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Acetylcholinesterase Activity in Fluorosis Adversely Affects Mental Well-being — An Experimental Study in Rural Rajasthan

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Abstract:

Fluoride toxicity is a burgeoning problem in worldwide and also in Rajasthan in India. In the state of Rajasthan, almost all districts have high fluoride (up to 18.0 ppm) in their drinking/ground water sources and about 11 million of the populations are at risk.

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Several clinical and experimental studies have reported that the fluoride induces changes in cerebral morphology and biochemistry that affect the neurological function. A. In the present study, 102 adults male (30.5 ± 9.8 yrs) were selected from the high fluoride region of the eastern regions of the Jaipur (Rajasthan, India), where fluoride content in water is 5.5 ± 1.2 ppm. Moreover, age matched controls (31.8 ±8.7) were selected from the Jaipur district where fluoride content in water was less than 1.5 ppm. The serum fluoride levels and acetylcholinesterase (AChE) were estimated, thereafter, General Health Questionnaire 60 (GHQ60) was used to assess behavioral changes in subject and controls. It observed that, the activity of AChE were found to be significantly (p<0.001) reduced in its directly correlate with scoring of GHQ60. On the basis of the results it may be concluded that fluoride exposure promotes the deterioration in sympathetic and parasympathetic neuron as evident by reduced AChE activity and it is positively correlates with cognitive changes. However, further in depth studies is required for the understanding of pathophysiology of fluoride neurotoxicity and its effect on cognitive decline in adults.

Key words: Fluoride; GHQ 60; Cognitive decline; Acetylcholinesterase

Introduction

High concentration of fluoride in ground water is a worldwide problem (WHO, 1984). The wide spread distribution of fluoride in nature is a direct source of adverse health effects in human populations. Fluorosis a progressive degenerative disorder resulted from excessive intake of fluoride. The fluoride contaminated drinking water is common cause of Fluorosis. Endemic fluorosis is prevalent in many parts of the world and often seriously impairs the health of human or animals (Chauhan et al., 2013). In Indian scenario approximately 66.6 million people (17 states in India) population are at risk from Fluorosis and of Rajasthan have high fluoride (up to 18.0 ppm) in their drinking/ground water sources and about 11 million of the populations are at risk (ICMR & RMRCT, 2004; Yadav et al

2003). Moreover in state of Rajasthan, people of 22 districts (out of 33 districts) are presently consuming Fluoride (Samal & Naik, 1988) greater than permissible limit.

Fluorosis causes damage not only to skeletal tissue and teeth but also to soft tissues such as brain (Chauhan et al., 2013; Bharti et al., 2014). Excessive ingestion of fluoride may be associated with central nervous system dysfunction. Animal studies have indicated that fluoride ingestion may lead to changes in behavior and neurodegeneration (Bhatnagar et al., 2002; Vann et al., 2012). Intelligence Quotient (IQ) was also reduced in children with exposure to high fluoride levels in drinking water (Singh et al., 2013).

Fluoride exposure exhibits rapid increased transport in blood stream and likely across the blood-brain barrier. While it appears that the toxicity of fluoride in brain may be due to excitotoxicity (Pellegrini-Giampietro et al, 1988) or free radicals and lipid peroxidation products generated by excitotoxicity have been shown to damage dendrites and synaptic connections, and lead to neuronal destruction (Isokawa & Levesque, 1991). The brain is affected to oxidative stress due to the presence of high levels of polyunsaturated fatty acids, relatively low antioxidant level, the presence of redox metal ions and high oxygen (Uttara et al, 2009). The free radical productions, lipid peroxidation, and distorted antioxidant defense systems are considered to play an important role in the toxic effects of fluoride (Sarkar et al., 2014; Morales-Gonzalez 2010). Fluoride, in different stages of life, the mode of action of F is debating and moreover its occurrence in the central nervous system (CNS) in adults is not well elucidated.

Keeping in view the paucity of information in relation to high F exposure in population residing in endemic areas and its impact on adult, the present study was undertaken. The significance of this study is to investigate the correlation of AChE activity and cognitive impairment in F exposed population in state of Rajasthan (India).

Methods

In the present study, 102 Adults (male, age- 18 to 45 years) were selected from the high F region of the Jaipur (Rajasthan, India), where F content in water is 5.5 ± 1.2 ppm. The affected adults were investigated clinically. The subjects were similar living conditions and differ minimally in terms of lifestyle, literacy, socioeconomic status, addiction and medical care (Table-1). Moreover, aged matched controls (N=87) were selected from the rural area of Jaipur district where fluoride content in water was less than 1.5 ppm.

Sample Collection

Blood sample of each subject and control were collected after clinical examination. 3.0 ml of blood sample was drawn from all adult under complete aseptic condition. The blood was collected in simple vial and allowed to clot at room temperature. The separated serum was used to measure serum fluoride concentration using specific fluoride ion selective electrode (Thermo Fisher Scientific Inc., Singapore).

