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OXIDATIVE STRESS AND LOW DOSE IONIZING RADIATION

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Abstract : The field of radiation biochemistry has provided tremendous impact in recent years as extensive research on free radicals has implicated them in radiation damage. An important offshoot of this field is the branch dealing with radiological protection in medical applications like diagnostic radiology. Hence, we decided to investigate the relationship between oxidative stress and low dose ionizing radiation (x-rays) in the work environment of x-ray technicians (radiographers), by comparing their RBC malondialdehyde, % hemolysis, catalase and plasma vitamin E levels with those of controls. There was a significant increase in the susceptibility of RBCs to hemolysis in radiographers compared to controls. Malondialdehyde and catalase levels were slightly increased in the radiographers, but this did not disturb the steady state concentration of their plasma vitamin E. These findings go in favour of theories stating that exposure to low dose ionizing radiation does cause a greater amount of oxidative stress, than that caused during normal routine metabolic processes.

Key words : X-rays radiographers oxidative stress

INTRODUCTION

X-rays belong to the indirectly ionizing electromagnetic group of radiations (1) and they have a very high penetrating power because of their low linear energy transfer (LET) (2). Exposure of eukaryotic cells to ionizing radiation results in the immediate formation of free radicals that last a matter of milliseconds (3) and cause oxidative stress through the radiolysis of body water which is often referred to as the indirect effect of radiation (1). This coupled with the 'oxygen effect' (1) enhances tissue injury through the process of lipid peroxidation (4). Both these effects are more pronounced for low LET radiations (5), accounting for more than 70% of molecular damage.

Daniel Billen (6) stated that free radical damage induced by low dose ionizing radiation was no greater than the free radical damage caused during routine metabolic chemistry itself, while J. F. Ward (7), argued against this concept.

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This argument and the paucity of data studying the effects of low dose ionizing radiation in humans *in vivo*, when compared to literature dealing with the different aspects of non-ionizing and high dose ionizing radiations, formed the basis of this work. Studies such as this are important because in developing country like ours, where biological security controls are not strict and extended work days are common, this type of monitoring may be useful as an indicator to detect early damage in order to demand more controls in radiation protection.

Oxidative stress in this work has been studied by estimating the levels of malondialdehyde (MDA), percentage hemolysis of RBCs, catalase and vitamin E in medical radiographers and comparing them with those of controls.

MATERIALS AND METHODS

The test group for this study consisted of 40 radiographers from different hospitals of Mangalore.

Inclusion criteria : The radiographers were primarily x-ray technicians, but some were also involved in other radiodiagnostic procedures like angiogram, CT scan and MRI. They were between the age group of 22–38 years of both sexes. They were further categorized as follows :

Group I : Consisted of 12 subjects with an occupational exposure of 5 years and below.

Group II : Consisted of 12 subjects with an occupational exposure of 5.1 to 10 years.

Group III : Consisted of 16 subjects with an occupational exposure of more than 10 years.

The control group included 40 healthy individuals, age and sex matched to the test group, who had no history of occupational exposure to low dose ionizing radiation.

Exclusion criteria : For both the test and control groups, care was taken to eliminate those with habits like smoking, tobacco chewing, alcohol consumption and also those with a history of tuberculosis, rheumatoid arthritis and diabetes mellitus, all of which play a vital role in contributing to oxidative stress injury.

Sample collection : Venous blood was collected in EDTA containers and centrifuged at 3000 rpm for 10 mins, within 3 hours of collection. Plasma was separated and used for the estimation of vitamin E. The separated cells were washed and suspended in an equal volume of 0.9% cold normal saline. This RBC suspension was then used for the assay of percentage hemolysis, malonaldehyde, catalase and hemoglobin.

Red cell lipid peroxidation was studied as thiobarbituric acid (TBA) reaction products by the method of Stocks and Dormandy (8). Oxidative hemolysis was measured by the method of Kartha and Krishnamurthy (9). Catalase activity of the hemolysate was determined by the method of Brannan et al (10). The hemoglobin content of erythrocytes was determined by the cyanmethemoglobin method and plasma vitamin E was measured using the Emmorie Engel reaction (11).

