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USING URINARY FLUORIDE AND DENTAL FLUOROSIS AS BIOMARKERS OF FLUORIDE EXPOSURE IN BRICK KILN WORKERS IN BALOCHISTAN, PAKISTAN

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ABSTRACT: A cross-sectional study was undertaken to determine the level of urinary fluoride and dental fluorosis in brick kiln workers (n=100) and a control group (n=20) in Balochistan, Pakistan. The fluoride level was also assessed in groundwater samples (n=30). The results showed the urinary fluoride level was significantly greater (p<0.05) in the brick kiln workers (0.17–0.30 mg/L) than in the control individuals (0.003 mg/L). Dental fluorosis was prevalent in both the brick kiln workers and the control group and the Community Fluoride Index (CFI) values were less than 0.42. The range of the groundwater fluoride levels was 0.87 to 1.59 mg/L with the upper part of the range being above upper limit recommended by the WHO (1.5 mg/L). Significant positive correlations (r=0.98, 0.90, 0.96) were determined between groundwater fluoride, urinary fluoride, and dental fluorosis. Consequently, it is assumed that the level of urinary fluoride and dental fluorosis could increase due to fluoride emissions from brick kilns and groundwater fluoride. The results of the current study suggest urinary fluoride and dental fluorosis are effective biomarkers for the monitoring of short-term and prolonged exposure to fluoride, although dental fluorosis is an indicator of fluoride exposure only while the teeth are developing up until the age of approximately 8 years. In order to avoid the long-term health-related consequences of fluoride exposure, regular monitoring of fluoride levels in the vicinity of brick kilns is recommended.

Keywords: Biomarkers; Brick kilns; Community Fluoride Index (CFI); Dental fluorosis; Pakistan; Urinary fluoride.

INTRODUCTION

Fluoride is ubiquitous in nature and its concentration is dependent upon the geological environment of an area.[1] Fluoride in the environment is usually related to natural sources such as weathering of rocks and volcanic eruptions and anthropogenic sources are also widely documented. Anthropogenic sources include emissions from phosphate fertilizer manufacturing plants, oil refineries, coal burning, steel production, and brick kilns.[2,3] Among all the sources of fluoride in the environment, those of anthropogenic origin are the most important. Chronic exposure of fluoride may result in dental, skeletal, and non-skeletal fluorosis. Intelligence may be reduced in children following intrauterine exposure to fluoride and students growing up in fluoride endemic areas of the world have been found to have comparatively less mental work capacity and a lower intelligent quotient (IQ) than students from nonendemic areas.[4] The soft tissues of the body affected by excessive fluoride include the brain, the thyroid leading to hypothyroidism, and the reproductive system.[5] Ingested fluoride entering into the body is partly excreted in the urine and partly retained in the tissues, particularly in bone. Anthropogenic sources of fluoride have increased its concentration in the environment and it poses a health threat to people living in areas with industrial fluoride pollution and fluoride endemic areas. There has been an increased demand for biomarkers to biomonitor human fluoride exposure.[6] Nowadays, this task has been widely accomplished through important biomarkers such as teeth, urine, nails, hair, and saliva. Urine and

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teeth are regarded as the most commonly used biomarkers due to their noninvasive and easy collection. Urinary fluoride (UF) has been measured as an early indicator of fluoride poisoning not only in fluoride-exposed workers but also in the population of the fluoride endemic areas.[7] Although systemic exposure to fluoride in drinking water, at a level of approximately 0.7 ppm, is used in several countries to provide protection from dental caries, there is concern about development of various adverse effects including reduced IQ with intrauterine exposure. The topical use of fluoride toothpaste for the prevention of dental caries is widespread. Dental fluorosis (DF) is the most sensitive indicator of the prolonged exposure of the fluoride and may result from exposure to excessive levels of fluoride while the teeth are developing, up to approximately the age of 8 years.[8]

