

Comparison of Fluoride Content in Drinking Water and Prevalence of Dental Fluorosis in 6 - 12-Year-Old Students in Mariwan (a Cold Region) and Behbahan (a Warm Region) during the 2013 - 2014 Educational Year

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Abstract

Background: Fluoride plays an important role in preventing dental caries. Low fluoride concentrations cannot prevent dental caries, but ingestion of very high concentrations of fluoride during enamel development and maturation could lead to fluorosis. Fluoridation of drinking water is the most effective and inexpensive method for preventing caries. The mandated concentration of fluoride incorporated into drinking water should consider the mean temperature of each region.

Objectives: The aim of the present study was to evaluate the prevalence of fluorosis in children aged 6 - 12 in Mariwan and Behbahan and determine the fluoride content of drinking water in these two towns.

Materials and Methods: In the present descriptive and cross-sectional study, 13 water samples were taken from homes in Behbahan, 1 sample from the town's water reservoir, 10 samples from homes in Mariwan (5 samples for each reservoir) and 1 sample each from the town's 2 reservoirs. The 26 samples (23 from homes and 3 from reservoirs) were taken in polyethylene containers. The SPANDS colorimetric technique was used to determine fluoride content. Homes that used home-based water purification systems were excluded from the study. In addition, 128 students (62 girls and 66 boys) in Behbahan and 90 students in Mariwan were randomly selected. Dean's index was used to determine dental fluorosis. The mean yearly temperatures of the two towns were obtained from the metrological bureaus of the two towns.

Results: The means fluoride content of water in Behbahan's reservoir and Mariwan's reservoirs 1 and 2 were 0.7, 0.24 and 0.036 ppm, respectively. The mean fluoride content of Behbahan's home waterlines and in the relevant home waterlines of reservoirs 1 and 2 in Mariwan were 0.67, 0.218, and 0.054 ppm, respectively. There were no significant differences between the relevant reservoirs. The prevalence of fluorosis in Behbahan was as follows: 84.4% healthy, 10.9% questionable, 1.6% very mild, 2.3% mild, and 0.8% moderate. In Mariwan, the prevalence in areas related to reservoir 1 was 96.7% healthy and 3.3% questionable; in areas related to reservoir 2 it was 94.4% healthy and 5.6% questionable.

Conclusions: The fluoride content of drinking water in reservoirs and at homes was below the optimal level in Mariwan. No differences were observed from the standard levels in Behbahan. There were no differences in fluoride content of water in reservoirs and in home pipelines, indicating no tangible changes in fluoride content from the reservoirs to the homes. In neither of the towns was severe fluorosis observed. There was no significant difference in the prevalence and severity of fluorosis between genders.

Keywords: Drinking Water Fluoride Content, Fluorosis, Dental, Temperature

1. Background

Dental caries is one of the most prevalent infectious diseases worldwide. One of the techniques to decrease the prevalence of caries is to make teeth more resistant to acid attack; this is possible through fluoride intake during development of teeth. High fluoride intake, however, results in dental fluorosis (1, 2). Four factors play major roles in the development of dental caries: presence of sugary food

in the oral cavity, presence of bacteria in the oral cavity, the physical and chemical structure of the teeth, and time (2, 3). Fluoride is the most important element that causes change in tooth structure and increases the strength of the enamel prism structure. The mechanisms of fluoride function are divided into local and systemic groups. Fluoridation of water, salt, and milk and the use of fluoride supplements have been introduced for systemic flu-

oridation, mainly using sodium fluoride. Solutions, gels, toothpastes, and rinses of sodium fluoride, stannous fluoride, amine fluorides, acidulated phosphate fluoride, and monofluorophosphate have been used for topical fluoridation (4).

Systemic fluoride reduces the incidence of tooth decay through several mechanisms. These include changing hydroxyapatite to fluoroapatite, reducing dissolution of enamel in acid and creating greater resistance to caries, reducing the production of acid by plaque, and increasing enamel remineralization. Fluoride secreted with saliva also provides local effects (5, 6).

