Statistical Analysis of Fluoride Levels in Human Urine and Drinking Water Samples of Fluorinated Area of Punjab (Pakistan)

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Summary: Increasing fluoride levels in drinking water of fluorinated areas of world leading to fluorosis. For bio-monitoring of fluorosis patients, fluoride levels were determined in drinking water and human urine samples of different individuals having dental fluorosis and bony deformities from fluorotic area of Punjab (Sham Ki Bhatiyan, Pakistan) and then compared with reference samples of non fluorotic area (Queens Road, Lahore, Pakistan) using ion selective electrode methodology. Fluoride levels in fluorinated area differ significantly from control group ($p < 0.05$). In drinking water and human urine samples, fluoride levels in fluorinated areas were: 136.192 ± 67.836 and 94.484 ± 36.572 $\mu molL^{-1}$ respectively, whereas in control samples, fluoride concentrations were: 19.306 ± 2.109 and 47.154 ± 22.685 $\mu molL^{-1}$ in water and urine samples correspondingly. Pearson's correlation data pointed out the fact that that human urine and water fluoride concentrations have a significant positive dose response relationship with the prevalence of dental and skeletal fluorosis in fluorotic areas having higher fluoride levels in drinking water.

Keywords: Fluorosis, fluorinated area, fluoride, human urine, drinking water.

Introduction

In Pakistan a mysterious disease was reported in 2000 in which more than 400 residents of a village called Kalalanwala were identified with bony deformities and dental mottling. The water fluoride content was found to be 29 mgL^{-1} which was much higher than the W.H.O recommended safe limit, i.e. less than 1.5 mgL^{-1}. In many parts of the world where the fluoride levels of drinking water are more than 1ppm, fluorosis is a serious health problem. Water, air and food are the three main sources of fluoride intake in human subjects. Though individual contribution of each is different from area to area but what is important is the total fluoride intake from all these three sources. Approximately 60 different types of industries like brick kilns, phosphate aluminum fertilizers, magnesium foundries, metal and steel works and ceramic industries cause fluoride intoxication by contaminating air and water. Among food products condiments, spices and table salt can contribute to total fluoride intake. Also beverages like tea have very high fluoride content [1-3]. Fluoride naturally present in environment in traces. Its concentration in water depends on various factors like: pH, TDS, alkalinity, temperature, depth (in case of ground water), porosity and acidic properties of soil [4-6].

Contaminated ground water with fluoride was noted in various Asian countries like: India, China, Siri-Lanka and Western countries like: Spain, Italy, West Indies and America [7-9]. Animals and vegetations of these areas are affected by fluorosis. Brick kilns in Dehli (India) have resulted in high fluoride levels in the ground water [10-15]. Dietary ingestion has recently been recognized as a route which can cause dental fluorosis however intake of fluoride from food can show great variations [16]. Main source of fluoride in children is ingestion or swallowing of fluoride rich toothpastes [17]. Ingestion of tobacco and Pan Masalah in India are assumed to be same as toothpaste ingestion [18]. Burning of low quality coal in a primitive way can also lead to fluorosis [19].

The role of fluoride in development of dental caries was noticed in early 20th century, when resident of certain areas of USA develop brown stains on their teeth and they were resistant to caries and dental decay [20]. It was discovered in 1930 that

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the severity and prevalence of this type of mottled enamel was in direct proportion to amount of fluoride in water [21]. Increase intake during the pre-eruptive stage of teeth leads to dental fluorosis and continuous ingestion over the years can finally cause skeletal fluorosis [22].

So, in the present study, concentration of fluoride has been determined in drinking water and human urine samples of fluorotic area patients of Pakistan (Sham Ki Bhatiyan, Punjab). For comparing them with non fluorinated area, water and human urine samples from non fluorotic area (Queens Road, Lahore, Punjab) were collected during same duration of study, i.e. December 2011-May 2012. Urines sample analysis for biomonitoring fluoride levels in human beings is chosen because of:

1. Ease of collection,
2. Less sophisticated instrumentation involved during sample collection and preservation,
3. Sample quantity is large for triplicate analysis which in turns increase precision,
4. No need of sample processing, as in case of blood, where during serum separation processing, most of the quantity is lost occasionally.

Results and Discussion

Fluoride Levels in Drinking Water Samples

Usually fluoride levels in drinking water is less than 1 ppm, but this value varied in different areas due to geographical and seasonal variations in various part of the world. Fluorine is a naturally occurring element commonly found in rocks animals, plants and soil. Erosion of rocks bearing fluorine minerals like fluoroapatite, cryolite and hydroxyapatite is the biggest natural source of fluoride in water and soil. The fluoride content of soil in high mountain areas is relatively low. It is transferred to lower plateaus or to sea via stream or rivers due to constant erosion of mountains. The ground water usually has high fluoride content as compared to surface water because fluoride from fluoride rich rocks is leached out when water percolates through these rocks [1, 2].

