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Oxidative Burden and Altered trace Elements as a Biomarker of Excessive Endemic Fluoride Exposure in School Children of Eastern Region in Rajasthan India

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Abstract

Fluoride Toxicity due to the presence of higher levels of fluoride in drinking water (>1.5ppm) may be serious problems in health of the children and adult in general. 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. In the present study, 53 children were selected from the rural area of the eastern regions (Dausa district) in the Rajasthan India, where fluoride content in water is 5.5 ± 1.2 ppm. Moreover, age matched controls were selected from the Jaipur district where fluoride content in water was less than 1.5 ppm. 3.0 ml of blood sample were taken to investigate oxidative stress parameters namely, lipid peroxide level (LPO), superoxide dismutase, catalase, glutathione peroxidase and reduced glutathione content. Moreover, serum Fe, Cu, Zn, Se and fluoride were investigated. Increased LPO and depleted antioxidant levels were observed in subjects along with the alteration in trace elements. The concentration of fluoride in serum was significantly correlates with their water concentration. On the basis of the results it may conclude that fluoride exposure promote oxidative stress and alteration in trace elements. These alterations may induce pathological conditions in fluoride exposed children. These biochemical markers may be used as the detection of early fluorosis. However, further in depth studies is required for the understanding of pathophysiological role of fluoride.

Keywords: Fluoride, oxidative stress, trace elements.

Introduction

Fluorosis is one of the manifestations of chronic poisoning from long-term exposure to high levels of fluoride, and is a serious health problem in many parts of the world where drinking water contains more than 1–1.5-ppm of fluoride[1]. There are various study has been reported that the water quality being reduced due to excess contamination of pollution and earth elements[2-4]. Moreover, various methods has reported to improve the quality of drinking water using natural resources[5].

An estimated 66.6 million people (17 states in India) including 6 million under 14 years children are at risk of acquiring fluorosis[6]. In Rajasthan, people of 22 districts (out of 32) are presently consuming fluoride[7] greater than permissible limit. Moreover, the reduced mental ability of adults with chronic fluorosis and the children's Intelligence Quotient (IQ) in the areas with endemic fluorosis were found to be lower than normal[8]. Sharma et al[9], has been reported that headache, lethargy and insomnia in population of high fluoride regions.

The mechanisms by which fluoride produce such effects are still not clear. Animal studies demonstrated that fluoride generates reacting oxygen species (ROS). Different enzymatic and non-enzymatic antioxidants regulated production of ROS by normal physiological processes but excessive production of ROS may leads to oxidative stress. Interactions between fluoride and free-radical reactions have been studied in various biological systems including fluorosis[10]. However, the relationship between fluorosis/fluoride toxicity and oxidative stress is still not clear.

Oxidative stress is a condition that indicates the imbalance between the pro-oxidants and antioxidants leading to the oxidative damage to lipids, proteins and DNA. Chinoy *et al*[11] reported that antioxidant supplementation with diet reverse the toxic effects of fluoride in the body. Selenium, at a certain concentration range, played a role in excreting high fluoride, adjusting the disorder of free radicals and lipid metabolism, thereby promoting the recovery of fluorosis in rats. Therefore, a greater understanding, at biochemical levels, of the fluoride toxicity is very important in clinical samples.

Keeping in view the paucity of information in relation to high fluoride exposure in population residing in endemic areas and its impact on school children, the present study was undertaken. The significance of this study is to investigate the antagonistic effect of trace metal and antioxidant of fluoride exposed school children in rural area of the Dousa district in Rajasthan.

Material and Methods

In the present study, 53 school children (male, age- 9 to 14 years) were selected from the high fluoride region of the eastern regions (Rural area of Dousa district) in Rajasthan India where fluoride content in water is more than 1.5 ppm. The affected children were investigated clinically. The subjects were similar living conditions and differ minimally in terms of lifestyle, parental education level, socioeconomic status, and medical care. The study proposal was approved by the Institutional ethical committee. 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 sources of drinking water of each family were investigated using fluoride selective electrode.

