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Association between fluoride exposure and kidney function in adults: A cross-sectional study based on endemic fluorosis area in China

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ABSTRACT

Background: The kidney toxicity of fluoride exposure has been demonstrated in animal studies, and a few studies have reported kidney function injury in children with fluoride exposure. However, epidemiological information for the effects of long-term fluoride exposure on adult kidney function remains limited.

Methods: We conducted a cross-sectional investigation in Wenshui County, Shanxi Province to examine the association between fluoride exposure and kidney function in adults, and a total of 1070 adults were included in our study. Urinary fluoride concentrations were measured using the national standardized ion selective electrode method. And markers of kidney function injury (urinary NAG, serum RBP, serum Urea, serum C3, serum UA and serum αl-MG) were measured using automatic biochemical analyzer. Multivariate linear regression analysis and binary logistic regression model were used to assess the relationship between urinary fluoride and markers of kidney function injury.

Results: Urinary fluoride was positively correlated with urinary NAG and serum Urea, negatively correlated with serum C3. In multivariate linear regression models, every 1 mg/L increment of urinary fluoride was associated with 1.583 U/L increase in urinary NAG, 0.199 mmol/L increase in serum Urea, 0.037 g/L decrease in serum C3 after adjusting for potential confounding factors. In the binary logistic regression model, higher levels of urinary fluoride were associated with an increased risk of kidney function injury. Determination of kidney function based on urinary NAG, every 1 mg/L increment in the urinary fluoride concentrations was associated with significant increases of 22.8% in the risk of kidney function injury after adjusting for potential confounding factors. Sensitivity analysis for the association between urinary fluoride concentrations and markers of kidney function (urinary NAG, serum Urea, and serum C3) by adjusting for the covariates, it is consistent with the primary analysis.

Conclusions: Our study suggests that long-term fluoride exposure is associated with kidney function in adults, and urinary NAG is a sensitive and robust marker of kidney dysfunction caused by fluoride exposure, which could be considered for the identification of early kidney injury in endemic fluorosis areas.

1. Introduction

Fluorine belongs to the halogen family in the periodic table being the lightest member and most electronegative and reactive of all elements (Rafique et al., 2015). It ranked 13th most abundant element, which makes up 0.06–0.09% of the total earth crust (Armienta and Segovia, 2008). Sources of fluoride in the body include food, water and air, but drinking water is the most common source (Dhar and Bhatnagar, 2009).

Abbreviations: NAG, N-acetyl-β-glucosidase; γ-GT, γ- glutamyltransferas; RBP, Retinol-binding protein; C3, the third component of complement; UA, Uric acid; α1-MG, α1-microglobulin; UC, Urinary creatinine; BMI, body mass index; UF, urinary fluoride.

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As we all know, excessive fluoride exposure will lead to the occurrence of fluorosis, and the most specific and serious clinical features of fluorosis are dental fluorosis and skeletal fluorosis (Srivastava and Flora, 2020; Wei et al., 2019). Because of it is a medical problem of special concern in 24 nations including China, fluorosis is a serious public health concern (Srivastava and Flora, 2020). In recent years, more and more studies have shown that fluoride exposure can not only damage the bone tissue, but also lead to a series of non-bone injury, including the vascular system, nervous system, reproductive system, urinary system, and so on (Amini et al., 2011; Ding et al., 2011; Liu et al., 2019; Wang et al., 2020; Xiong et al., 2007).

