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ORIGINAL ARTICLE

OXIDATIVE STRESS IN CASES OF CHRONIC FLUORIDE INTOXICATION

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ABSTRACT

This study was conducted to find out the level of oxidative stress and effect of supplementation of vitamin C, D and Calcium on levels of SOD, serum and urinary fluoride in children residing in endemic fluorosis area. For this the fluoride belt of Jaipur district was selected. The parameters selected were Super oxide dismutase, serum fluoride and urinary fluoride. The study was conducted on one hundred children, selected from four areas (25 from each area) consuming water containing 1.2, 2.4, 5.6 and 13.6 mg/l of fluoride. Drinking water fluoride, serum and urinary fluoride were measured by Ion selective electrode method. Serum SOD by Xanthine oxidase method using kit of Ransod (kit cat. No. SD125). The post treatment values showed a significant reduction in serum fluoride and SOD. Urinary fluoride levels increased significantly in post treatment stage. The results revealed a normal SOD levels in all groups but an increasing trend was observed with increasing fluoride concentration. Treatment with Calcium, Vitamin D and Vitamin C showed a significant reduction in serum fluoride and SOD and increase in urinary fluoride. A high positive correlation between pretreatment and post treatment group was observed in serum fluoride, SOD and urinary fluoride ($P < 0.05$). The study indicated an increasing oxidative stress in cases of fluorosis with increasing drinking water fluoride concentration. Treatment with Calcium, Vitamin D and Vitamin C resulted a significant reduction in serum fluoride and SOD and increase in urinary fluoride.

KEY WORDS

Fluorosis, Super oxide dismutase, Serum fluoride, Urinary fluoride, Drinking water fluoride.

INTRODUCTION

Chronic ingestion of fluoride in concentration above maximum permissible limits results in various pathological changes in organs and tissues especially in bones, teeth and musculoskeletal system. The mechanisms by which fluoride produce such effects are still not clear. It is reported to increase aging process (1), increase incidence of cancer and tumor growth (2), disrupt immune system (2), causes genetic damage (3) and interrupt DNA repair enzyme activity (2). Oxidative stress is implicated in a wide range of human diseases from cancer to diabetes, to brain disorders and even chronic fluoride toxicity. Reports regarding effects of fluoride on oxidative stress are conflicting. Measurement of antioxidant enzyme like glutathione peroxidase, Super oxide dismutase, catalase etc. and levels of lipid peroxidation have controversial results (3, 4, 5).

Vani et al reported accumulation of fluoride and altered activities of enzymes in whole brain and Gastrocnemius muscle of female mice treated with NaF (6). The enzymes SOD, GST and Catalase decreased significantly with moderately increased SDH, LDH, AIAT, AAT and CPK activities and membrane bound enzymes viz. Na^+, K^+, Mg^{++}, and Ca^{++} ATPase and AchK- were decreased significantly. The study showed that both brain and muscles are affected by fluorosis with inhibition of some enzymes associated with free radical metabolism, energy production and transfer, membrane transport and synaptic transmission (6). Shivanrayasthankara

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and colleagues (3) observed elevated Malondialdehyde in RBC including increased lipid per-oxidation in children aged 3-10 years with endemic skeletal fluorosis along with decreased levels of Glutathione and uric acid together with an increase in activity of Glutathione peroxidase, ascorbic acid along with a slight decrease in activity of SOD. The results of other studies (3, 4) also suggested enhanced oxidative stress and oxidative damage due to chronic fluoride ingestion. However Reddy et al (5) observed no significant difference in lipid per oxidation, Glutathione and vitamin C in the blood of human fluorotic patients and fluoride intoxicated rabbits as compared to respective controls. Neither any change in the activities of Catalase, Superoxide dismutase, Glutathione peroxidase or Glutathione S- transferase in blood was observed. Decrease in the activity of free radical scavenging enzymes (SOD, Glutathione peroxidase) also occurs in people living in areas of endemic fluorosis (7).

