CORRELATION ANALYSIS OF URINARY FLUORIDE LEVELS AND THE DAILY INTAKE OF FLUORIDE IN BRICK TEA TYPE FLUOROSIS AREAS

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ABSTRACT: Chronic exposure to the fluoride ion (F) can result in multisystem morbidity including skeletal fluorosis. The aim of this study was to investigate the relationship between urinary F levels and the daily intake of F in brick tea type fluorosis areas. The F levels were measured in brick tea infusions in six villages in Qinghai and five villages in Sinkiang, where the average intake of F from brick tea was >3.5 mg F/day and in which some residents had been diagnosed with having skeletal fluorosis using the WS/192-2008 standard of China. In addition, measurements were made of spot urine samples from the participants, who were aged over 16 yr, were born in the villages, and who continued to live in them. We found that differences may exist between Tibetans and Kazakhs in their F absorption and their sensitivity to F-induced damage. Although there was, in general, a significant positive correlation between the urinary F level and the daily intake of F from brick tea, this relationship was not consistently present when the subjects were stratified by ethnicity, age, and the presence or absence of skeletal fluorosis.

Keywords: Correlation; Fluoride load; Kazakhs; Tibetans; Urinary fluoride.

INTRODUCTION

Chronic exposure to the fluoride ion (F) can result in multisystem morbidity including skeletal fluorosis. Brick tea type fluorosis is a special type of fluorosis which is mainly found in the People's Republic of China.[1] It is caused by the exposure, over a long period, to a high concentration of F in brick tea infusions.[2] Over 30 million residents are threatened by brick tea type fluorosis and it is a serious public health problem. The affected residents are mainly the minority who follow the custom of drinking brick tea.[3] The concentration of F in the water of a brick tea infusion is well above the concentration of F in drinking water and food.[4] The F in the water of a brick tea infusion is the main source of the F exposure in the brick tea type of fluorosis [5].

It is estimated that approximately 36% of the F absorbed by adults becomes associated with the skeleton, while the rest is excreted in the urine. The level of urinary F excretion reflects the physiologic balance determined by the previous level of F intake, the extent of the recent F uptake, the rate of removal of F from bone, and the efficiency with which the kidneys can excrete fluoride.[6] Previous studies have confirmed that the level of urinary F is a good indicator for assessing

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the internal level of F exposure in the drinking water and the burning coal pollution types of fluorosis.[7-8]

The aim of this study was to investigate the relationship between urinary F levels and the daily intake of F in brick tea type fluorosis areas.

MATERIALS AND METHODS

Our survey, for assessing the relationship between urinary F and the daily intake of F in brick tea type fluorosis, was done from June 2012 to August 2012, using a cross-sectional design for the provision of reliable data.

Subjects: The brick-tea type fluorosis village was defined as one which the residents aged over 16 yr of age had an average intake of F from brick tea which was >3.5 mg F/day, and in which some of the residents had more than one sign of skeletal fluorosis confirmed by X-ray (GB17018, China). Eleven brick-tea type fluorosis villages were selected consisting of 6 villages in Ma Qin and Da Ri Counties, Qinghai Province, Guolo Tibetan Autonomous Prefecture, People's Republic of China in the northeastern part of the Tibetan Plateau, and 5 villages in Altay City and Habahe County, Sinkiang Province, People's Republic of China. Subjects were included in the study who were over 16 yr of age, had been born in the particular village, and had also grown up there. The study was approved by the Ethical Review Board of Harbin Medical University (HMUIRB20120021). All of the participants signed an informed consent form, and, for minors, written informed consent was also given by their guardians.

Sample collection and measurement: All of the participants completed an epidemic questionnaire survey form, which included family information, socio-demographic status, the amount of brick tea infusion consumed, and the past medical history. Meanwhile, a sample of the water in the brick tea infusion and a spot urine sample were also collected. The brick tea water was stored at –20°C until examination. We tested the concentration of F in the brick tea water and spot urine samples by the standard of China (GB19965-2005, WS/T 89-2006). In addition, all of the participants were assessed for the presence of skeletal fluorosis, using the standard of China (WS/192-2008), after taking X-rays of the forearm, leg (lower limb between the knee and the ankle), and pelvis. The subjects was classified as either having skeletal fluorosis, when more than one sign of skeletal fluorosis was present on X-ray examination, or not having skeletal fluorosis.