The kidney is one of the most important organs that remove fluoride from the body. Under normal physiological situations, about 60% of the daily fluoride absorbed by healthy adults is excreted through the kidney in the urine (Hefti, 1986). Thus, the kidney is one of the most exposed to fluoride concentrations of all soft tissues (Dharmaratne, 2019). A growing number of laboratory studies have shown that fluoride can cause kidney function injury, fluoride is destructive to the kidney cortex in the form of glomeruli, proximal and distal curved tubules, vacuolization of tubule cells, and cell infiltration (Alhusaini et al., 2018; Cárdenas-González et al., 2013; Dote et al., 2000). Experimental animal studies also have shown that fluoride causes kidney function injury through oxidative stress and apoptosis (Tian et al., 2019). In cell experiments, it was found that fluoride sensitivity of cells from different organs of rats showed that kidney cells being the most sensitive type (Hongslo et al., 1980). In the population epidemiological survey, the investigation on chronic kidney disease of unknown etiology in Sri Lanka found that the serum and urinary fluoride concentrations of patients were significantly higher than those of the healthy control group (Nanayakkara et al., 2020), which suggested that environmental fluoride exposure may have toxic effects on the kidney. Another research, concerning the effects of fluoride exposure on children's kidney injury in China showed that drinking water fluoride concentrations over 2.0 mg/L can cause kidney function injury in children, which mainly showed that water fluoride exposure was positively related to urinary N-acetyl-β-glucosidase (NAG) activity and urinary γ- glutamyltransferase (γ-GT) activity in children (Xiong et al., 2007). The results of another study on the health status of children aged 8–15 years in areas of fluorosis showed a significant increase in serum creatinine, which suggested significant kidney function injury in children in fluorosis areas (Khandare et al., 2017). Most fluoride ingested by the body is excreted by the kidney. In endemic fluorosis areas, people are exposed to fluoride from birth, and last for decades. However, epidemiological information for the effects of long-term fluoride exposure on adult kidney function remains limited, and sensitive and robust markers to identify early kidney damage are also lacking.

The kidney function can be evaluated by several indexes. Enzyme activity is usually low in urine and may increase when kidney tubular cells are injured (Skálová, 2005), so urinary enzymes is one of the commonly used index. NAG is a lysosomal enzyme that mainly existed in the proximal tubule, and increased activity of this enzyme in the urine may imply kidney function injury (Beker et al., 2018). Retinol-binding protein (RBP) is a 24 kDa liver-derived protein that has been deemed as the primary carrier of retinol in the circulation (Axelsson et al., 2009). The increase of RBP can reflect the damage of kidney tubule function early and can sensitively reflect the damage degree of proximal convoluted tubule (Ayatse, 1991). Urea, a small water-soluble molecule with a molecular weight of 60 g/mol (Vanholder et al., 2018), is the most common marker of kidney function injury (Kovalčíková et al., 2018). The third component of complement (C3), one of 19 blood plasma proteins, acts jointly to execute the membraneolytic, inflammatory and immunoregulatory activities of the complement system (Fishelson, 1991). Studies have shown that there is an association between excess C3 deposition and kidney disease (Sethi et al., 2017; Smith et al., 2019). Uric acid (UA) is a weak acid produced in the liver, muscles, and intestines, and about 70% of the daily UA production by the body is eliminated by the kidney. After kidney function injured, UA elimination is affected and blood UA levels are significantly increased. Serum UA is an indicator of kidney function injury (Maesaka and Fishbane, 1998; Mandal and Mount, 2015). α 1- microglobulin (α1-MG) is a glycoprotein synthesized from human liver and lymphocytes with a molecular weight of about 33,000 Daltons. The free α1-MG in the blood can freely pass through the glomerulus and be reabsorbed and metabolized by kidney tubules. The level of α1-MG in serum of kidney injury was significantly increased. Thus, urinary NAG, serum RBP, serum Urea, serum C3 and serum UA, and serum α1-MG were chosen as the markers of kidney function in this study.

The purpose of this study was evaluate the relationship between fluoride exposure and kidney function in adults through study the relationship between urinary fluoride levels and urinary NAG, serum RBP, serum Urea, serum C3 serum UA and serum α1-MG through a population cross-sectional survey.

2. Materials and methods

2.1. Study site and population

A cross-sectional study based on rural areas was conducted in 2019 in Wenshui County, Shanxi Province, which was identified as an area of drinking-water fluorosis according to long-term monitoring by the Shanxi Institute of Endemic Disease Prevention and Control. Excluding the exposure of other toxic and harmful substances that may damage kidney function, such as cadmium and lead, we finally selected four villages as our investigation sites. The participants included in our study were adults aged 18 years or above, who were villagers of the village and lived in the village since they were born. A total of 1070 adults were included in the study, the detection of some markers was slightly missing, and the number of people of each marker was determined according to the actual detection. All participants were provided with informed consent and signed the informed consent. The study protocol was approved by the Ethics Committee of Center for Endemic Disease Control, Chinese Center for Disease Control and Prevention, Harbin Medical University.

