ORIGINAL ARTICLE

Clinical Biomarkers for Diagnosis of Damages in Individuals with Long-Term Exposure to X-Rays

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ABSTRACT

Objective: To determine altered manifestation of plasma proteins in X-rays technicians who are regularly exposed to low doses of radiations over a long period during their job.

Study Design: Descriptive study.

Place and Duration of Study: District Headquarters Hospital and Mufti Mahmood Memorial Teaching Hospital; from January 2017 to January 2018.

Methodology: The study enrolled 70 individuals consisting of 50 X-ray technicians working 8 to 12 hours/day for five days per week and 20 unexposed healthy controls. The serum protein expression pattern (concentrations of various serum proteins) was evaluated through cellulose acetate electrophoresis and serum antioxidant status was measured through ferric reducing ability of plasma (FRAP) assay.

Results: The antioxidant assay showed significantly low trolox-equivalent antioxidant capacity (TEAC) status and FRAP value in X-ray technicians as compared to controls ($p<0.001$). Analysis of serum protein demonstrated a significantly reduced concentrations of albumin ($p<0.001$) and elevated level of the γ-globulins ($p<0.001$), while other globulins fractions like α1 and β remain unchanged. There was a strong negative correlation ($p<0.001$) according to Pearson coefficient ($r=87\%^{(-)}$) between albumin and γ-globulins fraction. Whereas, a positive correlation ($p<0.001$) ($r=46\%^{(+)}$) between alpha 1 globulin and albumin fraction was observed. A correlation between other globulin fractions and albumin was found statistically significant ($p<0.001$).

Conclusion: Elevated serum gamma globulins may be a potential protein biomarker for triage and detection of X-radiation induced damages.

Key Words: *Biomarkers, Cellulose acetate electrophoresis, Globulins, Occupational exposure, Alpha globulin.*

INTRODUCTION

X-rays are a type of high energy (100 eV to 100 KeV) radiations with a wavelength ranging from 0.01 to 10 nanometers, can cause cellular dysfunctions through ionization and genetic mutation upon interaction.[1,2] X-radiations originating from diagnostic tools (computed tomography, fluoroscopy and conventional X-ray radiography) are widely used in medical practice. Beside a number of medical benefits, X-ray imaging poses a high risk of the induction of cancer in the active workers.[2]

X-rays have been classified as carcinogenic by the World Health Organization's International Agency for Research on Cancer. Intervention radiology (IR) produces free radicals by liberating electron from water molecule which can cause irreversible damage to the cells and tissues of particular organ.[3,4] The longer exposure causes cancer due to excessive formation of reactive oxygen species (ROS) and nitric oxide (NO) in the body.[5] Antioxidant systems of the body protects the cells against damage initiated by free radicals, this system may be strengthened by the administration of antioxidant compounds, *i.e.* vitamin C and E.[6]

There are more than 500 different proteins in human blood which perform various functions, *e.g.* maintaining osmotic pressure, metabolic and hemostatic functions. Among these blood proteins, albumin, alpha 1, alpha 2, beta and gamma globulins are important subsets. One of the largest proteins found in blood is albumin which constitutes 60% of total blood proteins, is responsible for 80% of osmotic pressure and transportation of substances, maintaining plasma pH, blood viscosity and biophysical nature of blood flow.[5-7]

The interaction of radiations either causes ionization of DNA or other cellular components or radiolysis of cellular water through ROS that may lead to the oxidation of nucleic acid and other cellular components.[8-10] In *in vivo* studies, it is very difficult to assess the presence of free radicals directly due to their short life time. Thus one of the indirect measurements is made possible by analysis of changes induced by those radicals at plasma protein level. Irradiation also causes protein degradation and

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protein structure modifications.[8] An organism responds to radiation exposure by altering the expression or post translation modifications of proteins in cell, tissues or organic fluids of the body like serum, plasma or urine. So protein expression profiling in these specimen can be very useful to evaluate the detrimental effect of radiation exposures for long period of time.[9,10] Total serum proteins estimation is an important diagnostic technique to assess the health status of the organism. There are two major fractions of serum proteins (albumin and globulins) which can be separated by cellulose acetate-based electrophoresis. Globulins are a relatively smaller faction of total serum proteins which are sub-divided into 5 components (alpha1, alpha2, beta1, beta2 and gamma). The relative quantities of this subset are primarily studied in serum protein electrophoresis interpretation.