Fluoride prevents the production of glycosyl transferase enzymes and so reduces bacterial adhesion. It also prevents the formation of intracellular polysaccharides by limiting microbial metabolism (7). Topical fluoride can prevent the adhesion of bacteria to enamel surface proteins (8). The enamel of permanent teeth absorbs fluoride more readily than that of primary teeth because the pre-eruption period of permanent teeth is longer (9).

There are many places in the world where the fluoride content of groundwater is more than 1.5 milligrams per liter (10). Fluoride is present in the air, water and soil (11). The most effective and inexpensive method to prevent dental caries is fluoridation of drinking water (1).

Compared to the results of early fluoridation studies, differences in dental caries and fluorosis prevalence between fluoridated and non-fluoridated areas have markedly narrowed (12). Studies have shown that the maximum anti-cariogenic effect of water containing fluoride occurs during the period before tooth eruption. In addition, it has been shown that children who have drunk fluoridated water since birth exhibit 40% - 50% and 50% - 65% decreases in caries of the deciduous and permanent teeth, respectively (13).

In addition to determining the baseline fluoride content of drinking water in a region, the temperature of that region should also be taken into account when fluoridating its drinking water. In warm regions, more water is taken in and therefore less fluoride should be incorporated, and the reverse applies to cold regions (14). Based on WHO recommendations, the optimal fluoride content of drinking water should be 0.6 - 0.8 ppm in regions with a maximum daily temperature of 26.3 - 32.6°C and a yearly mean of 1.2 ppm in regions with a temperature of 10 - 12°C (WHO guidelines, 2006). The optimal fluoride content of drinking water is therefore 0.6 - 1.2 ppm, depending on the temperature of the region (14, 15).

Excessive fluoride in drinking water causes dental, skeletal, and non-skeletal fluorosis, which is endemic in several parts of the world (16). Dental fluorosis results from excessive fluoride intake at the time of tooth bud develop-

ment up to tooth eruption. Clinically, it first appears in its mild form in the form of white spots on the enamel. These gradually turn yellow or brown and finally become dark brown, indicating the most severe form of fluorosis and resulting in enamel destruction. The severity of fluorosis depends on the time and duration of exposure to fluoride, individual response, the degree of physical activity, nutritional factors, and bone growth and development. In this context, a similar fluoride dose might result in different severities of dental fluorosis. Other factors involved include latitude, malnutrition, and renal insufficiency (17-19).

The most important concern in relation to dental fluorosis is esthetic changes in tooth structure, which mostly occur in children at 20 - 30 months of age who are exposed to high fluoride doses (higher than 1.8 ppm daily). The critical period for exposure to high fluoride levels is 1 - 4 years of age; children of 8 years of age are at no risk. The safe fluoride dose has been reported to be 0.05 - 0.07 mg/kg daily (13-17).

We have found no studies to date that have evaluated the fluoride content of drinking water supplies in Behbahan and Mariwan.

2. Objectives

The aim of the present study was to determine the fluoride content of drinking water in Mariwan (a cold region) and Behbahan (a warm region) and compare them to optimal levels so that fluoridation measures could be adopted for the water supplies of the two towns if necessary and preventative measures would be adopted for dental fluorosis if necessary. Since the most important chronic complication of high fluoride levels in drinking water is fluorosis of permanent teeth, and the prevalence of such a condition has not been determined in these two towns, the prevalence of fluorosis in these two towns was also evaluated.

3. Patients and Methods

In this descriptive and cross-sectional study, the sample sizes were 13 samples from homes in Behbahan and 10 samples from homes in Mariwan that received their water from two water reservoirs there (5 samples from each reservoir). The samples were selected at random. To evaluate the effect of drinking water fluoride levels on dental fluorosis in children aged 6 - 12, schoolchildren of that age range in Behbahan and Mariwan were subjected to a stratified sampling technique; 62 girls and 66 boys were included in the study in Behbahan. In Mariwan, 45 students with equal sex distribution were selected at random for each water reservoir.

