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OXIDATIVE STRESS IN CHILDREN WITH ENDEMIC SKELETAL FLUOROSIS

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SUMMARY: In the village of Kheru Nayak Thanda in the Gulbarga district of Karnataka, India, 18 children aged 3 to 10 years with endemic skeletal fluorosis were shown to have oxidative stress as evidenced by elevated levels of malondialdehyde in their red blood cells, indicating increased lipid peroxidation. Significant alterations of antioxidant systems in the blood were confirmed by decreased levels of glutathione and uric acid together with an increase in the activity of glutathione peroxidase as well as the level of ascorbic acid along with a slight decrease in the activity of superoxide dismutase.

Keywords: Antioxidants, Ascorbic acid, Glutathione levels, Glutathione peroxidase, Gulbarga district, Karnataka, India, Kheru Nayak Thanda, Lipid peroxidation, Malondialdehyde levels, Skeletal fluorosis, Superoxide dismutase.

INTRODUCTION

Fluorosis is a well-defined clinical entity characterized by toxic effects of high-fluoride intake on teeth, bones and soft tissues.[1-9] Increased oxygen radical generation and lipid peroxidation have been implicated in the pathogenesis of many diseases and toxic action of a wide range of compounds.[10,11] This process has even been proposed to be an important mediating factor in the causation of detrimental effects of chronic fluoride toxicity.[12-14] Earlier we reported the prevalence of dental and skeletal fluorosis among children in Kheru Nayak Thanda, a remote village of Gulbarga district in Karnataka, India.[15] Out of 46 children, 41 (89%) had dental fluorosis, and 18 (39%) exhibited skeletal fluorosis. The water fluoride levels ranged from 0.5 to 12.6 ppm, with a mean of 5.53 ppm. The present study aimed to assess lipid peroxidation and levels of antioxidant defense systems in the blood of children afflicted with skeletal fluorosis.

MATERIALS AND METHODS

Eighteen children with skeletal fluorosis, in the age group of 3-10 years, were the subjects of this study.[15] Fifteen age- and sex-matched healthy children residing in other parts of Gulbarga with permissible levels (< 1.0 ppm) of fluoride in drinking water served as controls.

Blood samples of the children were collected by venipuncture into an acid-citrate-dextrose solution (1.0 mL per 4.0 mL of blood). Plasma and buffy coat (consisting of leukocytes and platelets) were removed by cen-

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trifugation at 3000 rpm for 15 min. Red blood cells were washed three times with buffered saline (0.9% saline in 0.01M phosphate buffer, pH 7.4). The packed cells were then suspended in an equal volume of the buffered saline.

Lipid peroxidation in the red blood cells was assessed by estimation of malondialdehyde (MDA) according to the method of Ohkawa *et al*.[16] Glutathione (GSH), glutathione peroxidase (GSH-P_x) and superoxide dismutase (SOD) were estimated in the red cell lysates. GSH was assayed by the method of Beutler *et al*,[17] GSH-P_x by the method of Pagilia and Valentine,[18] and SOD by the method of Beauchamp and Fridovich.[19] Hemoglobin (Hb) in the red cell lysates was estimated by the cyanmethemoglobin method.[20] In the plasma samples, ascorbic acid was determined by the method in the textbook by Tietz[21] and uric acid by the method of Trivedi *et al*.[22]

Statistical significance of the results was assessed by the Student's t test.

RESULTS

There was increased lipid peroxidation in the red blood cells of fluorotic children as evidenced by the elevated MDA levels. GSH levels were significantly lowered while the activity of GSH-P_x markedly increased in the red blood cells of fluorotic children, compared to those of healthy controls. A slight but significant decrease in the SOD activity of fluorotic children was also observed (Table 1).

The level of plasma ascorbic acid was markedly elevated, whereas that of uric acid was lower in fluorotic children compared to the controls (Table 2).

Table 1. Malondialdehyde (MDA) and antioxidants in the red blood cell lysates (Values: means ± SD)

Subjects (3-10 yr)	MDA (nanomoles/g Hb)	GSH (micromoles/g Hb)	GSH-P_x (units/g Hb)	SOD (units/g Hb)
Control (n=15)	219.3 ± 7.80	12.96 ± 0.61	30.42 ± 1.98	1545 ± 62.43
Fluorotic (n=18)	256.0 ± 9.04	8.63 ± 0.68	40.86 ± 1.71	1457 ± 46.25
Statistical significance	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$

Table 2. Ascorbic acid and uric acid in plasma (Values: mean ± SD)

Subjects (age 3-10 yr)	Ascorbic Acid (mg/dL)	Uric acid (mg/dL)
Control (n=15)	0.62 ± 0.09	2.92 ± 0.24
Fluorotic (n=18)	1.27 ± 0.36	2.35 ± 0.43
Statistical significance	$p < 0.001$	$p < 0.001$

DISCUSSION

A close association between chronic fluoride toxicity and increased oxidative stress has been reported in humans[23-25] and experimental animals.[13,14,26] Erythrocytes are more commonly employed in the evaluation of oxidative stress, since they are prone to oxidative reactions because of relatively high oxygen tension and the presence of polyunsaturated lipid-rich plasma membranes.[27] Fluoride has been demonstrated *in vivo* and *in vitro* to cause increased lipid peroxidation in erythrocytes of humans[23] and in blood and tissues of experimental animals.[13,14,26,28] The increased MDA levels in fluorotic children observed in this study are in accord with earlier findings.

Oxidant stress produced by free radicals and H_2O_2 is greater if fluoride impairs the production of free radical scavengers such as GSH, GSH-P_x, SOD, and ascorbic acid.[12] Studies have shown a decrease in the activities of SOD and GSH-P_x[25] and increased levels of GSH[24] in people living in areas of endemic fluorosis. In experimental animals, decreased levels of GSH, and GSH-P_x activity in erythrocytes[13] and a decreased activity of SOD in other tissues,[8,14,26] together with unaltered SOD activity in erythrocytes[13] were observed. Our study recorded increased GSH and GSH-P_x, and decreased SOD in the erythrocytes of rats exposed to 100-ppm fluoride in drinking water for four months.[28] The present study showed an increase in GSH-P_x activity and a decrease in GSH levels with a slight decrease in SOD activity in the red blood cells of fluorotic children. The decrease in the levels of GSH in red blood cells observed in our study may be due to increased utilization of GSH by GSH-P_x in detoxification of H_2O_2 generated by fluoride-induced oxidative stress. Uric acid is one of the important antioxidants of plasma,[29] and its level was decreased in fluorotic children. The results suggest that fluoride toxicity may involve a reduction of certain intrinsic scavengers resulting in an increased vulnerability to oxygen free radical toxicity.

Ascorbic acid is considered the most important antioxidant of the plasma[30,31] and also as an anti-stress factor.[32] Ingestion of inorganic fluoride in rats promotes synthesis and mobilization of ascorbic acid.[32] Ascorbic acid plays a significant role in the amelioration of fluoride-induced toxicity.[33] We have demonstrated an increase in plasma and brain ascorbic acid levels of rats exposed to chronic fluoride intoxication.[28] Increased plasma ascorbic acid levels in fluorotic children in our study may indicate increased utilization and/or mobilization of ascorbic acid from storage in response to fluoride-induced stress.

In conclusion, we found that chronic fluoride toxicity in children elicited increased lipid peroxidation associated with free radical mediated oxidative stress demonstrated by increased levels of MDA and increased or decreased levels or activities of antioxidants in the blood.

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