The aim of this study was to evaluate the X-rays-induced oxidative stress and its effects on serum proteins (albumin, α1, α2, β1, β2 and γ-globulins) expression in occupational operators of radio-therapeutic equipment.

METHODOLOGY

It was a comparative cross-sectional study conducted from January 2017 to Jan 2018. The current study was conducted according to the ethical guidelines and institutional approval of board of study of Gomal University, vide letter No. 4587-94.

The present study was carried out on 70 subjects which included 50 workers from radiology units of District Headquarters Hospital and Mufti Mahmood Memorial Teaching Hospital, Dera Ismail Khan, KPK, Pakistan and 20 healthy controls that had never been exposed to X-radiation. The study enrolled those X-rays technicians who worked for 48 hours to 72 hours per week. All subjects were enrolled after taking written inform consent. Venous blood samples (5 mls) were collected in plain blood collection tubes from all subjects between 9.0 and 11.0 a.m. All the serum samples were labelled with specific code to hide the identity of subjects. This study did not include those subjects who were suspected of having gross anemia, genetic disorders, diabetes mellitus, cardiopulmonary diseases, viral or bacterial infection, autoimmune diseases, malignancy or any medicine intake. FRAP assay (ferric reducing ability of plasma) was performed on blood serum to estimate the oxidative stress in X-rays exposed individuals.[11] The method is based on the formation of blue colored ferrous-tripyridyltriazine (Fe^{2+}-TPTZ) complex by reduction of ferric (Fe^{3+}) to ferrous (Fe^{2+}) ion at low pH. The optical density (OD) value was measured at 593nm wavelength.

FRAP value was calculated by equation:

$$\textit{FRAP } \mu\textit{Mole value} = \frac{\text{0 - to 4 - min } \triangle A_{593} \text{ nm test sample}}{\text{0 - to 4 - min } \triangle A_{593} \text{ nm test standard}} \times \text{FRAP}_{std}\ \mu\text{M}$$

The serum protein electrophoresis was performed on cellulose acetate strips. For serum protein electrophoresis, the strips were socked in ready-made buffer (pH 8.6) and placed between two Whatman No. 1 filter papers to remove the extra buffer.

Pearson correlation coefficient was used to determine the linear strength of interdependency. The coefficient of correlation with value more than 0.7 was set a value for strong relationship and less coefficient for a moderate interdependency. For unit change in the regress and was observed with the help of linear regression analyses in the presence of studied regressors in the mechanism observed. The descriptive statistics and inferential finding's significance was observed through t-statistics (student t-test for two sided comparison). For statistical analysis, SPSS version 20 (IBM, USA) was used. The statistical significance was observed on significant value set at 1% with p-values observed.

RESULTS

In this study, there was no statistically significant difference in ages of occupationally exposed radiology workers (36.98 ±8.07 years) and controls (36.80 ±7.78 years). The mean duration of X-radiation exposure was 57.12 ±9.25 hours per week.

The mean values of Trolox equivalent antioxidant capacity (TEAC) of serum in exposed group was significantly lower 40.99 ±13.34 µg/ml than control individuals 146.87 ±18.41 µg/ml with ($p<0.001$, Table I).

The serum protein electrophoresis revealed that total protein concentrations of exposed group were within normal range with the mean value 7.74 ±0.35 g/dl *vs*. 7.62 ±0.40 g/dl in control group (Table I).

Those radiology technicians, who worked for 60 and 72 hours per week, showed significant decline in serum albumin levels, while normal albumin levels were observed in technicians who worked for 48 hours per week. The mean values and standard deviation of albumin percentages in exposed group was 57.53 ±5.56 g/dl as compared with control group 63.47 ±1.54 g/dl (Figure 1, Table I).

The radiation exposed group showed serum alpha-1 globulin percentage within normal range (2.0 - 3.5%). The mean values of alpha-1 globulins in radiation exposed and control groups were 2.42 ±0.41 vs. 2.56 ±0.38 ($p=0.61$, Figure 1, Table I).

The mean values and standard deviation of serum percentage of alpha-2 globulins in both exposed and control groups were 7.00 ±1.27% vs. 7.8 ±0.98% .The radiation exposed group showed percentage of alpha-2 globulins within normal range (5.4 - 10.7%), ($p=0.026$, Figure 1, Table I).