2.2. General information collection

Participants were given face-to-face questionnaires and a general physical examination by trained doctoral and postgraduate students. General demographic data (age, sex, education, height, weight, waistline), socioeconomic status (family income), and disease history (hypertension) of the participants were collected. The body mass index (BMI) of the participants was further obtained by height and weight.

2.3. Sample collection

Blood samples were collected by professional nurses from the Affiliated Hospital of Shanxi Institute of endemic Disease Prevention and Control. 5 mL of fasting peripheral blood samples were collected from each participant. Centrifugation was performed at 3000 r/min (2 h after blood collection) for 10 min, and serum was divided into 1.5 mL EP tubes for detection of kidney function markers. All participants were given a polyethylene tube (50 mL) to collect morning urine samples, which were divided into 5 mL EP tubes. All samples were stored in a −80 ℃ refrigerator until analysis.

2.4. Determination of fluoride concentrations

Urine samples were used for determination of urinary fluoride concentrations using the national standard method ion selective electrode method. All reference solutions for the fluoride determinations were deionized water, and all chemicals used in the tests were reagents of analytical purity. Parallel samples were set for measurement and

averages were taken.

2.5. Determination of biomarkers for kidney function

In this study, urinary NAG, serum RBP, serum Urea, serum C3, serum UA, and serum α1-MG were detected using Automatic biochemical analyzer 3100 (Hitachi High-Technologies Corporation, Japan) for evaluating kidney function.

Urinary creatinine (UC) was measured by picric acid method (C011-1-1; Jiancheng Bioengineering Institute, Nanjing, China). UC was used to correct for urinary NAG and urinary fluoride in urine dilution during measurement. The concentration of creatinine-adjusted urinary fluoride concentration (UFC) and creatinine-adjusted urinary NAG (UNAG) were calculated using the formula UFC = UF/UC, UNAG = NAG/UC.

2.6. Quality assurance and quality control

The fluoride concentrations of urine samples were analyzed using the ion selective electrode method (WS/T 89-2015, Industry standard of the People's Republic of China). Standard fluoride solutions with concentrations of 1.0 mg/L and 10.0 mg/L were used to make standard curve series. The standard curve graph for F– was obtained by ion selective electrode with the calibration solutions range of 0.1–10.0 mg/L (0.1, 0.2, 0.5, 1.0, 2.0, 5.0 and 10.0 mg/L). Before measurement, 5 mL of total ionic strength adjustment buffer and 5 mL sample were added to each test cup, then a fluorine ion selected electrode (Shanghai Instrument Science Co., LTD, China) and a reference electrode (Shanghai Instrument Science Co., LTD, China) were connected to a fluoride ion meter (Shanghai Instrument Science Co., LTD, China). The fluoride concentration (mg/L) was measured while the solution was stirred at room temperature.

Automatic biochemical analyzer 3100 (Hitachi High-Technologies Corporation, Japan) was used to detect levels of urinary NAG, serum RBP, serum Urea, serum C3, serum UA, and serum α1-MG. Standards are used for experimental quality control before testing begins each day. All assays were performed using reagents provided by Medical system Biotechnology Co. Ltd (China) according to standard operating procedures. More information about reagents is available at https://www.nbmedicalsystem.com/. Before the test, all laboratory personnel involved in the test have undergone standardized training and passed the training examination.

2.7. Statistical analysis

Continuous variables were expressed as mean (± standard deviation) or median (P25-P75). Continuous variables with normal distribution were compared by student *t*-test or one-way analysis of variance, but continuous variables with non-normal distribution were compared by Mann-Whitney *U* test. Categorical variables were compared by chi-square test. Multivariable linear regressions were used to assess changes in markers associated with kidney function for every 1 mg/L increment in urinary fluoride, and associations between quartiles of urinary fluoride and kidney function-related markers were also performed. Binary Logistic regression models were used to further evaluate the relationship between urinary fluoride and kidney function, in which we determined kidney function with urinary NAG, serum Urea and C3, respectively, and divided them into two categories: kidney dysfunction and normal. According to the instructions, the range of urinary NAG in healthy people is 0.3–14.6 U/L, we believe that ≦ 14.6 U/L was normal kidney function, > 14.6 U/L was kidney dysfunction; serum Urea ranges from 1.43 to 7.14 mmol/L, and ≦ 7.14 mmol/L was considered normal kidney function, > 7.14 mmol/L was kidney dysfunction; serum C3 ranges from 0.79 to 1.52 g/L, ≧ 0.79 g/L was considered normal kidney function, < 0.79 g/L was kidney dysfunction. We used age, sex, education, family income, BMI, waistline, and hypertension as covariates. The effect estimates were presented as β or odds ratios (ORs) with their 95% confidence intervals (95% CIs).