Statistical analysis was done by t-test,

Analysis of repeated measures and One way analysis of variants depending on the nature of data. The tests used for different parameters are indicated under Tables I and II.

RESULTS

RBC MDA levels were higher in radiographers (tests) when compared to the controls (Table I). This is further strengthened by the fact that catalase levels were also higher in the radiographers (Table I). However the statistically significant (P=0.049) increase for catalase (Table II) was consistent with the above, only upto 10 years of occupational exposure, beyond which it became inconsistent. Mean vitamin E levels were higher in radiographers than controls. (Table I). A statistically significant increase (P=0.006) was seen on comparing the percent hemolysis between control and test group before incubation with H_2O_2 (Table I). An statistically insignificant increase was seen in the line graph comparing the percentage hemolysis before incubation with hydrogen peroxide with the years of occupational exposure (Table II). Susceptibility of RBCs to hemolysis showed a statistically significant increase in test group compared to the controls (P<0.001).

TABLE I: Levels of various parameters in radiographers and controls.

Parameter	*Test (n=40) Mean±SD*	*Control (n=40) Mean±SD*
RBC Catalase (units/g Hb)	418976±219944	313167±117415
RBC MDA (nmoles/ 100 ml RBC)	565.98±116.86	552.82±113.36
Plasma Vit E (mg/L)	8.38±2.16	7.68±1.58
% hemolysis before incubation with H_2O_2	3.2±1.4*	4.65±1.89
% hemolysis after incubation with H_2O_2	5.9±2.35	6.43±2.01
Susceptibility to hemolysis	2.7±0.9**	1.78±0.12

*P<0.05; **P<0.001.
Percentage hemolysis (before and after) intragroup comparison done by paired 't' test.
Test and control group compared with student 't' test.

TABLE II: Comparison of levels of various parameters and years of occupational exposure in radiographers.

Parameter	*Group I Mean±SD*	*Group II Mean±SD*	*Group III Mean±SD*
RBC MDA (nmoles/100 ml RBC)	536.47 ± 89.36	542.4 ± 124.74	605.77 ± 131.33
RBC Catalase (units/g Hb)	354057 ± 110115	597723 ± 292521*	333604 ± 148982
% hemolysis before incubation with H_2O_2	2.37 ± 1.54	2.87 ± 0.07	3.56 ± 1.38
n	12	12	16

Name of the test used: One way analysis of variance.
*P=0.049 (significant between groups I and II).

DISCUSSION

Increase in RBC MDA and catalase levels suggested that continued exposure to low dose ionizing radiation does induce a higher level of oxidative stress, as is seen by the increasing trend which is consistent with the findings of Deger et al in experimental animals (12). The inconsistency in the increase in levels of catalase in group III could be attributed to increasing age, as catalase activities were seen to decline considerably as a function of cell age in older animals (13), although the exact age at which such a decline starts has not been ascertained. This reasoning is supported by documentation stating that catalase levels show a negative correlation with Life span energy potential (LEP) (14).

The apparent increase in vitamin E level was probably because production of free radicals would need to be extensive for the steady state levels of antioxidants to be disturbed in vivo (15). Contrary to the popular finding that vitamin E is inversely related to the respective tissue MDA levels, the vitamin E level increased probably to overcome the effect of increased free radicals.

The statistically significant increase seen on comparing percentage hemolysis of radiographers with controls was probably because gross or increased tissue injury in vivo can be detected only when repeated small doses or dose fractions of radiation are given at intervals such that any adaptive response triggered by each small dose has already decayed away in the interval between the fractions (16). This was more likely to occur as the number of years of occupational exposure increased in the test group.

Thus we conclude by saying that the trends seen in this study strongly suggests that there is a possibility of a mild increase in oxidative stress occurring as a result of chronic occupational exposure to low dose ionizing radiation. Hence, more studies on the effects of both ionizing radiations and spontaneous events on DNA decay and repair need to be conducted to better understand the practical health consequences of low and protracted doses of radiation.

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