The diagnosis of the presence of skeletal fluorosis in an individual was based on the person having more than one sign of skeletal fluorosis present on X-ray. The signs of skeletal fluorosis may include those of osteoporosis, osteomalacia, sclerosis, altered bone turnover, and ossification of soft tissue and joint degeneration in the forearm, leg (lower limb between the knee and ankle), and pelvis.[9] Three degrees of severity of skeletal fluorosis can be distinguished:

MILD SKELETAL FLUOROSIS: (i) the normal trabecular pattern is replaced with gravel-like or granular bone spots, or (ii) the bone trabecula become thinner and sparse, and the spatial arrangement is irregular and obscured, or (iii) a hardened zone is present in a metaphysis of a long bone and a slight ossification is present in the soft tissues of the forearm or leg, or (iv) the radial crest is enlarged and the border is hardened, or (v) the interosseous membrane in the forearm or leg is ossified and shows the sign of bud break ground;

MODERATE SKELETAL FLUOROSIS: (i) the bone trabecula presents extensive diffuse coarse dense fusion, or (ii) extensive osteoporosis is present and the interosseous membrane in the forearm or leg is ossified, or (iii) the trabecular structure in the metaphyseal region of the limbs is obviously disordered and obscured, and osteoporosis is present in the cortical bone of the attachment of the pronator teres muscle, or (iv) obvious ossification is present in the tendons and ligaments of the pelvis and in the interosseous membrane of the forearm or leg;

SEVERE SKELETAL FLUOROSIS: (i) most of the bone trabecula are fused and present dentin-like osteosclerosis, or (ii) obvious osteoporosis or osteomalacia are present and the interosseous membrane in the forearm or leg is ossified, or (iii) the bone trabecula have a broken felt-like appearance, the bone structure presents a cotton-like appearance, or the cortical bone shows increased bone density, or (iv) multiple large joints present severe degenerative changes and deformity, and the soft tissue around the bone is obviously ossified.[9]

The daily intake of F from the water of the brick tea infusion, which is recognized as the principal F load in brick tea type fluorosis,[1] was calculated using the following equation:

$$\underset{\text{(mg F/day)}}{\text{Daily intake of F in brick tea}} = \underset{\text{(mg F/L)}}{\text{Concentration of F in brick tea}} \times \underset{\text{(L/day)}}{\text{Daily consumption of brick tea}}$$

Using the standard recommended values for the daily F intake from brick tea in China (GB17018-2011) and considering the size of the study population, we divided the participants into three groups based on their daily F intake from brick tea: a mild group (≤2.0 mg F/day), a moderate group (>2–<4 mg F/day), and a severe group (≥4 mg F/day).

Statistical Analysis: The data management and analysis were conducted with the SAS version 9.0 (SAS, Cary, NC). The intake of F in brick tea water per day was expressed as the median and the first and third quartiles (Q1 and Q3, respectively), and the urinary F was expressed as the geometric mean, Q1, and Q3 as they did not fit the normal distribution. The presence of significant differences in the demography characteristics were determined by t-tests, the Chi square test, and the Wilcoxon rank sum test. Correlation was tested by the Spearman Rank Correlation. All p values were two-sided, with <0.05 being considered statistically significant.

RESULTS

Two races, Tibetan and Kazakh, took part in our study and the descriptive statistics by ethnicity are presented in Table 1. The Tibetans (n=224) had a significantly higher incidence of skeletal fluorosis (χ^2=10.94, p=0.00) than the Kazakhs (n=221), although their urinary F levels were lower (z=6.26, p=0.00). No significant differences were present between the Tibetan and Kazakh ethnic groups in the gender proportions (χ^2=0.81, p=0.37), age (t=0.91, p=0.36), or the daily F intake from brick tea (z=0.10, p=0.92).

Table 1. Descriptive statistics in the Tibetans and Kazakhs, aged over 16 years, living in a brick tea fluorosis type village in Qinghai or Sinkiang

Parameters	Tibetans	Kazakhs	Statistical values
Skeletal fluorosis status			
Skeletal fluorosis not present [n (%)]	122 (54.47%)	154 (69.68%)	χ^2=10.94, p=0.00
Skeletal fluorosis present [n (%)]	102 (45.53%)	67 (30.32%)	
Age in years (mean±SD)	53.88±13.03	52.83±11.17	t=0.91, p=0.36
Sex [n (%)]			
Male	73 (32.59%)	81 (36.65%)	χ^2=0.81, p=0.37
Female	151 (67.41%)	140 (63.35%)	
Daily intake of fluoride from brick tea in mg F/day [median (Q1, Q3)]	7.33 (3.20,11.95)	6.55 (3.93,11.29)	z=0.10, p=0.92
Urinary fluoride in mg F/L, [geometric mean (Q1=first quartile, Q3=third quartile)]	2.26 (1.61,3.50)	3.29 (2.32,4.58)	z=6.26, p=0.00