Table-1 is showing the concentration of fluoride and pH levels of non fluorotic region, i.e. Queens Road, Lahore (Pakistan) and fluorotic region samples. Statistical analysis of fluoride and pH levels in water samples of endemic and non-endemic areas are shown in Table-3. It is clear from these data that fluoride levels along with pH are significantly different in both areas samples (in all cases Two tail P=0.000). Negative values of mean differences in case of fluoride levels in water indicate that concentration of fluoride is higher in fluorinated areas samples, whereas, negative values of pH mean difference indicate that the pH values are higher in fluorinated area as compared to non fluorinated area, means alkalinity level increases. This rise in fluoride level leads to dental and skeletal fluorosis in the patients of fluorinated area.

Fluoride Levels in Human Urine Samples

Predominant excretion of fluoride is done by the kidneys. Almost 90% of absorbed fluoride is excreted via kidneys in the urine while according to Laurance *et al* 40 to 60% percent fluoride is excreted. In a normal individual drinking water with a fluoride content of 0.3 ppm, the urinary fluoride can range between 0 and 1.2 ppm average being 0.4 ppm. Fluoride renal clearance is dependent on urinary pH and glomerular filtration rate. In alkaline urine, fluoride excretion is much more as compared to acidic urine [25].

In this study, urinary fluoride levels of normal individuals of non fluorinated area and patients of fluorinated areas are given in Table-2 and their statistical analysis is shown in Table-3. The data indicated that fluoride levels are significantly different in both areas samples (in all cases Two tail P=0.000). Negative values of mean differences in case of fluoride levels in urine indicate that concentration of fluoride is higher in fluorinated areas subjects.

Table-1: Water analysis of endemic and non fluorinated areas.

Sample parameter	Group Statistics				
	Group	Tested samples quantity)	Mean	Standard Deviation (±)	Standard Error Mean (±)
Water Fluoride Levels (µmol/L)	Non-fluorinated	120	19.306	2.109	0.193
	Fluorinated	120	136.192	67.836	6.193
pH	Non-fluorinated	120	7.473	0.292	0.027
	Fluorinated	120	8.328	0.257	0.023

Table-2: Urine analysis for fluoride levels of subjects from fluorinated and non fluorinated areas.

Sample parameter	Group Statistics				
	Group	Tested samples quantity (N)	Mean	Standard Deviation (±)	Standard Error Mean (±)
Urinary Fluoride Levels (µmol/L)	Non- fluorinated	120	47.154	22.685	2.071
	Fluorinated	120	94.484	36.572	3.339

Table-3: Statistical analysis of water data of fluorinated and non fluorinated areas.

Sample parameter	t-test for equality of means					
	t-value	*P* value (2-tailed)	Mean Difference	Standard Error Difference	95% Confidence Interval of the Difference	
					Lower	Upper
Water Fluoride Levels (µmol/L)	-18.866	0.000	-116.886	6.196	-129.153	-104.618
pH	-24.064	0.000	-0.856	0.036	-0.926	-0.786
Urinary Fluoride Levels (µmol/L)	-12.047	0.000	-47.330	3.929	-55.077	-39.583

Statistical Correlation Comparison of Samples of Fluorinated and Non Fluorinated Areas

Statistical comparison of samples of fluorinated and non fluorinated areas was done in terms of Pearson Correlation and results are shown in Table-4. From this table, it is clear that the fluoride levels in the urine samples of individuals of fluorinated area is significantly high (P-value < 0.01) as compared to control group. The same is true for water samples. Positive dose response is found in drinking water and human urine samples, i.e. with the increase in fluoride levels in drinking water, concentration of fluoride increases in human urine samples. A large difference in mean values of fluorinated and non fluorinated areas in Table-2 indicated that concentration of fluoride is very high in fluorotic area, especially in case of water samples, where this difference is more. Same trend is observed from t-values. So, some preventive measures should be taken for controlling it [26].

Fluorides are absorbed by passive diffusion in fasting states almost 100% is absorbed while in the presence of food fluoride absorption is reduced to 50 to 80 percent. Certain dietary elements can affect fluoride absorption from the gut. Salts of aluminum, calcium and magnesium can reduce absorption of fluoride due to formation of les soluble compounds. Increased fluoride absorption from gut occurs with the presence of substances like molybdenum sulphates and phosphates in the diet causing fluoride toxicity. The severity of fluorosis is increased by malnutrition and deficiency of calcium amino acids and vitamins [27-29]. Curing measures like water purification, use of calcium and vitamins (D, C and E) supplements, less use of tea and fluoride containing toothpastes should be adopted for controlling this higher concentration of fluoride.