Bricks kilns are recognized as a prime source of ambient air pollution.[9] In developing countries, the combustion of huge quantities of coal and other fuels may occur in brick kilns in a nonscientific manner and are considered to be a major source of fluoride emissions in the form of hydrogen fluoride. Brick kiln workers are at a great risk of developing fluorosis due to exposure to the fluoride emitted during the operation of the kilns.[9,10] It has been reported by many researchers that brick kiln workers engaged in various activities, ranging from making of Kachi bricks (wet bricks) to the firing of bricks, suffer from various health-related issues.[11,12] There is no information available on the monitoring of occupational fluoride exposure among the brick kilns workers in the study area in Balochistan, Pakistan. Therefore, the aim of the current study was to investigate the level of urinary fluoride and the prevalence of dental fluorosis in brick kiln workers and to determine the association between these two variables and the groundwater fluoride concentration.

MATERIALS AND METHODS

A cross-sectional study was conducted in three districts of Balochistan to assess the concentration of fluoride in urine samples and the prevalence of dental fluorosis in brick kiln workers and in a control group. The control group comprised individuals working in various offices and universities who lived in areas where there was no exposure to fluoride. The subject group were aged 17–45 years. Only male workers were recruited for the survey due to the strict culture present in Balochistan by which female workers were not allowed to work at workplaces such as brick kilns. A questionnaire was designed and administered to the subject group to assess their socioeconomic and demographic status. Prior to the sampling, the objectives of the study and a description of the complete procedure were presented to the brick kiln workers and the control group. Written consent was then given by each individual who agreed to participate. This study was approved by the Ethical Committee of Fatima Jinnah Women University, The Mall, Rawalpindi, Pakistan.

Water sampling: The water samples were collected in the vicinity of brick kilns from the study sites. Commonly, groundwater was obtained through tube wells, stored in storage tanks, and then used for brick kiln operations, agricultural activities, and for drinking purpose. The water samples for analysis were collected from storage tanks in sterile sampling bottles and then filtered prior to the analysis. The fluoride level in the water samples was analyzed using the US-EPA ion selective electrode method. To determine the concentration of fluoride in the groundwater, 10 mL of water sample was diluted with an equal volume of TISAB[13] and the concentration of fluoride was assessed through the ion selective electrode (CRISON, GLP 22^+).

Urine sample collection and analysis: Instant urine samples were collected in sterilized urine collection bottles from brick kiln workers and control groups from Quetta, Pishin, and Mastung. The sampling was carried out from August to September 2017. A total of 100 samples was collected from brick kiln workers in the three sites and 20 control samples were collected from people working in offices. All the samples were kept refrigerated at 18ºC until analysis. For the analysis, a 5 mL urine sample was mixed with 5 mL TISAB at pH 5, and a F-ion selective electrode was then used to detect the concentration of fluoride in the sample.[8]

Dental examination: The clinical examination for the presence of dental fluorosis was carried out using the WHO Dean's Index.[14] The examination was carried out using dental explorers and mirrors in daylight by a single dentist who was calibrated prior to the study of the subjects commencing. Dean's Index is based on grades according to the severity of the fluorosis.

Community Fluoride Index (CFI): The Community Fluoride Index (CFI) was calculated according to the standard formula:[15]

$$\text{Community Fluoride Index (CFI)} = \frac{\Sigma\,(\text{Number of people} \times \text{Dean numerical weight})}{\text{Total number of people examined}}$$

Calibration for dental fluorosis: The dental examination was conducted by a single dentist. The intra-examiner reproducibility was assessed through Cohen's kappa statistics.[15] The method was calibrated at the beginning and in the middle of the study by re-examination of 10% of the total examined population. The kappa index calculated was 0.85.

Quality control and quality assurance: All the chemicals used in the analysis were purchased from various reputable commercial sources and were of analytical grade. Deionized water was used throughout the experiment for the preparation of the calibration standards and reagents. The percentage mean recovery levels in the urine and the water samples were 95% and 94%, respectively.

Statistical analysis: One way analysis of variance (ANOVA) and Student's t-test were used to assess the variations in the fluoride level in the groundwater and the urine samples of the three districts of Balochistan. Similarly, Pearson's correlation was applied to the data set to determine the relationship between the fluoride concentration in the groundwater, urine samples, and dental fluorosis. All the statistical calculations were carried out by Excel, 2013.

