Effect of Fluorozis on the Erythrocyte Antioxidant Enzyme Activity Levels

[1]M. AKDOGAN*, [2]D. YILMAZ, [3]M. YONTEM,
[4]S. KALELI AND [1]I. KILIC
[1]*Department of Biochemistry, Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey.*
makdogan2009@gmail.com*
[2]*Women's Maternity and Child Diseases Hospital, Isparta, Turkey.*
[3]*Department of Biology, Faculty of Art and Science, Dumlupinar University, Kutahya,Turkey.*
[4]*Faculty of Health Sciences, Suleyman Demirel University, Isparta, Turkey.*

(Receıved on 27th May 2010, accepted in revised form 23rd December 2010)

Summary: While the flourine level of (drinking) water was higher than normal ranges in the center of Isparta region before 1995 year, this problematic situation is solved in later years. (However) the individuals who are staying in Yenice district are still expose to high levels of fluorine because of the usage of Andık spring water (3.8 mg/L flour level) as drinking water. In this study we aimed to investigate the harmful effect of floride on human erythrocytes via antioxidant defence system and lipid peroxidation. Therefore, we studied the activities of erythrocyte antioxidant enzymes such as Superoxide Dismutase (SOD), Glutathione Peroxidase (GSH-Px) and Catalase (CAT), and the level of erythrocyte Glutathione (GSH), thiobarbituric acid reactive substance (TBARS) and the level of urine floride in high floride exposed people (childen, adult and elderly).

The activities of SOD, GSH-Px and CAT and the level of GSH, TBARS and urine floride were higher in 3.8 mg/L floride exposed children (Group II) than 0.8 mg/L floride exposed control children (Group I) ($p<0.001$, $p<0.001$, $p<0.01$, $p<0.001$, $p<0.001$, $p<0.001$, respectively)

The activities of SOD and CAT and the level of TBARS and urine floride were higher in 3.8 mg/L floride exposed adult (Group IV) than 0.8 mg/L floride exposed control adult (Group III) ($p<0.001$). On the other hand, no significant changes were observed in the activity of GSH-Px and the level of GSH in Group IV and Group III ($p>0.05$).

The activities of SOD, GSH-Px and CAT were lower and the levels of TBARS and urine floride were higher in 3.8 mg/L floride exposed elderly people (Group VI) than 0.8 mg/L floride exposed control elderly people (Group V) ($p<0.001$). However, the decrease in GSH level was not significant ($p>0.05$).

As a result we thought that increased SOD, GSH-Px and CAT activities in floride exposed children and adult people, decreased activities of these enzymes in floride exposed elderly people, and increased TBARS in all groups may indicate floride caused oxidative damage in erythrocytes.

Introduction

Free radicals are atoms or molecules that contain one or more unpaired electrons and are highly chemically reactive. Free radicals are positively charged, negatively charged or neutral. Because radicals are highly reactive, they can harm the structure of proteins, lipids and neucleic acids in the cell . Free radicals play an important role in not only physiological events such as ageing but also in the physiopathology of many disorders, such as cancer, hypertension, atherosclerosis, inflamation, emphysema, oxygen toxicity, bronchopulmonary dysplasia, ischemia-reperfusion injury, skin disorders, reumatoid arthritis, diabetes mellitus, Alzheimer, hyperlipidemia, and Down's Syndrome [1-6]. Also, a close connection between chronic flouride toxicity and increased oxidative stress in both humans [7-9] and animals [10-12] has been reported. Flouride, which is a trace element, is a necessary halogen for the body. Flouride, of which 1 mg a day is consumed, is beneficial for the development of both the body and the teeth [13-16]. Fluorosis is a disorder clinically characterized by the toxic effects of excessive flouride intake in the teeth, bones and soft tissue [17-25]. Increased free oxygen radical production and lipid preoxidation play a role in the pathogenesis of many disorders and the toxic effect of numerous compounds [26-28]. This process is claimed to be a crucial factor in the formation of the harmful effects of chronic fluoride toxicity [10, 11, 29]. In acute flouride toxicity in humans and animals, various defects can appear resulting from decrease in calcium level, increase in potassium level and decrease in the level of usable oxygen in the cell, caused by the flouride's caustic, calcium's binding and various enzyme systems' suppressing effects in primarily the stomach, intestine, lung, heart, brain, kidney, neuron, and muscles [30-35]. The most important of these are decrease in the contraction ability of the heart muscles, rhythm disorders, sistolic and diastolic dysfunctions in connection to the decrease in the level of calcium in the heart [35-36]. Flouride toxicity can be observed in two ways