Estimations of AChE

AChE activity was estimated using 5, 5-dithio-bis (2- nitro benzoic acid) (DTNB). The serum cholinesterase with the Ellman reaction involves reaction of DTNB with thiocholine liberated from its esters by enzymatic hydrolysis. The yellow 5-thio-2-nitrobenzoate (DTNB) is formed that is detected by spectrophotometer at 412 nm (Ellman et al, 1961).

Cognitive Assessment

After taking consent from the subject and controls, cognitive assessments were carried out individually using Hindi version of general health questionnaire- 60 (GHQ60) [Goldberg et al 1976 & Chandrasekhar et al, 1980]. It is one of the most widely used and validated questionnaires to screen for high psychological stress and morbidity. The optimum threshold for case detection in general practice was found to be a score of 12 or more by Goldberg. Thus a person scoring 11 or less is a probable normal [Gautam et al, 1987].

Statistical Analysis

The results obtained from the study are expressed as mean ± SD. The statistical significance was determined by Mann-Whitney *p*-test. Probability, *p*-value less than 0.05 were considered statistically minimum significant.

Results

The concentration of fluoride in serum (figure-1) of the subjects were significantly ($p<0.001$) elevated and it is directly proportional to the concentration of fluoride in their drinking water (figure-2). The activity of AChE is depicted in figure-3. AChE was found to be markedly ($p<0.005$) decreased in the fluoride exposed population when compared with age matched healthy controls. The psychiatric GHQ questioner comprises with control and fluoride exposed population. It was found to be significantly ($p<0.001$) raised (figure-4) in subjects when compared with the controls. Significant correlation was observed (figure- 5) positive correlation between serum fluoride and water fluoride level (5A), urine fluoride and water fluoride level (5B) negative correlation between serum fluoride and AChE (5C) and serum fluoride and GHQ60 (5D).

Discussion

It is well documented that fluorine is an essential trace element for the body, but excessive fluoride ingestion of a long period may result in dental and skeletal fluorosis, as well as the decline of the learning and memory ability and reduced IQ (Chioca et al., 2008).

In the present study, demographic distribution in term of age and BMI was insignificant ($p>0.05$) change in control and subjects (Table -1). The concentration of fluoride water was found to be increased significantly in subjects when compared with the controls (figure-1). The concentration of fluoride in serum was markedly elevated in subjects than that of age matched controls (figure-2). The concentration of serum fluoride has recognized as a consistent marker of fluoride exposure and can be also used as one of the biomarkers to assess the effect of endemic fluorosis. The large difference between fluoride concentrations in serum of control and subjects correlated with concentration of F in drinking water of them. It is suggestive that fluoride directly incorporated into the blood and it may deposit in different body organs, bones, teeth, liver, kidney and brain (Ailani et al, 2009).

Previously, we reported that increased fluoride exposure delineated the IQ in school children (Singh et al, 2013). In addition, fluoride neurotoxicity is supported by various animal studies, which show cognitive disorders are associated with fluoride exposure and behavioral change and delayed learning and memory ability (Zhao et al, 1996, Lu et al, 2000, Xiang et al, 2003, Trivedi et al, 2007). Moreover, Sharma et al. (2009) reported that the headache, lethargy and insomnia in population of high fluoride regions. In this study, we found increased behavioral impairment, estimated by GHQ- 60 in subject and control. Significant elevated impairment score was recorded in subjects when compared with the controls (figure-4). Although the relation between fluoride exposure with decline in cognitive functions and the cholinergic system is not clear, considerable experimental evidence exists for a relation between the declines in cholinergic functions (Rupniak et al, 1991). As we observed significant reduction in AChE in fluoride exposed population (figure-3). The correlation between cognition and fluoride reinstated the idea that the fluoride interferes with functioning of cholinergic system is involved in learning and memory and boosted research into the involvement of the cholinergic.

Conclusion

On the basis of the results it is concluded that the correlation between the cholinergic deficiency and the cognitive decline in fluorosis led to the simple conclusion that fluoride reduced cholinergic function underlies the cognitive deficit. However, further studies are required for the understanding of pathophysiology of Fluorosis.

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Table-1: Difference of age and BMI in control and subjects

	Control (102)	**Exposed (87)**
Age	30.5 ± 9.8	31.8 ±8.7
BMI	23.2 ± 2.1	22.8 ± 2.2
Socio-economic status	Lower (100%)	Lower (100%)
Literacy (H.Sc.)	100 %	100 %
Smokers	58%	51%
Alcoholic	4% (occasionally)	3.5% (occasionally)

The demographic data including age and BMI of control and subjects.

