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A correlation between Serum Vitamin, Acetylcholinesterase Activity and IQ in Children with Excessive Endemic Fluoride exposure in Rajasthan, India

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Abstract

Fluoride is widely distributed in nature and a direct source of adverse health effects in human populations. Fluoride poisoning attributed by long-term exposure to high levels of fluoride called fluorosis. The present study was carried out among 9-14 years old school children of Dausa district, Rajasthan India. The subjects were selected from high fluoride region (>2.0 ppm) and age matched were selected from Jaipur district where fluoride content was (<1.5 ppm). A set of question was used to collect information from the children's personal character, clinical health outcome, residential record, educational achievements and family socioeconomic status. Fluoride in serum, urine and drinking water were estimated in both subjects and controls along with serum vitamin (A, C, D and E) and acetylcholinesterase (AChE). The Raven's Standard Progressive Matrices was used to measurement of children's intelligence. IQ scores and serum F levels was directly correlated with the concentration of serum F level. Reduced concentration of vitamin (C, D and E) and AChE activity is the potent markers of neuro-degeneration. The conclusion of the study is that the excessive fluoride delineates the neuronal impairment which were evident by reduce IQ score and serum AChE activity. Moreover, the altered vitamin concentrations which may further lead to brain and bone damage.

Keywords: adverse health effects, human populations, fluoride, socioeconomic status, serum vitamin, acetylcholinesterase.

Introduction

Elevated level of naturally occurring fluoride (F) in our drinking water is a universal problem. However, there are various study has been reported that the water quality being reduced due to excess contamination of pollution and earth elements[1,2,3]. Moreover, various methods have also been developed to improve the quality of drinking water using natural resources[4]. Among these pollutant, wide spread distribution of fluoride in nature is a undeviating source of undesirable health effects in human populations. The health effects in humans associated with exposure to F (skeletal and dental fluorosis) are well documented. Fluorosis is one of the most common problems from long-term exposure to high levels of fluoride, and is a more chronic health problem in different parts of the world where fluoride is more than 1–1.5-ppm in drinking water[5].

In Indian context an estimated 66.6 million people (17 states in India) including 6 million under 14 children are at risk of acquiring fluorosis (ICMR, RMRCT, Update 2004)6. Moreover in Rajasthan, people of 22 districts (out of 32) are presently contaminated fluoride[7,8] greater than permissible limit which lead to cognitive problem.

In children, cognitive capacities, particularly intelligence reduction are reported. It is also evident by animal studies, which show cognitive deficits are associated with F exposure and motor and learning disability [9,10,11]. On the other hand Sharma[12] and associates reported that headache, lethargy and insomnia in population of high fluoride regions. Fluoride exposure exhibit increased transport into the blood stream and likely across the blood-brain barrier. The brain is more susceptible to ROS induced oxidative stress due to the presence of high concentration of polyunsaturated fatty acids and relatively low antioxidant status [13]. Fluoride, in the developmental stages of life, the mode of action of fluoride is debating and moreover its occurrence in the developing central nervous system (CNS) during childhood fluoride exposure is not well elucidated.

Keeping in view the paucity of information in relation to high fluoride exposure in population residing in endemic areas and its impact on children, the present study was undertaken. The significance of this study is to investigate the correlation between acetylcholinesterase and IQ and its association with the concentration of serum vitamin and fluoride levels in childrens with high fluoride region in Dausa district of Rajasthan, India.

Material and Methods

In the present study, 73 school children (male, age- 9 to 14 years) were selected from the high fluoride region of the eastern

regions (Rural area of Dausa district) in Rajasthan India where fluoride content in water is more than 2.0 ppm. The affected children were investigated clinically. The subjects were similar in living conditions, parental literacy, socioeconomic status, and health history. Moreover, age and sex matched controls were selected from the rural area of Jaipur district where fluoride content in water was less than 1.5 ppm. The proposed study was approved by the Institutional ethical committee.

Sample collection: Drinking Water sample: A sample of 200 ml of drinking water was collected in a sterilized polyethylene bottle at each child's home. The fluoride levels were analyzed using fluoride ion selective electrode (Thermo Fisher Scientific Inc., Singapore). Urine and Blood sample of each subject were collected after clinical examination of subjects and controls. The 3.0 ml of blood sample was withdrawn from all the subjects and control under complete aseptic condition. The blood was collected in simple vial for the separation of serum. The separated serum was used for the estimation of serum fluoride and vitamins concentration. While, the concentration of fluoride was measured using specific fluoride ion selective electrode (Thermo Fisher Scientific Inc., Singapore)

Psychological Assessment: The Intelligence Quotient (IQ) of each subject and control were measured with the Raven's Test[14,15]. It was a set of standardized questionnaire, completed by subjects and controls individually.

