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Research Article

Trends on oxidative stress and antioxidant status in fluoride affected areas in Kolar district, India

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ABSTRACT

Background: Fluoride in cellular respiratory processes and its association in free radical generation are extensively studied. Cell permeability generates the oxidative stress through free radical species entry that sequentially affects the cellular biomolecules and cause toxic effects. Present study intended to determine the trend of oxidative stress and antioxidant status in the fluoride affected area that may raise the need for supplementation of antioxidants. Oxidative stress denoted in terms of ischemia modified albumin and antioxidants by vitamin C & total antioxidant capacity.
Methods: Sixty subjects were divided into two groups. Group I (control) from fluoride unaffected areas (n=30) and group II subjects from fluoride affected area (n=30). Serum was assayed for Ischemia Modified Albumin (IMA), plasma for Total Antioxidant Capacity (TAC) and vitamin C.
Results: The mean ± SD of IMA, TAC, and vitamin C of individuals subjected in fluoride affected areas are 2.403 ± 0.543, 210 mmol/l ± 82.2 and 0.62 mg/dl ± 0.23 respectively. The values were compared to individuals from fluoride unaffected areas have IMA (1.054 ABSU ± 0.851), TAC (312 mmol/l ± 62.1) and vitamin C (0.93 mg/dl ± 0.14) with P <0.001.
Conclusion: The present study revealed that, individuals subjected to fluoride affected areas have more oxidative stress and decreased levels of antioxidant status indicates the risk of generation of free radicals intensity as a chief component of oxidative stress. This research outcome necessitates dietary supplementation of nutrient antioxidants to minimize the consequences of oxidative damage to vital biomolecules in fluoridated affected areas.

Keywords: Fluoride, Oxidative stress, Vitamin C, Ischemia modified albumin (IMA)

INTRODUCTION

In the global scenario, twenty three nations have the problem of excess fluoride in drinking water and resulting endemicity for fluorosis.[1] India lies in a geographical fluoride belt; fluorosis is an endemic condition prevailed in 17 states of India. The highest endemicity rate has been reported in Andhra Pradesh, Rajasthan, Punjab, Tamilnadu and Karnataka.[2,3] In Karnataka, totally 16 districts are endemic viz Dharwad, Gadag, Bellary, Belgaum, Raichur, Bijapur, Gulbarga, Chitradurga, Tumkur, Chikmagalur, Mandya, Bangalore Rural, Mysore, Mangalore, Shimoga and Kolar.[4]

Fluorosis is a disease state caused by excess intake of Fluoride through drinking water, food, or inhalation. Acute high-level exposure to fluoride is rare and usually due to accidental contamination of drinking-water or due to fires or explosions. Moderate-level & chronic exposure of above 1.5 mg/L of water as per the WHO guideline value of fluoride in water is more common. Fluoride is also known to cross the cell membranes and to enter soft tissues.[5]

Fluoride regulates enzyme activity in glycolysis and citric acid cycle and also an activator of G-proteins in signal transduction process in eukaryotic cells. Studies have produced conflicting results on the relationship between free radical generation, lipid peroxidation, and antioxidant functions have been investigated extensively in both human and animal fluorosis.[6-8] It is worthwhile to study the implications of fluoride toxic effect[9] *in vivo* in terms of health and disease are necessary. Reactive Oxygen Species (ROS) produced in aerobic metabolism in the prevailing fluoride-induced toxicity potentially results in lipid peroxidation as damage to cell membranes and eventually alters the functions of cellular biomolecules. This might be the consequence of apoptosis of cell under oxidative stress during fluorosis.[10]

The protecting feature of Super Oxide Dismutase (SOD) one of the enzyme, antioxidant play essential role in life that significantly combats the superoxide levels in vivo and minimizing oxidative damage. In the present study, we investigated the tendency of oxidative stress & antioxidant status in fluoride affected and unaffected areas.