*To whom all correspondence should be addressed.

depending on flouride intake: acute and chronic fluorosis. Acute fluorosis is formed as a result of a very high level of flouride intake in a very short period of time. Toxicities resulting from flourine compounds (sodium flouride) that melt in water easily are more frequently encountered [37]. Chronic flouride toxicity occurs as a result of the intake of flouride compounds for a long period of time in amounts above normal levels. It shows its impact specifically on the teeth and bones. Chronic flouride toxicity develops slowly. It may take months, even years for the symptoms to occur [38].

Yet, in chronic flouride toxicity, in addition to the disorders observed in acute flouride toxicity, disorders specifically in the bones, kidneys, thyroid gland, hypophysis, testes and teeth are noticeable [39, 40]. Thus, this study aimed at evaluating the erythrocyte antioxidant defense system and lipid peroxidation [41-49] in order to investigate the toxicity effect on the erythrocytes of three different groups (child, adult, and elderly people) subject to chronic fluorosis exposure.

Results and Discussion

The increase in erythrocyte SOD, GSH-Px, CAT acitivity level, erythrocyte GSH, erythrocyte TBARS and urinal flouride levels in the child group subject to flourosis (Group II), who consumed drinking water with a flourine concentration of 3.8 mg/L, was found to be significant when compared to the child control group in which children consumed drinking water with a flourine concentration of 0.8 mg/L ($p<0.001$, $p<0.001$, $p<0.01$, $p<0.001$, $p<0.001$, $p<0.001$, respectively) (Table-1). While the increase in erythrocyte SOD, GSH-Px, CAT acitivity level, erythrocyte TBARS and urinal flouride levels of the adult group subject to flourosis (Group IV), who consumed drinking water with a flouride concentration of 3.8 mg/L, was found to be significant ($p<0.001$) when compared to the adult control group (Group III), who consumed drinking water with a flouride concentration of 0.8 mg/L, the increase in erythrocyte GSH-Px acitivity level and the decrease in erythrocyte GSH were found to be insignificant ($p>0.05$) (Table-1). The decrease in erythrocyte SOD, GSH-Px and CAT activity level, and the increase in erythrocyte TBARS and urinal flouride levels of the elderly group subject to flourosis (Group VI), who consumed drinking water with a flouride concentration of 3.8 mg/L, were found to be statistically highly significant when compared to elderly control group (Group V), who consumed water with a flouride concentration of 0.8 mg/L ($p<0.001$). On the other hand, the decrease in erythrocyte GSH level was low ($p>0.05$) (Table-1). A close connection between chronic flouride toxicities and increased oxidative stress in both humans [7-9] and animals [10-12] have been reported. Because of the existence of rich plasma membranes from polyunsaturated lipids and tendencies to oxidative reactions due to high oxygen density, erythrocites are prevalently used in the examination of oxidative stress [50]. Oxidant stress caused by H_2O_2 and free radicals is high if it distorts the production of such free radical scavengers as flouride, GSH, GSH-Px, SOD and Ascorbic acid [51]. In this study, the increase in erythrocyte SOD, GSH-Px, CAT activity levels and erythrocyte GSH levels of the children (Group II) and adults (Group IV) subject to chronic flouride, and the decrease in erythrocyte SOD, GSH-Px, CAT actitivity levels and erythrocyte GSH levels of the elderly group subject to flourosis (Group VI) indicate the distortion of free radical scavengers.