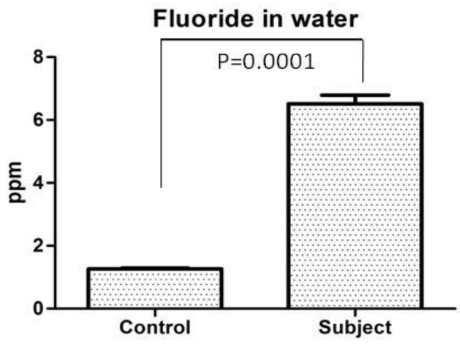

Figure -1. Concentration of fluoride in water is expressed as mean ± SD for control and subjects. The statistical significance was determined by Mann-Whitney *p*-test. The p< 0.05 were considered minimum statistical significant between groups.

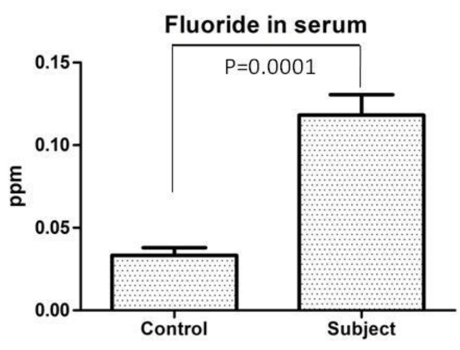

Figure -2. Concentration of fluoride in serum is expressed as mean ± SD for control and subjects. The statistical significance was determined by Mann-Whitney *p*-test. The p < 0.05 were considered minimum statistical significant between groups.

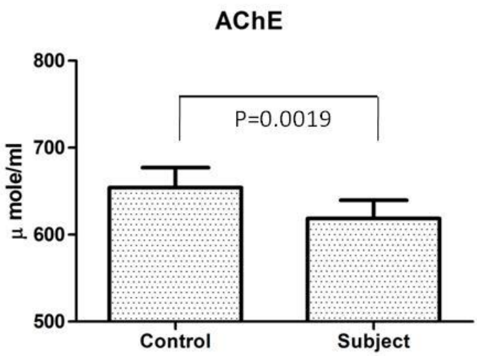

Figure -3. Activity of acetylcholinesterase is expressed as mean ± SD for control and subjects. The statistical significance was determined by Mann-Whitney *p*-test. The $p < 0.05$ were considered minimum statistical significant between groups.

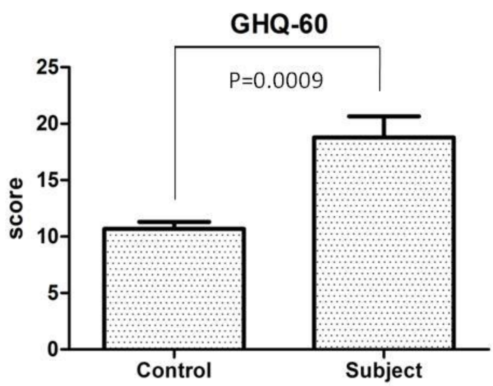

Figure -4. GHQ60 scoring is expressed as mean ± SD for control and subjects. The statistical significance was determined by Mann-Whitney *p*-test. The $p < 0.05$ were considered minimum statistical significant between groups.

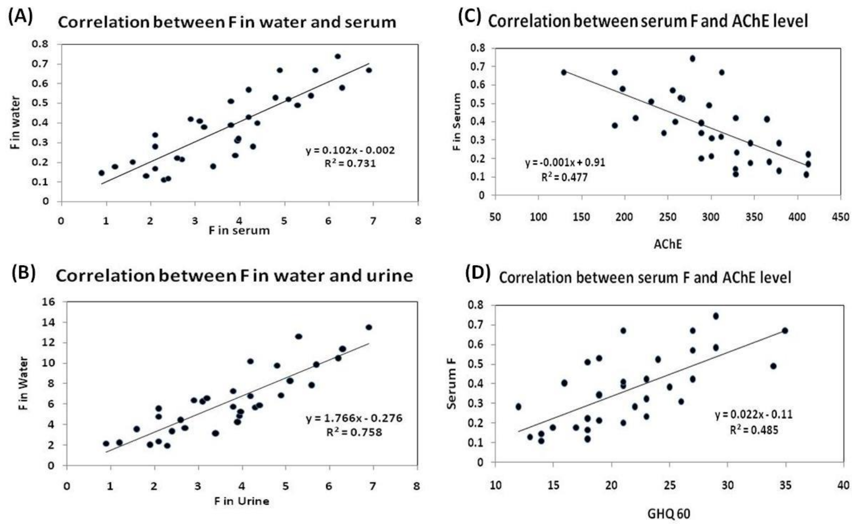

Figure-5. Correlation graphs shows significant between serum fluoride and water fluoride level was significant (A), urine fluoride and water fluoride level (B) negative correlation between serum fluoride and AChE (C) and serum fluoride and GHQ60 (D).