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The cholinergic system, intelligence, and dental fluorosis in school-aged children with low-to-moderate fluoride exposure

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ABSTRACT

Disruption of cholinergic neurotransmission can affect cognition, but little is known about whether low-to-moderate fluoride exposure affects cholinergic system and its effect on the prevalence of dental fluorosis (DF) and intelligence quotient (IQ). A cross-sectional study was conducted to explore the associations of moderate fluoride exposure and cholinergic system in relation to children's DF and IQ. We recruited 709 resident children in Tianjin, China. Ion selective electrode method was used to detect fluoride concentrations in water and urine. Cholinergic system was assessed by the detection of choline acetyltransferase (ChAT), acetylcholinesterase (AChE) and acetylcholine (ACh) levels in serum. Compared with children in the first quartile, those in fourth quartile the risk of either developing DF or IQ < 120 increased by 19% and 20% for water and urinary fluoride. The risk of having both increased by 58% and 62% in third and fourth quartile for water fluoride, 52% and 65% for urinary fluoride. Water fluoride concentrations were positively associated with AChE and negatively associated with ChAT and ACh, trends were same for urinary fluoride except for ACh. The risk of either developing DF or having non-high intelligence rose by 22% (95%*CI*: 1.07%, 1.38%) for the fourth quartile than those in the first quartile of AChE, for having the both, the risk was 1.27 (95%*CI*: 1.07, 1.50), 1.37 (95%*CI*: 1.17, 1.62) and 1.44 (95%*CI*: 1.23, 1.68) in second, third and fourth quartiles. The mediation proportion by AChE between water fluoride and either developing DF or IQ < 120 was 15.7%. For both to exist, the proportion was 6.7% and 7.2% for water and urinary fluoride. Our findings suggest low-to-moderate fluoride exposure was associated with dysfunction of cholinergic system for children. AChE may partly mediate the prevalence of DF and lower probability of having superior and above intelligence.

1. Introduction

Fluoride is widely distributed in the water, soil and atmosphere in our daily life. Appropriate fluoride intake is useful for the development of bone and teeth, however, long-term excessive fluoride exposure can lead to fluorosis that are characterized by dental fluorosis (DF) and skeletal fluorosis (Death et al., 2015; Kebede et al., 2016; Srivastava and Flora, 2020). It is estimated that endemic fluorosis areas involved 25 nations over the world, and about 200 million people may suffer from fluorosis due to fluoride contamination in groundwater including China

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(Chen et al., 2012; Jha et al., 2013; Rahman et al., 2020; Verma et al., 2017). Although defluoridation projects have been implemented by local governments, the prevalence of DF is still a serious social problem in some areas of the world (Irigoyen-Camacho et al., 2016; Mandinic et al., 2010; Martignon et al., 2021; Saha et al., 2021).

In addition to skeletal damage, excessive fluoride can also cause serious lesions to other soft tissues such as brain, liver, and so on (Malin et al., 2019; Sun et al., 2014), and fluoride neurotoxicity has attracted extensive attention in recent years. Researches from animal experiments showed that excessive fluoride exposure in drinking water can lead to decreased learning and memory ability in rats (Jiang et al., 2014; Qiu et al., 2020). Other studies showed that fluoride exposure caused neuronal degeneration and neurotransmitter activity alteration (Dong et al., 2015). Consistently, accumulating epidemiological investigations showed that the intelligence quotients (IQ) of children were negatively associated with fluoride concentrations (Choi et al., 2012; Duan et al., 2018; Wang et al., 2020). Moreover, our previous findings further suggested that fluoride concentrations were negatively associated with the odds of having excellent intelligence in girls (Zhou et al., 2021). Nevertheless, a prospective study of Broadbent et al. showed that no clear IQ differences caused by fluoride exposure were noted (Broadbent et al., 2015). Till date, the associations between fluoride exposure and IQ scores were controversial, which could be attributed to the differences in sample size, exposure concentration, and geographical location of the subjects. To reduce the health damage caused by fluoride, the World Health Organization (WHO) sets 1.5 mg/L as the upper limit of fluoride concentration in human drinking water (World Health Organization, 2017). China has even stricter standard that set 1.0 mg/L as the maximum concentration (Zhu et al., 2006).

Synapses are the most basic functional units of the brain that transmit information by mediating effective electrical or chemical signals (Rizalar et al., 2021). Acetylcholine (ACh) is the first identified cholinergic neurotransmitter, and the alteration of its content can fully reflect the growth and development of the body and the ability of learning and memory (Picciotto et al., 2012). ACh is synthesized by acetyl CoA and choline in the cytoplasm catalyzed by choline acetyltransferase (ChAT) and then transferred to synaptic vesicle. When the presynaptic membrane is depolarized, ACh enters the synaptic cleavage and binds to receptors on the postsynaptic membrane, which was then hydrolyzed by acetylcholinesterase (AChE). Studies have shown that disruption of cholinergic neurotransmission can affect cognition and behavioral processes (Hampel et al., 2018; Pabst et al., 2016; Solari and Hangya, 2018). More importantly, it has been found that excessive fluoride exposure can lead to decreased degree of synaptic connection and insufficient ability of synaptic neurotransmitters (Niu et al., 2018). Our recent study also found that excessive fluoride exposure during development led to decreased learning and memory ability in rats, accompanied by reduced dendritic branches, lower density of dendritic spines, and decreased expression of synaptic structural and functional marker proteins in CA1 region of rat hippocampus (Chen et al., 2018). Enamel dysplasia caused by excessive fluoride intake during tooth embryo development is the cause of DF (Fejerskov et al., 1990). Studies have shown that inflammatory cytokines can interfere with the maturation of ameloblast (Noda et al., 2018; Yamazaki et al., 2018). In recent years, the anti-inflammatory effects of the cholinergic system have been increasingly reported (Han et al., 2017; Hoover, 2017; Li et al., 2020). However, epidemiological analyses of the associations between fluoride exposure, cholinergic system, DF, and IQ were not found.