RESULTS

Fluoride in the groundwater of the study area: The range of the fluoride concentration in the groundwater samples from Quetta, Pishin, and Mastung was 0.87–1.59 mg/L (Figure 1). The highest concentration of the fluoride was reported in the groundwater samples of the Mastung and the variations in the groundwater fluoride among the study sites were statistically significant ($p>0.05$).

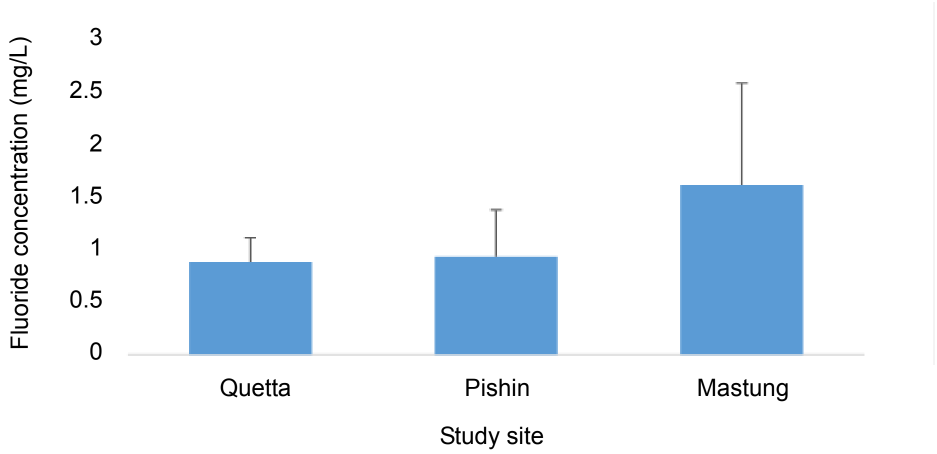

Figure 1. Fluoride concentration in the groundwater of the three sites of the study area.

Fluoride in the urine samples of the studied population: The urinary fluoride varied between 0.17 to 0.30 mg/L in the studied population. The concentration of urinary fluoride was comparatively less in the control group. Student's t test results showed that the variations in the concentration of the urinary fluoride of the exposed group (brick kiln workers) and the control group were statistically significant ($p<0.05$).The highest urinary fluoride was reported in the urine samples of the Mastung population (Table 1).

Table 1. Urinary fluoride concentration (mg/L) in the studied population

Study site	Range	Mean±SD
Quetta (n=25)	0.013–0.54	0.17*±0.15
Pishin (n=50)	0.002–0.842	0.19*±0.21
Mastung (n=25)	0.092–0.811	0.30*±0.19
Control (n=20)	0.0003–0.007	0.003±0.002

*p is significant at level of 0.05

Prevalence of dental fluorosis: A dental examination was carried out to determine the level of prevalence of dental fluorosis in both the exposed and the control populations. Overall, the results of the study showed that Quetta, Pishin, and Mastung exhibited 80%, 94% and 84%, respectively, of dental fluorosis in the exposed populations. The percentage of severe dental fluorosis was in the range of 32–46% in the three sites. Dental fluorosis was also noted in the control group. Fifty percent of the individuals in the control group exhibited mild dental fluorosis (Figure 2).

