the concentration in maternal plasma, foetal plasma and foetal deciduous tooth enamel also increases [5]. After consumption, ~90% of fluoride is absorbed from the gastrointestinal tract, and the remaining

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10% is excreted in faeces. In humans, 99% of the fluoride is deposited in bones and teeth, and the remaining 1% is found in soft tissues [6]. The free passage of fluoride to the foetus through the placenta has been disputed by researchers. Although the mechanisms of maternal–foetal transmission of fluoride are poorly understood, many studies have proven that fluoride can readily pass across the placenta and ultimately increase fluoride levels in foetal tissue. Many articles pertaining to the advantages and adverse effects of fluoride on pregnant women and foetuses have been published [5, 7].

Vitamin D is a steroid hormone that plays a significant role in maintaining normal calcium and phosphorous levels in the blood. It improves bone health status by enhancing mineralisation through the absorption of calcium and the secretion of parathyroid hormone [8]. Worldwide, vitamin D deficiency is a pandemic, but in most populations this deficiency is underdiagnosed and undertreated. Vitamin D deficiency is seen in all age groups irrespective of gender, race and location [9]. Sufficient 25(OH)D levels are important for pregnant women to sustain vitamin D levels for themselves and their foetuses. Deficient 25(OH)D levels among mothers may be associated with some adverse outcomes in neonates, such as impaired bone development, insulin-dependent diabetes, impaired immune system function, multiple sclerosis cancer, asthma and atrophy, but the results obtained from previous studies were inconsistent [10].

Serum calcium plays diverse roles in maintaining homoeostasis, muscle contraction, cellular function, nerve conduction and cellular membrane stability. Serum calcium levels depend on parathyroid hormone (PTH), vitamin D and calcitonin secretion in the body [11]. Low calcium levels in pregnant women were associated with hypertensive disorders, particularly preeclampsia. During pregnancy, calcium and vitamin D metabolism undergo many adaptations. PTH is a very essential hormone in calcium homoeostasis. Maternal PTH is positively associated with birth weight and foetal upper arm and calf circumference. Parathyroid hormone regulates foeto-placental mineral homoeostasis and skeletal development and stimulates placental calcium transfer [12].

Since 1948, studies have shown a relationship between fluoride and calcium [13]. To date, many works have confirmed that fluoride could modify calcium homoeostasis in the human population and that calcium also plays a significant role in the cellular alterations induced by fluoride [14]. Long-term consumption of fluoridated drinking water increases fluoride plasma levels, which are related to a decrease in calcium transport across the renal tubule endoplasmic reticulum and plasma membrane, as well as to a reduction in the amount of calcium pump proteins in isolated kidney membranes [15]. In the present study, it was hypothesised that varying levels of fluoride present in drinking water are associated with serum calcium, vitamin D and PTH levels in pregnant women and newborn infants. Hence, the study was conducted to investigate the relationship of varying fluoride concentrations in drinking water with calcium and vitamin D levels in pregnant women and newborn infants.

Methodology

In the present study, two groups were established based on the fluoride concentration in drinking water. One group, in which the fluoride concentration in drinking water was <1 ppm, was considered low/optimum, and the other group was considered a high fluoride group, in which the fluoride concentration was ≥1 ppm as per the Indian Bureau of Standards [16]. In each group, 90 pregnant women were recruited from the hospital during delivery. The study sample consisted of pregnant women recruited from JSS Hospital, Mysore, during a prenatal visit approximately one month before their due date. Only primiparous mothers who were healthy, non-smokers, aged between 18 and 45 years were included. Uneventful, singleton foetuses and full-term pregnancies were included. Women with a history of renal stones or hypercalcaemia or any serious pregnancy complications at the time of enrolment were excluded from the study. All subjects gave written informed consent in accordance with the Declaration of Helsinki. Before the beginning of the study, the protocol was approved by the institutional ethics committee at JSS Dental College and Hospital.

Sample size

The sample size was calculated using a 95% confidence interval and 80% power assuming a mean difference of 0.5 mg calcium in the optimum/low and high levels of fluoride in drinking water with a standard deviation of 1.16. The sample size obtained was 85 for each group. To overcome the loss to follow-up and due to any other reasons, the sample size was increased to 90 for each group. Therefore, the total sample size for the present study was 180 (hypothesis testing for two means equal variance- N master sample size calculation software).

