

## FLUORIDE LEVEL IN THE BREAST MILK IN KOOHBANAN, A CITY WITH ENDEMIC DENTAL FLUOROSIS

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**ABSTRACT:** Dental fluorosis is a major health issue in Koohbanan, a city in Kerman province, Iran, and the aim of this study was to determine the amount of fluoride ion (F) in women's breast milk. Sixty-two mothers who breast-fed their infants participated in the study and were separated in two groups according to whether or not they had dental fluorosis. Two mL of the breast milk from each mother was sent to a laboratory in standard conditions and the amount of F measured with a special fluoride ion electrode. The data were analyzed with independent t-test using the SPSS version 20. The mean amount of F in the breast milk of mothers without dental fluorosis was 0.006 ppm while in the mothers with dental fluorosis it was markedly elevated at 0.550 ppm ( $p < 0.001$ ). The high level of F in the breast milk of mothers with dental fluorosis, is a concern as it may be a risk factor for their children also developing dental fluorosis.

Key Words: Breast milk; Dental fluorosis; Fluoride.

### INTRODUCTION

In recent years changes have occurred in the feeding patterns of newborns and infants in many countries. The breastfeeding of infants has increased as a result of recommendations promoting breastfeeding instead of feeding with formulas.<sup>1</sup> Children receive the fluoride ion (F) from many sources including water and food. Studies in Iran have been shown that F may affect the birth height and weight of infants, may adversely affect the IQ of children, and may increase the prevalence of dental caries in children.<sup>2-4</sup> The concentration of F in the mother's milk has a role in determining the amount of fluoride in the infant's body.<sup>5-6</sup>

Studies by Dean in 1938, 1942, and 1945 on the link between low levels of F and the high prevalence of dental caries culminated in the recommendation that the optimal concentration of fluoride in drinking water should be 0.7–1.2 mg/L (ppm), depending on the mean temperature of each city.<sup>7-9</sup> However, on 24 April 2015 the US Department of Health and Human Services Federal Panel on Community Water Fluoridation replaced their earlier recommendation for community water systems, based on the outdoor air temperature of geographic regions and involving

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a range of 0.7–1.2 mg F/L, with a new recommendation of a level of 0.7 mg F/L.<sup>10</sup> Although the World Health Organization set, in 1984 and reaffirmed in 1993, a guideline of 1.5 mg F/L as a “desirable” upper limit for F in drinking water, it also allows countries to set country standards<sup>11</sup> and the lower national standards have been set for Senegal, West Africa, (0.6 mg/L),<sup>12</sup> and India (1 mg/L), with a rider for the Indian standard being “lesser the fluoride the better, as fluoride is injurious to health.”<sup>12</sup> The minimum concentration of fluoride which results in dental fluorosis has been reported to be 0.06–0.07 mg/kg of body weight per day.<sup>13</sup>

Although many studies have been conducted on the amount of F in drinking water, bottled water, and black tea,<sup>14–18</sup> and its removal from drinking water when present in excess,<sup>19</sup> only a few studies have been carried out on the concentration of F in breast milk, formula, and infant foods.<sup>6,20,21</sup> Therefore, given the importance of the subject, the authors decided to carry out a study to determine the concentration of F in mothers’ breast milk in Koohbanan, a city in Kerman province, Iran, where dental fluorosis is prevalent. The present study complements previous studies in the Koohbanan region.<sup>13,22–25</sup>

The prevalence of fluorosis in guidance school students’ permanent teeth in Koohbanan has been reported to be 93%.<sup>22</sup> The concentrations of F in the drinking tap water in Koohbanan and in the water used for agricultural purposes is are 2.36 and 3.10 mg/L, respectively.<sup>23</sup> The level of F in the food produced in the region is 0.02–8.85 mg/L.<sup>23</sup> In addition, it has been found that for Koohbanan preschool children, aged 4–5 yr, the F intake is 1.71 mg/day and the urinary excretion of F is 0.41 mg/day.<sup>13</sup> Another study in Koohbanan showed that the prevalence of fluorosis in the deciduous teeth of children was 76.5% and the amount of F in the enamel of the deciduous teeth was 108.7 µg/mL.<sup>24</sup>