Sensitivity analysis was carried out by adjusting different covariates to observe the effects of the exclusion and inclusion of different variables on the model, and to further test the robustness of the relationship

Table 1
Basic characteristics of general population.

Variables	Serum sample (αl-MG, C3, RBP) N = 1070	Urine sample (NAG) N = 955	Serum sample (Urea) N = 964	Serum sample (UA) N = 965
Age[a] (years)	58.21(10.87)	57.90(11.04)	57.91(11.08)	57.90(11.08)
Gender[c]				
Man	370(34.6%)	322(33.7%)	324(33.6%)	324(33.6%)
Women	700(65.4%)	633(66.3%)	640(66.4%)	641(66.4%)
Education[c]				
Primary and below	433(40.5%)	345(36.1%)	352(36.5%)	352(36.5%)
Junior high school	510(47.7%)	486(50.9%)	488(50.6%)	489(50.7%)
Senior high and above	104(9.7%)	101(10.6%)	101(10.5%)	101(10.5%)
Don't know	23(2.1%)	23(2.4%)	23(2.4%)	23(2.4%)
Family income[b] (Ten thousand RMB/year)	1.00(0.25–3.00)	1.00(0.30–3.00)	1.00(0.30–3.00)	1.00(0.30–3.00)
BMI[a] (kg/m^2)	26.26(12.15)	26.32(12.84)	26.30(12.78)	26.30(12.78)
Waistline[b] (cm)	88.0(81.0–94.0)	87.0(80.0–94.0)	87.0(80.0–93.5)	87.0(80.0–93.5)
Hypertension[c]				
No	616(57.6%)	556(58.2%)	560(58.1%)	561(58.1%)
Yes	402(37.6%)	369(38.6%)	374(38.8%)	374(38.2%)
Don't know	52(4.8%)	30(3.1%)	30(3.1%)	30(3.1%)
Urinary fluoride[a] (mg/L)	1.62(1.00)	1.53(0.94)	1.51(0.92)	1.51(0.92)
Serum α1-MG[a] (mg/L)	38.26(10.98)	–	–	–
Serum C3[b] (g/L)	1.17(1.01–1.33)	–	–	–
Serum RBP[b] (μmol/L)	34.85(30.50–39.70)	–	–	–
Urinary NAG[a](U/L)	–	10.39(9.18)	–	–
Serum Urea[a](mmol/L)	–	–	5.54(1.46)	–
Serum UA[a] (μmol/L)	–	–	–	358.68(96.71)

[a] Data were presented as mean(± standard deviation)for continuous variables.
[b] Data were presented as median (P25-P75)for continuous variables.
[c] Number (percentage) for categorical variables.

between independent variables and dependent variables. Excel 2016 (Beijing Kingsoft Office Software, Inc., China) and SPSS25.0 software (SPSS, Inc., Chicago, IL, USA) were used for data processing and analysis. Hypothesis testing for all analyses was based on two-tailed rejection regions, and P-value < 0.05 was applied to declare statistical significance.

3. Results

3.1. Basic characteristics of the participants

Table 1 shows the basic characteristics of the 1070 participants in this study. Among them, there were many more females than males, the proportion was 65.4% and 34.6%, respectively. The participants' mean (± SD) age was 58.21(± 10.87), and most of them reported their educational background as middle school and below. The mean (± SD) BMI of the participants was 26.26(± 12.15).

Some samples were not detected during testing, and the samples actually included are shown in Table 1. The mean urinary fluoride concentration was 1.62 mg/L. The mean (± SD) or median (P25-P75) concentrations of markers kidney function injury (NAG, RBP, Urea, C3, UA and αl-MG) were 10.39 ± 9.18 U/L, 34.85(30.50–39.70) mg/L, 5.54 ± 1.46 mmol/L, 1.1(1.01–1.33) g/L, 358.86 ± 96.32 μmol/L, and 38.26 ± 10.98 mg/L, respectively.