Estimations of Vitamins: Vitamin A was estimated using high pressure liquid chromatography (HPLC; (Waters Limited Mulford USA) system[16]**.** Retinyl acetate internal standard was used and retinol levels were expressed as µg/dl. Serum vit-C was measured using using 2,4 dinitrophenyl hydrazine[17] in presence of mild reducing agent thiourea reagent to form a red colour compound bis-hydrazone, measured at 520nm in spectrophotometer. The vitamin D concentrations were measured by competitive radioimmunoassay after acetonitril extraction using commercially available kit (Immune diagnostic Germany). The concentration was represented as ng/ml in serum. The concentration of vitamin-E in serum was estimated using xylene extraction and reduction of ferric to ferrous ions[18]**.** The red colored complex with the α-α' Dipyridyl. The colour absorbance was read at 460nm.

Estimations of AChE: AChE activity was investigated by Ellman method[19]. Reaction involves, 5, 5-dithio-bis (2-nitrobenzoic acid) (DTNB) with thiocholine liberated from its esters by enzymatic hydrolysis. The yellow cloured complex of 5-thio-2-nitrobenzoate (TNB) is formed that was detected by spectrophotometer at 412 nm.

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 significant.

Results and Discussion

The demographic distribution in term of age and BMI was insignificant ($p>0.05$) change in control and subjects in table-1. The concentration of fluoride in drinking water, urine and serum of control and subject groups presented in table-2. Significant ($p<0.001$) difference were observed in subjects serum fluoride levels and fluoride in their drinking water. The fluoride concentration serum and urine was directly proportional to the concentration of fluoride in drinking water. The IQ scores of the subjects were comparatively reduced as compared to controls. However, subjects exhibited lower IQ. The maximum subjects exhibited the range 80-90 (35%) followed by 90-109 (29%) and 70-79 (22%), while, the maximum controls were exhibited 90-109 (47%) followed by 80-89 (30%) and 70-79 (10%). The cardiac physiology was observed in subject and control in figure-1. The systolic blood pressure were found to be significantly ($p=0.05$) in subject as compared with controls. While, heart rate and systolic blood pressure was insignificantly ($p>0.05$) change in subjects as compared to controls. The concentration of hemoglobin was found to be significantly ($p<0.05$) reduced in subject when compared with age matched controls. The concentration of vitamin A, C, D and E were found to be changes in subjects as compared to controls depicted in figure -2. The concentration of vitamin C, E and D was found to be significantly ($p<0.05$) reduced in subject when compared with the age matched controls while the concentration of vitamin A was insignificant ($p>0.05$) changed as compared to controls**.** The activity of AChE was found to be markedly ($p<0.05$) reduced in subjects in fluoride exposed subjects when compared with the age matched healthy subjects in figure-3.

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

	Control	Subjects	p-value
Age	12.2 ±1.4	12.3 ±1.5	0.5685
BMI	22.9 ± 3.2	23.2 ± 2.9	0.6125

Data are expressed as mean ± SD in control and subjects.

Table-2
The Concentration of Fluoride in drinking water, urine and serum

	Control	Subjects	P-value
Water Fluoride	1.03 ± 0.15	6.8 ± 1.6	<0.001
Urine Fluoride	1.79 ± 0.2	5.23 ± 2.1	<0.001
Serum fluoride	0.029 ± 0.012	0.986 ± 0.03	<0.001

Data are expressed as mean ± SD in control and subjects

In the present study, the concentration of serum fluoride was proposed as a consistent marker of fluoride exposure and can be also used as one of the potent biomarkers of endemic fluorosis. The large difference between fluoride concentrations in serum of control and subjects correlates with concentration of intake

fluoride. Kidneys are among sensitive body organ in their physiological functioning to removal of excessive amount of fluoride[20]. Very few studies are reported that impaired renal function in fluoride exposure. On the other hand, it has been reported that urinary F excretion in morning in children same as the total daily average consumption of fluoride[21].

Table-3
The IQ distribution in subjects and controls

	Control (N=72)		Subjects (N=70)	
	Score	***(%)***	***Score***	***(%)***
>130	0	0	0	0
120-129	2	2.8	1	1.4
110-119	5	6.9	2	2.8
90-109	34	47.2	21	29.2
80-89	22	30.6	25	34.7
70-79	7	9.7	16	22.2
<69	2	2.8	5	6.9
Total	**72**	**100**	**70**	**100**

