

## EFFECT OF FLUORIDE ON OXIDATIVE STRESS AND BIOCHEMICAL MARKERS OF BONE TURNOVER IN POSTMENOPAUSAL WOMEN

A Sivanarayana Goudu,<sup>a</sup> M Dhananjaya Naidu<sup>b</sup>  
Andhra Pradesh, India

**SUMMARY:** The present study was conducted on 42 postmenopausal women subjects in Vailapally village, Nalgonda district, Andhra Pradesh, India, an endemic fluorotic area (water fluoride >4 ppm) and 34 postmenopausal women of nonfluorotic villages (water fluoride <0.4 ppm) of the Nalgonda area. The age group of the recruited subjects was 48–58 years and their years since menopause (YSM) was <10 years. Serum levels of fluoride (F), total alkaline phosphatase (ALP), tartarate resistant acid phosphatase-5b (TRAP-5b), catalase (CAT), glutathione-S-transferase (GST) and malondialdehyde (MDA) were estimated for bone mineral antioxidant and lipid peroxidation status. Significantly increased bone turnover markers ALP, TRAP-5b ( $p<0.01$ ), and oxidative stress were observed with decreased levels of CAT and GST ( $p<0.01$ ) activity in postmenopausal women residing in the fluorotic village. Significantly elevated levels of MDA ( $p<0.01$ ) in these women compared to those in the nonfluorotic village indicated an increase in lipid peroxidation under fluoride stress.

Keywords: Antioxidant status; Biochemical fluorosis markers; Bone turnover; High F Vailapally village; Lipid peroxidation; Low F villages; Nalgonda district, Andhra Pradesh, India; Postmenopausal women.

### INTRODUCTION

In many parts of the world, fluoride (F) causes damage not only to hard tissues like teeth and bones, but also to soft tissues, such as brain, liver, kidney, and spinal cord.<sup>1</sup> Human exposure to F occurs mainly through drinking water, and, although not universally accepted, the World Health Organization has recommended 1.5 mg F/L drinking water as the safe limit.<sup>2</sup> Detrimental effects of F on skeletal tissues are characterized by dental mottling, crippling deformities and osteoporosis.<sup>3</sup> Intake of F is also known to promote the formation of reactive oxygen species (ROS) that can lead to oxidative damage to the vital cell components.

Under normal conditions, damage by toxic free radicals is physiologically counteracted by the intracellular antioxidant systems: antioxidant enzymes and endogenous free radical scavengers.<sup>4,5</sup> However when the rate of free radical generation exceeds the capacity of antioxidant defenses, it induces oxidative stress leading to damage of DNA, proteins, and lipids and inhibition several groups of enzymes.<sup>6,7</sup> ROS are produced in cells at various sites including the plasma membrane, mitochondria, and cytoplasm. The largest amounts of ROS are produced in mitochondria because of electron leaks in the respiratory chain. At the surface of the cell, NADPH oxidases transport electrons across the plasma membrane to generate superoxide ( $O_2^{2-}$ ). Superoxide is unstable and so is rapidly converted into  $H_2O_2$ , which can diffuse through the cell membrane.<sup>8</sup> Many studies reported F as anabolic agent and capable of affecting osteoblast cells *in vitro*<sup>9</sup> and *in vivo*.<sup>10</sup> ROS generated with high F intake enhance osteoclastogenesis<sup>8</sup> and

<sup>a</sup>Department of Biotechnology, Sri Venkateswara University, Tirupati 517502 Andhra Pradesh, India; <sup>b</sup>For Correspondence: Prof M Dhananjaya Naidu, Department of Zoology, Yogi Vemana University, Kadapa 516003, Andhra Pradesh, India; E-mail:asngoudbio@gmail.com

affect bone mineralization.<sup>11</sup> ROS initiate the peroxidation of membrane lipids,<sup>12</sup> and produce, as one of the end products, MDA, which is an indicator of oxidative stress.<sup>13</sup>

The aim of the present study was to investigate the effect of F on antioxidant enzymes, lipid peroxidation, and bone turnover markers in postmenopausal women.

### MATERIALS AND METHODS

Postmenopausal women (n=42) with a mean age of 56.8 years and <10 years since menopause (YSM) were voluntarily selected as the study population from the endemic fluorotic village (F >4 ppm) Vailapally, Nalgonda district, Andhrapradesh, India. Menopausal women volunteers (n=34) with a mean age 54.3 years and <10 YSM from other parts of Nalgonda district (F <0.4 ppm) were recruited as controls. All the procedures were approved by the institutional ethical committee in accordance with the Helsinki Declaration of 1975, as revised in 2000. After obtaining informed consent from the women in both groups, 4 mL of venous blood was drawn into sterile disposable syringes and transferred into centrifuge tubes. The samples were centrifuged at 3000 RPM for 20 min, and the serum was collected for further analysis. Red blood cells were washed with 0.9% saline in 0.01M pH 7.4 phosphate buffer. Serum and water F levels were measured with a F ion selective electrode (Orion-940).