METHODS

Subject selection

Sixty voluntary individuals were divided into two groups. Group I (control) from fluoride unaffected areas (n=30) and group II subjected to fluoride affected area (n=30) in Kolar district, Karnataka India. The study was conducted during February to August 2013 at the division of proteomics; central research laboratory, Sri Devaraj Urs academy of higher education and research, Kolar by obtaining institutional ethical clearance and individual inform consent form with the patients. Individuals with known history of cardiovascular disease, renal disorders, diabetic patients, viral & bacterial infections were excluded from the study.

Sample collection and storage

Three ml of heparinized and non-heparinized fasting blood samples were collected from both the groups and transferred into clean, dry, sterile centrifuge tubes and centrifuged at 3000 X g for 15 minutes to obtain Plasma and Serum. The plasma and serum was separated and stored at -80°C for further analysis.

Biochemical analysis

Serum was assayed for Ischemia Modified Albumin (IMA); a normal albumin molecule has a metal binding site. There are few free binding sites available. During ischemia, this metal binding capacity is reduced due to modification of the N-terminal binding residues. A cobalt containing reagent when added to ischemic blood finds binding sites scarce, and hence excess free cobalt will color the sample and a positive test for ischemia is diagnosed which can be measured at 470 nm in ABS units.

Determination of vitamin C

Vitamin C or L-ascorbic acid levels were estimated using 2,4 DNPH method by spectrophotometric method. 0.1 ml of serum was deproteinated by the addition of 1 ml of methanol, vortexed for 30 Sec then centrifuged at 3000 RPM for 30 min to separate the proteins. To the clear supernatant, 1.5 ml of methanol and 0.5 ml of DNPH solution was added mixed thoroughly and absorbance was read at 517 nm against blank. Ascorbic acid was used as a reference standard.

Total antioxidant capacity (TAC)

Antioxidant power converts ferric to ferrous ion. Reduction at low pH causes a colored ferrous tripyridyltriazine complex to form Ferric Reducing Ability of Plasma (FRAP) values are obtained by comparing the absorbance change at 593 nm in mixture (test), with those containing ferrous ion in known concentration (standard).

Statistical analysis

Mean and standard deviation of each of the measured parameters were calculated in different groups separately and compared to those in healthy individuals. The differences observed if any will be tested for significance by the unpaired t-test taking a P value <0.05 to be significant. Whole statistical analysis was performed on MS excel spreadsheets, Microsoft windows, version 2013.

RESULTS

The mean ± SD levels of Ischemia Modified Albumin levels (IMA) with individuals in fluoride affected areas are (2.403 ± 0.543), total antioxidant levels are (210 mmol/l ± 82.2), the levels of Vitamin C are (0.62 mg/dl ± 0.23) when compared to the normal with individuals in fluoride unaffected areas IMA (1.054 ± 0.851), total antioxidant levels (312 ± 62.1 mmol/l) and vitamin C (0.93 ± 0.14 mg/dl) were statistically significant with P <0.001. However, there are no variations in the blood pressure. For all the individuals in the fluoride affected area, presents the normal blood pressure (~120/80 mm of

Hg). The mean Body Mass Index (BMI) of individuals in the fluoride affected area is 21.28 kg/m^2.