Sample collection: After clinical examination of subjects and controls, 3.0 ml of blood sample was drawn from all children under complete aseptic condition. One ml of blood was transferred to vial containing heparin which was used for the estimation of oxidative stress parameter and rest of blood was collected in simple vial and was allowed to clot at room temperature. The separated serum was used to measure serum fluoride levels using specific fluoride electrode (Thermo Fischer, Singapore) and trace elements using atomic absorption spectrophotometer.

Hematological test: The hematological tests were carried out using commercially available Qualigens kit (Mumbai, India). The hematological parameters namely hemoglobin (Hb; g/dl), red blood corpuscles (RBC; X 10^6 cells/ mm^3 of blood), white blood corpuscles (WBC; X 10^3 cells/ mm^3 of blood), mean cell volume (μ^3), mean corpuscular hemoglobin (g/dl) and hematocrit (%) were carried out in blood samples of subjects and controls.

Oxidative stress markers: Blood lysate was prepared to estimate oxidative stress parameter. The protein content was measured[12] using bovine serum albumin (BSA) as standard. The lipid peroxide (LPx) levels were measured[13] using thiobarbituric acid reacting substances (TBARS), estimated by spectrophotometrically at 532 nm and expressed as nmole of MDA /mg protein. The superoxide dismutase (SOD EC 1: 15.1.1) activity was determined from its ability to inhibit the reduction of NBT in presence of PMS[14]. The reaction was monitored spectrophotometrically at 560nm. The SOD activity was expressed as U/mg protein (1 unit is the amount of enzyme that inhibit the reduction of NBT by one half in above reaction mixture). Catalase (CAT, EC 1.11.1.6) activity was assayed as per the previously described method[15] using hydrogen peroxide as substrate; the decomposition of H_2O_2 was followed at 240nm on spectrophotometer. The CAT activity was expressed as U/mg protein. The glutathione peroxidase (GSHPx, EC 1.11.1.0) was assayed using GSH, NADPH and H_2O_2 as reactants[16]. The oxidation of GSH into GSSG was measured in terms of oxidation of NADPH to $NADP^+$ and assayed as decrease in the absorbance of reaction mixture at 340 nm on spectrophotometer. The activity of GSHPx was expressed as n moles of NADPH oxidized / min / mg protein. Reduced glutathione was measured in deprotonized supernatent in lysate with tetrachloroacetic acid, centrifuged and supernatant was used for the estimation of reduced glutathione (GSH) by the use of Ellman reagent (5, 5' dithiobis (2-nitro benzoic acid)[17] . The optical density of the pale colour was measured on the spectrophotometer on 412 nm. An appropriate standard (pure GSH) was run simultaneously. The level of GSH was expressed as µmole GSH / mg protein.

Trace element analysis: The serum trace elements (Fe, Cu, Zn and Se) were estimated using atomic absorption spectrophotometer (Perkin Elmer Analyst- 300). Serum sample were digested with a mixture of HNO_3: H_3PO4 (6:1) till residue remained. The residue was dissolved in an appropriate amount of 0.1N HNO_3 for the estimation of metals. The metal contents were measured in mg /L serum. Known amount of each metal was processed identically so as to serve as standard control.