Table-1: Levels of SOD, GSH-Px and CAT erythrocyte activities and concetrations of erythrocyte GSH, erythrocyte TBARS and urine fluoride and in all groups

Groups	Age	SOD U/g Hb	GSH-Px U/g Hb	CAT k/g Hb	GSH μmol/g Hb	TBARS nmol/mg Hb	Fluoride mg/L
Group I	11.46±1.64	1505±88	30.42±2.33	1.61±0.17	5.92±1.3	2.29±0.14	0.22±0.08
Group II	11.13±1.24	1778±114	37.80±1.64	1.79±0.14	8.02±1.20	2.59±0.18	3.10±0.69
Group III	30.73±5.17	1352±73	40.73±2.35	1.26±0.09	6.16±1.17	3.30±0.16	0.27±0.11
Group IV	28.80±5.03	1474±84	42.39±2.48	1.58±0.15	5.47±1.13	3.83±0.20	3.81±0.25
Group V	67.66±7.96	1434±79	44.00±2.72	1.62±0.11	3.52±0.83	4.57±0.28	0.25±0.08
Group VI	64.73±6.87	1101±88	34.82±2.05	1.24±0.14	3.18±0.82	5.69±0.28	4.04±0.22

Groups	SOD	GSH-Px	CAT	GSH	TBARS	Fluoride
Group I- Group II	p<0.001	p<0.001	p<0.005[1]	p<0.001	p<0.001	p<0.001
Group III-Group IV	p<0.001	p>0.05[1]	p<0.001	p>0.05[1]	p<0.001	p<0.001
Group V-Group VI	p<0.001	p<0.001	p<0.001	p>0.05[1]	p<0.001	p<0.001

[1] In normally distributed groups Student's t test was used, in not normally distributed Mann Whitney U test was used. $p<0.05$ was considered significant

Studies showed an increase in GSH [51] and a decrease in SOD and GSH-Px [9] activities in people living in regions of endemic flourosis. In experimental studies, GSH levels and GSH-Px activity in erythrocytes were found to be low and SOD activity unchanged [10], while in other tissues, the SOD level was found to be low [12, 52, 53]. In another study, it was found that GSH level and GSH-Px activity in rat erythrocytes that were exposed to drinking water with a flouride concentration of 100 mg/L for 4 months increased, while SOD activity decreased [12]. In this study, in the child (Group II) and adult (Group IV) groups with chronic flouride exposure, SOD in erythrocytes which catalyzed the tranformation of erythrocyte superoxide radicals (O_2^-) caused by flouride into H_2O_2, was activated in H_2O_2 even in high concentrations, while the excess O_2^- found in the area activated GSH-Px and CAT activity.

While SOD erythrocytes, which catalyzed the transformation of erythrocyte superoxide radicals (O_2^-), caused by flouride, into H_2O_2 in old-aged group subject to flourosis (GroupVI), are inhibitted in high H_2O_2 concentrations, the excess O_2^- found in the area inhibitted GSH-Px and CAT acitivity. Flouride in human erythrocytes [7] and the tissues and blood of experimental animals [10-12] were shown to have increased as *in vivo* and *in vitro* and caused lipid preoxidation. In this study, the significant increase in the erythrocyte TBARS levels of the three different groups (child, adult, elderly) subject to chronic flourosis due to the consumption of Andık water (with an average flouride concentration of 3.8 mg/L) as drinking water shows consistency with previous findings. These results indicate that while flouride toxicity results in the increase in some intrinsic defense mechanisms resulting in increased free oxygen radical toxicity in child and adult groups subject to flourosis, it can be the cause of the decrease in the elderly group subject to flourosis. We are of the opinion that the increased level of TBARS in chronic flouride toxicity may be the cause of the increase in lipid peroxidation together with oxidative stress, by free radical shown by increased or decreased antioxidant enzyme activities in the blood

Experimental

Working Groups

Control group was selected from district of Istiklal resident of the Isparta, their dirinking water have contained 1.4 mg/L fluoride. Fluorosis group was selected from Yenice district of the Isparta resident, their drinking water ensured from the Andık creek, and contained 3.8 mg/L fluoride. The participants of this study were comprised of 90 individuals from Isparta, a province in Turkey: there were 15 male children aged between 9-15 years (average: 11.46±1.64) in the child control group (Group I), 15 male children aged between 9-13 years (average: 11.13±1.24) in the child group subject to flourosis (Group II), 15 male adults the aged between 25-39 years (average: 30.73±5.17) in the adult control group (Group III), 15 male adults aged between 21-33 years (average: 28.80±5.03) in the adult group subject to flourosis (Group IV), 15 elderly men aged between 57-72 years (average: 67.66±7.96) in the elderly control group (Group V), and 15 elderly men aged between 56-71 years (average: 64.73±6.87) in the elderly group subject to flourosis (Group VI).