Fluorosis is a preventable disease and once a person is affected by it no cure is available, however, various workers have reported a decrease in the severity of the manifestations of fluorosis by using various therapeutic interventions including administration of vitamin C,D and salts of calcium, magnesium, glutamic acid or aluminum (8,9). Gupta et al reported partial reversal in the dental and skeletal manifestations of fluorosis after supplementation of vitamin C, D and calcium for 6 months in fluorosis affected children in endemic fluoride zone of Rajasthan (10). Susheela et al also reported a reversal in the manifestations of fluorosis after using nutrients like calcium, vitamin E, C and antioxidants (11). The aim of the present study was to find out the level of oxidative stress and effect of supplementation of vitamin C, D and Calcium on levels of SOD, serum and urinary fluoride in children residing in endemic fluorosis area

MATERIALS AND METHODS

Drinking water fluoride concentration was determined in 150 villages of Jaipur district. The fluoride in water was estimated using ISE (Ion selective electrode) method. Out of these, three affected areas were selected based on different fluoride concentrations. One more area (served as control) was also selected, where drinking water fluoride concentration was less then 1.5 ppm. Group I Jaipur City (Control group)-1.2 ppm; Group II Ramsagar ki Dhani-2.4 ppm; Group III Shivdaspura-5.6 ppm; Group IV Raipuria-13.6 ppm.

The criteria for the selection was different levels of fluoride above 1.5 ppm in drinking water in these villages, proximity to the investigators working place and compliance of the children to therapeutic intervention. The study was conducted on 100 children. Twenty five children were selected from each of three effected areas (presenting with dental fluorosis), and 25 children were selected from control (Area with < 1.5 ppm fluoride). The age group was from 5 to 12 years. An informed consent was obtained from parents of all children after explaining the purpose of the study.

Clinical examination was done by a pediatrician for the presence of any chronic disease and by a dental surgeon for presence of dental fluorosis. 3 ml of blood sample was drawn from all children under complete aseptic precaution at start of the study, before beginning of supplementation and at the end of six months of the supplementation. One ml of blood was transferred to vial containing heparin which was used for the estimation of Super oxide dismutase (12) 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 (13). Samples of urine were also collected at the time of obtaining blood samples for estimation of urinary fluoride (14). Supplementation was provided to all children included in study consisting of 500 mg twice daily of ascorbic acid (vitamin C) and 1 gm of elemental calcium given in form of calcium carbonate packaged in gelatin capsules in two divided doses morning and evening immediately following meals. Vitamin D was given in the doss of 60000 IU biweekly. Samples of blood and urine were also collected at the end of six months of supplementation for the analysis.

Statistical Analysis: Coefficient of correlation (r) was applied using Microsoft Excel software, Microsoft Corporation, USA on a personal computer. The p-value for significance of correlation analysis were also calculated (p value < 0.05 was considered significant). The relevant p values of correlation analysis (r) are discussed.

RESULTS

The values of Serum fluoride, SOD and urinary fluoride are depicted in Table 1. It was observed that serum and urinary fluoride increases with drinking water fluoride levels. The SOD levels were well with in normal range but an increasing trend was observed with increasing fluoride concentration. The post treatment values showed a significant reduction in serum fluoride and SOD. Urinary fluoride levels increased significantly in post treatment stage.

DISCUSSION

Serum fluoride levels were found to be elevated in group II,

Table 1: Pre and Post Supplementation levels of serum fluoride, urinary fluoride and SOD in studied groups

Groups	Serum Fluoride		P value	SOD		P value	Urinary Fluoride		P value
	Pre Supplementation	Post Supplementation		Pre Supplementation	Post Supplementation		Pre Supplementation	Post Supplementation	
I	0.16±0.05	0.14±0.05	NS	1104±75.0	1127±75.0	NS	0.48±0.11	0.45±0.11	NS
II	0.7±0.1	0.6±0.1	<0.01	1079±200.0	995.5±200.0	<0.05	9.95±0.78	10.9±0.78	<0.01
III	0.6±0.15	0.38±0.15	<0.01	1175±165.4	998.5±165.4	<0.01	15.0±0.72	17.06±0.72	<0.01
IV	1.03±0.13	0.81±0.13	<0.01	1525±194.0	1210±194.0	<0.01	13.3±1.83	14.64±1.83	<0.01