3.1. Water Sampling and Storage of Samples

Polyethylene containers were used to collect and store samples. The containers were rinsed several times and dried because chlorine in water interferes with the determination of fluoride content. The chlorine was therefore neutralized. Two water reservoirs supply the drinking water in Mariwan, taking their supplies from 4 wells, 2 of which supply the first reservoir while the 2 others supply the second. Each reservoir supplies drinking water to a part of the town. In Behbahan, there is only one water supply, which is a combination of the Maroun and Kheirabad rivers; it is mixed and collected in one reservoir to supply the drinking water of the whole town. The samples were collected in fall, 2013, from the two reservoirs in Mariwan and the one reservoir in Behbahan. Homes that provided samples did not use any systems for home water purification. The volume of each sample was 250 mL. Each sample was stored in capped containers away from light at room temperature. The samples were then coded and transferred to the laboratory of the faculty of health, Hamadan University of Medical Sciences, Hamadan, Iran.

3.2. Procedural Steps

A Hach DR/5000 spectrophotometer was used to determine the fluoride content of water samples (Figures 1 and 2). During each test, 10 mL of deionized distilled water was placed in the special cell of the device and 2 mL of SPANDS reagent was added to it to prepare a control solution. The 10 mL of water sample was placed in another special cell and 2 mL of SPANDS reagent was added to it. The temperatures of the demonized water and the samples were the same, with a maximum difference of $\pm 1^\circ\text{C}$. The caps of the cells were secured and the cells were gently shaken so that the reaction would occur. The reaction time was one minute. The cell containing the control solution was placed in the spectrophotometer to adjust the reading to zero. Then the cell containing the sample was placed in the device and the reading was recorded (Figure 3). The spectrophotometer was adjusted at 580 nm throughout the whole test. For higher accuracy of fluoride content determination, each sample was separately tested 3 times and the mean value of the fluoride content of the sample was taken. The metrological bureaus of Mariwan and Behbahan provided the mean temperatures of the two towns. Mariwan's mean temperature was 55.04°F and Behbahan's was 86°F (Figures 1 - 3).

To determine the prevalence of dental fluorosis, we first determined the number of male and female elementary schools and samples sizes, and then selected the subjects at random. Informed written consent was obtained from the children's parents and then a checklist was used



Figure 1. Hach DR/5000 Spectrophotometer



Figure 2. The Special Cell of the Hach DR/5000 Spectrophotometer

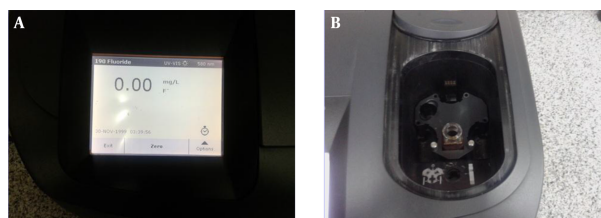


Figure 3. Placement of the Cells Containing the Control and the Test Samples in the Spectrophotometer

to collect data. Clinical examinations of the teeth were carried out under proper natural light with the use of dental mirrors by two educated and calibrated examiners; cotton rolls or gauze pieces were used to clean and dry tooth surfaces. All subjects brushed their teeth before examinations to ensure an accurate diagnosis of fluorosis. Students who

had drunk water from other water sources, had lived in another region for some time, had used home water purification systems in their houses, or had used fluoride supplementation were excluded from the study.

Dean's index was then used to diagnose dental fluorosis. With this index, each tooth receives a score and each subject's final score is the highest (the most severe) score of 6 scores given to at least two teeth. In other words, the score registered should correspond with the fluorosis status of at least two teeth; if only one tooth exhibits fluorosis and the other teeth are sound and normal, the whole dentition is considered sound. The scores of Dean's index are presorted in the Table 1.