The correlations between the urinary F and daily intake of F from brick tea infusions stratified by sex, race, age group, the state of illness, and the levels of F intake from the brick tea infusions are shown in Table 2. Significantly positive correlations were present in the groups stratified by different sex (male: r=0.29, p<0.05; female: r=0.20, p<0.05), race (Tibetan: r=0.24, p<0.05; Kazakh: r=0.24, p<0.05), and state of illness (without skeletal fluorosis: r=0.30, p<0.05; with skeletal fluorosis: r=0.20, p<0.05). A significant positive correlation was also found in some age groups (50–59 yr: r=0.36, p<0.05; 60–69 yr: r=0.27, p=0.01). No significant association was found in the groups stratified by different levels of daily F intake from brick tea.

Table 2. Correlation index between the daily intake of fluoride from brick tea and the urinary fluoride in the Tibetans and Kazakhs, aged over 16 years, living in a brick tea fluorosis type village in Qinghai or Sinkiang. Values for the daily intake of fluoride from brick tea are in mg F/day [median, first quartile (Q1), third quartile (Q3)] and the values for the urinary fluoride are in mg F/L (geometric mean, Q1, Q3)

Parameters	Number	Daily intake of fluoride from brick tea in mg F/day [median (Q1, Q3)]	Urinary fluoride in mg F/L [geometric mean (Q1, Q3)]	Correlation index	p
Total study group	445	9.75 (3.64,11.84)	2.73 (1.82,4.05)	0.23	<0.05
Sex					
Male	154	10.45 (3.98,12.01)	3.03 (1.96,4.38)	0.29	<0.05
Female	291	9.38 (3.50,11.48)	2.58 (1.75,3.87)	0.20	<0.05
Race					
Tibetan	224	9.93 (3.20,11.95)	2.26 (1.61,3.50)	0.24	<0.05
Kazakh	221	9.57 (3.93,11.29)	3.29 (2.32,4.58)	0.24	<0.05
Age group (yr)					
≤39	56	8.98 (2.88,12.16)	2.03 (1.29,3.17)	0.16	0.24
40~49	138	10.07 (4.49,10.81)	2.87 (1.95,4.39)	0.13	0.12
50~59	103	8.97 (3.78,10.84)	2.80 (1.79,4.49)	0.36	<0.05
60~69	91	10.60 (3.63,11.91)	2.84 (2.05,4.17)	0.27	0.01
≥70	57	9.77 (3.50,13.65)	2.89 (2.12,4.15)	0.25	0.06
State of illness					
Skeletal fluorosis not present	276	9.91 (4.04,11.95)	2.70 (2.51,2.92)	0.20	<0.05
Skeletal fluorosis present	169	9.49 (3.37,11.43)	2.76 (1.85,4.15)	0.30	<0.05
Daily intake of fluoride from brick tea in mg F/day					
≤2	54	1.31 (0.87,1.75)	2.09 (1.69,3.05)	–0.04	0.76
2~4	69	3.03 (2.58,3.50)	2.17 (1.22,3.56)	–0.03	0.83
4~8	124	5.76 (4.84,6.59)	2.84 (1.87,4.28)	0.10	0.25
>8	198	16.89 (9.86,18.99)	3.10 (2.12,4.54)	0.08	0.29

With further stratified analysis, we also found some significant correlations between the urinary F and the daily intake of F from brick tea. In the Tibetan population, there was a positive correlation between the urinary F and the daily intake of brick tea F in three stratification groups: (i) males aged 40–49 yr with skeletal fluorosis ($r=0.81$, $p<0.05$); (ii) males aged 50–59 yr without skeletal fluorosis ($r=0.58$, $p<0.05$); and (iii) females aged 60–69 yr with skeletal fluorosis ($r=0.56$, $p<0.05$) (Table 3).

Table 3. Further stratified analysis of the correlation index between the daily intake of fluoride from brick tea and the urinary fluoride in the Tibetans, aged over 16 years, living in a brick tea fluorosis type village in Qinghai or Sinkiang

Parameters	Correlation index in the different age groups Age groups (yr)				
	≤39	40~49	50~59	60~69	≥70
Male					
Skeletal fluorosis not present	–0.50	–0.13	0.58*	0.40	–†
Skeletal fluorosis present	–†	0.81*	–0.43	0.20	0.61
Female					
Skeletal fluorosis not present	0.30	–0.02	0.11	0.09	–0.14
Skeletal fluorosis present	0.50	0.41	0.41	0.56*	0.35

Comparing the groups with and without skeletal fluorosis: *$p<0.05$, †the sample size was too small for analysis.