In addition to the high prevalence of dental fluorosis in Koohbanan children, the mean IQ of 7–9-yr-old children in this high drinking water F city (mean 2.38 ppm) of 91.37±15.63 was significantly lower than in the low drinking water F city Baft (mean 0.41 ppm) where the mean IQ was 97.80±15.95 (p=0.028).<sup>25</sup>

Fluoride readily crosses the placenta.<sup>26,27</sup> However, no study is available on the amount of F in mothers’ milk in a region with a high concentration of F in the drinking water and/or food products.

Studies have shown that there is a relationship between the F concentration of mothers’ milk and that in their plasma, with an increase in the F concentration in the plasma being followed by an equal increase in the secretion of F in mothers’ milk.<sup>28,29</sup> Therefore, it is possible to make a rough estimate of the amount of F an infant receives by determining the amount of F in mother’s milk. In addition, it is possible to determine the amount of F received by a mother and to determine the risk of fluorosis in an infant.

The objective of the present study was to determine the F concentration of the breast milk in mothers who themselves had dental fluorosis and had children with dental fluorosis. It was anticipated that the results of the study might contribute to the prevention of fluorosis in the permanent teeth in children living in Koohbanan

and increase the knowledge of the association between the F concentration in the environment and that in mothers' milk.

### MATERIALS AND METHODS

The students, aged 8–12 years, were clinically examined in several schools in Koohbanan, and students with fluorosis of permanent incisors were selected. The mothers of these children were then called and, if the mother was still breastfeeding an infant and she herself had dental fluorosis (such as Figures 1 and 2), the mother was given information about the study procedures and aims and informed consent for taking part in the study requested. All the participants gave informed consent.

**Figure 1.** A mother and her baby belonging to group 2 (with dental fluorosis).



**Figure 2.** Teeth of the older child of the mother shown in Figure 1.



Two mL of breast milk were then obtained from each mother and sent to the laboratory in sterile test tubes at 1–5°C under standard laboratory conditions. The fluoride concentrations of the milk samples were determined.

Based on similar studies and the high prevalence of fluorosis in Koohbanan,<sup>13,22,24</sup> the sample size was determined as 30. Thirty-one milk samples were taken from Koohbanan mothers with dental fluorosis. In addition, a total of 31 milk samples were also collected from mothers in Koohbanan, who were breastfeeding an infant, but neither they nor their schoolchildren had dental fluorosis. The F concentrations of these samples were also determined. To select these mothers, 8–12-yr-old children in the same schools who had no dental fluorosis were selected and their mothers were called. If these mothers and their older children did not have dental fluorosis and the mothers were breastfeeding an infant, they were selected for sampling of their milk after providing information on the study and obtaining informed consent. To determine the concentration of F in all the milk samples, the silicon-facilitated diffusion technique with a specific fluoride ion electrode (Metrohm Co., Swiss) was used. The electrode is referred to as a potentiometer. This method was used in similar studies.<sup>20,21,29</sup> The examiner was blind to the samples. Data were analyzed with the SPSS 20 using the independent-samples t-test. Before the statistical analyses, Kolmogorov-Smirnov test was used to evaluate whether or not a normal distribution of data was present in the two groups.

## RESULTS

There was statistically significant difference in the amount of F in the breast milk samples of the two groups ( $p < 0.001$ ) (Tables 1 and 2).

**Table 1.** Comparison of the fluoride levels (ppm) in the breast milk of the mothers with and without dental fluorosis

Group	No. of mothers	Breast milk fluoride level (mean±SD ppm)
Mothers without dental fluorosis	31	0.0061±0.0028)*
Mothers with dental fluorosis	31	0.55±0.248)*

Comparing the fluoride levels in the mothers with and without dental fluorosis: \* $p < 0.001$ .