Although there are many studies on the effects of fluoride on DF and IQ, the exact mechanism is not clear. In the present study, a cross-sectional study was performed to explore the associations between low-to-moderate fluoride exposure and cholinergic system in relation to DF and IQ, most important, the combined toxicity of fluoride in children was reflected by integrating DF and IQ into the outcome.

2. Materials and methods

2.1. Study design and population

The subjects were from a cross-sectional study in rural areas of Tianjin, China in 2015. Surveillance data from the local Center for Disease Control and Prevention shows that fluoride concentrations in water remained stable over the past years. Stratified multi-stage random sampling and cluster sampling methods were used for sampling. First, seven towns were selected by simple random sampling method (SRS), and there were three historical high fluoride areas and four non-endemic areas, then 24 villages were randomly selected from each town. Finally, 709 subjects were recruited by the cluster sampling method. All participants and their guardians signed the informed consent with full knowledge of our program. The study was approved by the Review Board of Huazhong University of Science and Technology and Ethical Committee of Tianjin Center for Disease Control and Prevention.

2.2. General data collection

Trained professionals collected basic characteristics of subjects and their parents through face-to-face interview, including age, gender, physical residence, paternal education, maternal education, parental occupation, family incomes, health condition during pregnancy and delivery conditions such as premature birth, post-term birth, hypoxia, dystocia and low birth weight. Body mass index (BMI) calculated from height and weight was used to assess the children's development status.

2.3. Sample collection

All subjects' fasting peripheral blood samples (5 mL) were collected with polyethylene Na-EDTA tubes. Besides, drinking water samples were collected using a polyethylene tube (50 mL) from the local source of water supply in each village. In addition, spot (early-morning) urine samples were collected using a polyethylene tube (50 mL). All samples were transported with icebox to the laboratory within 2 h, and stored at − 80 °C for next analysis.

2.4. Determination of fluoride concentrations

F^- concentrations of water and urine were measured by ion analyzer that with a fluoride selective electrode (INESA, Shanghai, China). The minimum detection value of the F^- selected electrode was 0.01 mg/L. Measure machine were calibrated by standard fluoride solutions prepared from 1000 mg/L stock solution. The range of the calibration solutions were 0.1–50.0 mg/L (0.1, 0.2, 0.5, 1.0, 2.0, 5.0, 10.0, 20.0 and 50.0 mg/L). Double-distilled deionized water was used for all the reference regents. Parallel samples were set for each subject and then averages were calculated for analysis.

2.5. Measurement of urine creatinine

Creatinine determination kit (Mindray, Shenzhen, China) was used to detect the concentration of urine creatinine. Frozen urine samples were defrosted at room temperature and centrifuged at 3000 rpm for 10 min, then 100 μL supernatant were picked up from each sample and taken into a 1.5 mL centrifuge tube and diluted with 900 μL double-distilled deionized water. Each round of testing included 39 samples, standard A, standard B, and blank control. The dilution was placed into the automatic biochemical analyzer (Mindray, Shenzhen, China) for testing according to the kit instructions.

2.6. Assessment of IQ

Combined Raven's Test-The Rural in China (CRT-RC2) (Liu et al., 2009) was used to measure children's IQ scores. CRT-RC2 is widely used for cognitive ability verification test, because of less influenced by language, culture, ethnic, and religion differences. Briefly, it comprises of six groups A, AB, B, C, D and E in total of 72 questions. The test was completed within 40 min according to the instruction manual. For each round of testing, 40 children were assigned to a clean, bright classroom randomly, and taken the test independently, under the strict supervision of trained teachers who were blinded to the children's exposure status. IQ scores of the participants were categorized as follows: dull normal and below ($\leq$ 89), normal (90–109), high normal (110–119), superior and above ($\geq$ 120).

2.7. Assessment of DF

The diagnosis of DF was estimated by Dean's classification system (Molina-Frechero et al., 2015). Each subject was diagnosed by two trained and professional dentists who were blinded to the children's exposure status independently. The diagnosis of dental conditions is performed under bright natural light after the teeth were cleaned and dried, CPI probes were used according to the recommendations of the WHO criteria. The grade of DF was divided into the following levels: severe, moderate, mild, very mild, questionable and normal. Only when the judgements of two dentists were in agreement will a conclusion be reached, otherwise, another dentist will join to make recommendations.

2.8. Measurements of cholinergic system

The serum was stored at − 80 °C until measured. Enzyme-linked immunosorbent assays (Shanghai Enzyme-linked Biotechnology, Shanghai, China) were used to detect the expression of cholinergic system. All detection was performed using reagents provided by Enzyme-linked Biotechnology (Shanghai, China) according to the manufacturer's operating procedures, the detection was completed by multifunctional enzyme marker (BioTek, Winooski, USA). Cholinergic system was assessed by choline acetyl transferase [ChAT, (pg/mL)], acetylcholine esterase [AChE, (nmol/L)] and acetyl choline [ACh, (nmol/L)].