3.2. Multivariate linear regression analysis between urinary fluoride and markers of kidney function

In continuous analyses, every 1 mg/L increment of urinary fluoride was associated with 1.583 U/L increase in NAG after adjusting for covariates. Every 1 mg/L increment of urinary fluoride was associated with 0.199 mmol/L increase in Urea and 0.037 g/L decrease in C3 after adjusting for potential confounding factors.

In the categorical analysis, urinary fluoride concentrations were positively associated with urinary NAG levels (P-trend<0.001). Urinary NAG showed an increased trend with increasing urinary fluoride quartiles, with adjusted β of 2.385 (95%CI: 0.799, 3.993) with 1.38–2.01 mg/L urinary fluoride concentration (quartile 3) and 4.876 (95% CI:3.319, 6.614) with urinary fluoride concentration $>$ 2.01 mg/L (quartile 4), when compared with urinary fluoride concentration $\leq$ 0.90 mg/L (quartile 1). Urinary fluoride concentrations were also positively associated with serum Urea levels (P-trend < 0.001), with adjusted β of 0.414 (95%CI: 0.149, 0.678) with $>$ 2.01 mg/L urinary fluoride concentration (quartile 4) when compared with urinary fluoride concentration $\leq$ 0.90 mg/L (quartile 1). Serum C3 showed an downward trend with increasing urinary fluoride quartiles (Table 2).

In addition, we analyzed the relationship between urinary fluoride and markers of kidney function in different genders, respectively (Table S1). We observed that urinary fluoride was positively associated with urinary NAG and serum Urea in male and female groups, and negatively associated with C3 in male and female groups. However, the relationship between urinary fluoride and serum α1-Mg was different in different sex groups. We also stratified by age to study the relationship between urinary fluoride and markers of kidney function, and the results were consistent with previous studies. And an interesting phenomenon was discovered that in the 46–60 years old group, the increase in urinary fluoride concentration of 1 mg/L resulted in higher increments ($\beta = 1.955$, $P < 0.001$) in urinary NAG compared with the other two groups (Table S2).

Before and after adjusting for urinary fluoride and urinary NAG with urinary creatinine, we found a consistent trend about relationship between urinary fluoride and urinary NAG. The detailed results are shown in Tables S3 and S4.

Table 2
Association between urinary fluoride and markers of kidney function.

UF (mg/L)	Markers of kidney function, *β* (95% *CI*) [d]											
	Urinary NAG	*P*	Serum RBP	*P*	Serum Urea	*P*	Serum C3	*P*	Serum UA	*P*	Serum α1-MG	*P*
Quartile1(≤ 0.90)	Reference		Reference		Reference		Reference		Reference		Reference	
Quartile2 (0.91–1.37)[a]	1.471(− 0.125, 3.067)	0.071	-0.040(− 1.271, 1.192)	0.949	0.021(− 0.219, 0.260)	0.866	-0.026(− 0.066, 0.013)	0.190	0.297(− 14.878, 15.471)	0.969	-1.166 (− 2.963, 0.632)	0.203
Quartile3 (1.38–2.01)[a]	**2.385(0.766, 4.004)**	**0.004**	0.356(− 0.897, 1.610)	0.577	0.234(− 0.012, 0.479)	0.062	-0.022(− 0.062, 0.018)	0.279	3.235(− 12.303, 18.772)	0.683	-1.453(− 3.283, 0.376)	0.376
Quartile4(> 2.01)[a]	**4.876(3.319, 6.614)**	**< 0.001**	0.023(− 1.264, 1.311)	0.971	**0.414(0.149, 0.678)**	**0.002**	**-0.103(− 0.144, −0.061)**	**< 0.001**	14.666(− 2.032, 31.363)	0.085	-1.933(− 3.813, − 0.054)	-0.054
P-trend[b]		**< 0.001**		0.989		**< 0.001**		**< 0.001**		0.300		0.760
Continuous[c]	**1.583(1.092, 2.352)**	**< 0.001**	-0.108(− 0.561, 0.346)	0.642	**0.199(0.102, 0.297)**	**< 0.001**	**-0.037(− 0.051, − 0.022)**	**< 0.001**	3.859(− 2.314, 10.032)	0.220	-0.328(− 0.992, 0.335)	0.332

[a] The assessments of β and 95% *CI* for every quartile increment of urinary fluoride.
[b] P for trend were estimated by One-way ANOVA polynomial linear trend.
[c] The assessments of β and 95% CI for every 1 mg/L increment of urinary fluoride.
[d] Adjustment: age, gender, education, family income, BMI, waistline and hypertension.