Table 1. The scores of Dean's Index

| Index | Quality | Scores |
|---|--------------|--------|
| The enamel exhibits a normal and glossy translucent structure | Normal/Sound | 0 |
| There are some changes in the enamel translucency with some white flecks and rare white spots | Questionable | 1 |
| Paper-thin white opaque areas are dispersed irregularly on the tooth surface | Very mild | 2 |
| There are more extensive opaque white areas in the enamel, but less than 50% of the tooth surface is involved | Mild | 3 |
| All tooth surfaces are involved and subject to wear, with an unpleasant brown appearance | Moderate | 4 |
| All the tooth surfaces are involved and discrete or confluent pits are present along with brown spots | Severe | 5 |

One-way ANOVA was used to compare the mean scores between the groups after normalizing data and the equality of variances in each group were confirmed by the Kolmogorov-Smirnov and Levene's tests, respectively. Post hoc Tukey tests were used for two-by-two comparisons of the water reservoirs. An independent t-test was used to compare the means of the two independent variables (fluoride content of water sources in Mariwan and Behbahan). The t-test was used to compare the means for each town with a constant numeric value after the normality of data was confirmed. An ordinal logistic regression model was used to determine the risk factors affecting the severity of dental fluorosis, including gender and city. This model was used due to the ordinal nature of the variable, i.e. the severity of dental fluorosis. SPSS 20 was used for the statistical analyses of data and statistical significance was defined at $P < 0.05$.

4. Results

As shown in Table 2, the mean fluoride content of drinking water in Behbahan was higher than that in Mariwan.

Table 2. Mean Fluoride Levels in the Water Reservoirs of Mariwan and Behbahan

| Town | Number of Tests | Mean |
|-----------------|-----------------|-------|
| Mariwan | | |
| Reservoir 1 | 3 | 0.240 |
| Reservoir 2 | 3 | 0.036 |
| Behbahan | | |
| | 3 | 0.700 |

To compare the fluoride means, the ANOVA test was performed. As noted, normality and equality of the variances were checked before ANOVA (using Kolmogorov-Smirnov and Levene's tests, respectively). These tests, which are necessary assumptions of ANOVA, did not show significance; P values were 0.301 and 0.626, respectively.

The result of ANOVA did show a significant difference between the mean fluoride levels ($P < 0.001$). A post hoc Tukey test was used to clarify the difference pattern. According to this test, the mean fluoride levels in water samples collected from homes served by the two reservoirs in Mariwan were significantly lower than the reservoir in Behbahan ($P < 0.001$), while there was no difference between the mean fluoride levels in water samples collected from homes served by the two reservoirs in Mariwan. Following the analysis, the information from the two reservoirs in Mariwan was combined and then the two towns were compared using the independent sample t-test. The test indicated that fluoride levels in the two cities were statistically different ($P < 0.001$). Table 4 presents the results of comparing mean fluoride levels in drinking water from Mariwan and Behbahan.

4.1. Comparison of Fluoride Levels in Drinking Water Samples from Mariwan and Behbahan in Relation to Mean Yearly Temperature

Table 5 presents the results of a one-sample t-test to compare the fluoride levels in water samples collected from homes to the global standard.

Comparison of the mean fluoride level of water samples collected from homes in Mariwan with the global standard using the one-sample t-test showed a significant difference between the two values, with the fluoride levels in Mariwan home samples being significantly lower than the global standard level at the 95% confidence level as evidenced by the very low P Value.