The result of serum electrophoresis revealed no abnormality in serum beta globulin percentage when compared with the normal values. The mean values of beta globulins levels in radiation exposed individuals

Table I: Effects of X-radiation exposure on serum proteins and FRAP ability in human subjects with respect to duration.

	Exposed Group (n=50) Duration of exposure (Hours per week)			Control group (n=20)	p-value
	48 (n=23)	60 (n=12)	72 (n=15)		
TEAC(μg/ml)	52.37 ±8.84	37.65 ±2.54	26.21 ±6.23	146.60 ±18.41	<0.0001
FRAP value (μM)	237.99 ±32.5	180.93 ±13.51	144.33 ±25.64	585.15 ±67.66	<0.0001
Total protein (g/dl)	7.61 ±0.36	7.81 ±0.36	7.90 ±0.24	7.61 ±0.40	<0.0001
Albumin band					
Low	-	12	15	-	<0.0001
Normal	23	-	-	20	
High	-	-	-		
Mean ±SD albumin (%)	63.22 ±2.98	53.66 ±0.41	52.55 ±1.17	63.20 ±1.54	
Alpha-1 band					
Low	-	-	-	-	0.61
Normal	23	12	15	20	
High	-	-	-	-	
Mean ±SD alpha1 (%)	2.23 ±0.34	2.5 ±0.32	2.62 ±0.37	2.22 ±0.38	
Alpha-2 band					
Low	-	-	-	-	0.026
Normal	23	12	15	20	
High	-	-	-	-	
Mean ±SD alpha 2 (%)	7.08 ±1.26	7.04 ±1.17	6.84 ±1.43	7.8 ±0.98	
Beta band					
Low	-	-	-	-	0.026
Normal	23	12	15	20	
High	-	-	-	-	
Mean ±SD beta (%)	10.97 ±2.01	11.93 ±1.20	11.86 ±1.38	10.13 ±1.34	
Gamma band					
Low	-	-	-	-	<0.0001
Normal	23	-	-	20	
High	-	12	15	-	
Mean ±SD gamma (%)	16.20 ±1.41	19.18 ±0.41	21.38 ±1.38	15.56 ±1.04	
Albumin / globulin (A/G)		1.498		1.717	<0.0001

1. FRAP assay and cellulose acetate electrophoresis was performed with serum obtained from X-radiation exposed group and controls.
2. The exposed group was divided on the basis of duration of radiations exposure.
3. To observe the effect of radiations exposure duration, the serum protein fractions of exposed group were divided into low, normal & high levels.
4. Similarly, effect of duration of exposure on TEAC & FRAP value was observed. The values are mentioned in mean and standard deviation.
SD: Standard deviation.

Table II: Correlation between serum proteins fractions of exposed subjects.

	Albumin	Gamma	Alph1	Alph2	Beta
Albumin					
Pearson correlation	1	-.871**	-.477**	.001	-.465**
Sig. (2-tailed)	-	.000	.000	.995	.001
N	50	50	50	50	50
Gamma					
Pearson correlation	-.871**	1	.438**	-.228	.177
Sig. (2-tailed)	.000	-	.001	.111	.220
N	50	50	50	50	50
Alpha 1					
Pearson correlation	-.477**	.438**	1	.053	.118
Sig. (2-tailed)	.000	.001	-	.717	.416
N	50	50	50	50	50
Alpha 2					
Pearson correlation	.001	-.228	.053	1	.081
Sig. (2-tailed)	.995	.111	.717	-	.575
N	50	50	50	50	50
Beta					
Pearson correlation	-.465**	.177	.118	.081	1
Sig. (2-tailed)	.001	.220	.416	.575	-
N	50	50	50	50	50

*** Correlation is significant at the 0.01 level (2-tailed).*
Note: Sig. (2-tailed): 2- tailed t-test was used.
N: Total subjects.
*** Significant relationship at p=0.01% or less.*

versus control groups were 11.44 ±1.68% *vs*. 11.13 ±1.34% with (p=0.129, Figure 1, Table I).