**Table 2.** Fluoride level (ppm) in the breast milk samples of the mothers

Group code*	Amount of fluoride in breast milk (ppm)	Group code*	Amount of fluoride in breast milk (ppm)	Group code*	Amount of fluoride in breast milk (ppm)	Group code*	Amount of fluoride in breast milk (ppm)
1 a	0.004	1 q	0.008	2 a	0.891	2 q	0.383
1 b	0.005	1 r	0.003	2 b	0.723	2 r	0.126
1 c	0.004	1 s	0.008	2 c	0.842	2 s	0.825
1 d	0.003	1 t	0.006	2 d	0.987	2 t	0.687
1 e	0.008	1 u	0.009	2 e	0.423	2 u	0.427
1 f	0.001	1 v	0.007	2 f	0.312	2 v	0.654
1 g	0.009	1 w	0.008	2 g	0.462	2 w	0.791
1 h	0.008	1 x	0.003	2 h	0.371	2 x	0.908
1 i	0.009	1 y	0.005	2 i	0.420	2y	0.729
1 j	0.003	1 z	0.009	2 j	0.519	2 z	0.922
1 k	0.002	1 ab	0.010	2 k	0.672	2 ab	0.385
1 l	0.001	1 ac	0.008	2 l	0.898	2 ac	0.318
1 m	0.004	1 ad	0.005	2 m	0.428	2 ad	0.252
1 n	0.004	1 ae	0.009	2 n	0.381	2 ae	0.371
1 o	0.010	1 af	0.008	2 o	0.482	2 af	0.125
1 p	0.008			2 p	0.335		

Group code: 1= the mothers without dental fluorosis, 2= the mothers with dental fluorosis.

## DISCUSSION

In the present study, the F concentration in the milk of mothers who were breastfeeding an infant, had fluorosis in their anterior teeth, and had an older child with fluorosis of their permanent anterior teeth, was determined to be 0.55 ppm. However, the F concentration of the milk of mothers, who were breastfeeding an infant, did not have fluorosis and had an older child without dental fluorosis in the permanent anterior teeth, was found to be significantly lower at 0.006 ppm ( $p < 0.001$ ).

Sener et al. reported that the mean F concentration of the milk of mothers living in a region with no reports of fluorosis was 0.006 ppm,<sup>29</sup> consistent with the result of the present study, in which the breast milk of breast feeding mothers without dental fluorosis and with children without fluorosis in their permanent anterior teeth was 0.006 ppm.

In a 1981 study by Ekstrand et al., five mothers, aged 27–36 yr, were given F by mouth on the third postpartum day.<sup>30</sup> A dose of 1–5 mg was given as an aqueous solution of sodium fluoride in the morning after fasting for 10 hr.<sup>30</sup> Blood and breast milk were sampled simultaneously in a non-glass system before and 30, 60, 90, and 120 min after the dose.<sup>30</sup> The F concentration in the plasma was measured with a fluoride-sensitive electrode, and in the milk was determined using a modified microdiffusion technique.<sup>30</sup> Recovery experiments showed no evidence that F binds to the constituents of breast milk.<sup>30</sup> Hence the figures given for breast milk represented exclusively free fluoride.<sup>30</sup> The plasma F concentration in all subjects rapidly increased after the F intake.<sup>30</sup> The highest plasma concentrations appeared 30 min after dosing, when they varied between 3–6 and 4–5  $\mu\text{mol/L}$  (70 and 86 ng F/mL, 0.070 and 0.086 mg F/L or ppm).<sup>30</sup> This rapid absorption was in line the authors' results from oral single-dose studies in man.<sup>30</sup> There was no corresponding increase in the F concentrations in the breast milk, the values varying between 0–1 and 0–4  $\mu\text{mol/L}$  (2 and 8 ng F/mL, 0.002 and 0.008 mg F/L or ppm).<sup>30</sup> These findings showed that plasma F was poorly transferred to breast milk and infants thus received almost no F during breast feeding.<sup>30</sup>

