EFFECT OF FLUORIDE AND REACTIVE OXYGEN SPECIES ON HEALTHY POSTMENOPAUSAL WOMEN

Kumari Minakshi[1*] , Sharma Sunil[2], Pahwa Manjubala[3], Menka K.[4], Singh Veena[5], Meena Suresh[6]

1.6.Assistant professor, Department of Biochemistry, JNU Medical College and Hospital, Jaipur 2.Associate Cunsltant ,Narayana hospital Jaipur. 3.Professor, Department of Biochemistry, Pt. B.D.Sharma Pgims, Rohtak.4 Resident, Department of Biochemistry, Pt. B.D.Sharma Pgims, Rohtak. **5.** Professor and Head, Department of Biochemistry, Pt. B.D.Sharma Pgims, Rohtak

***Email id of corresponding author-** drminakshi29@gmail.com

Received: 14/01/2016 ***Revised: 06/09/2016*** ***Accepted: 19/08/2016***

ABSTRACT

Objective:-To determine the effects of fluoride level & reactive oxygen species on healthy postmenopausal women. **Material & methods:-**Seventy five volunteer healthy postmenopausal women from Rohtak city (Haryana) were recruited for the study. The mean age of the patients studied in the present study was 50 years. Measurement of the biochemical parameters viz. serum calcium, inorganic phosphorus and alkaline phosphatase, fluoride & total antioxidant capacity was done. **Result: -** There was no significant difference was observed in biochemical parameters during study period in the postmenopausal subject's viz. serum fluoride (0.025±0.047 ppm), serum T-AOC (128.023±115.36), and serum calcium (9.69±0.873 mg/dl) and serum phosphorus level (3.5±0.593 mg/dl). **Conclusion:-**The results of the present study suggest although the change in various biochemical parameters during study period was not statistically significant but increase in the level of fluorine indirectly stimulated oxidative stress in postmenopausal women.

Key words : Fluorosis, Postmenopausal women, Oxidative stress, Reactive oxygen species

INTRODUCTION:

Endemic fluorosis (skeletal and dental) is a serious public health problem in many parts of the world, especially in India. In India about 62 million people are consuming excess of fluoride (F) in drinking water and the problem has reached alarming proportions affecting at least 20 states of India (1). In Haryana the total number of 12 districts endemic for fluorosis are Rohtak, Bhiwani, Faridabad, Gurgaon, Jind, Karnal, Kurukshetra, Kaithal, Mohindergarh, Sonipat, Sirsa and Rewari (2) and villages are categorized as high fluoride villages: 1.52-4 mg F/L and low/normal fluoride village: 0.30-1.0 mg F/L (3).The problem of excessive fluoride in groundwater in India was first reported in 1937 in the State of Andhra Pradesh (4).

Chronic ingestion of fluoride in concentration above maximum permissible limits (more than 1 parts per million (ppm) of fluoride) results in various pathological changes in organs and tissues especially in bones, teeth and

musculoskeletal system. The mechanisms by which fluoride produces such effects are still not clear. It is reported to increase aging process, increase incidence of cancer and tumor growth, disrupt immune system, causes genetic damage and interrupt DNA repair enzyme activity (5). Oxidative stress is implicated in a wide range of human diseases from cancer to diabetes, to brain disorders and even chronic fluoride toxicity.

Oxidative stress is a biochemical disequilibrium precipitated by excessive production of free radicals and reactive oxygen species (ROS), which provoke oxidative damage to biomolecules and which cannot be counteracted by antioxidative systems. This biochemical alteration has been linked with ageing and more of 100 chronic degenerative diseases, among which osteoporosis is found. It has been demonstrated recently that free radicals intervene in bone resorption; promoting osteoclastic differentiation in such a manner that bone resorption is increased with oxidative stress (6). Oxidative stress has been considered to play a key role in many chronic degenerative diseases (7). It occurs when the generation of reactive oxygen species (ROS) in a system exceeds the system's capacity to neutralize and eliminate them.

Estrogens help to prevent bone loss by increasing antioxidant defence in bone. Postmenopausal women residing in an endemic fluorotic area have the additional influence of F on bone in addition to that of estrogen deficiency. Bone fluorine content was decreases with increase in age, which suggests that bone metabolism changes associated with menopause. F supplementation stimulates new bone formation but does not decrease the risk for fracture (8).

As the studies related to this are very few & to gain a better understanding of the problem, the present study is planned to determine the effects of fluoride levels & reactive oxygen species on healthy postmenopausal women.