At the time of recruitment, the participants were given a validated questionnaire regarding their medical history, sunshine exposure duration, lifestyle factors and supplement use. The questionnaire included a food frequency questionnaire (FFQ) to calculate the intake of vitamin D and calcium [17]. Before using the questionnaire was validated, and a final version was used for the study. The questionnaire was collected from the participants before labour and checked by one of the researchers. Records on pregnancy follow-ups and the birth report, including infant length, weight, and head circumference, were assessed by research staff using the standardised WHO protocol. The duration of the pregnancy was recorded.

Fluoride analysis

Study participants were asked to obtain water samples that they consumed during the course of the pregnancy. The serum and urine fluoride levels of the pregnant women were assessed prior to delivery. Cord blood fluoride levels were assessed post delivery. The fluoride levels were measured according to the American Public Health Association (APHA) guidelines with a standardised instrument. To sensitise the 9609BNWP fluoride electrode, a 10 ppm standard fluoride solution and 0.5 ml of total ionic strength adjustment buffer (TISAB III) were used. The electrode was dipped in the solution for 20 min prior to calibration every day.

Laboratory measurements

First, blood samples collected from a pregnant woman for a routine investigation before delivery were used to assess fluoride, vitamin D, PTH, and calcium levels. Then, after delivery, the same parameters were assessed again in the cord blood samples. After blood sample collection, the samples were immediately placed in tubes and submitted to the hospital's clinical analysis laboratory, where they were centrifuged and then transported under refrigeration. 25 (OH)D and PTH were analysed in a fully automated immunoassay system Roche-Coba e601. Calcium was analysed using a fully automated chemistry analyser (Toshiba TBA120 FR). All the samples were stored at −20 °C.

Data management and statistical analysis

SPSS-23 version was used for the statistical analysis. For descriptive analysis, the mean, standard deviation, frequency and percentages were calculated. For inferential statistics, if the data followed a normal distribution comparison of two independent groups, an unpaired 't' test was used. For skewed distribution data, a nonparametric Mann–Whitney test was used to compare two independent groups. To test the normality of the data, the Kolmogorov–Smirnov test and Shapiro-Wilk test were used. For the comparison of frequencies and percentages, the Pearson chi-square test was used. To find the association between the groups, Spearman's correlation test was used.

To identify one-to-one associations between drinking water fluoride levels and serum calcium, vitamin D and parathyroid hormone levels, simple linear regression was used. In the model, drinking water fluoride level was considered an independent variable, and vitamin D, parathyroid hormone and serum calcium were entered as dependent variables. To control for the confounders of socioeconomic status, maternal education and sunlight exposure duration, multivariate linear regression was performed. From the multivariate regression analysis model, an equation was used to predict the parameters vitamin D, parathyroid hormone and serum calcium with each ppm increase in fluoride in drinking water. $P < 0.05$ was used for statistical significance.

Results

The mean age of the low/optimum group was 23.88 (3.57), and that of the high fluoride group was 24.13 (3.85); the age difference between the groups was not significant. Apart from age, the following variables were also considered: socioeconomic status, maternal education, calcium and vitamin D supplement usage, calcium and vitamin D dietary intake, gestation length, sunlight exposure, birth weight and newborn length. Among these, socioeconomic status, maternal education and sunlight exposure showed statistically significant differences between the two groups. The mean (SD) fluoride concentrations in drinking water in the low/optimum group was 0.50 (0.28) and that in the high fluoride group was 2.65 (1.29). The fluoride concentrations found in urine, pregnant mother's blood and cord blood were 0.20, 0.014 and 0.0110 ppm in the low/optimum group and 1.91, 0.15 and 0.10 ppm, respectively, in the high fluoride group. The comparison of the mean fluoride concentrations in water, urine, pregnant mother's blood and cord blood among the two groups was found to be statistically highly significant ($p < 0.001$) (Table 1).

Table 2 depicts the mean values and categories of 25 (OH)D, PTH and calcium levels in pregnant mothers' blood and cord blood. In maternal blood, the mean values of 25 (OH)D and PTH were significantly different between the low/optimum and high fluoride groups, whereas in cord blood, 25(OH)D and serum calcium showed significant differences both in mean values and categorisation. All the values are presented in Table 2.

Table 3 explains the strength of the association between the fluoride present in drinking water, urine, maternal blood and cord blood. The fluoride concentration in drinking water was positively associated with urine, maternal blood, and cord blood ($r = 0.897$, 0.897 and 0.768) and negatively

Table 1 Descriptive characteristics.