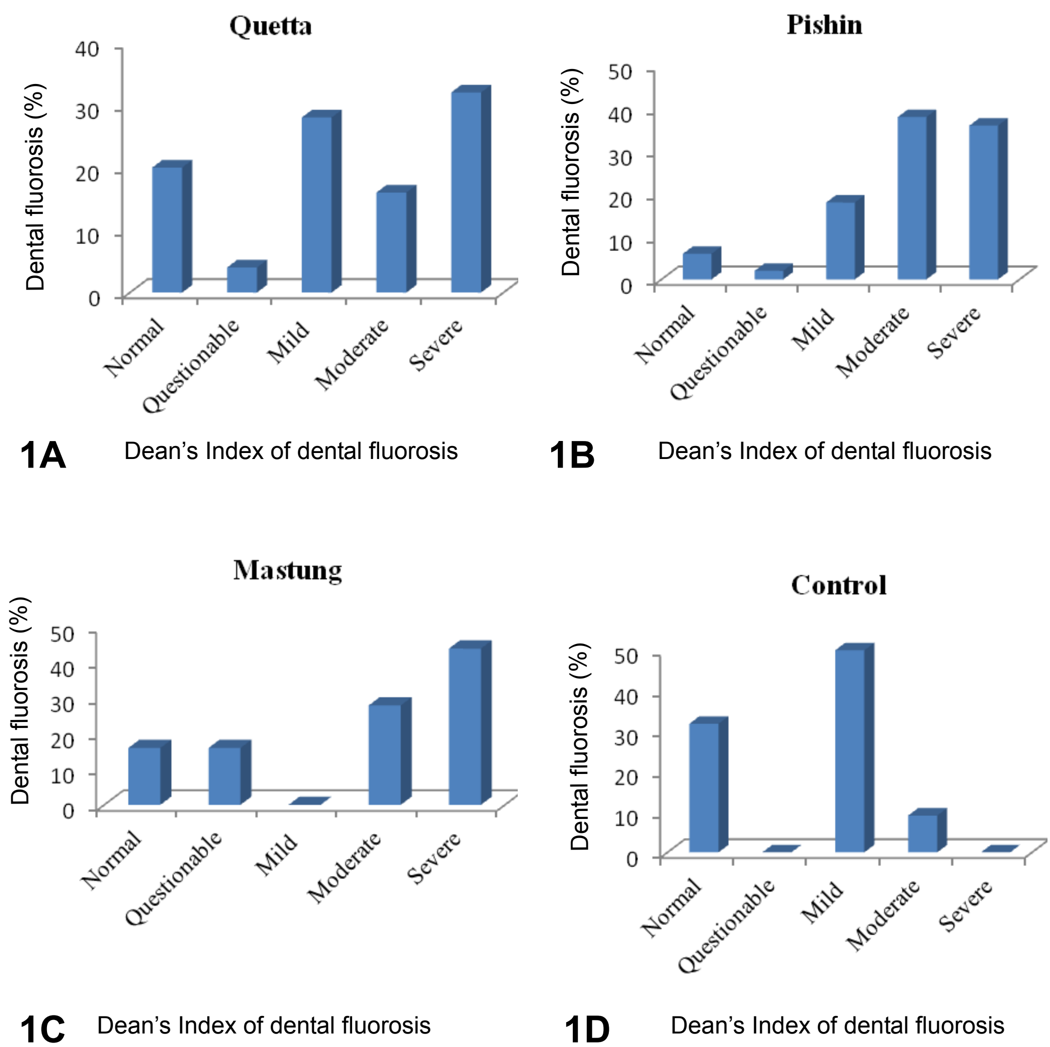

Figure 2. Percentage (%) prevalence of dental fluorosis in the studied population of the study sites. 1A: Quetta, 1B: Pishin, 1C: Mastung, and 1D: Control group.

The relationship among the groundwater fluoride concentration, urinary F, and dental fluorosis was assessed through Pearson's correlations. A strong positive relationship was determined by the aforementioned parameters (groundwater F, urinary F, and dental fluorosis) (Table 2).

Table 2. The Pearson's correlation among the studied parameters of groundwater fluoride (F), urinary F, and the Community Fluoride Index (CFI)

Parameter	r
Groundwater F and urinary F	0.98
Groundwater F and CFI	0.90
Urinary F and CFI	0.96

Socio-demographic status of the studied population: The socio-demographic data of the brick kiln workers and the control group are presented in Table 3. The age range of the brick kiln workers and the control group was 17 to 47 years. All the participants in the current study were males. The working hours of the brick kiln workers were more than those of the control group. The brick kiln workers were at a greater risk of fluoride exposure due to a lack of personal protective equipment (PPE) and of occupational exposure standards. All of the brick kiln workers were using groundwater for drinking and were regularly exposed to the operational activities of the brick kilns. The brick kiln workers were uneducated and most of them were unaware of the health risks associated with the brick kiln operations. They suffered from various health-related issues including toothache, muscle pain, coughing, and fever. They frequently took 4 to 5 cups of tea in a day in order to relax from the workload. In comparison to the brick kiln workers, the control group was highly educated, was health conscious, and had a good knowledge of the impact of brick kiln pollution on the surrounding ambient environment.