2.9. Statistical analysis

Descriptive statistics of the basic demographic information in the analysis according to the types of the variables, such as age, gender, height, weight, BMI, parent education level, family income level, etc. Mean ± standard deviance (SD) for continuous variables and frequency [proportion (%)] for categorical variables. As for the descriptive analysis of water fluoride, urine fluoride, urine creatinine, ChAT, AChE and ACh, they were presented with minimum, 10 th, 25 th, 50 th, 75 th, 90 th percentiles and maximum. Studies have shown that the prevalence of DF in the fluoride-exposed populations is > 10%, so log-binomial model was used to evaluate the relationship between fluoride exposure and DF, so as cholinergic system and DF (Martinez et al., 2017). Multiple linear regression models were used to assess the associations between quartiles of fluoride exposure and cholinergic system and IQ scores, as well as associations between quartiles of cholinergic system and IQ scores. Trend tests were conducted by setting the median of each quartile as continuous variable in the multiple linear regression models (Greenland, 1995). The association between fluoride exposure and IQ levels was assessed by multiple logistic regression model, so as cholinergic system and IQ levels. In the logistic regression model, children presented 31% and 33% lower probability of developing superior and above intelligence with every 1 mg/L increase of water and urinary fluoride respectively. As showed in our previous study, the distributions of IQ scores between normal group (water fluoride $\leq$ 1.0 mg/L) and high-fluoride-exposure group (water fluoride > 1.0 mg/L) were notably different, especially for the proportion of IQ scores over 120 (Yu et al., 2018). Then, to study the effect of fluoride on children's IQ level of superior and above we set 120 as the cut-off point of IQ scores, and combined with the prevalence of DF (very mild, mild, moderate dental fluorosis that presented in our subjects were included), the children are divided into the following groups: control group (IQ $\geq$ 120, normal), either DF or IQ < 120, DF and IQ < 120.

Log-binomial regression model was used to assess the relationship between fluoride exposure and DF/IQ, as well as association between cholinergic system and DF/IQ. Moreover, in order to explore the impact of cholinergic system in the association between fluoride exposure and the prevalence of DF and lower probability of developing superior and above intelligence, mediation analysis was conducted, the details were obtained from https://www.hsph.harvard.edu/donna-spiegelman/software/mediate/ (Lin et al., 1997). Confounders were selected based on current literature of covariates that could influence cholinergic system, DF and IQ in children (Green et al., 2019; Yu et al., 2018; Zhao et al., 2021). Finally, we kept age, gender, BMI, parental education level, financial situation, low birth weight and urine creatinine (for urinary fluoride) as the covariates. Results were presented as regression coefficients (β) or odds ratios (*OR*s) or prevalence ratios (*PR*s) with 95% confidence intervals (95% *CI*s).

We used epidata version 3.0 for database build. Data analysis was performed with SPSS (version 25.0), Stata (version 15.0) and the SAS software package (version 9.4, SAS Institute Inc). Two-sided tests were performed for all analyses and $\alpha = 0.05$ was used as the test criterion.

3. Results

3.1. Characteristics of the participants

A total of 709 school children aged 6.70–13.00 were recruited by random sampling. The geographic characteristics of the subjects were listed in Table 1. The proportion of boys and girls was 53.74% and 46.26%. The mean ± SD of age and BMI (kg/m^2) was 9.86 ± 1.16 years and 17.47 ± 3.50 kg/m^2. There were 3.39% of the subjects (n = 24) having low birth weight. Their average living time in the studied area was 9.60 ± 1.52 years. Most of their parents have a primary and below school education level (99.44%) with yearly households below 30,000 (97.46%).

Table S1 presents the descriptive statistics for concentrations of water fluoride, urinary fluoride, urine creatinine, cholinergic system (ChAT, AChE, ACh). The water fluoride concentration ranged from 0.20 mg/L to 3.90 mg/L, with a mean value of 1.20 ± 0.95 mg/L, and the median was 1.00 mg/L. The mean ± SD of the urinary fluoride was 0.66 ± 0.67 mg/L, with the range from 0.02 mg/L to 5.41 mg/L. Meanwhile, the urine creatinine ranged from 0.30 mg/L to 2.99 mg/L, with a mean value of 1.16 ± 0.62 mg/L. The mean ± SD of cholinergic systems were 210.12 ± 75.44 pg/mL for ChAT, 158.86 ± 35.44 nmol/L for AChE and 376.12 ± 77.78 nmol/L for ACh.

3.2. Association between fluoride exposure and DF/IQ

The association between fluoride exposure and IQ scores was presented in Table S2. In categorical analyses, compared with the subjects in the first quartile of water concentration, those in the third and highest quartiles presented decrease of 2.77 and 4.10 in IQ scores [$\beta = -2.77$ (95% *CI*: −5.44, −0.10) for third quartile and $\beta = -4.10$ (95% *CI*: −6.71, −1.48) for the highest quartile, $P < 0.001$ for trend test]. As for urinary fluoride, compared with the first quartile, participants in the third and highest quartiles presented decrease of 3.02 and 4.49 in IQ scores [$\beta = -3.02$ (95% *CI*: −5.71, −0.33) for third quartile and $\beta = -4.49$ (95% *CI*: −7.21, −1.77) for fourth quartile, $P < 0.001$ for trend test]. Similar associations between fluoride concentration and IQ scores were observed in male and female respectively.

In logistic regression model, children presented 31% (95% *CI*: 0.10, 0.46) and 33% (95% *CI*: 0.03, 0.54) lower probability of developing superior and above intelligence with every 1 mg/L increase of water and urinary fluoride. (Table S3).

Table 1
Basic characteristics of general population.