Table 3. Mean fluoride levels in water samples collected from homes in Mariwan and Behbahan

| Town | Number | Mean | SD | Mean confidence interval | P Value of ANOVA |
|-----------------|--------|-------|--------|--------------------------|------------------|
| Mariwan | | | | | |
| Reservoir 1 | 5 | 0.218 | 0.0396 | 0.180 - 0.280 | |
| Reservoir 2 | 5 | 0.054 | 0.0266 | 0.023 - 0.095 | < 0.001 |
| Behbahan | 13 | 0.670 | 0.164 | 0.180 - 0830 | |

Table 4. Mean Fluoride Levels of Drinking Water in Mariwan and Behbahan

| | Mean Difference | SD | P Value |
|-------------------------|-----------------|-------|---------|
| Mariwan-Behbahan | -0.534 | 0.058 | < 0.001 |

4.2. Distribution of Dental Fluorosis Based on Dean's Index

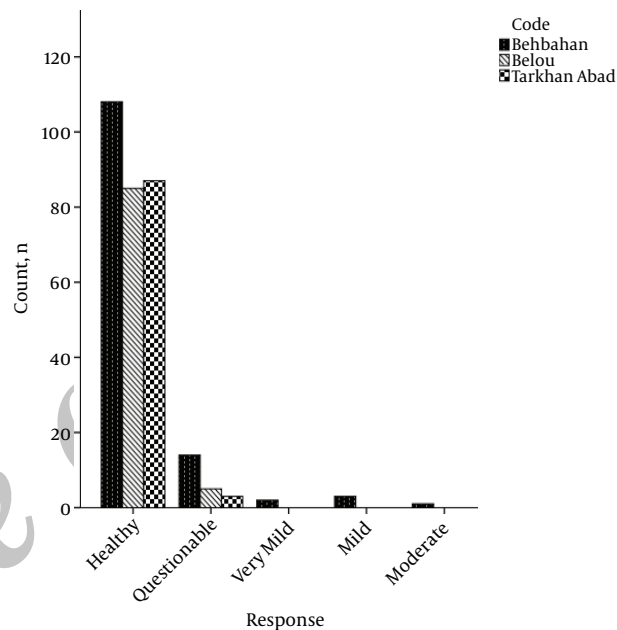
Table 6 presents the distribution of dental fluorosis in Behbahan and Mariwan based on Dean's index.

According to Table 6, in Behbahan, the normal and the moderate fluorosis rates were the maximum and minimum rates, respectively. In the Bilow region of Mariwan, the normal group exhibited the maximum number of subjects and the questionable group comprised 5.6% of the subjects, with no subjects in other categories. In the Tarkhanmabad region of Mariwan, similar to the Bilow region, the normal group comprised the maximum number of subjects, with 3.3% being questionable. On the whole and based on the index used for evaluations, 90.9% of the subjects did not exhibit fluorosis and were considered normal, with the moderate group exhibiting the minimum number of subjects (Figure 4, Table 5).

To fit a generalized cumulative logistic regression model, the three categories of mild, very mild, and moderate were combined because of sparse data. According to this model, the children's gender was not significantly related to the severity of fluorosis ($P = 0.851$) but the city was recognized as a risk factor dental fluorosis ($P < 0.001$). After excluding the gender covariate from the model, the results showed that the cumulative odds of increasing fluorosis severity by as much as one unit in Behbahan are 4.07 times that of Mariwan.

5. Discussion

In the present study, the fluoride content of drinking water supplies and the prevalence of dental fluorosis were evaluated in Mariwan and Behbahan, which are two towns in different regions of Iran with different climatic conditions. Variations in fluoride levels from the water reservoirs to homes were also simultaneously assessed. The

**Figure 4.** Distribution of Fluorosis in Mariwan and Behbahan Based on Dean's Index

mean fluoride levels in drinking water samples collected from homes in Behbahan and those related to reservoirs 1 and 2 in Mariwan were 0.67, 0.218, and 0.054 ppm, respectively; from the perspective of the climatic conditions of Mariwan, these are considered low compared to optimal fluoride levels. Fluoride levels of drinking water supplies in Behbahan, however, were not significantly different from optimal levels. In addition, comparison of fluoride levels of samples collected directly from the reservoirs and those collected from homes related to the same water reservoir did not reveal any significant differences. It can therefore be concluded that the fluoride levels did not undergo any tangible changes from the reservoir(s) to homes.