Table 3
Association between urinary fluoride and kidney function was studied by NAG.

UF (mg/L)	≤ 14.6 (Normal) N (%)	> 14.6 (Kidney dysfunction) N (%)	Crude, OR (95% *CI*)	*P*	Adjusted[d], OR (95% *CI*)	*P*
Quartile1(≤ 0.90)	221(28.9%)	36(18.9%)	Reference		Reference	
Quartile2(0.91–1.37)[a]	207(27.1%)	50(26.3%)	1.483(0.928, 2.369)	0.099	1.385(0.863, 2.223)	0.177
Quartile3(1.38–2.01)[a]	193(25.2%)	49(25.8)	1.559(0.973, 2.498)	0.065	1.393(0.863, 2.250)	0.175
Quartile4(> 2.01)[a]	144(18.8%)	55(28.9%)	**2.345(1.466, 3.750)**	**< 0.001**	**2.014(1.248, 3.250)**	**0.004**
P-trend[b]				**0.001**		**0.001**
Continuous[c]	765	190	**1.279(1.095, 1.493)**	**0.002**	**1.228(1.047, 1.439)**	**0.011**

OR: odds ratio; *CI*: confidence interval.
[a] The assessments of OR and 95% *CI* for every quartile increment of urinary fluoride.
[b] P-trend were estimated by binary logical regression.
[c] The assessments of OR and 95% *CI* for every 1 mg/L increment of urinary fluoride.
[d] Adjustment: age, waistline.

Table 4
Association between urinary fluoride and kidney function was studied by serum Urea.

UF (mg/L)	≤ 7.14 (Normal) N (%)	> 7.14 (Kidney dysfunction) N (%)	Crude, OR (95% *CI*)	*P*	Adjusted[d], OR (95% *CI*)	*P*
Quartile1(≤ 0.90)	241(28.3%)	24(21.4%)	Reference		Reference	
Quartile2(0.91–1.37)[a]	233(27.3%)	28(25.0%)	1.207(0.680, 2.143)	0.521	1.097(0.610, 1.971)	0.757
Quartile3(1.38–2.01)[a]	212(24.6%)	30(26.8%)	1.421(0.806, 2.507)	0.225	1.302(0.729, 2.328)	0.373
Quartile4(> 2.01)[a]	166(19.5%)	30(26.8%)	**1.815(1.024, 3.215)**	**0.041**	1.526(0.844, 2.760)	0.162
P-trend[b]				**0.033**		**0.033**
Continuous[c]	852	112	**1.244(1.029, 1.504)**	**0.024**	1.162(0.954, 1.416)	0.135

OR: odds ratio; *CI*: confidence interval.
[a] The assessments of OR and 95% *CI* for every quartile increment of urinary fluoride.
[b] P-trend were estimated by binary logical regression.
[c] The assessments of OR and 95% *CI* for every 1 mg/L increment of urinary fluoride.
[d] Adjustment: age, gender.

3.3. Binary logical regression analysis between urinary fluoride and markers of kidney function

Table 3 shows the relationship between urinary fluoride and kidney function based on urinary NAG. The basic characteristics of the population are presented in Table S5. In continuous analyses, we observed significant increases of 22.8% in the risk of kidney function injury (the OR was1.228) for every 1 mg/L increment in the urinary fluoride concentrations after adjusting for potential confounding factors. In categorical analyses, we observed an increasing trend of the risk of kidney function injury with increasing quartiles of urinary fluoride concentrations ($P < 0.001$ for trend over quartiles).

Table 4 shows the relationship between urinary fluoride and kidney function based on Urea. The basic characteristics of the population are presented in Table S6. In continuous analyses, we observed significant increases of 24.4% in the risk of kidney injury (the OR was 1.244) for every 1 mg/L increment in the urinary fluoride concentrations (Significant only before adjusting for confounders). However, there was a significantly positive trend that increased risk of kidney function injury with increasing quartiles of urinary fluoride concentrations ($P = 0.033$ for trend).

