ORIGINAL ARTICLE

Expression analysis of tumour necrosis factor alpha (TNF-α) and alkaline phosphatase in occupational workers exposed to low dose of X-radiation: A case-control study

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Abstract

Objective: To evaluate liver and inflammatory biomarkers in occupationally exposed radiology workers.

Method: The descriptive study was conducted at Mufti Mehmood Memorial Teaching Hospital and Gomal Centre of Biochemistry and Biotechnology, Gomal University, Dera Ismail Khan, Pakistan, from September 2017 to May 2018, and comprised X-ray technicians working 48-72 hours per week, and a group of age- and gender-matched unexposed healthy controls. The exposed group was divided into three sub-groups based on their radiation work duration. Liver health status involved estimation of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, gamma-glutamyl transferase GGT and bilirubin through automated chemistry analyser, while serum tumour necrosis factor-alpha and interleukin-6 levels through enzyme-linked immunosorbent assay technique. Relative gene expression analysis of tumour necrosis factor-alpha and alkaline phosphatase was performed through reverse transcription-polymerase chain reaction. Data was analysed using SPSS 20.

Results: Of the 70 subjects, 50(71.4%) were cases with a mean age of 36.98±8.07 years and 20(28.6%) were controls with a mean age of 36.80±7.78 years. Serum alanine aminotransferase and alkaline phosphatase levels showed significant elevation in the cases compared to the controls ($p<0.0001$), although alanine aminotransferase levels were within the normal range. The difference in aspartate aminotransferase, gamma-glutamyl transferase and bilirubin levels was not significant ($p>0.05$). Tumour necrosis factor-alpha concentration was significantly high in the cases compared to the controls ($p<0.0001$). In contrast with proteomic analysis, relative gene expression analysis revealed reduced level of alkaline phosphatase and tumour necrosis factor-alpha in the cases compared to the controls ($p<0.05$).

Conclusion: Serum proteomic analysis of X-ray technicians indicated acute inflammatory conditions, while genomic analysis exhibited down-regulation of alkaline phosphatase and tumour necrosis factor-alpha genes.

Keywords: TNF-alpha, X-ray technicians, ALP, Genomic and proteomic analysis.

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Introduction

Medical radiation exposure has exponentially increased over the past 10 years by routine diagnostic procedures like computed tomography (CT) scans, X-rays, mammography and fluoroscopy.[1] X-rays are categorised as low-dose ionizing radiation (LD-IR) and their exposure for extended period may lead to significant health risks.[2] Medical radiology workers are passively exposed to LD-IR that could damage the living tissues. Therefore, it is presumed that medical profession is a major victim of Ld-IR exposure.[3] X-radiations are also classified as carcinogenic by the World Health Organisation's (WHO) International Agency for Research on Cancer and the National Institute of Environmental Health Sciences of the United States Department of Health and Human Services.[4] Previously, the cancer risk related to LD-IR was extrapolated from documented high dose (HD) IR exposure, which is the risk to the biological system using a linear no-threshold model, but recent studies suggest that both LD-IR and HD-IR have different mechanisms of action over the biological system.[5-10]

Liver is considered a radiation-sensitive organ and several studies have suggested change in liver enzymes, including alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), whose activities reflect the radiation-induced hepatocellular damage.[11] A study on protracted LD-IR effect on liver function of occupational radiation workers confirmed significant increase in serum levels of ALT and AST compared to unexposed workers.[12] Several studies have reported that patients without liver diseases developed hepatic dysfunction when they undergo radiation therapy.[13-15] In an animal model study, after exposure of rats to a single dose of radiation, a significant elevation in ALT was observed.[16] It has been established that LD-IR (0.02-1.0Gy) exposure causes liver inflammation.[17]

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Studies have suggested that radiation-induced alteration in normal serum enzymes/protein levels. After irradiation, change in protein expression or post-translational modifications occur in cells, tissues or in fluids i.e. serum, plasma or urine. This change in protein levels can be used to differentiate between irradiated or non-irradiated individuals.[18] The identification of change in expression of specific protein is suitable to find IR-induced biological markers.[19,20]