Concerning the prevalence of dental fluorosis, clinical examinations based on Dean's index showed that, for male subjects, the normal and moderately affected subjects represented the maximum and minimum number of subjects. For female subjects, the normal and mildly af-

Table 5. One-sample T-Test Comparing Fluoride Levels in Water Samples Collected From Homes to the Global Standard

| Town | Number | Standard Level | Mean Difference | t-Value | P Value | Bootstrap Confidence Interval | |
|----------|--------|----------------|-----------------|---------|---------|-------------------------------|-------------|
| | | | | | | Lower Bound | Upper Bound |
| Mariwan | 10 | 1.1 | -0.936 | -33.16 | < 0.001 | 0.08 | 0.19 |
| Behbahan | 13 | 0.7 | -0.029 | -0.641 | 0.534 | 0.57 | 0.74 |

Table 6. Distribution of Dental Fluorosis in Behbahan and Mariwan Based on Dean's Index^a

| | Behbahan | Mariwan (Bilow) | Mariwan (Tarkhanabad) | Total |
|-----------|------------|-----------------|-----------------------|------------|
| Healthy | 108 (84.4) | 85 (94.4) | 87 (96.7) | 280 (90.9) |
| Suspected | 14 (10.9) | 5 (5.6) | 3 (3.3) | 22 (7.1) |
| Very mild | 2 (1.6) | 0 | 0 | 2 (0.6) |
| Mild | 3 (2.3) | 0 | 0 | 3 (1) |
| Moderate | 1 (0.8) | 0 | 0 | 1 (0.3) |
| Total | 128 (100) | 90 (100) | 90 (100) | 308 (100) |

^aValues are expressed as No. (%).

ected subjects represented the maximum and minimum number of the subjects. On the whole, and based on the index used, 90.9% of the subjects were normal; the moderately affected subjects represented the minimum number of subjects. Concerning the prevalence of dental fluorosis in relation to the Behbahan water reservoir, 84.4% of the subjects were normal, 10.9% were questionable, 1.6% were very mildly affected, 2.3% were mildly affected, and 0.8% were moderately affected. Concerning reservoir No. 1 in Mariwan, 96.7% of the subjects were normal and 3.3% were questionable. Concerning reservoir No. 2 in Mariwan, 94.4% were normal and 5.6% were questionable. Severe fluorosis was not observed in either town. These results might indicate low levels of fluoride in the water supplies of Mariwan, but not optimal levels. In addition, the fluoride levels of Behbahan's water supply in relation to the development of fluorosis were in the optimal range when considering the town's mean temperatures.

One of the advantages of the present study was taking account of the climatic conditions of the regions under study. These conditions can affect the intake of water, and consequently the amount of fluoride received as well. By considering this factor, the water supplies in Mariwan were deficient in fluoride, which might result in a higher prevalence of dental caries in that region. Further evaluations and comprehensive examinations, however, are necessary. Based on the results of the present study, fluoride in the water supplies in Mariwan should therefore be provided from other sources, and measures should be adopted to prevent excessive fluoride intake in Behbahan. One lim-

itation of the present study could be that differences in the usual and customary nutrition of the two regions were not taken into account; such nutritional habits might affect the amount of fluoride received from other sources.

An epidemiological study on fluorosis and dental caries by Franzolin et al. in 2008 examined different types of water resources and concluded that dental caries and fluorosis were risks in all water sources for both sexes. No difference was seen in the severity of fluorosis among different water sources (20).

Amouei et al. studied all groundwater wells in rural areas of Khaf city, Razavi Province, northeastern Iran between 2009 and 2010. They found that in rural areas, fluoride concentrations ranged from 0.11 to 3.59 ppm. The level was less than the permissible limit in 31% of the studied samples, higher than the permissible limit in 4% of the samples, and within the optimum limit of 1 to 1.5 ppm in 65% of the samples (21).