Variables	Low/optimum	High	*p* value
Age (years) Socioeconomic status	23.88 (3.57)	24.13 (3.85)	0.645 NS
	n (%)	*n* (%)	
	Class I—13 (14.4)	Class I—3 (3.3)	
	Class II—26 (28.9)	Class II—18 (20.0)	0.000 HS
	Class III—31 (34.4)	Class III—23 (25.6)	
	Class IV—15 (16.7)	Class IV—27 (30.0)	
	Class V—5 (5.6)	Class V—19 (21.1)	
Education	Illiterate—4 (4.4)	Illiterate—10 (11.1)	0.000 HS
	Primary (1 to 4)—10 (11.1)	Primary (1 to 4)—23 (25.6)	
	Middle (5 to 7)—16 (17.8)	Middle (5 to 7)—26 (28.9)	
	High school and PUC—32 (35.6)	High school and PUC—23 (25.6)	
	Degree and Diploma—28 (31.1)	Degree and Diploma—8 (8.9)	
Calcium and vitamin D supplements	Yes—89 (98.9)	Yes—85 (94.4)	0.211 NS
	No—1 (1.1)	No—(5.6)	
Sunlight exposure (hours/month)	42.00 (17.84)	58.50 (34.95)	0.000HS
Gestation length (weeks)	38.31 (1.13)	38.47 (0.97)	0.321 NS
Gender	Male—47 (52.2) Female—43 (47.8)	Male—44 (48.9) Female—46 (51.1)	0.766 NS
Birth weight (kg)	2.69 (0.57)	2.60 (0.56)	0.274 NS
Birth length (cm)	49.24 (1.38)	49.14 (1.37)	0.599 NS
Dietary intake of calcium mg/d	895.41 (213.70)	836.10 (233.24)	0.077 NS
Drinking water fluoride level ppm	0.50 (0.28)	2.65 (1.29)	0.001 HS
Urine fluoride ppm	0.20 (0.24)	1.92 (1.19)	0.001 HS
Pregnant mothers blood fluoride ppm	0.014 (0.014)	0.153 (0.113)	0.001 HS
Cord blood fluoride ppm	0.011 (0.011)	0.11 (0.10)	0.001 HS

associated with 25(OH)D, PTH and calcium levels ($r = -0.289$, -0.279, -0.385 and -0.175). All indexes were significantly associated, except parathyroid hormone in cord blood and calcium in maternal blood.

Simple linear regression analysis and multivariate regression analysis adjusted with covariates are presented in Table 4. Vitamin D was significantly associated with drinking water fluoride in maternal and cord blood in both the univariate ($\beta = -1.041$ and -0.918, $p = 0.006$ and 0.009) and multivariate regression ($\beta = -0.943$ and -0.847, $p = 0.019$ and 0.023) analyses. Parathyroid hormone was significantly associated with maternal serum ($\beta = -1.731$, $p = 0.002$) and serum calcium in cord blood ($\beta = -0.212$, $p = 0.044$) with drinking water fluoride.

Discussion

The present study was designed to estimate the fluoride concentration in the drinking water, urine, blood before delivery and cord blood after delivery of pregnant mothers. Another objective of the present study was to investigate the association of the serum calcium, vitamin D, and parathormone levels of pregnant mothers just before delivery with the same parameters in the cord blood after delivery. To the best of our knowledge, this is the first study to evaluate the association of fluoride with the abovementioned parameters in groups of pregnant women exposed to both low/optimum levels and high levels of fluoride in the drinking water.

The study conducted by Grinaldo et al. showed that risk factors associated with human fluoride exposure were mainly through drinking water [18]. In the present study, the low/optimum level group of pregnant women mainly used river water (surface water), and the high fluoride group used tap water. In addition, this study shows that as the fluoride concentration increases in drinking water, an increase in fluoride levels is observed in pregnant mothers' blood and urine. This result is in accordance with previous studies by Iftekhar Ahmed et al., LizetJarquin-Yanez et al. and Christine till et al. [19–21].

Very few studies have been performed to associate fluoride levels in the urine and serum of pregnant mothers

Table 2 Biochemical characteristics.