Among the exposed groups, those subjects having 60 and 72 hours exposure time showed hypergammaglobulinemia (normal values 8.0%-18.0%). The mean values and standard deviation of gamma globulins of serum in exposed groups and controls were 18.43 ±2.57% *vs*. 15.56 ±1.04% (p <0.001, Figure 1, Table I). In comparison to control group, significantly reduced albumin/globulin (A/G) ratio was observed (p<0.001) in X-rays exposed group (Table I).

Statistical analysis between albumin and gamma globulins showed a highly negative correlation. The linear simultaneous change in both serum proteins have statistically significant interdependence (p<0.001). The correlation coefficient between albumin and gamma globulin was 0.871 with the inverse relationship (p<0.001, Table II).

The linear interdependence among alpha1 and gamma globulins (r = 50%) and alpha1 and albumin (r = 48%) are statistically significant (p<0.001). The correlation coefficient for respective interdependency indicated a

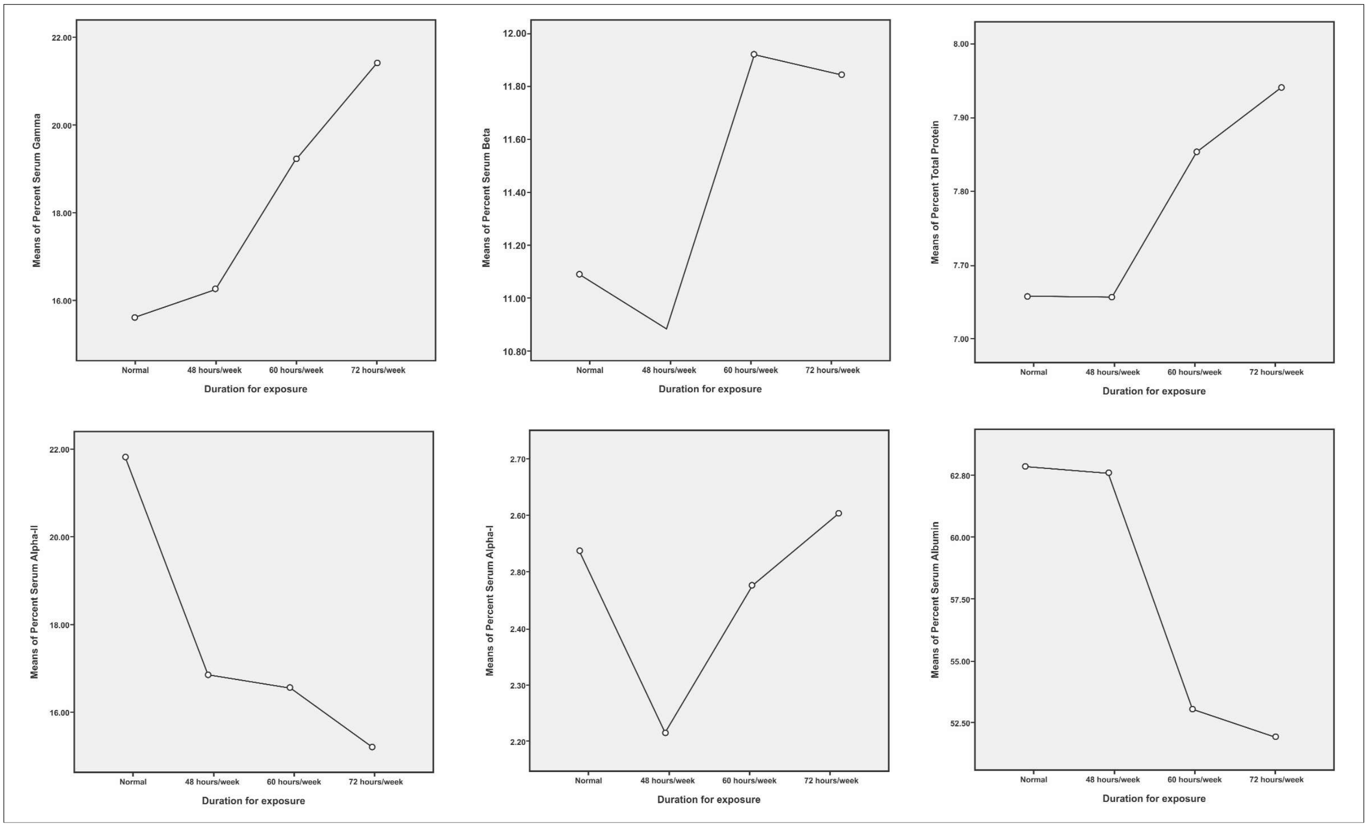

Figure 1: Variations in electrophoretic serum proteins fraction (Albumin, Alpha1, Apha2, Beta and Gamma). Expression patterns with respect to duration of radiation exposure as compared to control have been presented.

moderate nature of relationship. Albumin and alpha1 had an inverse relationship; while the relationship between alpha1 and gamma globulins was direct (r = 44%, Table II).