Variables	Means ± SD or n (percentage)
Sample size	709
Age (years)[a]	9.86 ± 1.16 (6.70–13.00)
Gender[b]	
Boys	381 (53.74%)
Girls	328 (46.26%)
Height (cm)[a]	142.22 ± 8.76
Weight (kg)[a]	35.86 ± 10.26
Body mass index (kg/m^2)[a]	17.47 ± 3.50
Low birth weight	24 (3. 39%)
Years of residence (years)[a]	9.60 ± 1.52
Household income (RMB/year)[b]	
< 10,000	57 (8.04%)
10,000–30,000	634 (89.42%)
> 30,000	18 (2.54%)
Paternal education[b]	
Primary and below	704 (99.30%)
High school	4 (0.56%)
Junior college and above	1 (0.14%)
Maternal education[b]	
Primary and below	706 (99.58%)
High school	2 (0.28%)
Junior college and above	1 (0.14%)
IQ scores[a]	106.86 ± 12.66
IQ levels[b]	
Superior and above (≥ 120)	115 (16.22%)
High normal (110–119)	171 (24.12%)
Normal (90–109)	368 (51.90%)
Dull normal and below (70–89)	55 (7.76%)
DF level[b]	
Normal	308 (43.44%)
Very mild	112 (15.80%)
Mild	250 (35.26%)
Moderate	39 (5.50%)

Abbreviation: RMB, renminbi, is the official currency of the People's Republic of China, and its basic unit is yuan.
[a] Data were presented as mean ± SD for continuous variables.
[b] Number (percentage) for categorical variables.

As for DF, in categorical analyses, compared with the first quartile for water fluoride, the risk of developing DF was 3.79 (95% *CI*: 2.90, 4.95) and 3.97 (95% *CI*: 3.04, 5.17) in third and fourth quartile respectively. Similar association between the risk of developing DF and urinary fluoride was observed, with the PR were 1.66 (95% *CI*: 1.28, 2.14), 2.73 (95% *CI*: 2.17, 3.44) and 3.24 (95% *CI*: 2.58, 4.07) for the second, third, and fourth quartiles (Table S4).

Taken DF and IQ together, in the categorical analyses, compared with the first quartile, those in the fourth quartile the risk of either developing DF or IQ < 120 were increased by 19% (95% *CI*: 1.06, 1.34) and 20% (95% *CI*: 1.05, 1.37) for water and urinary fluoride respectively. In addition, as for the risk of both having DF and IQ < 120, compared with the first quartile the *PRs* were 1.58 (95% *CI*: 1.29, 1.94) and 1.62 (95% *CI*: 1.33, 1.98) in third and fourth quartiles for water fluoride, 1.52 (95% *CI*: 1.28, 1.82) and 1.65 (95% *CI*: 1.38, 1.96) for urinary fluoride (Table 2).

3.3. Association between fluoride exposure and cholinergic system

Adjusted assessments for fluoride exposure and cholinergic system were presented in Table 3. In the categorical analyses, water fluoride concentrations were positively associated with AChE ($P < 0.001$ for trend test) and negatively associated with ChAT and ACh ($P = 0.008$ and 0.017 for trend test, respectively), in addition, urinary fluoride concentration was positively associated with AChE ($P = 0.013$ for trend test) and negatively associated with ChAT ($P = 0.013$ for trend test) (Table 3).

3.4. Association between cholinergic system and IQ

In our present study, AChE concentrations were negatively

Table 2
Association between fluoride exposure and DF/IQ.

Fluoride content	Crude		Adjusted[a]	
	PR (95% *CI*)	*P* value	*PR* (95% *CI*)	*P* value
Either DF or IQ < 120				
Water fluoride				
Q1 (≤ 0.30)	Reference		Reference	
Q2 (0.30–1.00)	0.88 (0.78, 1.23)	0.061	0.89 (0.79, 1.00)	0.050
Q3 (1.00–1.60)	1.08 (0.95, 1.23)	0.240	1.08 (0.95, 1.21)	0.245
Q4 (> 1.60)	**1.15 (1.05, 1.27)**	**0.004**	**1.19 (1.06, 1.34)**	**0.002**
Urinary fluoride				
Q1 (≤ 0.20)	Reference		Reference	
Q2 (0.20–0.48)	0.94 (0.81, 1.07)	0.346	0.97 (0.85, 1.10)	0.628
Q3 (0.48–0.90)	1.08 (0.95, 1.22)	0.254	1.13 (0.98, 1.31)	0.090
Q4 (>0.90)	**1.15 (1.03, 1.29)**	**0.017**	**1.20 (1.05, 1.37)**	**0.007**
DF and IQ < 120				
Water fluoride				
Q1 (≤ 0.30)	Reference		Reference	
Q2 (0.30–1.00)	0.93 (0.71, 1.22)	0.595	0.92 (0.71, 1.20)	0.558
Q3 (1.00–1.60)	**1.60 (1.30, 1.97)**	**< 0.001**	**1.58 (1.29, 1.94)**	**< 0.001**
Q4 (> 1.60)	**1.63 (1.33, 2.00)**	**< 0.001**	**1.62 (1.33, 1.98)**	**< 0.001**
Urinary fluoride				
Q1 (≤ 0.20)	Reference		Reference	
Q2 (0.20–0.48)	1.13 (0.92, 1.39)	0.253	1.19 (0.98, 1.44)	0.083
Q3 (0.48–0.90)	**1.44 (1.20, 1.72)**	**< 0.001**	**1.52 (1.28, 1.82)**	**< 0.001**
Q4 (> 0.90)	**1.50 (1.26, 1.79)**	**< 0.001**	**1.65 (1.38, 1.96)**	**< 0.001**

Abbreviation: Q, quartile; *PR*, prevalence ratio; *CI*, confidence interval. Bold indicates statistically significant.
[a] Adjustment: age, gender, BMI, low birth weight, paternal education, maternal education, family incomes, urine creatinine (for urinary fluoride).