MATERIALS AND METHODS

Seventy five volunteer healthy postmenopausal women having natural /surgical menopause & amenorrhea less than1 year residing in Rohtak city (Haryana) were recruited in the study. The present study was conducted in the Department of Biochemistry, Pt. B.D. Sharma PGIMS, Rohtak. The mean age of the subjects was 50. On the basis of age subjects were divided into two groups- Group 1(45-49years) and Group 2(50-55years).Women with a history of chronic diseases like diabetes mellitus, hypertension, cardiovascular disease, chronic renal failure, and liver disease, and those who smoked or were on any medications were excluded from the study. After obtaining informed consent from each subject, venous blood samples were collected after overnight fasting between 0800 to 0900 hr from the most accessible peripheral vein in the arm without applying a tourniquet for the estimation of serum F, calcium, phosphorus& alkaline phosphatase (ALP). Special investigation performed were serum fluoride level (ppm) & serum total antioxidant capacity (T-AOC) (ng/ml) to measure oxidative stress. Specimens were allowed to clot for 30 minute at room temperature and then sera were separated by centrifugation of the sample and was used for the routine assays on the same day and for special investigation specimen were stored in plastic storage vials at -80^0 C till analysis.Serum F levels were determined using a F ion selective electrode (Fluoride ionplus® Sure-Flow® Solid State Combination ISE). Serum calcium was measured by the Arsenazo III kit method. Serum inorganic phosphorus (P) levels were determined by the Ammonium molybdate kit method. Serum alkaline phosphatase (ALP) activity was

measured by the Paranitrophenyl phosphate kit method. Total antioxidant capacity was measured by enzyme linked sorbent assay (ELISA) using Human total antioxidant capacity (T-AOC) assay kit.

The statistical analysis was performed using SPSS Software 20 version. The descriptive results are expressed as mean, standard deviation (SD) and standard error (SE) of mean (SEM). Significance of difference between the two groups observed is assessed by using the Student's t test. The p values are expressed along with mean values and standard deviation. The p Values less than 0.05 were considered statistically significant.

TABLE-1: Biochemical parameters of postmenopausal women (n=75)

Parameter	Mean	±SD	SEM
Fluorid (ppm)	0.025	0.047	0.0055
T-AOC (ng/ml)	128.023	115.36	13.32
Ca (mg/dl)	9.69	0.873	0.1
P (mg/dl)	3.5	0.593	0.06
ALP (U/L)	81.01	24.73	2.85

TABLE 2: Correlation between F & T-AOC in study subjects (n=75)

Fluoride (ppm)	T-AOC (ng/ml)	
	Pearson Correlation	-0.186
	Sig. (2-tailed)	0.110
	N	75

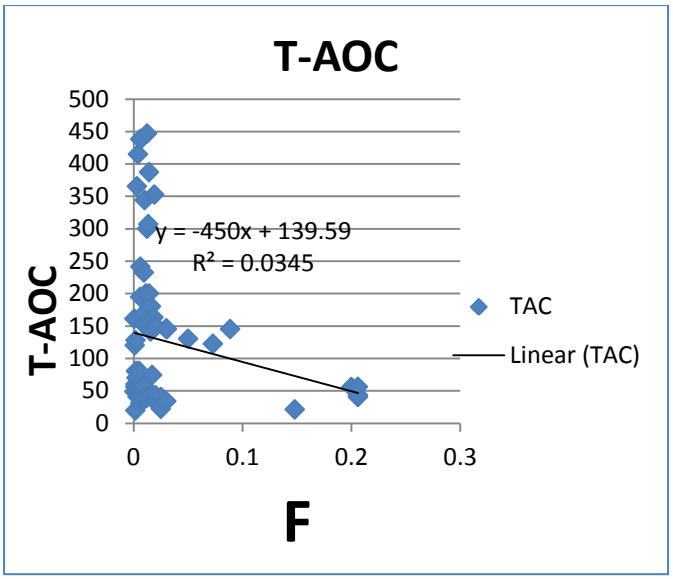

Fig: 1-Correlation between F & T-AOC in study subjects

RESULTS

The mean age of the patients studied in the present study was 50 years. Various, biochemical parameters viz. serum fluoride (0.025±0.047 ppm), serum T-AOC (128.023±115.36), serum calcium and serum phosphorus level were studied in the postmenopausal subjects in the present study and no significant difference was observed in these biochemical parameters during study period (Table1). Negative but not statistically significant correlation between fluoride & T-AOC (Table2 and Figure1) was observed in the present study.

DISCUSSION

In postmenopausal women estrogen deficiency is associated with bone loss. In fluorosis there is bone resorption indirectly stimulated by oxidative stress. In a normal cell, there is an appropriate pro-oxidant: antioxidant balance. F induces oxidative stress leading to the generation of ROS, thus resulting in a shift of the pro-oxidant and antioxidant balance towards the pro-

oxidants wherein the production of ROS is increased and the levels of antioxidants are diminished. Although ROS did not appear to be directly involved, hydrogen peroxide (H_2O_2) has been found to causes bone resorption by stimulating osteoclasts H_2O_2-induced bone resorption can be suppressed by catalase (CAT) (9). Various animal studies and a few human studies have reported an association between F and oxidative stress (10-12).

Oxidative stress is an independent risk factor for osteoporosis. The role of reactive oxygen species in bone metabolism is unique and dual considering their effect under physiological and pathological conditions (13). In the present study, oxidative stress was studied in terms of T-AOC. Oxidative stress increases H_2O_2 levels and thus can stimulate the osteoclast precursor cells leading to increased osteoclast proliferation. Because of the decreased CAT activity level, the inhibitory effect on osteoclasts is not seen, thereby leading to increased bone resorption.