Data are expressed as Number of children and their scores

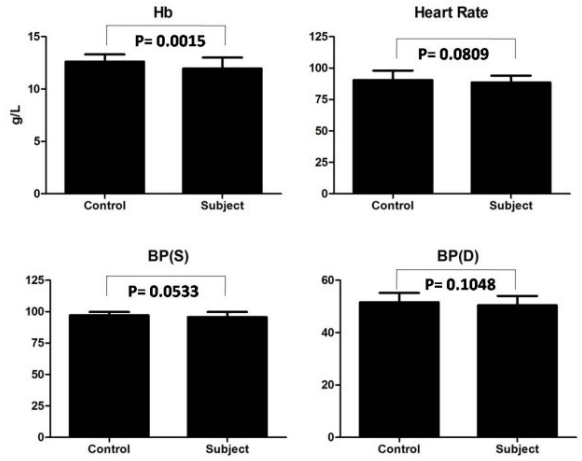

Figure-1
The measurement of heamoglobin and cardiac physiology (Heart rate, Blood pressure systolic and diastolic) expressed as mean ± SD for control and subjects

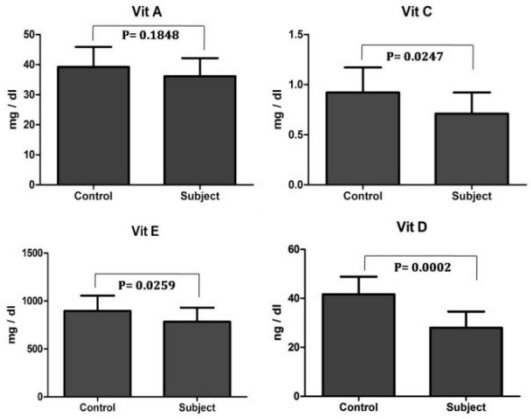

Figure-2
The concentration of serum vitamins (A, C, E and D) expressed as mean ± SD for control and subjects

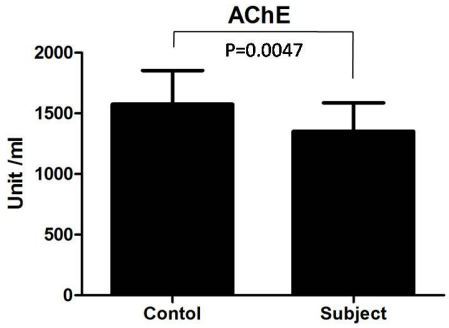

Figure-3
The concentration of serum acetylcholinesterase expressed as mean ± SD for control and subjects

It is suggestive that fluoride may directly incorporate into the blood stream and deposit in different body organs and tissues, bones and teeth[22]. In the present study Fluoride intoxication decreases Hb levels. These results suggested that fluoride may interfere to erythropoiesis either combination on mature erythrocytes or altered cellular metabolism in erythroid progenitors. Therefore, hemoglobin synthesis hinders due to inhibition in erythropoiesis. We observed insignificant changes in heat rate and blood pressure. On the contrary, it is found that fluoride slows heart beat rate and abnormalities in heart rate[23]. It has also been reported that the increased high blood-fluoride levels is also responsible for calcification of aorta and arteries[24,25].

In the present study, we observed decreased concentration of vitamins namely vit E and C. Vitamin C and E are the potent exogenous antioxidant that may help to reduce free radical injury. The reduced form of ascorbic acid has high electron-donating power to free electron. There are several studies demonstrated that vitamin C has ameliorating properties against free radical injury to brain in neurodegenerative disorders[26]. Moreover, vitamin C supplimentation also reduces the risk a number of acute and chronic diseases, cardiovascular, cancer and neurological disorders etc[27]. On the otherhand, vit D is important for neonatal and adult brain development and its regulatory function, several vitamin D receptors in the brain are involved in cognition[28]. As we observed reduced AChE activity in subjects which may be directly correlate with the reduced intelligence score of the subjects.

In the case of children, supplementation of vitamins and minerals increases intelligence score[29]. Vit E is also a potent natural antioxidant which protects brain and other organs from the ROS that are produced by various environmental stressors. Various animal studies has been demonstrated that fluoride toxicity and fluorosis can be minimized by the dietary supplementation of vit E. Vit E and C improves antioxidant defense mechanism of the body through increased rate of renal clearance of fluoride as evident by our study. While the vit D is necessary for bone mineralization and also protect bone from

fluorosis. Vit D also improves the renal perfusion by proper functioning of the fluoride induced secondary hyperparathyroidism[30].

Conclusion

The results of the present study may conclude that fluoride exposure promote oxidative stress and alteration in trace metal analysis. These alterations may induce pathophysiological activities due to lack of proper drinking water source. High fluoride content in the drinking water (bore well water) and associated fluoride induced health problem among the children, our team would like to advised the administration to provide an alternative water sources and to provide some specific antioxidant source for preventation of fluoride toxicity in children and other population of Rajasthan who are suffering from fluoride toxicity. However, further in depth studies is required for the understanding of pathophysiology of fluorosis.

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