Table 3
Association between fluoride exposure and cholinergic system.

Fluoride content (mg/L)	Cholinergic systems, β (95% *CI*)[a]		
	ChAT (pg/mL)	AChE (nmol/L)	ACh (nmol/L)
Water fluoride			
Q1 (≤ 0.30)	Reference	Reference	Reference
Q2 (0.30–1.00)	− 1.49 (−16.64, 13.67)	2.82 (−4.23, 9.88)	− 8.00 (−23.50, 7.51)
Q3(1.00–1.60)	**−19.14 (−35.30, −2.98)**	**13.04 (5.51, 20.58)**	**−17.37 (−33.92, −0.83)**
Q4(> 1.60)	**−18.96 (−34.81, −3.12)**	**16.42 (9.04, 23.81)**	**−19.21 (−35.43, −3.00)**
Trend test	**0.008**	**< 0.001**	**0.017**
Urinary fluoride			
Q1 (≤ 0.20)	Reference	Reference	Reference
Q2 (0.20–0.48)	− 9.39 (−25.31, 6.54)	2.05 (−5.45, 9.56)	− 0.91 (−17.31, 15.50)
Q3 (0.48–0.90)	− 11.05 (−27.24, 5.13)	4.89 (−2.74, 12.52)	− 5.57 (−22.24, 11.10)
Q4 (> 0.90)	**−23.57 (−39.93, −7.22)**	**9.04 (1.33, 16.75)**	− 10.52 (−27.37, 6.33)
Trend test	**0.013**	**0.013**	0.365

Abbreviation: Q, quartile; β, regression coefficient; *CI*, confidence interval. Bold indicates statistically significant.
[a] Adjustment: age, gender, BMI, low birth weight, paternal education, maternal education, family incomes, urine creatinine (for urinary fluoride).

associated with IQ scores ($P < 0.001$ for trend test), with adjusted β were − 3.18 (95% *CI*: −5.79, −0.56) for second quartile, − 2.73 (95% *CI*: −5.53, −0.13) for third quartile and − 4.09 (95% *CI*: −6.72, −1.46) for the highest quartile (Table 4). Similar associations between IQ scores and AChE concentration were observed in male and female respectively. However, the association between ChAT and IQ scores was not significant, nor was ACh.

Multiple logistic regression analyses showed that compared with children with the first quartile of AChE, those in the third and fouth quartile presented 53% (95% *CI*: 0.06, 0.76) and 46% (95% *CI*: 0.00, 0.71) lower probability of developing superior and above intelligence. At the same time, children with the highest AChE quartile presented 68% higher likelihood of having IQ scores that fell in the dull normal and below range (Table S5).

3.5. Association between cholinergic system and DF

Table 5 presented the association between cholinergic system and DF prevalence, the prevalence of DF was positively associated with AChE concentration ($P < 0.001$ for trend test) and negatively associated with ChAT and ACh contents ($P < 0.001$ for trend test respectively).

3.6. Association between cholinergic system and DF/IQ

Table 6 presented the association between cholinergic system and DF/IQ. In categorical analyses, when adjusting for potential confounding factors, children in the fourth quartile presented 22 (95% *CI*: 1.07, 1.38) higher probability of developing DF or IQ < 120 than those in the first quartile. For the risk of both developing DF and IQ < 120 the *PRs* were 1.27 (95% *CI*:1.07, 1.50), 1.37 (95% *CI*: 1.17, 1.62) and 1.44 (95% *CI*: 1.23, 1.68) for children in the second, third and fourth quartiles when compared to those in the first quartile.

3.7. The mediation effect of AChE in the association between fluoride exposure and DF/IQ

Significant mediation by AChE concentration was observed in the models that associating fluoride exposure with DF and IQ (Fig. 1). The proportion of mediation by AChE between water fluoride and either developing DF or IQ < 120 was 15.7% (95% *CI*: 4.4%, 43.2%), as for urinary fluoride, the mediation effect was not significant. For the children who both having DF and IQ < 120 the proportion of mediation were 6.7% (95% *CI*: 2.8%, 15.0%) and 7.2% (95% *CI*: 2.2%, 20.9%) for water and urinary fluoride respectively.

3.8. Sensitivity analysis

Sensitivity analyses were conducted for the association between

Table 5
Association between cholinergic system and the prevalence of DF.