For the control groups, the individuals who are staying in Istiklal district and using water (1.4 mg/L flour level) as drinking water were selected. For the study groups, the individuals who are staying in Yenice district and using Andık spring water (3.8 mg/L flour level) as drinking water were selected .

The participants that made up both the control and experimental groups had no health problems nor any clinic complaints. For flouride diagnosis of the experimental group, urinal flouride levels and discoloration on the teeth were taken into consideration. In the process of group formation, the anamnesis of each case was taken and their systemic examination was conducted. The participants of these groups had no organic or mental disorders, and were not smoking nor using drugs and alcohol. Before starting to work from medical school ethics committee approval was taken. The consent of the families of children in both the control group and the child group subject to flourosis was obtained. All the groups were comprised of volunteers.

Biochemical Analysis

Blood samples were centrifuged (3000 rpm, 10 min) and the plasma was separated. Erythrocytes were washed three times with both 140 nM NaCl and phosphate buffer (7.4 pH) [44]. The erythrocyte suspension was haemolysed with mercaptoethanol and used to measure haemolysate TBARS and GSH and haemoglobin levels and SOD, GSH-Px and CAT activities. Draper and Hadley's method was used to measure the TBARS level of erythrocytes [45] and Beutler et al.'s method was used to measure the Reduced GSH [46], whereas Sun et al.'s method was

used to detect SOD activity [44], Paglia and Valentine's method to detect GSH-Px activity [47], Aebi's method to detect CAT activity [48], and Fairbanks and Klee's method to detect haemoglobin level [49]. Obtained results were calculated as nmol/mg Hb, μmol/g Hb, U/g Hb, U/g Hb, k/g Hb, respectively, TBARS, GSH levels and SOD, GSH-Px and CAT activities.

Orion Model SA (USA) combined fluoride electrodes were used in the measurement of urine fluoride levels. The results were expressed in mg/L.

Statistical Analysis

For statistical analysis, SPSS for Windows 9.05 statistics packet program was used. The data was evaluated with Mann-Whitney U and Kruskal-Wallis tests. $p<0.05$ was considered significant.

Conclusions

These results indicate that while flouride toxicity results in the increase in some intrinsic defense mechanisms resulting in increased free oxygen radical toxicity in child and adult groups subject to flourosis, it can be the cause of the decrease in the elderly group subject to flourosis. As a result, we are of the opinion that the increased level of TBARS in chronic flouride toxicity may be the cause of the increase in lipid peroxidation together with oxidative stress, by free radical shown by increased or decreased antioxidant enzyme activities in the blood