In the present study, biochemical parameters studied in the postmenopausal subjects (Table1) which showed that although study subjects are residing in the area of endemic fluorosis, they had normal levels of fluoride, T-AOC, calcium and P. This could be due to use of reverse osmosis (RO) water.

Further it was observed in the present study (Table 2 and graph 1) that the Pearson correlation between fluoride & T-AOC was found to be negative but the difference was not to be statistically significant, which shows that F induces oxidative stress it leads to decrease in T-AOC. This increase in oxidative stress causes elevation in malondialdehyde levels and decreases in the activity of catalase and -SH groups. The concentration of thiobarbituric acid reactive substances also increases in all investigated tissues suggesting an increased lipid per oxidation under chronic fluoride intoxication (14). Hence, our observations suggest that increased level of F indirectly stimulates the oxidative stress in postmenopausal women.

Limitations: Although there is much remains to be done, our work generate important finding in the field of fluorosis. In other words, we are having acknowledged the limitation of data processing. The main limitations are expressed as follows: the first limitation concerns the lack of pre-menopausal group. Secondly, some other parameters which affect bone status e.g. FSH, estrogen were not measured.

CONCLUSION

The present study suggests that although the results were not statistically significant in the present study but the increase in fluoride level induces oxidative stress in postmenopausal women. ROS derived from fluoride induced oxidative stress appear to stimulate bone resorption. Accordingly, despite the fact that serum calcium and serum inorganic phosphorus levels were obviously kept up within normal limits due to ROS-induced bone resorption. However, more studies are required to have better understanding of the other biochemical parameters contributing for the changes occurring during menopause.

Acknowledgment:

We are indebted to the staff of the Department of Biochemistry, Pt. B.D. Sharma PGIMS, Rohtak for their technical assistance. No financial aid or any other type of grant was accepted for this work.

Competing Interests:

Authors have declared that no competing interests exist.

REFERENCES

1. Reddy GB, Khandare AL, Reddy PY, Rao GS and Srivalli I. Antioxidant defence system and lipid peroxidation in patients with skeletal fluorosis and in fluoride intoxicated rabbits. Toxicological sciences 2003; 72:363-8.
2. Fluorideandfluorosis.com. New Delhi: Fluorosis research and rural development foundation online resource. Available from: URL:http://www.fluorideandfluorosis.com/fluorosis/prevalence.html Accessed on: August 11, 2012.
3. Yadav JP, Lata S, Kataria SK, Kumar S. Fluoride distribution in ground water and survey of dental fluorosis among school children in the villages of the Jhajjar District of Haryana, India. Environ Geochem Health 2009; 31:431-8.
4. Short HE, Mc Robert GR, Bernard TW, Nayyar ASM. Endemic fluorosis in the Madras Presidency. Indian J Med Res 1937; 25:553-61.
5. Ailani V, Gupta RC, Gupta SK and Gupta K. Oxidative stress in cases of chronic fluoride intoxication. Indian J Clin Biochem 2009; 24(4):426-9.
6. Martha A. Sanchez-Rodriguez, Mirna Ruiz-Ramos, Elsa Correa Munoz. Oxidative stress as a risk factor for osteoporosis in elderly Mexicans as characterized by antioxidant enzymes. BMC Musculoskeletal Disorders. 2007; 8:417-25.
7. Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol. 2007; 39(1):44-84.
8. Dequeker J, Declerck K. Fluor in the treatment of osteoporosis. An overview of thirty years clinical research. Schweiz Med Wochenschr 1993; 123(47):2228-34.
9. Fraser JH, Helfrich MH, Wallace HM, Ralston SH. Hydrogen peroxide, but not superoxide, stimulates bone resorption in mouse calvariae. Bone. 1996;19(3):223-6.
10. Shan KR, Qi XL, Long YG, Nordberg A, Guan ZZ. Decreased nicotinic receptors in PC12 cells and rat brains influenced by fluoride toxicity—a mechanism relating to a damage at the level in post-transcription of the receptor genes. Toxicology 2004; 200(2-3):169-77.
11. Mostafaei F, McNeill F.E., Chettle D R, Wainman B C, Pidruczny A E and Prestwich W V. Measurements of fluorine in contemporary urban Canadians: a comparison of the levels found in human bone using *in vivo* and *ex vivo* neutron activation analysis. Physio Measurement, 2015; 36, (3).
12. Susheela AK, Bhatnagar M. Reversal of fluoride induced cell injury through elimination of fluoride and consumption of diet rich in essential nutrients and antioxidants. Mol Cell Biochem 2002; 234-235(1-2):335-40.
13. Kataraki P, Rao P. Fluoride and oxidative stress in postmenopausal women. IOSR J Pharmacy 2012; 2(5):57-9.

14. Kandare AL, Rao GS and Balakrishna N. Dual x-ray abosrptiometry(DXA) study of endemic skeletal fluorosis in a village of Nalgonda district, Andhra Pradesh. Fluoride, 2007;40(3):190-7.