Serum ALP activity was measured by the 4-amino-antipyrene method.<sup>14</sup> The TRAP-5b activity was measured by the ELISA method with a kit supplied by Immuno Diagnostic Systems (IDS), UK. The lipid peroxidation product MDA was measured as thiobarbituric acid reacting substances (TBARS).<sup>15</sup> The resulting rise in pink color complex was measured by a spectrophotometer.<sup>16</sup> Erythrocyte CAT activity was assessed by the method of Aebi<sup>17</sup> and GST activity by that of Habig et al.<sup>18</sup>

Statistical analysis was performed using SPSS software (Version 11.5). Data are expressed as mean and standard deviation. Significance of difference between the study and control groups was observed by using the Student t-test. P values <0.05 were considered statistically significant.

### RESULTS

Both the endemic fluorotic group of women residing in the high F village since birth and the nonfluorotic women in the low F village had <10 YSM. The biochemical parameters of the two groups of women are shown in the table below. Serum levels of F were significantly (p<0.001) elevated in the fluorotic village subjects compared to those in the nonfluorotic village. Serum levels of the osteoclast specific activity marker TRAP-5b and bone formation marker ALP were significantly (p<0.001) higher in the fluorotic village. The significantly increased (p<0.001) lipid peroxidation product, MDA (p<0.001), and decreased CAT and GST activity (p<0.001) were observed in the fluorotic study group compared to the nonfluorotic group.

**Table.** Biochemical markers of postmenopausal women residing in a nonfluorotic and a fluorotic village  
(Values are mean  $\pm$  SD)

| Parameter            | Nonfluorotic (n=34) | Fluorotic (n=42)               |
|----------------------|---------------------|--------------------------------|
| Serum fluoride (ppm) | 0.08 $\pm$ 0.01     | 0.27 $\pm$ 0.04 <sup>*</sup>   |
| Serum ALP (KA units) | 6.72 $\pm$ 1.08     | 8.16 $\pm$ 1.76 <sup>*</sup>   |
| Serum TRAP-5b (U/L)  | 3.06 $\pm$ 0.14     | 4.18 $\pm$ 0.26 <sup>*</sup>   |
| MDA (nmol/mL)        | 2.58 $\pm$ 0.21     | 3.84 $\pm$ 0.25 <sup>*</sup>   |
| CAT (KU/g Hb)        | 76.15 $\pm$ 8.34    | 48.52 $\pm$ 12.96 <sup>*</sup> |
| GST (IU/L)           | 64.3 $\pm$ 14.25    | 42.15 $\pm$ 12.2 <sup>*</sup>  |

<sup>\*</sup>Compared with the control,  $p < 0.001$ .

## DISCUSSION

Estrogen deficiency leads to increased bone turnover in postmenopausal women. In this study, significantly elevated levels of bone formation alkaline phosphates (ALP) and bone resorption marker (TRAP-5b) were observed in fluorotic postmenopausal women compared to their nonfluorotic controls. According to Sreelakshmi et al.,<sup>19</sup> increased oxidative stress exerted by F-derived ROS contribute to bone resorption in postmenopausal women. Here we found a significantly increased activity of TRAP-5b and ALP representing increased bone turnover rate in the fluorotic group.

Catalase is a hemeprotein, which catalyses the dismutation of H<sub>2</sub>O<sub>2</sub> into H<sub>2</sub>O and O<sub>2</sub>.<sup>20</sup> Contradictory results have been reported on influence of F on the activity of CAT. Some studies have reported decreased activity<sup>5,21</sup> and others increased<sup>7</sup> and unchanged<sup>22</sup> CAT activity. Reddy et al. did not find any difference in the activity of CAT in red blood cells of F-intoxicated rabbits.<sup>11</sup> Earlier studies from our laboratory, however, confirm increased lipid peroxidation and decreased activity of CAT and GST in males with skeletal fluorosis.<sup>23</sup> ROS react with methylene groups of polyunsaturated fatty acids, initiating the peroxidation of membrane lipids and producing MDA as one of the end products.<sup>12</sup> Increased MDA from F toxicity generates ROS and thereby higher levels of H<sub>2</sub>O<sub>2</sub> are formed in cells. H<sub>2</sub>O<sub>2</sub> at high concentration is deleterious to cells, and its accumulation causes oxidation of cellular targets such as proteins, lipids, and DNA leading to mutagenesis and cell death.<sup>24</sup> Removal of H<sub>2</sub>O<sub>2</sub> from cells is, therefore, necessary for protection against oxidative damage. The present study showed increased MDA and decreased activity of CAT and GST in postmenopausal women with skeletal fluorosis. Similar trends with MDA and antioxidant enzyme status have also been observed by others in humans with fluorosis.<sup>11,12</sup>

In summary, the present findings suggest that accelerated bone turnover rate and increased lipid peroxidation are detrimental effects of F toxicity. More extensive

studies are needed to explore the implications of F-induced oxidative stress on metabolic bone disorders in postmenopausal women.

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