References

1. E. F. Elstner, *Oxygen Radical–Biochemical Basis for their Efficacy*. Journal of Molecular Medicine., **69**, 949 (1991).
2. B. Halliwell, *Free Radicals, antioxidants and human disease: curiosity, cause, consequence?* The Lancet., **344,** 721-724 (1194).
3. I Akkus, *Serbest Radikaller ve Fizyolojik Etkileri*. Mimoza Yayınları Konya (1995).
4. D. A. Barber and S. R. Harris, *Oxygen free radicals and antioxidants: A review*. American Pharmacy., **34**, 26 (1994).
5. K. S. Satish and H. A. Naseem, *The effect of oxidants on biomembranes and cellular metabolism.* Molecular and Cellular Biochemistry., **91**, 149 (1989).
6. J. A. Escobar and M. A. Rubio, *SOD and Catalase Inactivation by Singlet Oxygen and Peroxyl Radicals*. Free Radical Biology & Medicine., **20**, 285 (1996).
7. D. Saralakumari and P. Ramakrishna Rao, *Red cell membrane alterations in human chronic fluoride toxicity*. Biochemistry and Molecular Biology International., **23**, 639 (1991).
8. L. R. Jeji, Sharma, S. S. Jolly and S. Pamnani, *Implication of glutathione in endemic fluorosis.* Fluoride., **18**, 117 (1985).
9. J. Li and S. Cao, *Recent studies on endemic fluorosis in China*. Fluoride **27**, 125-8 (1994).
10. G**.** Zhi-Zhong, Y. Pei-si, Y. Nai-den and Z. Zong-Jie, *An experimental study of blood biochemical diagnostic indices for chronic fluorosis,* Fluoride., **22**, 112 (1989).
11. A**.** Sharnia and N. J. Chinoy, *Role of free radicals in Fluoride-induced toxicity in liver and kidney of mice and its reversal*. Fluoride., **31**, S26 (1998).
12. P. D. Patel and N. J. Chinoy, *Influence of fluoride on biological free radical reactions in ovary of mice and its reversal.* Fluoride., **31**, 527 (1998).
13. E. J. Underwood, Fluoride. *Trace elements in human and animal nutrution*, 2nd Ed, Academic Press, New york (1962).
14. Anon 1. *Fluorine and fluorides*. Environmental Health Criteria No:36. WHO, Geneva, (1984), p.1.
15. Anon 2. *Review of Fluoride: Benefits and risks. Report of the Ad. Hoc. Subcommittee on fluoride of the Committee to Coordinate Environmental Heath and Related Programs, Public Health Service.*, DC, Dept. of Health and Human Services, Washington (1991).
16. J. J. Murray, Appropriate use of fluorides for human health, World Health Organization, Geneve (1986), p.131.
17. K. Krishnamachari, *Trace elements in human nutrition and health,* World Health Organization, Geneva (1996), p.187.
18. B. R. Bhussry, V. Demole, H. C. Hodge, S. S. Jolly, A. Singh and D. R. Taves, *Toxic ef fects of larger doses of fluoride*. *In: Fluorides and human health,* World Health Organization, Geneva (1979), p.225.
19. M. Teotia, S. P. S. Teotia, K. B. Kunwar, *Endemic skeletal fluorosis*. Archives of Disease in Childhood, **46**, 686 (1971).
20. S. S Jolly, B. M. Singh, O. C Mathur and K. C. Malhotra, *Epidemiological, clinical and biochemical study of endemic dental and skeletal fluorosis in Punjab*. British Medical Journal, **4**, 427 (1968).