In a 2014 study in a low natural drinking water F area, with approximately 0.3 ppm F in drinking water, in Sassari, Sardinia, Italy, Campus et al.<sup>28</sup> reported a value for F in breast milk of breastfeeding mothers, who were given a non-sucrose food supplement containing fluoride, of  $515 \pm 105 \mu\text{g F/L}$  (0.515 ppm) which was similar to the value of 0.55 ppm found in the present study in the milk of mothers who were breastfeeding an infant, had fluorosis in their anterior teeth, and had an older child with fluorosis of their permanent anterior teeth. The mothers in the study by Campus who received the F supplement dissolved 2.17 g of a supplement powder into 100–150 mL of water and drank it once a day after breakfast.<sup>28</sup> The water used to dissolve the supplement did not contain more than 40  $\mu\text{g F/L}$  (0.04 ppm).<sup>28</sup> The dose was described as “1,500  $\mu\text{g F/L}$  for each dose” (1.5 mg F/L or 1.5 ppm) but this definition appears to be a concentration rather than a dose.<sup>28</sup> However, the value of 0.515 pm found by Campus et al. is not comparable to the value of 0.55 pm found in the present study. Campus et al. note that the F values

they found were higher than those in the literature.<sup>28</sup> A control group of mothers given “a non-sucrose food supplement without fluoride content” had F in breast milk value at the end of the study after 6 weeks of  $468 \pm 104 \mu\text{g F/L}$  (0.468 ppm) ( $p=0.040$ )<sup>28</sup> which is much higher than the usual values for F in breast milk in control mothers, such as the range of 0.002–0.008 in the study by Ekstrand et al.<sup>30</sup> Campus et al. measured the breast milk F levels with an ion specific electrode, model 960a, coupled to a potentiometer, model 710a.<sup>28</sup> They measured the inorganic free F, the inorganic bonded F, the casein organic F, and the globulin organic F.<sup>28</sup> The value for the total F content consisted of the sum of these measurements.<sup>28</sup> They considered that probably the method they used for the analysis contributed to the higher values they found.<sup>28</sup> Thus, the value of 0.515 ppm F in breast milk found by Campus et al. is not comparable to the value of 0.55 ppm F in breast milk found in the present study.

The breastfeeding mothers with dental fluorosis in the present study received F naturally on a daily basis through drinking water or different food items. A previous study showed that 4–5-yr-old children in Koohbanan received a daily dose of 1.7 mg of F through drinking water and different foods.<sup>13</sup> Adults in Koohbanan, who drink more water daily and eat more food, would receive even higher doses of fluoride.

In the present study, the mean F concentration in the milk of the mothers with fluorosis was almost ninety times than that of the control mothers. This significant difference between the two groups might be explained by the fact that, based on the demographical and biological data provided by the mothers without fluorosis in their teeth and with children without dental fluorosis, they were from highly educated families who had immigrated to Koohbanan due to their jobs. Therefore, by considering the knowledge available in relation to the prevalence of dental fluorosis on Koohbanan and the fact that the occurrence of dental fluorosis is related to the drinking water and foods available in that region, these residents do not drink the tap water in Koohbanan, drink processed bottled water, and procure raw materials for foods such as vegetables, etc., from regions out of Koohbanan. As a result, they receive less F and less F enters their milk. However, the mothers who had dental fluorosis, who had children with dental fluorosis, and who had a high concentration of F in their milk, had lived in Koohbanan from the time they started their families and were from farming and cattle-raising families who normally drank tap water and produced their own raw materials for their food or procured them from that region. Previous studies have shown that the drinking water and agricultural water F concentrations in Koohbanan are 2.36 and 3.10 ppm, respectively, and of all the foodstuffs prepared in that region, raw vegetables have the highest F content, with values in excess of 5 ppm in some cases.<sup>23</sup>