Cholinergic system	Crude		Adjusted[a]	
	PR (95%*CI*)	*P*	*PR* (95%*CI*)	*P*
DF				
ChAT (pg/mL)				
Q1 (≤ 154.12)	Reference		Reference	
Q2 (154.12–210.87)	0.91 (0.79, 1.06)	0.215	0.87 (0.75, 1.01)	0.061
Q3 (210.87–267.83)	**0.71 (0.60, 0.85)**	**< 0.001**	**0.68 (0.57, 0.82)**	**< 0.001**
Q4 (> 267.83)	**0.60 (0.50, 0.74)**	**< 0.001**	**0.57 (0.47, 070)**	**< 0.001**
Trend test		**< 0.001**		**< 0.001**
AChE (nmol/L)				
Q1 (≤ 133.66)	Reference		Reference	
Q2 (133.66–157.97)	**1.51 (1.21, 1.93)**	**< 0.001**	**1.55 (1.22, 1.97)**	**< 0.001**
Q3 (157.97–184.03)	**1.69 (1.35, 2.12)**	**< 0.001**	**1.74 (1.39, 2.18)**	**< 0.001**
Q4 (> 184.03)	**2.03 (1.64, 2.51)**	**< 0.001**	**2.06 (1.66, 2.54)**	**< 0.001**
Trend test		**< 0.001**		**< 0.001**
ACh (nmol/L)				
Q1 (≤ 316.73)	Reference		Reference	
Q2 (316.73–374.85)	0.90 (0.77, 1.06)	0.200	0.91 (0.78, 1.07)	0.273
Q3 (374.85–429.19)	**0.82 (0.70, 0.97)**	**0.023**	**0.81 (0.68, 0.96)**	**0.014**
Q4 (> 429.19)	**0.64 (0.52, 0.78)**	**< 0.001**	**0.62 (0.51, 0.75)**	**< 0.001**
Trend test		**< 0.001**		**< 0.001**

Abbreviation: Q, quartile; *PR*, prevalence ratio; *CI*, confidence interval. Bold indicates statistically significant.
[a] Adjustment: age, gender, BMI, low birth weight, paternal education, maternal education, family incomes.

Table 4
Association between cholinergic system and IQ scores.

Cholinergic system	IQ scores , β (95%CI)		
	All[a]	Male[b]	Female[b]
ChAT (pg/mL)			
Q1 (≤ 154.12)	Reference	Reference	Reference
Q2 (154.12–210.87)	0.81 (−1.83, 3.44)	0.31 (−3.85, 4.47)	1.03 (−2.80, 4.86)
Q3(210.87–267.83)	2.28 (−0.36, 4.92)	3.11 (−0.90, 7.12)	2.43 (−1.63, 6.48)
Q4 (> 267.83)	1.97 (−0.67, 4.61)	3.04 (−0.98, 7.06)	0.77 (−3.24, 4.77)
Trend test	0.84	0.07	0.554
AChE (nmol/L)			
Q1 (≤ 133.66)	Reference	Reference	Reference
Q2 (133.66–157.97)	**−3.18 (−5.79, −0.56)**	− 5.03 (−8.88, −1.19)	− 2.69 (−6.78,1.40)
Q3 (157.97–184.03)	**−2.73 (−5.53, −0.13)**	− 1.69 (−5.44,2.06)	**−4.24 (−8.44, −0.04)**
Q4 (> 184.03)	**−4.09 (−6.72, −1.46)**	**−5.14 (−9.06, −1.22)**	**−6.12 (−10.16, −2.08)**
Trend test	**< 0.001**	**0.035**	**0.002**
ACh (nmol/L)			
Q1 (≤ 316.73)	Reference	Reference	Reference
Q2 (316.73–374.85)	− 0.13 (−2.75, 2.49)	− 2.88 (−6.82, 1.06)	2.64 (−1.38, 6.66)
Q3 (374.85–429.19)	− 1.09 (−3.72, 1.53)	− 1.66 (−5.70, 2.37)	− 0.44 (−4.37, 3.49)
Q4 (> 429.19)	1.47 (−1.17, 4.11)	− 0.13 (−4.10, 3.84)	3.25 (−0.81, 7.30)
Trend test	0.440	0.873	0.274

Abbreviation: Q, quartile; β, regression coefficient; *CI*, confidence interval. Bold indicates statistically significant.
[a] Adjustment: age, gender, BMI, low birth weight, paternal education, maternal education, family incomes.
[b] Adjustment: age, BMI, low birth weight, paternal education, maternal education, family incomes.

Table 6
Association between cholinergic system and DF/IQ.

Cholinergic system	Crude		Adjusted[a]	
	PR (95% *CI*)	*P* value	*PR* (95% *CI*)	*P* value
Either DF or IQ < 120				
AChE (nmol/L)				
Q1 (≤ 133.66)	Reference		Reference	
Q2 (133.66–157.97)	1.09 (0.94, 1.26)	0.253	1.06 (0.92, 1.22)	0.427
Q3 (157.97–184.03)	1.14 (1.00, 1.31)	0.054	1.12 (0.97, 1.28)	0.112
Q4 (> 184.03)	**1.21 (1.06, 1.38)**	**0.004**	**1.22 (1.07, 1.38)**	**0.002**
DF and IQ < 120				
AChE (nmol/L)				
Q1 (≤ 133.66)	Reference		Reference	
Q2 (133.66–157.97)	**1.29 (1.08, 1.54)**	**0.004**	**1.27 (1.07, 1.50)**	**0.006**
Q3 (157.97–184.03)	**1.37 (1.16, 1.62)**	**< 0.001**	**1.37 (1.17, 1.62)**	**< 0.001**
Q4 (> 184.03)	**1.46 (1.25, 1.72)**	**< 0.001**	**1.44 (1.23, 1.68)**	**< 0.001**

Abbreviation: Q, quartile; *PR*, prevalence ratio; *CI*, confidence interval. Bold indicates statistically significant.
[a] Adjustment: age, gender, BMI, low birth weight, paternal education, maternal education, family incomes.

fluoride exposure, DF, IQ, and cholinergic system by adjusting for the covariates among demographics, development, socioeconomics, and delivery conditions (Tables S6–S12). We obtained similar results to what we found in the present analyses.