21. M. Michael, W. Barot and N.J. Chinoy, *Investigations of soft tissue functions in fluorotic individuals of North Gujarat.* Fluoride, **29**, 63 (1996).
22. N. J. Chinoy, *Effects of fluoride on physiology of animals and human beings.* Indian Journal of Environment and Toxicology, **1**, 17 (1991).
23. E. Gnicka-Mariczar, Z. Machoy, R. Tamawski, E. Birkner and A. Mamczar, *Influence of long-term sodium fluoride administration on selected parametsrs of rat blood serum and liver function.* Fluoride, **30**, 157 (1979).
24. M.L. Vani and K.P. Reddy, *Effects of fluoride accumulation on some enzymes of brain and gastrocnemius muscle of mice.* Fluoride, **33**, 17 (2000).
25. P. A. Monsour and B. J. Kruger, *Effect of fuoride on soft tissues in vertebrates (A review)* Fluoride, **18**, 53 (1985).
26. B. Halliwell and J. M.C. Gutteridge, *Oxygen free radicals and iron in relation to biology and medicine: some problems and concepts.* Archives of Biochemistry and Biophysics, **246**, 501 (1986).
27. A. L. Tappel, *Lipid peroxidation damage to cell components*. Federation Proceedings, **32**, 1870 (1973).
28. R. Rzeuski, D. Chlubek and Z. Machoy, *Interactions between fluoride and biological free radical reactions.* Fluoride, **31**, 43 (1989).
29. R. Yolken, P. Korecny and Mc Carty, *Acute fluoride poisining.* Pediatrics, **58**, 90 (1976).
30. S. Hirano, M. Ando and S. Kanno, *Inflammation responses of rat alveolar macrophages following exposure to floride.* Archives Toxicolology, **73**, 310 (1999).
31. J.A. Varner, K. F. Jensen, W. Horvath and R. L. Isaacson, *Chronic administration of aluminium-fluoride or sodium-fluoride toratsin drinking water: Alterations in neuronal and cerebrovascular integrity*. Fluoride, **784**, 284 (1998).
32. K. Usuda, K. Kono, T. Dote, K. Nishiura, K. Miyata, H. Nishiura, M. Shimahara. and K. Sugimoto, *Urinary biomarkers monitoring for experimental fluoride nephrotoxicity*. Archives Toxicolology, **7**, 104 (1998).
33. A. Rigalli, M. Morosano and R. C. Puche, *Bioavailability of fluoride administered as sodium fluoride or sodium monofluorophosphate to human volunteers*. Arzneimittel-Forschung, **46**, 531 (1996).
34. J. P. Morgan, R. E. Emy, P. D. Allen, W. Grosman and J. K. Ghathmey, *Abnormal intracellular calcium handling. A major cause of systolic and diastolic dysfunction in ventricular myocardium*. Circulation, **81**, 21 (1980).
35. O Strubelt, H. Iven and M. Younes, *The pathophysiological profile of the acute cardiovascular toxicity of sodium fluoride.* Toxicology, **24**, 313 (1982).
36. N. Çetin, V. Sağmanlıgil, B. Emre, A. Bilgili, and M. Toker, *Effect of Acute Fluoride Poisoning on Some Echocardiographic Parameters in Rabbits.* Turk Journal of Veterinary and Animal Sciences, **25**, 45 (2001).
37. K.C. Walton, Environmental fluoride and fluorosis in mammals**.** Mammal Review, 18, 77 (1988).
38. D. C. Blood, O. M. Radostitis, J. H. Arundel, C. C. Gay, Veterinary Medicine, in O. W. Schalm, N. J. Jain, A. Carroll (Ed.) Textbook of Diseases of Cattle, Sheep, Pigs, Goats and Horses, 8th Edition, Balliere Tindall Cassel London (2006).
39. S. Kaya and F. Akar, *Metaller ve diğer inorganik ve radyoetkin maddeler. In: Veteriner Hekimliğinde Toksikoloji* Ed: Kaya S, Pirinçi İ. ve Bilgili A. Medisan Yayınevi, Yayın No: 35, Ankara (1998).
40. M. A Boillat, J. Garcia and L. Velebit, *Radiological criteria of industrial fluorosis*. Skeletal Radiology, **5**, 161 (1981).
41. D. Shahwar, M. A. Raza, M. A. S. Mughal, M. A. Abbasi, V. U. Ahmad, *Journal of the Chemical Society of Pakistan*, **32**, 357 (2010).
42. M. Yontem, S. Kaleli, M. Akdogan, R. Kayapinar, F. Gultekin, *Journal of the Chemical Society of Pakistan*, **32**, 353 (2010).
43. C. Yuan, X. J. Zhang, Y. D. Du, Y. H. Guo, L. Y. Sun, Q. Ren, Z. T. Zhao, *Journal of the Chemical Society of Pakistan*, **32**, 189 (2010).
44. Y. Sun, W. O. Larry and Li. Ying, *Clinical Chemistry*, **34**, 497 (1988).
45. H. H. Drapper and M. Hadley, *Methods Enzymology*, **186**, 421 (1990).
46. E. Beutler, O. Duron and B. M. Kelly, *Improved method for the determination of blood glutathione.* Journal of Laboratory and Clinical Medicine, **61**, 882 (1963).
47. D. E. Paglia and W. N. Valentine, *Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase.* Journal of Laboratory and Clinical Medicine, **70**, 158 (1967).
48. H. Aebi*, Catalase in vitro. Enzymology,* **105**, 121 (1984).
49. V. F. Fairbanks and G. G. Klee, *Biochemical aspects of hematology.* In: Burtis, C.A., Ashwood, E. R. (Ed.), Tietz Textbook of Clinical

Chemistry, third ed. WB Saunders, Philadelphia (1991), p.1690.

50. A. Stern, *Red cell oxidative damage, ın* A. Sies (Ed.) *Oxidative s*tress, Academic Press, London (1985), p.331.
51. R. Rzeuski, D. Chlubek and Z. Machoy, *Interactions between fluoride and biological free radical reactions.* Fluoride, **31**, 43 (1988)
52. M. L. Vani and K. P. Reddy, *Effects of fluoride accumulation on some enzymes of brain and gastrocnemius muscle of mice.* Fluoride, **33**, 17 (2000).
53. A. Sharnia and N. J. Chinoy, *Role of free radicals in Fluoride-induced toxicity in liver and kidney of mice and its reversal,* Fluoride, **31**, S26 (1998).