Based on scientific data, a 1-yr-old healthy infant weighs about 10 kg and drinks almost 1 L of mother’s milk in 24 hr.<sup>31</sup> Therefore, such an infant in Koohbanan, who has a mother and elder brother or sister with dental fluorosis, receives approximately 0.55 mg of F from breast milk daily. On the other hand, it has been reported that the maximum permissible dose of F for the prevention of dental

fluorosis, received from different routes, is 0.05 mg daily per kg of body weight,<sup>13,32</sup> i.e., the maximum permissible dose of F received daily through the different routes by a 1-yr-old infant weighing 10 kg is 0.5 mg, which is very close to the 0.55 mg received daily by the Koohbanan infants being breast-fed by mothers with dental fluorosis. Therefore, such a child apparently receives a permissible dose of F from the mother's milk and should not be affected by fluorosis in the anterior permanent teeth in the future. The crowns of anterior permanent teeth begin to form at 3–4 months of age.<sup>33</sup> As a result, these teeth are susceptible to the presence of high concentrations of F during infancy with the risk of dental fluorosis developing. If, in fact, dental fluorosis was detected on the surface of the crowns of these permanent teeth in such children, there might be three reasons for the occurrence of this abnormality:

1. The child begins eating foods from the ages of 1 to 4 years, which is the time when the formation of the crowns of permanent teeth ends<sup>33</sup> and drinks more water, too. Water, semi-solid foods, and solid foods in Koohbanan usually contain large amounts F as discussed above.<sup>23</sup> Therefore, such a child will receive a further high intake of F, resulting in the fluorosis of the permanent anterior teeth. A study in Koohbanan showed that 4–5-yr-old children who were the native residents of Koohbanan received 1.71 mg of F daily.<sup>13</sup> By considering the results of that study and the present study, it can be concluded that from the age under 2 yr up to 4–5 years the amount of F received daily by these children increases from 0.55 to 1.71 mg, which is almost 3 times the maximum permissible limit of 0.05 mg daily per kg of body weight, or 0.5 mg F/day for a 1-yr-old infant weighing 10 kg.<sup>13,32</sup>

2. It is possible that the child has suffered from relative malnutrition when the permanent anterior teeth were developing, i.e., up to 4 yr of age, because studies have shown that malnutrition results in a decrease in proteins in the body. Therefore, the quality of the enamel matrix of permanent teeth which were developing was affected. Based on the results of previous studies, the enamel of developing teeth with a weaker matrix is more profoundly affected by F and is more susceptible to fluorosis.<sup>34</sup> As a result, fluorosis will be more severe in children with more severe malnutrition.

3. Studies have shown that a high altitude of a residential area or region, such as a city or town, results in a relative hypoxia in the body tissues, a decrease in the excretion of F from the body, and increase in the F concentration in the hard tissues, such as teeth and bone, which increases the occurrence of fluorosis and its severity.<sup>13,23</sup> The altitude of Koohbanan is more than 2000 m and is considered a high-altitude region. Therefore, F is excreted from the body at a lower rate, resulting in its concentration in the body tissues, including the teeth. Therefore, the odds for the occurrence of fluorosis increase even when the F concentrations are not higher than the permissible levels. A study by Poureslami and Khazaeli showed that only about 24% of the F entering the body of 4–5-yr-old children in Koohbanan was excreted in their urine while other studies in low-altitude regions showed that almost 30, 39, and 51% of the ingested F was excreted in the urine.<sup>13</sup>



## CONCLUSION

Based on the results of the present study, the F concentration in the breast feeding mothers' milk in Koohbanan, who themselves and their children suffered from dental fluorosis, was significantly higher than that in those without fluorosis. Therefore, considering the fact that a breast-fed child is growing gradually and will eat solid and semi-solid foods with a high F content and will drink more high F water, there is a high risk of fluorosis affecting the anterior permanent teeth in such children.

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- 494 Research report  
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