Table 3. Demographic description of the study population (brick kiln workers and control group)

Demographic parameter	Brick kiln workers	Control group
Age (years)	17–47	17–47
Drinking water source	Tubewell water	Tubewell water Mineral water
Dental care	None	Regular brushing of teeth using toothpaste
Dental diseases	Frequent tooth ache and sensitivity to heat and cold	None
Skeletal and other diseases	Whole body pain Muscle pain Fever Coughing	None
Working hours	9 hours per day	7 hours per day for 6 days/week
Salary (Pakistani rupees)	30,000–35,000	60,000–80,000
Tea drinking	4 to 5 cups per day	2 to 3 cups per day

DISCUSSION

Fluorine is neither an essential trace element for humans nor necessary for the development of healthy teeth and bones.[16] An excessive intake will cause chronic fluoride poisoning, such as dental fluorosis, skeletal fluorosis, and non-skeletal fluorosis.[17] Although the World Health Organization set, in 1984 and reaffirmed in 1993, a guideline of 1.5 mg F/L (1.5 ppm) as a "desirable" upper limit, it also allows countries to set Country Standards, their own national standards or local guidelines.[18] The limit of 1.5 mg F/L has been seen to be unsuitable in some countries and lower Country Standards have been set of 1 mg/L in India and 0.6 mg/L in Senegal, West Africa.[19] A rider to the Indian limit is that the "lesser the fluoride the better, as fluoride is injurious to health."[19] In the current study, the concentrations of fluoride in the groundwater of the study area near the upper end of the range of 0.87–1.59 mg/L were above the WHO guideline for an upper limit of 1.5 mg/L.[18,20]

The highest concentration of ground water fluoride found in the study might be attributed to the dissolution of calcite and fluorite rocks and the precipitation of fluoride. The study area is characterized by an arid climate with mineral deposits and spatial variations. Other possible reasons include agricultural inputs, combustion of coal in brick kilns, industrial emissions, and atmospheric deposition.[21] Groundwater contamination with an elevated level of fluoride has also been reported in other parts of the country. In Pakistan, fluoride contamination is regarded as a significant health hazard, second in importance to arsenic. The results of the present study are in line with the study by Chandio et al.[22] who confirmed the presence of elevated fluoride levels in the groundwater of Mastung. The results of the current study suggest that the water of the study area might be causing long-term exposure to excessive fluoride levels and that the situation needs ongoing monitoring but not immediate community-based interventions.

The biomonitoring of the human fluoride exposure in the current study was accomplished through urinary fluoride analysis and examination for dental fluorosis. These two biomarkers can be used to assess the impact of fluoride on human health. The daily level of urinary fluoride has been found to be the most reliable indicator of early fluoride exposure.[23] The results of the current study showed that brick kiln workers had a higher concentration of the urinary fluoride than the control group. These results are consistent with those of other studies showing a higher urinary fluoride concentration in phosphate fertilizer workers exposed to excessive fluoride emissions.[24,25]