Results and Discussion

The concentration of fluoride in drinking water and serum of control and subject groups presented in figure-1(A and B). Significant ($p<0.001$) difference were observed in subjects serum fluoride levels and fluoride in their drinking water. The f concentration serum was directly proportional to the concentration of fluoride in drinking water. Moreover, the age and BMI of the control and subjects were found to be insignificant ($P>0.05$) changed in table-1. The hematological profiles were compared between control and subjects in table-2. The concentration of Hb, RBCs, MCV and Hct were found to be significantly ($p<0.05$) reduced in subjects when compared with age matched healthy control. On the other hand, the concentration of WBCs was found to be increased significantly ($p<0.001$) in subjects as compared with the control. The oxidative stress markers namely, LPO, SOD, CAT, GPx and GSH compared with control and subjects in table-3. The lipid peroxide levels in subjects were found to be increased significantly ($p<0.05$) in subject as compared with control. The activity of enzymatic antioxidant i.e., SOD, CAT and GPx were found to be reduced significantly ($p<0.01$) in subjects when compared with control. Non enzymatic antioxidant GSH was also found to be significantly ($p<0.05$) depleted in subjects. The concentration of different trace metals (Fe, Cu, Zn and Se) in subjects and controls is presented in table-4. The concentration of Fe, Zn and Se were found to significantly ($p<0.05$) reduced in subjects while the concentration of Cu was increased significantly in subjects when compared with age matched controls.

Chronic fluoride can severely damage human health, but its pathogenesis is poorly understood. As in the case of many acute and chronic degenerative diseases, increased rate of ROS productions and lipid peroxide levels have even been considered to play an important role in the pathogenesis of chronic fluoride toxicity.

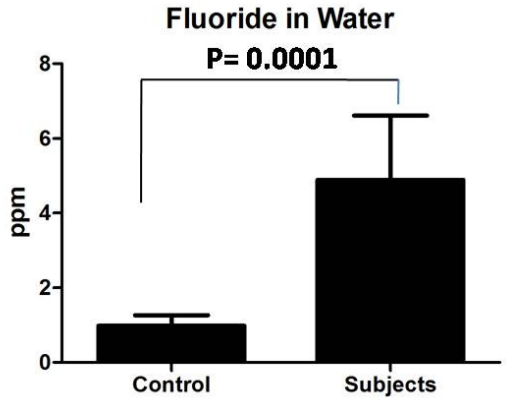

Figure-1
Levels of fluoride in drinking water of control and subjects, The results are expressed as mean ± SD for control and subjects

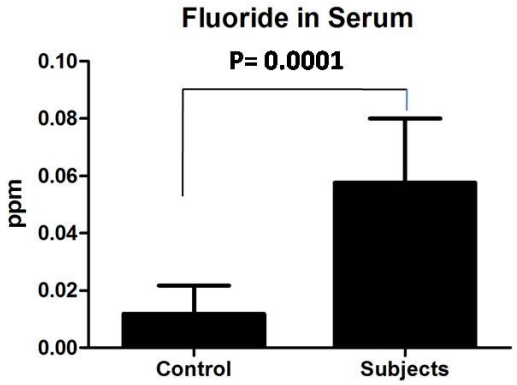

Figure-2
Figure-1: Levels of fluoride in serum of control and subjects. The results are expressed as mean ± SD for control and subjects

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

	Control	Subjects	p-value
Age	11.9±1.5	12.1±1.7	0.5685
BMI	23.6± 3.6	23.93±2.5	0.6125

Data are expressed as mean ± SD in control and subjects

Table-2
Comparison of hematological profile in control and subjects

	Control	Subjects	p- value
Hb	11.76±1.4	10.9 ±0.8	0.0314
RBCs	4.349 ± 0.32	4.13 ± 0.27	0.0065
WBCs	8.069±0.73	9.036±0.8	0.0001
MCV	81.4 ±3.26	77.9 ± 5.02	0.0053
Hct	36.3 ± 2.0	34.1±2.7	0.0017

Data are expressed as mean ± SD in control and subjects

Table-3
Oxidative stress parameters in control and subjects

	Control	Subjects	p- value
LPO	2.52±0.4	4.27±0.6	0.0214
SOD	1.79 ±0.5	1.27±0.3	0.0006
CAT	45.6 ± 10.1	31.4 ± 6.1	0.0025
GPx	32.8 ± 5.3	27.9 ± 3.4	0.0066
GSH	417.4 ± 71.9	365.3 ± 51.1	0.0289