Variables	Low/optimum	High	*p* value
Mean (SD)			
Vitamin D			
Maternal blood	12.31 (7.87)	9.11 (6.39)	0.001 HS
Cord blood	10.96 (7.5)	7.9 (5.62)	0.001HS
Parathyroid hormone (PTH)			
Maternal blood	19.47 (10.73)	12.29 (8.75)	0.000 HS
Cord blood	10.63 (15.16)	9.69 (14.38)	0.523 NS
Calcium			
Maternal blood	7.42 (1.45)	7.25 (1.53)	0.812 NS
Cord blood	7.69 (2.10)	7.11 (1.69)	0.020 S
Frequency (%)			
Vitamin D			
Maternal blood <10 ng/ml (Deficient)	40 (44.4)	55 (61.1)	
10–32 ng/ml (Insufficient)	49 (54.4)	34 (37.8)	0.079 NS
>32 ng/ml (Adequate)	1 (1.1)	1 (1.1)	
Cord blood			
<10 ng/ml (Deficient)	45 (50.0)	65 (72.2)	
10–32 ng/ml (Insufficient)	42 (46.7)	24 (26.7)	0.008 HS
>32 ng/ml (Adequate)	3 (3.3)	1 (1.1)	
Parathyroid Hormone (PTH)			
Maternal blood			
<10 pg/Ml (Hypo)	17 (18.9)	43 (47.8)	
10–55 pg/Ml (Normal)	72 (80.0)	47 (52.2)	0.000 HS
>55 pg/Ml (Hyper)	1 (1.1)	0 (0)	
Cord blood			
<10 pg/Ml (Hypo)	72 (80.0)	74 (82.2)	
10–55 pg/Ml (Normal)	16 (17.8)	14 (15.6)	0.923 NS
>55 pg/Ml (Hyper)	2 (2.2)	2 (2.2)	
Calcium			
Maternal blood			
<8.6 mg/dl (Hypo)	74 (82.2)	81 (90)	
8.6–10 mg/dl (Normal)	14 (15.6)	9 (10)	0.182 NS
>10 mg/dl (Hyper)	2 (2.2)	0 (0)	
Cord blood			
<8.6 mg/dl (Hypo)	54 (60.0)	73 (81.1)	
8.6–10 mg/dl (Normal)	31 (34.4)	15 (16.7)	0.008 HS
>10 mg/dl (Hyper)	5 (5.6)	2 (2.2)	

just before delivery with those in cord blood. According to the present study, the placenta does not provide a complete barrier to the passage of fluoride to the foetus (cord blood). As the fluoride concentration increased in the drinking water, the fluoride levels in the mothers' serum and cord blood also increased. In high fluoride areas, there are chances of increased fluoride exposure to the foetus, which in turn may cause potential negative effects on foetal development. The results were consistent with previous studies by Opydo-Szymaczek and Borysewicz, Ahmed et al. [19, 22]. The acceptable (recommended) concentration of fluoride is 1 mg/l in urine and 0.15 ppm in serum. In the present study, both urine and serum fluoride concentrations among the pregnant women in the high fluoride group were higher than the recommended values [23].

The majority of studies have shown that vitamin D deficiency is highly prevalent among pregnant women and the general population. Previous studies have reported that 61 to 93.3% of pregnant women have vitamin D deficiency/insufficiency in different parts of India [24–34]. In the present study, vitamin D deficiency (VDD < 10 ng/ml) was found in 61.1% of pregnant mother blood samples and 72.2% of cord blood samples in the high fluoride group compared to 44.4% and 50%, respectively, in low/optimum fluoride group. Vitamin D deficiency in pregnant mother blood and cord blood samples was found to be more common among the high fluoride group than the low/optimum fluoride group.

The major source of vitamin D for humans is diet and exposure of the skin to sunlight [35]. In the present study, the high fluoride group consisted of pregnant women with low socioeconomic status who had more sunlight exposure than the women in the low/optimum group, as they had to work in agricultural fields. The low/optimum group consisted of an urban population who had lower sunlight exposure, and pollution also decreased the synthesis of vitamin D. Both groups were on the same dosage of calcium and vitamin D supplements for the same period of time. However, the results were contrary to expectation. Even with exposure to more sunlight and less pollution, the high fluoride group had significantly more pregnant women with vitamin D deficiency than did the low/optimum group.

Regarding supplementation, the pregnant women in both the high and low/optimum fluoride groups regularly took calcium and vitamin D tablets. In addition, in India, the antenatal care programme provides free supplements containing calcium and vitamin D to pregnant women.