In the Kazakh population, aged 50–59 yr without skeletal fluorosis, positive correlations were present between the urinary F and the daily intake of brick tea F in both males ($r=0.57$, $p<0.05$) and females ($r=0.61$, $p<0.05$) (Table 4).

DISCUSSION

Since the brick tea type of fluorosis was firstly reported in Aba Zhou of Sichuan Province in 1983, reports of it have come from other provinces in China[1] as well as from other countries.[10-11] Brick tea fluorosis not only damages the hard tissues but also the soft tissues.[12-13]

McClure reported a closely linear relationship between the concentration of F in drinking water and urinary F.[14] In addition, when the concentration of F in drinking water was low, the intake of F also had a significant positive correlation with urinary F in the burning coal pollution type of fluorosis.[15-16]

Table 4. Further stratified analysis of the correlation index between the daily intake of fluoride from brick tea and the urinary fluoride in the Kazakhs, aged over 16 years, living in a brick tea fluorosis type village in Qinghai or Sinkiang

Parameters	Correlation index in the different age groups Age groups (yr)				
	≤39	40~49	50~59	60~69	≥70
Male					
Skeletal fluorosis not present	0.20	0.12	0.57*	0.48	–0.04
Skeletal fluorosis present	–†	0.20	–0.06	–0.36	0.70
Female					
Skeletal fluorosis not present	0.28	0.22	0.61*	0.08	0.55
Skeletal fluorosis present	–†	0.26	–0.29	0.50	–†

Comparing the groups with and without skeletal fluorosis: *$p<0.05$, †the sample size was too small for analysis.

We found that while the external exposure to F, from the F in brick tea, was similar for the Tibetans and Kazakhs, the internal fluoride exposure to F, as reflected in the urinary F levels, was higher in the Kazakhs. However, despite the Kazakhs having a higher internal F exposure, their rate of developing skeletal fluorosis was less than in the Tibetans. This suggests that the absorption of F and the sensitivity to developing F-induced damage may be different between the Tibetans and the Kazakhs.

Although there was, in general, a significant positive correlation between the urinary F and the daily intake of F from brick tea, it appeared that this significant positive correlation was not consistently present when the subjects were stratified by race and age. For example, the relationship was significant ($r=0.81$, $p<0.05$) for male Tibetans with skeletal fluorosis, aged 40–49 yr, but not ($r=0.20$, not significant) for male Kazakhs with skeletal fluorosis, aged 40–49 yr (Tables 3 and 4). Similarly, the relationship was significant ($r=0.61$, $p<0.05$) for female Kazakhs without skeletal fluorosis, aged 50–59 yr, but not ($r=0.11$, not significant) for female Tibetans without skeletal fluorosis, aged 50–59 yr (Tables 3 and 4).

Four possible explanations for this are:

(i) Different routes for F intake might contribute to the difference. The main intake route for the intake of F in the drinking water type of fluorosis is through drinking water, while, in the burning coal pollution type fluorosis, F intake may be through air, food, and vegetables. In the brick tea type of fluorosis. the intake of F is through the ingestion of brick tea water To our knowledge, there are a lot of tea polyphenols in brick tea, with a total weight which is almost 15–30% higher than the weight of the F in the brick tea infusion.[17] The tea polyphenols could not only antagonize the kidney damage caused by F[18-19] but also have an influence on glomerular filtration.[20]

(ii) The brick tea type of fluorosis has a higher level of F exposure than that in both the drinking water and the coal burning pollution types of fluorosis. Wu et al. found that the concentration of F in brick tea ranged from 261.7 mg F/kg to 875.8 mg F/kg,[4] which resulted in a higher concentration of F in the brick tea water than in both the drinking water and coal burning pollution types of fluorosis. Furthermore, the excretion of F in the kidney was different with a high F load than with a low F load.[20]

(iii) The brick tea type fluorosis has unique epidemiologic features. The residents living in the wards with the brick tea type of fluorosis were mainly a minority group with their own unique food culture, which was rich in protein, e.g. meat and milk,[21] and this may have influenced the excretion of F. In addition, most of these wards were situated at a high altitude of over 2000 m. High altitude can influence the renal excretion of F.[22]

(iv) Finally, the sample size was small in this study which may result in insufficient statistical power and make the finding of a correlation between the urinary F level and the F load from brick tea impossible. A further survey with a large sample size will be conducted to test our hypotheses in the future.

CONCLUSIONS

In summary, differences may exist between Tibetans and Kazakhs in their F absorption and their sensitivity to F-induced damage. Although there was, in general, a significant positive correlation between urinary F and the daily intake of F from brick tea, this relationship was not consistently present when the subjects were stratified by ethnicity, age, and the presence or absence of skeletal fluorosis.

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