Data are expressed as mean ± SD in control and subjects

Table-4
Concentration of trace elements in control and subjects

	Control	Subjects	p- value
Fe	91.5 ± 6.0	63.8 ±6.1	0.0483
Cu	68.7 ± 16.4	79.9 ± 11.5	0.0023
Zn	13.7 ± 3.5	11.8 ± 2.5	0.035
Se	7.43 ± 2.4	6.49 ± 1.2	0.0001

Data are expressed as mean ± SD in control and subjects

In the present study, the concentration of serum fluoride has been 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 fluoride in drinking water of control and subjects. It is suggestive that fluoride directly incorporated into the blood and it may deposit in different body organs, bones and teeth. These findings are concomitant with observation of Gerber et al[18]. Moreover, hemtolological study revealed that altered peripheral blood composition (table-2) is a reflection of disrupted haematopoietic process. Blood, which rapidly and constantly flows through the tissues and play in important role in the transportation of nutrients, antioxidants, hormones and some other chemicals. These chemical are required to for the physiological functioning of the body. Fluoride intoxication decreases Hb levels, RBCs, MCV and Hct while WBCs increased. These results suggested that fluoride may disturb erythropoiesis through combined effects on mature erythrocytes and cellular metabolism in late erythroid progenitors. Also, the inhibition in erythropoiesis and iron metabolism due to fluoride treatment probably hinders haemoglobin synthesis and erythroid Humans are uniformly exposed to fluoride that is present in the drinking water.

Lipid peroxide levels and antioxidant i.e., SOD, CAT, GPx and GSH were estimated in RBCs lysate in subjects and control. Oxidant and antioxidant balance is required for the physiological activity and function of the cell. Increase rate of free radical generation causes dysfunction and promotes oxidative damage to tissue and cells) and it is more associated with their pathological effects that ultimately lead to protein and cellular damage as well as cell death[19]. ROS has been related with the pathologies of over one hundred diseases. It has been reported that excessive fluoride exposure can damage the redox balance of the cells in tissues, decrease antioxidant defense

capacity[20,21]. As evident by our study, we observed significant increment of lipid peroxidation and reduced antioxidant status. Present finding are concomitant with the previous observation[22,23]. They are reported a close association between chronic fluoride toxicity and increased oxidative stress in humans and in experimental animals. Vani and associates[24] has also reported that fluoride induces inhibition of antioxidant enzymes associated with free radical metabolism.

In the present study, trace element concentrations in serum were investigated (table-4). The antagonistic effects were observed in subjects with fluoride intoxication when compared with controls. Fe, Zn and Se were found to be reduced and Cu was increased in serum. It is suggestive that cations of Cu, Fe, Mn, and anions of Se have unpaired electrons that allow their participation in redox reactions involving mostly one electron loss (oxidation) or gain (reduction)[25]. These elements can be explained by their capacity to catalyze the initiation of free radical reactions or the decomposition of peroxides and other unstable molecules, allowing the propagation of deleterious free radical reactions. Following the recognition of the participation of free radicals in a number of biological processes and pathological states, metals were identified as participants in most of the free radical reactions, acting as pro-oxidant or antioxidant entities[26]. The role the metal plays depends on its chemical structure (iron can act as pro-oxidant and antioxidant; selenium is usually an antioxidant), as well as on the molecule that is chelating the metal[25]. Zn is involved in the activity of about 100 enzymes, e.g. RNA polymerase, carbonic anhydrase, Cu–Zn superoxide dismutase, angiotensin I converting enzyme. Also it is present in Zn-fingers associated with DNA[27]. Se is incorporated into proteins to make selenoproteins, which are important antioxidant enzymes. Se is found in glutathione peroxidase, thioredoxins, and selenoprotein[28].

Conclusion

On the basis of the results it 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. In view of high fluoride content of the bore well water and associated fluoride toxicity among the children, Our team has advised the local administration to provide an alternative water supply 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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