DISCUSSION

X-radiation technicians who do not follow the radiation protection protocol, are considered as the most appropriate candidates for findings the effects of low-dose radiations exposures over the longer periods of time.[12] So investigating these occupationally workers is an important source of gaining information about exposure to IR of low doses and related risk assessment.[13,14] Radiations-induced chronic oxidative stress leads to various pathological effects.[15] With this background information, the authors intended to investigate the antioxidant capacity and expression pattern of different serum proteins in those radiology technicians.

The results of this study revealed significantly low TEAC in X-ray technicians. The significant reduced TEAC values were observed in X-ray technicians who work for 72 hours per week. Similar findings have been reported in previous studies, serum total antioxidant level (TAL) in radiology workers were found to be reduced as compare to control.[15-17] The pathological role of oxidative stress is well established and IR-induced oxidative stress lasts for longer duration of time in the body is called as late effects. In an animal study, significantly elevated oxidative stress was also observed in mice after six months of exposure to IR.[18] Similarly, elevated oxidative stress was also observed in mice in post-irradiated lungs. It is confirmed that ROS disrupts biomolecules to produce detrimental effects such as cancer.[19,20] Low doses of radiation do not produce direct deterministic effects like skin burn or erythema. However, its longer exposure causes certain types of neoplasms.[16] The results of current study confirmed a negative correlation between radiation work duration and oxidative stress. The oxidative products of plasma proteins was also elevated in radiology staff of hospital.[19] Previous studies have reported a significant high level of lipid peroxidation with reduced activity of erythrocyte glutathione peroxidase, superoxide dismutase and catalase in erythrocytes of X-rays workers as compared to control.[21]

The radiations exposure can also alter the expression of serum proteins. Therefore, protein expression profiling has been quite helpful to develop radiation-induced serum biomarkers.[22] In current study, the largest fraction, albumin is found to be declined (hypoalbuminemia) in X-ray technicians, those having work duration of 60 and 72 hours per week (Table I). Previous studies have reported hypoalbuminemia in radiology workers and attributed to the radio-sensitivity of liver, that leads to hepatocytes damages which cause reduced synthesis of albumin in liver.[23] Acute inflammatory conditions also decreases the production of albumin by liver, because interleukin-6 engages hepatocytes in increased productions of acute-phase reactants *i.e*. C-reactive protein, complement factors and serum amyloid A.[24] In animal studies, serum albumin levels was declined after irradiation on total body of rats.[25]

The alpha1, alpha 2 and beta globulins were in normal ranges in both, X-ray technicians and control groups. Technicians of longer work duration 60 and 72 hours per week, demonstrated most frequent abnormal bands in gamma region (Table I). In animal model studies, elevated gamma globulin fraction was observed in cattle after X-ray irradiation on whole body.[22,24] In radiology staff, the status of serum immunoglobulins, which perform natural protective functions of body, was found to be significantly elevated.[21] Low dose radiation-induced enhanced Th2 immune response was observed in X-ray technicians indicating increased immunoglobulin (IgG, IgA, and IgM) production.[20] In the current study, it is observed that long-term exposure to low doses of X-radiations increases serum gamma globulin levels, comprised of immunoglobulins and C-reactive protein, indicates a notable impact on immune system and development of inflammations in the body of radiology workers. The authors recommended the adaptation and implementation of technical preventive measures by the X-rays department workers such as wearing appropriate protective equipment, like lead apparel, lead goggles, thyroid shield etc. It is also suggested that the X-ray technicians must undergo periodic medical surveillance tests to identify the more susceptible workers.

CONCLUSION

The outcome of current study revealed that the long term exposure (60 to 72 hours per week) to low doses of X-rays may cause increase in oxidative stress and changes the expression pattern of albumin and gamma globulins, which in clinical terms may be correlated with acute/chronic inflammation.

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