Ramazani et al. collected 36 samples from the water supplies of Shiraz at the closest possible locations to the water sources and reported mean fluoride levels of 0.15 - 0.35 ppm, which were below the optimal level in all the cases (22).

Nazemi et al. evaluated the fluoride levels of drinking water and the DMF index in 7-year-old children in Shahrud. They randomly selected 138 students and followed them for the 6 years of 2004 to 2009. They also collected 120 samples from the town's water supplies for the evaluation of fluoride levels by the ion-selective technique. The mean fluoride levels were reported to be 0.45 - 0.75

ppm, with a DMF index of 3.18 - 3.81. A significant correlation was found between the DMF index and mean fluoride levels in different years (23).

Azami et al. selected 29 out of 617 articles on dental fluorosis in Iran. Those 29 articles reported on the evaluation of fluoride levels in 4,434 water samples that had been collected from surface and underground water sources and tap water from 17 provinces in Iran during a period of 236 months in all seasons. The mean fluoride level was 0.17 ± 0.43 ppm with a range of 0 - 3.06. The fluoride levels were in the standard levels in only three provinces. The prevalence of fluorosis was reported to be 61%, with 1% affected with severe fluorosis. Despite the low levels of fluoride concentration compared to standard values, a high rate of fluorosis was reported in Iran, consistent with the results of the present study, in which a high prevalence rate of cases with questionable fluorosis was reported in Mariwan despite low fluoride levels (24).

In a study by Ramazani et al. in 2004, students 13 - 16 years of age were evaluated to determine the prevalence of fluorosis; 43% of the subjects had mild fluorosis, 25% had moderate fluorosis, and 11.5% exhibited severe fluorosis (25). In the present study, however, mild fluorosis, moderate fluorosis, and severe fluorosis were reported in 1%, 0.3%, and 0% of the subjects.

An epidemiological study by Franzolin et al. in 2008 investigated fluorosis and dental caries, evaluating them in terms of different water sources, and concluded that dental caries was present irrespective of the water source and fluorosis was detected in both genders. No significant difference was observed in the severity of fluorosis among different water sources (20). In the present study, however, Mariwan's mean fluoride levels of 0.218 and 0.054 ppm were correlated with questionable fluorosis in only 3.3% and 5.6% of the subjects, respectively, with no cases of severe fluorosis. By contrast, in Behbahan with its higher fluoride levels, 4.7% of the subjects exhibited very mild, mild, or moderate fluorosis.

5.1. Conclusion

The results of the present study showed a higher prevalence rate of fluorosis in Behbahan, which had a higher fluoride content in its drinking water than did Mariwan. Despite low levels of fluoride in Mariwan, however, questionable cases of fluorosis were observed. These might be attributed to drinking significant amounts of tea or consuming other foodstuffs that result in excessive fluoride intake. None of the subjects in the present study exhibited severe fluorosis, which is consistent with the amount of fluoride received. Given the low fluoride levels in Mariwan's water supplies, a high rate of dental caries was expected; fluoride should therefore be prescribed under the

supervision of dentists or it should be provided from other sources. In Behbahan, however, since fluoride levels are favorable and the region is hot, it is expected that if care is not exercised concerning the amount of fluoride received, excessive amounts of fluoride might be received from other sources, resulting in a high prevalence rate of fluorosis. The organs responsible should therefore be consulted, dental caries rates should be evaluated, and fluoride levels in drinking water should be evaluated periodically to prevent dental caries and fluorosis.

5.2. Recommendations

1. The prevalence of dental caries should be evaluated in the two towns to modify the amount of fluoride received.
2. Other drinks and foodstuffs commonly consumed in the two towns should be evaluated for their fluoride levels to help prescribe a proper concentration of fluoride.
3. The knowledge of parents, healthcare instructors in schools, and students should be promoted concerning fluorosis prevention, the etiologic factors involved, its side effects, and its treatment.

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Footnote

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