4. Discussion

Annual surveillance data from the local CDC revealed that fluoride concentration in drinking water maintained stable during the past decades in our study area. The range of the water fluoride concentration in our study scope was 0.20–3.90 mg/L, and the median was 1.00 mg/L, that was equal to the drinking water quality standards in China (Zhu et al., 2006) and within the fluoride limit recommendec by WHO (World Health Organization, 2017), suggesting that the fluoride concentrations of our subjects were at low-to-moderate levels. The long-term external fluoride exposure levels could represent by water fluoride.

As one of the most common hazard of children exposure to fluoride, DF has always been the focus of fluoride toxicity research. Both water fluoride and urinary fluoride were positively associated with the prevalence of DF in our study, and this was consistent with the previous animal and epidemiological studies (Das et al., 2020; Demelash et al., 2019; Guner et al., 2016; Macey et al., 2018). Fluoride can cross the blood-brain barrier and accumulate in brain tissue, causing irreversible damage on the nervous system. The central nervous system of children is more vulnerable than adults for fluoride exposure, and the study of it is more meaningful. Fluorosis among children is still a public health problem in some areas of the world (Chen et al., 2014). The effect of fluoride on children's IQ have been investigated from areas with high fluoride exposure (Tang et al., 2008), and results showed that children's intelligence were associated with high concentrations of fluoride (Ding et al., 2011). Similar results were found from study among Canada and Mexico (Bashash et al., 2017a; Till et al., 2020). Epidemiological studies in Canada also showed that excessive maternal exposure to fluoride during pregnancy has been associated with lower IQ scores in children aged 3–4 years (Green et al., 2019). In the present study, we found that higher fluoride concentrations were accompanied with lower IQ scores, and fluoride concentrations were negatively associated with the probability of developing superior and above intelligence.

In the logistic regression model, children presented 31% and 33% lower probability of developing superior and above intelligence with every 1 mg/L increase of water and urinary fluoride. As showed in our previous study, the distributions of IQ scores between normal group (water fluoride ≤ 1.0 mg/L) and high-fluoride-exposure group (water fluoride > 1.0 mg/L) were notably different, especially for the proportion of IQ scores over 120 (Yu et al., 2018). Then, to study the effect of

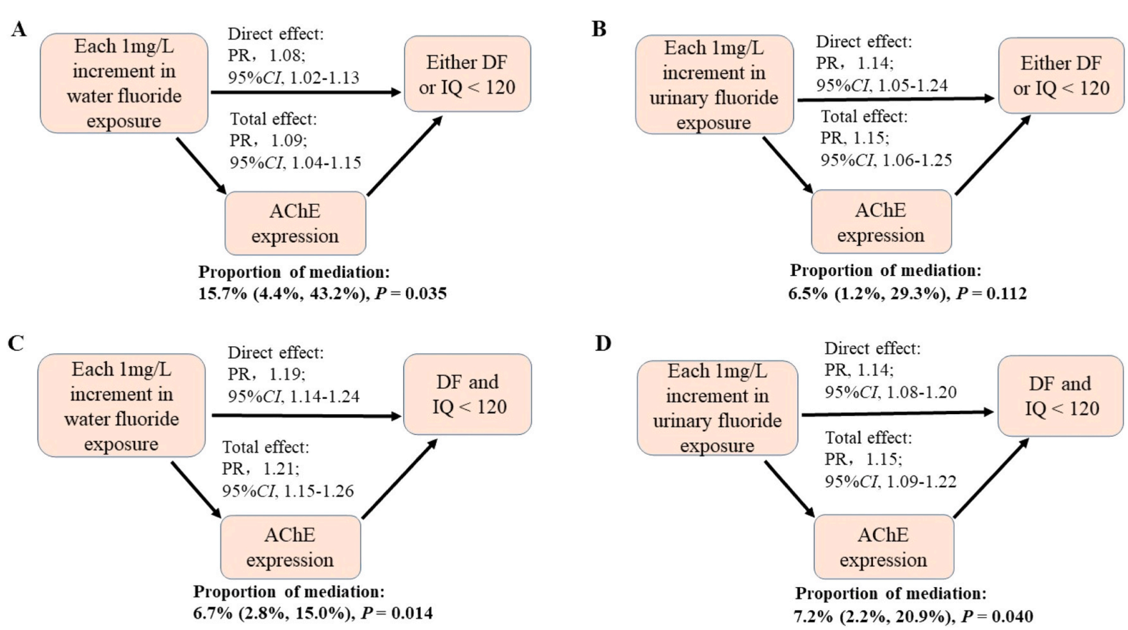

Fig. 1. The mediation effect of AChE in the association between fluoride exposure and DF/IQ. Mediation analysis of the estimated effect (95% *CIs*) of fluoride exposure (mg/L) on DF/IQ through the expression of AChE. (A) Mediation analysis suggested AChE mediated 15.7% (95% *CI*: 4.4%, 43.2%) of the association between water fluoride and the prevalence of either DF or IQ < 120. (B) Analysis suggested that mediation effect of AChE between urinary fluoride and the prevalence of either DF or IQ < 120 was not statistically significant. (C) Mediation analysis suggested AChE mediated 6.7% (95% *CI*: 2.8%, 15.0%) of the association between water fluoride and the prevalence of DF and IQ < 120. (D) Mediation analysis suggested AChE mediated 7.2% (95% *CI*: 2.2%, 20.9%) of the association between urinary fluoride and the prevalence of DF and IQ < 120. Models were adjusted for age, gender, BMI, low birth weight, paternal education, maternal education, family incomes, urine creatinine (for urinary fluoride).

fluoride on children's IQ level of superior and above we set 120 as the cut-off point of IQ scores. Contrary to previous studies that set individual either IQ or DF as the ending of the analysis model, we put the two together to study the comprehensive effect of fluoride on children's health, in addition to linear regressions, categorical analyses were conducted in our study, which makes more sense to analyze the effect of fluoride on children's health. Our study showed that, compared with the control group, with the increment of fluoride levels, children with both had a high percentage than those who having either DF or non-high intelligence alone. That means, children sensitive to toxicity of fluoride were more likely to have both DF and a lower probability of developing superior and above intelligence at the same time.