III and IV as compared to group I (control) (10) in pre supplementation group. After supplementation the levels came down significantly in all the test groups, but the changes in the control group was non significant (Table 1). The significant changes observed in the test group after supplementation of calcium, vitamin C and vitamin D may be due to decreased absorption of fluoride from the gastro-intestinal tract as well as increased renal clearance. Calcium, vitamin C and vitamin D supplementation have definite role in improving renal clearance. It has been reported that rea**c**tive oxygen spe**c**ies play a major role in the development of endothelial dysfun**c**tion of the renal vas**c**ulature and Vitamin C being an anti oxidant improves the renal clearance in conditions with oxidative stress. The calcium and vitamin D improves the compromised renal perfusion by correcting the secondary hyperparathyroidism induced by high fluoride ingestion.

Few workers considered urinary fluoride excretion as one of the best indices of fluoride intake (9, 17). In present study the mean urinary fluoride was 0.48 ppm in control and 9.95 ppm, 15.0 ppm and 13.3 ppm in group II, III and IV respectively. A significant increase in urinary fluoride excretion was observed in all test groups after supplementation. These findings suggests that as the level of fluoride in drinking water increases the excretion of fluoride also increases in urine and supplementation with calcium, vitamin C and D increases the renal clearance of fluoride. Further a significant positive correlation has been observed between serum fluoride and urinary fluoride excretion in control and group IV (r value= +0.684 and +0.47 respectively). Various workers have reported (9, 15) increased fluoride excretion following increased ingestion of fluoride, although severity of disease and urinary fluoride levels has no correlation (11). Teotia et al (18), Gupta et al (19) also reported similar findings but no reports are available regarding effect of such supplementation therapy as used in this study on urinary fluoride excretion. Reduction in levels of serum fluoride and concomitant increase in excretion of fluoride in urine indicates a definite role of supplementation of vitamin C, D and calcium in reducing fluoride accumulation in body.

Oxidative stress is implicated in wide range of human diseases, including cancer and diabetes. Various workers have found strong evidence for oxidative stress in fluorosis in animal and human beings. Vani et al (6) reported inhibition of some enzymes associated with free radical metabolism, energy production and transfer, membrane potential and synaptic transmission but with an enhance activity of SOD in fluorosis affected animals. Normally the value of SOD ranges between 1102 to 1601 IU/gm of hemoglobin. In present study levels of SOD were found to range between 1079 U/gm of hemoglobin to 1525 U/gm of hemoglobin in pre supplementation category which are well within normal limits. After supplementation a significant decrease was observed in all the groups while there was no change in control group. Such decrease in the levels of SOD after supplementation in all the test groups reflects an overt state of oxidative stress in these children.

Birkner et al (20) also observed increased oxidative stress in terms of elevated malondialdehyde levels, in children with endemic skeletal fluorosis. Same authors in 2002 again reported enhanced oxidative stress in the brain of fluoride treated rats. On the other hand Reddy et al (5) while studying the antioxidant defense system in human from endemic fluorosis area found no significant difference in lipid peroxidation, glutathione and vitamin C in blood of human fluorotic patients, neither there was any change observed in the activity of catalase, superoxide dismutase and glutathione peroxidase or glutathione s. transferase due to fluoride intoxication. Various authors have reported a differential effect of fluorosis on various antioxidants defense system; such differential effects may be due to involvement of these systems at different levels of dealing with the generated reactive oxygen species (R.O.S). Latest study in year 2006 (21) also reported a decrease in the activity of catalase and -SH groups, while the concentration of thiobarbituric acid reactive substances increased in all investigated tissues suggesting an increased

lipid per oxidation under chronic fluoride intoxication. In present study the normal SOD levels in all the studied groups and a significant fall after supplementation raises the question about the most debatable issue of oxidative stress in fluorosis necessitating more elaborate studies involving measurement of other indicators of oxidative stress along with superoxide dismutase.

The study indicated an increasing oxidative stress in cases of fluorosis with increasing drinking water fluoride concentration. Treatment with Calcium, Vitamin D and Vitamin C showed a significant reduction in serum fluoride and SOD and increase in urinary fluoride.

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