Even though supplements were provided to all the pregnant women, those who drank drinking water with high fluoride levels exhibited significantly low levels of vitamin D, which could be due to many reasons. As previously stated, the calcium concentration may regulate vitamin D in healthy adults and in pregnant women. Previous studies have stated that fluoride ingested in high concentrations forms insoluble complexes with calcium, which can markedly decrease gastrointestinal fluoride absorption, causing hypocalcaemia [36]. Another study stated that an increase in fluoride plasma levels causes a reduction in calcium transport across the renal tubule endoplasmic reticulum (ER) and plasma membrane and a reduction in calcium pump proteins in isolated kidney membranes [15]. In addition, fluoride may act in many ways at the molecular level, such as vitamin D-binding proteins, epimers, calcium homoeostasis, etc [37].

The effect of fluoride on vitamin D may be direct or indirect. Much molecular research is required to determine the exact mechanism underlying the reduction of vitamin D in maternal blood. Another alarming issue from the present

Table 3 Spearmans correlation between vitamin D, parathyroid hormone (PTH) and calcium in maternal serum and cord blood and fluoride present in drinking water, urine, maternal serum and cord blood.

		Fluoridewater	Fluoride urine	Fluoride blood	Fluoride cord blood	Vitamin-D Blood	Vitamin-D cord blood	Parathyroid hormone Blood	Parathyroid hormone Cord blood	Calcium blood	Calcium cord blood
Spearman's rho											
Fluoride Water	Correlation coefficient	1.000									
	Sig. (two-tailed)	.									
	N	180									
Fluoride Urine	Correlation coefficient	0.897 (**)	1.000								
	Sig. (two-tailed)	0.000	.								
	N	180	180								
Fluoride Blood	Correlation coefficient	0.897 (**)	0.834 (**)	1.000							
	Sig. (two-tailed)	0.000	0.000	.							
	N	180	180	180							
Fluoride Cordblood	Correlation coefficient	0.768 (**)	0.734 (**)	0.901 (**)	1.000						
	Sig. (two-tailed)	0.000	0.000	0.000	.						
	N	180	180	180	180						
Vitamin-D Blood	Correlation coefficient	−0.289 (**)	−0.302 (**)	−0.260 (**)	−0.200 (**)	1.000					
	Sig. (two-tailed)	0.000	0.000	0.000	0.007	.					
	N	180	180	180	180	180					
Vitamin-D Cordblood	Correlation coefficient	−0.279 (**)	−0.283 (**)	−0.268 (**)	−0.239 (**)	0.737 (**)	1.000				
	Sig. (two-tailed)	0.000	0.000	0.000	0.001	0.000	.				
	N	180	180	180	180	180	180				
Parathyroid hormone Blood	Correlation coefficient	−0.385 (**)	−0.365 (**)	−0.362 (**)	−0.304 (**)	0.273 (**)	0.238 (**)	1.000			
	Sig. (two-tailed)	0.000	0.000	0.000	0.000	0.000	0.001	.			
	N	180	180	180	180	180	180	180			
Parathyroid hormone Cordblood	Correlation coefficient	0.044	0.027	0.052	0.048	−0.087	−0.066	0.120	1.000		
	Sig. (two-tailed)	0.556	0.720	0.491	0.521	0.244	0.382	0.108	.		
	N	180	180	180	180	180	180	180	180		
Calcium Blood	Correlation coefficient	−0.030	−0.007	−0.131	−0.193 (**)	0.211 (**)	0.081	0.170 (*)	−0.121	1.000	
	Sig. (two-tailed)	0.688	0.926	0.079	0.009	0.004	0.282	0.023	0.106	.	
	N	180	180	180	180	180	180	180	180	180	
Calcium Cord blood	Correlation coefficient	−0.175 (*)	−0.134	−0.285 (**)	−0.306 (**)	0.054	0.077	0.119	−0.053	0.540 (**)	1.000
	Sig. (two-tailed)	0.019	0.073	0.000	0.000	0.474	0.305	0.113	0.478	0.000	.
	N	180	180	180	180	180	180	180	180	180	180

**Correlation is significant at the 0.01 level (two-tailed).

*Correlation is significant at the 0.05 level (two-tailed).

Table 4 Simple and multivariate regression analysis table.