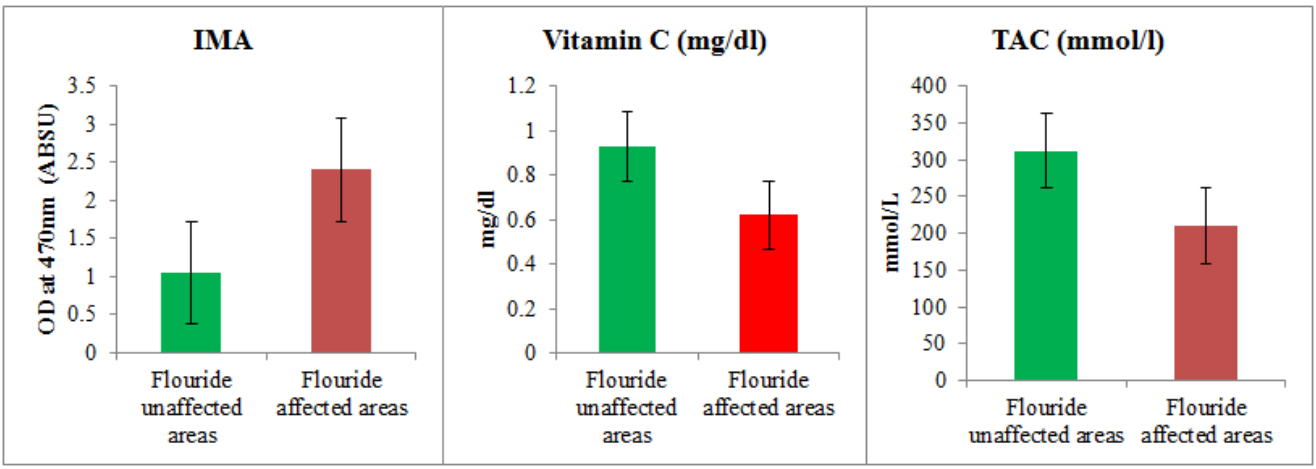

Figure 1: Shows the levels of IMA, vitamin C and TAC in the study groups.

Table 1: Shows the biochemical parameters in the study groups.

Groups	Parameters		
	IMA (ABSU)	TAC (m mol/l)	Vitamin C (mg/dl)
Fluoride unaffected areas	1.054 ± 0.851	312 ± 62.1	0.93 ± 0.14
Fluoride affected areas	2.403 ± 0.543	210 ± 82.2	0.62 ± 0.23

DISCUSSION

Ischemia modified albumin levels were found to be elevated in the individuals subjected to the fluoride affected areas (Group 2) as compared to subjects with fluoride unaffected areas (Group 1). The significant changes observed in the group 2 when compared to group 1 (Table 1) may be because of the individuals living in extremely fluoridated affected areas certainly prone to more oxidative stress measured by the means of increased levels of protein oxidation marker Ischemia Modified Albumin (IMA) which acts as an oxidative stress marker as presented in this study. Fluorosis is a serious public health problem in many parts of the world where drinking water contains more than 1 ppm of fluoride. A study conducted by Vinita Ailani et al. also observed increased oxidative stress in cases of fluorosis with increasing drinking fluorine water concenrations.[11]

In general mechanism, vitamin C plays an important role in the orderly deposition of fluoride into various tissues and prevents the development of fluorosis. A significant decrease in the vitamin C levels was observed in the individuals living in the fluoride affected area (Group 1) when compared to the individuals living in the fluoride unaffected area (Group 2) in the study. Teotia M et al.[12] and Gupta et al.[13] also reported similar findings of our study, but no reports are available regarding the effect of vitamin C supplementation therapy.

Reduction in levels of serum fluoride and increase in excretion of fluoride in urine indicates a definite role of supplementation of vitamin C, D and calcium in reducing fluoride accumulation in the body therefore vitamin C is a good reducing agent known as antioxidant in clinical research. Our study results convince that decreased levels of vitamin C in fluoridated affected individuals clearly demonstrate the risk of exposure to free radicals toxicity.

Similarly, the total antioxidant capacity is measured in terms of the Ferric Reducing Ability of Plasma (FRAP) indicated decreased levels of plasma antioxidants in fluoridated affected individuals in comparison with fluoride affected individuals.

Our study also states the inverse relation between oxidative stress and antioxidant levels with respect to the toxic effects of fluoride could be due altered antioxidants. This observation possibly demonstrates the need of dietary antioxidant supplementation in scavenging the propagation of free radicals generated in order to prevent the risk associated with oxidative stress in individuals at fluoride affected areas. However, further studies are essentially at multi-centric level of fluoride hit zones to arrive data using large population.

The current study concludes that, increased oxidative stress in terms of generation of free radicals in the population of fluoride affected areas is due to the

increased cellular permeability of fluoride, which is in contrast with the fluoride unaffected areas. Similarly antioxidant status in terms of vitamin C and Total antioxidant capacity in the affected areas suggests the need of oral supplementation of the nutrient antioxidants to minimize the harmful effects of oxidative stress inducer.

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