The human body is also exposed to fluoride through dietary sources with various ingestion patterns.[26] Fluoride ingestion has been reported from dietary products such as toothpaste, tea, pan masala (a mixture of nuts, seeds, herbs, and spices), and tobacco. It was observed in the current study that none of the brick kiln workers were using toothpaste suggesting that these workers were not exposed to fluoride-containing toothpaste but, however, they were commonly consumed tea and tobacco. Dental fluorosis is most prevalent in fluoride endemic areas, where the groundwater has evaluated levels of the fluoride.[27,28] The increased prevalence of the dental fluorosis found in the present study with the increased concentration of the fluoride in the groundwater is in agreement with the findings of other studies.[29,30] In the current study, the fluoride exposure from the brick kilns, especially in the form of HF, is likely to be responsible for greater urinary fluoride in the brick kiln workers and the mild dental fluorosis in the control group might be due to fluoride ingestion from drinking water and other sources of exposure like food and dental products.[31] The absorption of fluoride in the body depends on the chemical nature of the ingested fluoride. When fluoride is ingested, 90% is absorbed into the blood stream and 10% is excreted in the faeces.[32] Nearly half of the absorbed fluoride is quickly taken up by the calcifying tissues of bone and growing teeth where it forms fluorapatite, 45% of the fluoride in the blood is filtered in the kidneys, stored in the bladder and eventually excreted in the urine.[32] Only a small amount, approximately 0.05%, is excreted in other bodily fluids (breast milk, sweat, and saliva).[32] Some fluoride is also stored in the soft tissues of the body and a small, but functionally significant amount because of its effect on enzymatic function, is taken up by the pineal gland which may also calcify.

Fluoride is absorbed through passive diffusion in both the stomach and small intestine. Fluoride in the stomach, is usually, present in the form of the weak acid hydrogen fluoride (HF). HF diffuses from the stomach into the blood and circulates, predominately as the fluoride ion, to other parts of the body. In the plasma, fluoride is present in both ionic and nonionic forms. The ionic form of the fluoride has less affinity to bind to the plasma proteins and is thus excreted through the urine. The amount of urinary fluoride reflects the level of the ingested fluoride.[33]

The positive significant correlations between the level of groundwater fluoride and presence of dental fluorosis in the studied population confirms the fact that the dental fluorosis in the study area may be due to the fluoride in the groundwater. These results are consistent with the findings of Kumar et al.[34] In the present investigation, the significant positive correlation obtained between the CFI and the groundwater fluoride levels are in line with the findings in the other studies.[35,36] Dean suggested that CFI values greater than 0.4 are of public health importance and a CFI above this level may indicate a need for immediate community-based interventions to provide fluoride free water to the local people. In the current study, the CFI values were less than this threshold level and therefore, no immediate community-based interventions are required. Similarly, the CFI values found in the current investigation were less than the CFI values reported by Kumar et al.[34] Various studies have confirmed that there is a positive relationship between the intake of fluoride via drinking water and the prevalence of dental fluorosis.[8] The severity of dental fluorosis is dependent on several other diverse factors such as the duration and the timing of the exposure.[37] Teeth can only develop dental fluorosis if the fluoride exposure occurs while the teeth are still developing, approximately up to the age of 8 years.

The socio-demographic results of the current study showed that the brick kiln workers are at a greater risk of exposure to fluoride, from brick kiln pollution, as compared to the control group. The results of our study are in accordance with the study conducted by Sanjel et al.[38] in Kathmandu Valley, Nepal. They associated the high prevalence of respiratory diseases with the brick kiln occupational exposure and regarded brick kilns as major source of pollution.

CONCLUSION

In the current investigation, we found that there was a statistically significant difference in the urinary fluoride level in the brick kiln workers as compared to the control group of office workers. Dental fluorosis was prevalent in both the brick kiln workers and the control population, but because the CFI values were less than 0.42 no immediate community based interventions were indicated. Similarly, the upper part of the range of the fluoride concentration in the groundwater samples from Quetta, Pishin, and Mastung, of 0.87–1.59 mg/L, was above the upper limit guideline of the WHO of 1.5 mg/L. A positive correlation was obtained between the groundwater fluoride level, the urinary fluoride level, and the presence of dental fluorosis. It is assumed that brick kiln workers are at high risk of fluoride exposures due to the fluoride emissions from brick kilns and fluoride in groundwater. Urinary fluoride and dental fluorosis are excellent biomarkers of fluoride exposure and can be used to assess long and short term fluoride exposure although dental fluorosis is an indicator of fluoride exposure only while the teeth are developing up until the age of approximately 8 years. It is recommended, that regular fluoride monitoring of the groundwater of the study area should be carried out in order to prevent the occurrence of dental fluorosis and skeletal fluorosis in the future and that the workplaces for the brick kiln workers should be improved by regulating occupational exposure standards for exposure to fluoride.

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