Table 5 shows the relationship between urinary fluoride and kidney function based on C3. The basic characteristics of the population are presented in Table S7. In continuous analyses, we observed significant increases of 34.4% in the risk of kidney function injury (the OR was 1.344) for every 1 mg/L increment in the urinary fluoride concentrations. However, the results were reversed in categorical analyses.

3.4. Sensitivity analysis

Sensitivity analysis for the associations between urinary fluoride concentration and markers of kidney function (urinary NAG, serum

Table 5
Association between urinary fluoride and kidney function was studied by serum C3.

UF (mg/L)	≧ 0.79 (Normal) N (%)	< 0.79 (Kidney dysfunction) N (%)	Crude, OR (95% *CI*)	*P*	Adjusted[d], OR (95% *CI*)	*P*
Quartile1(≤ 0.90)	255(25.0%)	11(21.6%)	Reference		Reference	
Quartile2(0.91–1.37)[a]	264(25.9%)	10(19.6%)	1.139(0.475, 2.728)	0.771	1.133(0.471, 2.726)	0.780
Quartile3(1.38–2.01)[a]	254(24.9%)	7(13.7%)	1.565(0.597, 4.102)	0.362	1.556(0.592, 4.090)	0.370
Quartile4(> 2.01)[a]	246(24.1%)	23(%)	**0.461(0.220, 0.967)**	**0.040**	**0.435(0.206, 0.922)**	**0.030**
P-trend[b]				**0.033**		**0.033**
Continuous[c]	1019	51	**1.321(1.020, 1.711)**	**0.035**	**1.344(1.034, 1.748)**	**0.027**

OR: odds ratio; *CI*: confidence interval.
[a] The assessments of OR and 95% *CI* for every quartile increment of urinary fluoride.
[b] P-trend were estimated by binary logical regression.
[c] The assessments of OR and 95% *CI* for every 1 mg/L increment of urinary fluoride.
[d] Adjustment: gender, hypertension.

Urea, and serum C3) by adjusting for the covariates were similar results with the primary analyses (Table S8).

4. Discussion

We examined the relationship between urinary fluoride concentrations and levels of markers of kidney function (urinary NAG, serum RBP, serum Urea, serum C3 serum UA and serum α1-MG) in a cross-sectional study of endemic fluorosis in China. We found that the levels of urinary NAG and serum Urea increased significantly with the increase of urinary fluoride concentration, urinary fluoride was negatively correlated with C3 in adults. Specifically, an increment of 1 mg/L in urinary fluoride was associated with 1.583 U/L increase in urinary NAG and 0.199 mmol/L increase in serum Urea after adjusting for potential confounding factors. And higher levels of urinary fluoride were associated with an increased risk of kidney function injury.

NAG is mainly distributed in the cytoplasm of the proximal tubular cell, and there was a significant increase in NAG in urine when renal tubule injury. Because of its sensitivity and accurate prediction, NAG has been widely concerned and used in clinical nephropathy examination (Yan et al., 2019). In both the linear regression model and the binary logistic regression model, there was a significantly positive relationship between urinary NAG activity and urinary fluoride concentrations in our study, which is consistent with previous studies (Xiong et al., 2007). Thus, urinary NAG can be used for early assessment of renal injury in areas of endemic fluorosis.

Urea is a waste product of nitrogen-containing compounds, metabolized by the liver and excreted in urine. High serum Urea levels may reflect kidney dysfunction. Our study found a positive correlation between urinary fluoride concentrations and serum Urea level($\beta = 0.199$, $P < 0.001$) , but a study in the United States has shown the opposite which in higher water fluoride concentrations were associated with lower serum Urea ($\beta = -0.93$, $P = 0.007$) among teenagers after linear regression models adjusted for covariates (Malin et al., 2019). The possible reason for the inconsistent results is the difference in the study population. Our study subjects were adults with an average age of 58.21 (10.87), while their study subjects were adolescents with an average age of 15.32 (0.07). The duration of fluoride exposure of these two groups is different, and the body's ability to metabolize fluoride is also different. Another difference is that they studied the relationship between fluoride concentrations in water and serum Urea, not urinary fluoride concentration. Urinary fluoride, as a reaction of internal exposure in the body, can better reflect the real situation of fluoride exposure and metabolism.