Abnormal synaptic structure or function in childhood can lead to central nervous system dysfunction, which is manifested as impaired learning and memory ability and intellectual defect. Multiple linear regression results showed that fluoride exposure was positively associated with the expression of AChE, while negatively associated with the levels of ChAT and ACh. Coincidentally, epidemiological studies showed that the neurotransmitter dopamine polymorphism has modifying effects of fluoride exposure on children's IQ (Zhao et al., 2021). These studies indicate that the role of neurotransmitters in fluoride neurotoxicity is gradually being recognized. Additionally, fluoride influences synaptic neuron plasticity in the hippocampus of rats were found in animal experiments (Yang et al., 2018). Moreover, it has been shown that fluoride affects dopamine metabolism and causes changes in the expression of dopamine receptors (Kupnicka et al., 2020). We further explored the association between cholinergic system and IQ scores and found that AChE concentrations were negatively associated with IQ scores, in addition, logistic regression analyses showed that compared with children in the first quartile of AChE, those in the third and fouth quartiles presented 53% and 46% lower probability of developing superior and above intelligence. However, the association between ACh and IQ scores was not significant, nor was ChAT. AChE was evolved to regulate cholinergic neurotransmission by hydrolyzing synaptic ACh. Evidence is rapidly mounting that cholinesterases play a role in the development of neuron (Brimijoin and Koenigsberger, 1999; Paraoanu and Layer, 2008), and AChE inhibitor was the most widely investigated target for the development of AD therapeutics (Gabr and Brogi, 2020; Pan et al., 2019). Epidemiological studies showed erythrocyte AChE could be a promising marker for early recognition of environmental lead exposure and lead-neurotoxicity in children (Nwobi et al., 2019), which is also used as an environmental exposure with neurotoxicity, AChE might serve as a marker of fluoride-related intellectual impairment.

In addition, the association between AChE and DF/IQ was conducted. Fluoride was positively associated with the concentration of AChE and negatively associated with ChAT and ACh. Furthermore, AChE was associated with the prevalence of DF and the lower probability of developing superior and above intelligence. We speculate whether the increment of AChE is an intermediate linking that mediate fluoride exposure and DF/IQ. To our surprise, significant mediation by AChE was observed in the models associating fluoride exposure with DF and IQ. What interesting in our study is that we first put DF and IQ as one ending in our analysis and investigated the mediation effect of AChE on the association between fluoride exposure and DF/IQ. The introduction of cholinergic system is also unprecedented in the analyses of fluoride and DF/IQ. Compared to the previous studies, more information on the health of low-to-moderate fluoride exposure was provided in our research, which could further complete the epidemiological evidence of fluoride exposure and health damage.

There are some limitations in our study. Due to the cross-sectional study design, it is not possible to show individual differences in development, insufficient to establish a causal relationship between fluoride exposure and health damage. Besides, spot urine samples were used in our study, the levels of internal fluoride exposure in children are not adequately reflected, but, association between fluoride concentrations of spot (early morning) and 24-hour samples has been observed (Zohouri et al., 2006). We don't have past data on water fluoride, but according to the monitoring data of CDC over the past years, the amount of F^- in drinking water remained stable in the village where our subjects resided, which made our findings more reliable. Last but not least, exposure to toxic and heavy metals occur simultaneously as a mixture in real-life (Valeri et al., 2017). In our study the lack of control for other exposures is a limitation, therefore more comprehensive epidemiology studies of relationship between fluoride exposure and health effects need to be carried out.

5. Conclusions

In conclusion, our study showed that low-to-moderate fluoride exposure was associated with the alteration of cholinergic system, DF and IQ, and AChE partly mediated the elevated prevalence of DF and the lower probability of developing superior and above intelligence caused by fluoride. Therefore, long-term monitoring of fluoride level is necessary for the prevention and control of endemic fluorosis and the implementation of defluoridation projects in areas of endemic fluorosis.

CRediT authorship contribution statement

Su-mei Wang: Conceptualization, Investigation, Writing – original draft, Writing – review & editing. **Qian Zhao:** Conceptualization, Investigation, Validation, Writing – review & editing. **Gao-chun Li:** Formal analysis, Writing – review & editing. **Meng-wei Wang:** Formal analysis, Writing – review & editing. **Hongliang-Liu:** Formal analysis, Writing – review & editing. **Xing-chen Yu:** Formal analysis, Writing – review & editing. **Jing-wen Chen:** Formal analysis, Writing – review & editing. **Pei Li:** Formal analysis, Writing – review & editing. **Li-xin Dong:** Methodology, Writing – review & editing. **Guo-yu Zhou:** Formal analysis, Writing – review & editing. **Yu-shan Cui:** Formal analysis, Writing – review & editing. **Meng-ru Wang:** Formal analysis, Writing – review & editing. **Li Liu:** Conceptualization, Investigation, Writing – original draft, Writing – review & editing. **Ai-guo Wang:** Conceptualization, Visualization, Resources, Supervision, Writing – review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declared 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.2021.112959.

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