Parameters	Constant	β	SE	p value
Vitamin D				
Maternal serum				
*	12.351	−1.041	0.376	0.006
#	13.950	−0.943	0.398	0.019
Cord blood				
*	10.881	−0.918	0.349	0.009
#	12.149	−0.847	0.370	0.023
Parathyroid hormone				
Maternal serum				
*	19.203	−2.108	0.522	0.001
#	28.147	−1.731	0.538	0.002
Serum calcium				
Cord blood				
*	7.706	−0.197	0.100	0.049
#	6.256	−0.212	0.104	0.044

*= simple linear regression analysis without adjusting any variables. Drinking water fluoride considered as independent variable and parameters vitamin D, parathyroid hormone and serum calcium, were considered as dependent variable.

#= multivariate linear regression analysis adjusting socioeconomic status, maternal education and duration of sunlight exposure.

study is that vitamin D levels in pregnant mothers' blood and in cord blood were positively correlated. A vitamin D deficiency in the mothers' blood will be carried to the foetus, and a 20% decrease was observed in the cord blood. Long-term studies need to be conducted to investigate risks among infants and pre-primary school children whose mothers consumed drinking water with high fluoride levels while pregnant.

In the present study, the PTH concentration in pregnant mothers' blood was significantly different between the high and low/optimum groups. The high fluoride group showed lower levels of PTH in pregnant mothers' blood than the low/optimum fluoride group. Fluoride ions alone or in combination with aluminium ($Al3+$) have been shown to enhance the activity of guanine nucleotide-binding proteins (G proteins) in cell membrane preparations from a variety of cell types and in intact hepatic cells. Previous studies have shown that G proteins have a role in the regulation of PTH secretion and intracellular second messengers that modulate PTH secretion. The studies also explained the possible role of intracellular second messengers and G proteins during deficient PTH secretion [38].

Unlike pregnant mothers' blood, in cord blood, PTH levels anywhere not significantly different between the high and low/optimum fluoride groups of pregnant women. The mean cord blood PTH levels were lower than the maternal blood PTH levels. This result may be due to the degradation of the perfused hormone during passage through the placenta [39].

In cord blood, the proportion of hypocalcaemia among cord blood samples was significantly higher in the high fluoride group than in the low/optimum. The amount of calcium actively transferred to the foetus during the third trimester across the placenta when the collagen matrix is rapidly ossified. In neonates, ~30 g of calcium is present at birth [40]. The calcium content in foetal serum increases exponentially during gestation. The literature indicates that the calcium concentration in foetal blood is higher than that in maternal serum [41]. The increase in foetal serum calcium content occurs through an active transport mechanism. However, in the present study, the calcium concentration was lower in cord blood than in maternal serum in the high fluoride group. In the low/optimum group, the calcium concentration was higher in cord blood than in maternal serum. The exchange of nutrients and gases occurs through the syncytiotrophoblast layer of the placenta between the mother and foetus. The calcium concentration in the foetal blood is regulated by foetal parathyroid hormone and plasma concentration of 1,25 (OH)2 or vitamin D3. Several studies have shown that vitamin D plays a key role in calcium transport through syncytial cells [41]. Vitamin D-dependent calcium binding protein is present in the placenta. In the present study, vitamin D was significantly lower in the high fluoride group than in the low/optimum fluoride group. The decreased levels of vitamin D might play a role in the control of this transport. The exact mechanism through which calcium transport through the placenta is blocked in the high fluoride group remains unclear.

Our study had some limitations. The present study was a cross-sectional design. Therefore, a cause–effect relationship could not be identified. The present study was conducted during the winter season. There is a lack of information regarding vitamin D and other element levels during other seasons. Data regarding exposure to sunlight and diet history were self-reported, and more chances of recall bias were possible.

Conclusion

The present study concludes that high fluoride levels in drinking water are negatively associated with calcium and the associated hormones vitamin D and parathyroid hormone. Government policies were undertaken to provide safe drinking water and strict follow-up of vitamin D, calcium and PTH and other elements during pregnancy. We can spread awareness regarding the effect of high fluoride on calcium and vitamin D in pregnant women and newborn infants.

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Author contributions HMT was responsible for project conception, development of overall research plan and study oversight. DD was responsible for study oversight and data collection. MNK was responsible for primary responsibility for final content. MMW and SNP were responsible for hands-on conduct of the experiments and data collection.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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