Experimental

Reagents and Instruments

Following chemicals and instruments were used during this study for quantification of various analytes: Ion Meter–Jenway (3345 Bench Ion Meter), Fluoride ion selective combination electrode–Jenway (924-305), Fluoride TISAB–Jenway (025-107), calcium ISAB–Jenway (025-097), micropippette 100-1000 µl– Biohit (720060), BD Vacutainers for collection of blood samples, de-ionized water (>18MΩ/cm) for dilutions.

Selection of Subjects

Human urine and drinking water samples were collected from two areas of Punjab, 60-80 Km apart, namely: Sham ki Bhatiyan (fluorotic area) and Queens Road, Lahore (Non fluorotic area). As the reference values of fluoride in drinking water and urine samples of different areas of world differ significantly, so for comparative purpose, control samples were collected from non fluorotic area.

A group of 120 patients of different sex, ranging from the age of 8 to 17 years were selected on the basis of physical examination for viewing prevalence of brown stains (dental fluorosis symptoms) along with bony deformities (skeletal fluorosis symptoms) as shown in Fig. 1 and 2. A general physical examination of all the patients was done to evaluate any bony deformities or joint stiffness. None of the subjects had limitation of movements, but all of them complained from mild to severe bone pains and stiffness of joints. Only two subjects had bowing of legs while rest of the subjects was without any bony deformities [17-22]. A group of 120 healthy volunteers were selected from non fluorotic area in the age range of 8-17 years as a reference / control group.



Fig. 1: Stained teeth of patients of fluorinated area (symptoms of dental fluorosis).

Table-4: Pearson correlation between urine and pH levels of fluorinated and non fluorinated areas.

Dependent parameter	Statistical parameter	Independent parameter		
		Water Fluoride Levels (µmol/L)	pH	Urinary Fluoride Levels (µmol/L)
Water Fluoride Levels (µmol/L)	Pearson Correlation	1	0.657**	0.514**
	P value (2-tailed)	N.C	0	0
	N	240	240	240
pH	Pearson Correlation	0.657**	1	0.503**
	P value (2-tailed)	0	N.C	0
	N	240	240	240
Urinary Fluoride Levels (µmol/L)	Pearson Correlation	0.514**	0.503**	1
	P value (2-tailed)	0	0	N.C
	N	240	240	240

**: Correlation is significant at the 0.01 level (2-tailed).
N.C: Not compared with itself.



Fig. 2: Bony deformities in patients of fluorinated area (symptoms of skeletal fluorosis).

Sample Collection

Water Samples

The samples of drinking water were collected from each subject's household tap, were kept in clean polythene bottles and labeled as W.1 to W.120 (non fluorotic area) and W.121-W.240 (fluorotic area). These were analyzed for fluoride concentration using ion selective electrode methodology. pH of samples were noted before fluoride analysis.

Urine Samples

Samples for non endemic area were labeled as U.1 to U.120 and for endemic area were labeled as U.121 to U.240. They were stored at in dark, air conditioned rooms and subsequently analyzed within one week.

Fluoride Analysis

Water Samples

For analyzing water samples with ion selective electrode, samples were prepared by taking 7.0 mL drinking water sample and mixing it with 3.0 mL of fluoride TISAB. Same procedure is followed with all water samples. Fluoride standards range is kept in between of 0.1-10.0 mgL^{-1}. Double distilled water is used for dilutions. Ion selective electrode (composite type) is calibrated first with standard solutions of fluoride and then all samples were analyzed subsequently. Electrode was washed and dried twice intermittently before dipping into next sample [23, 24].

Urine samples

For urine samples, 0.01-0.04 mgL^{-1} standard fluoride solutions range is used. Urine samples were prepared by taking 1.0 mL urine and 0.3 mL TISAB in sample tubes. Then they were analyzed with ion selective electrode with same methodology as for analyzing water samples.

Statistical Comparison of Fluorotic and Non Fluorotic Area Samples

For comparing the concentration of fluoride in fluorotic and non fluorotic area samples of urine and drinking water, paired sample't' test was applied for data analysis using SPSS 17 software. 95% confidence interval was applied. Pearson correlation test is applied for comparing means and finding positive dose response. *P* value of <0.05 was considered to be statistically significant in case of t-test and *P* value of <0.01 was considered to be statistically significant in case of Pearson correlation test [25-27].

Conclusion

It is clear from these results that fluoride levels in drinking water and human urine samples of fluorinated area significantly varied from control samples. In drinking water and human urine samples, fluoride levels in fluorinated areas were: 136.192 ± 67.836 and 94.484 ± 36.572 $\mu molL^{-1}$ respectively, whereas in control samples, fluoride concentrations were: 19.306 ± 2.109 and 47.154 ± 22.685 $\mu molL^{-1}$ in water and urine samples correspondingly. So,

precautionary measures like: water purification, use of calcium and vitamins (D, C and E) supplements, less use of tea and fluoride containing toothpastes, should be adopted to reduce fluoride levels in drinking water because it leads to dental and skeletal fluorosis in severe conditions.

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