C3 plays an important role in the progression of kidney injury in human hypertensive nephropathy (Cui et al., 2017). A complement-mediated C3 glomerulopathy with predominant C3 deposition was proved to be one of the mechanisms of membranoproliferative glomerulonephritis (Riedl et al., 2017; Sethi and Fervenza, 2011). Our study found a weakly negative correlation between urinary fluoride concentrations and serum C3 ($\beta = -0.037$, $P < 0.001$), which may be related to glomerular deposition of C3. It is necessary to further explore serum C3 as a marker of kidney injury in subsequent studies.

In contrast, urinary fluoride levels were not significantly associated with serum RBP and serum UA of markers of kidney function. Studies have shown that serum RBP and UA level is a biomarker of patients with acute or chronic kidney disease, mainly used for clinical stage of patients (Andreucci et al., 2017; Goodman, 1980; Kang, 2018). Our study population is a natural population, not patients with clinical kidney disease. The study examined the relationship between fluoride exposure and kidney damage, which is more likely to be expressed as early (subclinical) damage.

Our findings showed that age and gender modified the association between fluoride exposure and marker of kidney function. We found that the increase in urinary fluoride concentrations of 1 mg/L was associated with higher increments ($\beta = 1.955$, $P < 0.001$) in urinary NAG in the 46–60 years old group compared with the other two groups. Urinary fluoride levels were not significantly associated with serum αl-MG of markers of kidney function. But the relationship between urinary fluoride and serum α1-MG was significant in the women group, which is an interesting phenomenon and can be studied in more detail in the future. Animal studies have shown that the activity of NAG in mouse kidneys, urine and plasma was correlated with age and sex (Funakawa et al., 1987). The 46–60 years of age is the stage of menopause for most people, and their specific physiological changes may lead to higher urinary NAG, which needs to be confirmed by further studies.

Our study has several strengths. Our study shows a natural population-based study in China to examine the relationship between chronic fluoride exposure and kidney function related markers among adults, and investigate fluoride exposure and early impairment of kidney function. Our study found a very stable linear correlation between urinary fluoride concentrations and urinary NAG and serum Urea in adult after sensitivity analysis, which will provide a scientific basis for the establishment of a more accurate kidney function screening program in fluorosis areas. Our study provides a basis for further evaluation and formulation of safety guidelines for fluoride exposure.

This study had several limitations. Our study was a cross-sectional investigation and is incomplete adequacy as evidence to explain the kidney toxicity of fluoride, and more longitudinal studies are needed. In our study, known factors for kidney function injury were not all collected for covariate control, which makes our results likely to be questioned by other researchers. However, this is also the disadvantage of population research compared with animal experimental research, that is, the influencing factors caused by individual differences are difficult to be controlled artificially or fully understood by researchers. Fortunately, we did our best to collect important covariates.

5. Conclusions

In conclusion, our study suggests that long-term fluoride exposure is associated with kidney function in adults. Our study found that urinary fluoride concentrations were positively correlated with both urinary NAG and serum Urea in adult, and every 1 mg/L increment of urinary fluoride was associated with 1.583 U/L increase in urinary NAG and 0.199 mmol/L increase in serum Urea after adjusting for potential confounding factors. And urinary NAG, a sensitive and robust marker of kidney dysfunction caused by fluoride exposure, could be considered for the identification of early kidney injury. Therefore, these findings will provide a theoretical basis for countries and regions with fluorosis to establish relevant health policies.

CRediT authorship contribution statement

Liaowei Wu: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Visualization. **Chenlu Fan:** Methodology, Validation, Resources, Writing – original draft. **Zaihong Zhang:** Formal analysis, Resources, Validation. **Xin Zhang:** Formal analysis, Resources, Validation. **Qun Lou:** Investigation. **Ning Guo:** Investigation. **Wei Huang:** Investigation. **Meichen Zhang:** Investigation. **Fanshuo Yin:** Investigation. **Zhizhong Guan:** Supervision, Writing – review & editing. **Yanmei Yang:** Conceptualization, Methodology, Writing – original draft, Writing – review & editing, Project administration. **Yanhui Gao:** Conceptualization, Supervision, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2021.112735.

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