

## LIPID PEROXIDATION AND ANTIOXIDANT ENZYME STATUS OF ADULT MALES WITH SKELETAL FLUOROSIS IN ANDHRA PRADESH, INDIA

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**SUMMARY:** Blood samples from 24 adult males, age 25 to 40, with endemic skeletal fluorosis, living in the Vaillapally village of the Nalgonda district, Andhra Pradesh, India, were examined and compared with samples from 15 matched controls for their antioxidant enzyme activity and lipid peroxidation. Elevated malondialdehyde (MDA) levels indicated an increase in lipid peroxidation products, and decreased activity levels of catalase (CAT) and glutathione-S-transferase (GST) reflected significant alterations in their antioxidant status. These results, in agreement with recent findings by others, demonstrate that chronic fluoride intoxication in adult males elicits increased lipid peroxidation associated with a significant decrease in the activities of CAT and GST.

Keywords: Andhra Pradesh, India; Antioxidant status; Catalase; Glutathione-S-transferase; Lipid peroxidation; Malondialdehyde; Skeletal fluorosis.

### INTRODUCTION

Fluorosis is a metabolic hard tissue disease caused by ingestion of excessive amounts of fluoride (F), mainly through drinking water but also from food in endemic F areas.<sup>1</sup> Excess intake of fluoride (F), apart from causing dental and skeletal abnormalities, can inhibit the activity of many enzymes.<sup>2,3</sup> F is also known to cross cell membranes and enter soft tissues, causing impairment of soft-tissue function in F-intoxicated animals.<sup>4-6</sup> Generation of free radicals, lipid peroxidation products, and altered antioxidant defense systems are also regarded as toxic effects of F.<sup>6-10</sup>

The present study aimed to assess the status of lipid peroxidation and levels of antioxidant enzyme status in the blood of males afflicted with skeletal fluorosis in Andhra Pradesh, India.

### MATERIALS AND METHODS

Twenty-four male volunteers, 25–40 years of age, with severe manifestations of skeletal fluorosis, from the Vaillapally village of the Nalgonda district, Andhra Pradesh, India, were chosen as the experimental group for this study. All had been consuming drinking water with a high F content of 5.5 to 7 ppm since birth. Fifteen age-matched healthy males, residing in other parts of Nalgonda district with much lower F levels (<1.0 ppm) in their drinking water, served as controls.

Blood samples of the subjects were collected by arm venipuncture into an EDTA solution (1.0 mL per 4.0 mL of blood). Plasma and buffy coat (consisting of leukocytes and platelets) were removed by centrifugation at 3000 rpm for 20 min. Red blood cells were washed three times with 0.9% saline in 0.01 M pH 7.4

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phosphate buffer. The packed cells were then suspended in an equal volume of the buffered saline. Blood samples without anti-coagulant were also collected.

Lipid peroxidation in blood serum was assessed by estimation of malondialdehyde (MDA) according to the method of Ohkawa et al.<sup>11</sup> Catalase (CAT) activity in red cell lysates was determined by the method of Aebi,<sup>12</sup> and glutathione-S-transferase (GST) activity in serum was assessed by the method of Habig et al.<sup>13</sup> Hemoglobin (Hb) content in red cell lysates was estimated by the Drabkin method.<sup>14</sup> Serum F was measured with a F ion selective electrode (Orion-940).

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

## RESULTS

Increased lipid peroxidation was observed in the fluorotic males as shown by the elevated MDA levels. Activities of CAT and GST were significantly decreased in these men compared to those of healthy controls, and the decrease was statistically significant (Table).

**Table.** Malondialdehyde (MDA) and fluoride (F) levels and activities of antioxidant enzymes CAT and GST in blood of control and fluorotic subjects (values: mean  $\pm$  SE)

Subjects (Age 25-40)	MDA (nmol/ml)	CAT (KU/gm Hb)	GST (IU/L)	Serum F (ppm)
Controls (n = 15)	2.06 $\pm$ 0.02	87.14 $\pm$ 4.07	75.14 $\pm$ 2.63	0.07 $\pm$ 0.001
Fluorotics (n= 24)	3.49 $\pm$ 0.19	64.98 $\pm$ 3.82	47.07 $\pm$ 2.56	0.26 $\pm$ 0.004
Statistical significance	p<0.001	p<0.001	p<0.001	p<0.001

## DISCUSSION

Reactive oxygen species (ROS) are implicated as important pathological mediators in many disorders. Increased generation of ROS and enhanced lipid peroxidation are considered responsible for the toxicity of a wide range of metabolic products.<sup>15-17</sup> Various authors have reported relationships between F and oxidative stress caused by free radicals.<sup>6-10,18-21</sup> Increased lipid peroxidation in human blood induced by F has also been demonstrated *in vivo* and *in vitro*.<sup>21</sup> The elevated MDA levels in fluorotic males observed in this study are thus in accord with previous findings.

Oxidative stress produced by free radicals and hydrogen peroxide is greater if F impairs the production of free radical scavengers such as GSH (glutathione), CAT, GSH-Px (GSH peroxidase), SOD (superoxide dismutase), and GST (glutathione-S-transferase).<sup>6</sup> Decreases in the activities of SOD, CAT, GST, and GPX have been found in people living in areas of endemic fluorosis<sup>9,22</sup> and in tissues of experimental animals subjected to F intoxication.<sup>3,23</sup> The decrease in the activities of CAT and GST observed in our study is therefore very likely to be due to oxidative stress exerted by F intoxication. Our results indicate that F intensifies lipid peroxidation and reduces antioxidant potential in living cells.

Some investigators, however, have reported that F does not impair antioxidant defense systems.<sup>24-26</sup> Such differences in results are possibly due to many factors such as age, sex, calcium intake, dose and duration of F intake, renal efficiency in handling F, and methods used for biochemical assay.<sup>6,27</sup> It is therefore worth noting that Susheela et al.<sup>28</sup> and Chinoy et al.<sup>29-31</sup> found that supplementing the

diet with antioxidants reversed the toxic effects of F on antioxidant defense systems.

In conclusion, we have found that the blood of adult males with skeletal fluorosis living in an endemic fluorosis area exhibited increased lipid peroxidation associated with significantly decreased activity of the free radical-scavenging enzymes CAT and GST.

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