Cytokines are intracellular messengers that are released by stimulated cells and tissues, which may play an important role in radiation biology research. Currently, researchers are mainly focussing on biological response modifiers (proteins) as antineoplastic agents. A multipotent cytokine, tumour necrosis factor-alpha (TNF-α), produced by activated macrophages was identified as a tumouricidal protein that can affect haemorrhagic necrosis. TNF-α has the ability to mediate cytotoxicity both in vitro and in vivo.[21] Many studies on animals have reported several cytokines that are involved in innate defence and protection from lethal effects of IR, i.e., pro-inflammatory cytokines like interleukin-6 (IL-6) and TNF-α. TNF-α is an intracellular messenger that is involved in the leukocytes' adhesion to vascular endothelium during cellular immune response to inflammatory process.[22] The cytotoxicity due to TNF-α is related to the generation of free radicals and deoxyribonucleic acid (DNA) fragmentation. Studies on medical staff of radiology department showed elevated levels of serum TNF-α IL-2, while IL-4 level remained reduced.[23] After exposure of cell lines to different IR doses, increasing level of TNF-α messenger ribonucleic acid (mRNA) expression was observed. This increase in gene expression depicts increased production of TNF-α protein, which boost up radiation lethality.[24]

IR induces expression of several cytokines and growth factors in cell lines, like TNF-α, IL-1α, IL-1β, IL-6, type I Interferons (IFN), and granulocyte macrophage-colony stimulating factor (GM-CSF), after IR irradiation.[25] Animal studies have confirmed that radiation-induced inflammatory reaction is mediated by various inflammation-related cytokines genes, such as TNF-α, IL-8, IL-6, IL-1, IFN-γ, vascular endothelial growth factor (VEGF) and epidermal growth factor (EGF) receptor (EGFR), through exogenous stress signal. TNF-α and IL-1 levels were found to be elevated after irradiation of mammalian cells, while excessive production of IL-6 and IL-8 have been reported in fibroblasts and keratinocytes after exposure to X-ray or ultraviolet (UV) radiations.[26]

Gene expression analysis using quantitative real-time polymerase chain reaction (PCR) was performed and found an abnormal pattern of inflammation-related genes on human peripheral blood after irradiation with X-rays (0.5, 3 and 10 Gy doses).[27]

The current study was planned to evaluate the passive effect of X-radiation by evaluating the serum levels of pro-inflammatory cytokines TNF-α and IL-6, and liver biomarkers ALT, ALP, AST, gamma-glutamyl transferase (GGT) and bilirubin in occupational operators of radio-diagnostic equipments.

Subjects and Methods

The descriptive study was conducted at Mufti Mehmood Memorial Teaching Hospital (MMMTH) and Gomal Centre of Biochemistry and Biotechnology (GCBB), Gomal University, Dera Ismail (D.I.) Khan, Pakistan, from September 2017 to May 2018, and comprised X-ray technicians from the radiology units of MMMTH and District Head Quarter (DHQ) Hospital, D.I. Khan, and some private clinics in the study area who were working for 48-72 hours per week. The radiation-exposed cases were divided into three subgroups on the basis of work duration being 48 hours, 60 hours and 72 hours per week. Age- and gender-matched group of healthy volunteers were enrolled as controls. Suspected cases of gross anaemia, genetic disorder, diabetes mellitus, cardiopulmonary diseases, viral or bacterial infection, autoimmune diseases, malignancy were excluded, and so were those taking any medicines.[28] The sample was raised after taking written informed consent from the subjects. To hide the identity of individuals, the samples were labelled with specific codes.

After approval from the institutional ethics review board, the sample size was calculated was performed in line with literature, but reliability coefficient was set at 3.48 with 0.002 level of significance. The cumulative error, however, was kept at 0.05.[29]

Initially, 5ml peripheral blood was collected from each subject by venipuncture; 1ml was immediately shifted into 1ml trizol reagent tube for mRNA extraction, and 4ml into plain blood collection tube for serum analysis. Whole blood was incubated for one hour at room temperature to allow clotting followed by centrifugation at 3000rpm for 10 minutes. Serum was separated and kept at -10^{O}C till further investigation.

Pro-inflammatory cytokine serum TNF-α was quantified through quantitative sandwich anzyme-linked imunosorbent assay (ELISA) technique using a commercial kit (96T; WKEA Med supplies Corp, China; Catalogue No: WH-110). Briefly, 10 micro wells on the microtiter plate were used to make serial dilutions of standard protein standard curve preparation. The sample dilution (40µl) was added into each sample well before the addition of serum sample

(10µl). After gentle mixing, plate was incubated in closure membrane for 30 minutes at 37°C. Thereafter, incubated mixture contents were aspirated and 5 times washing of each well was performed with the wash solution 30-fold diluted with distilled water. The plate was blot-dried by hitting onto absorbent paper, and enzyme conjugate reagent (50µl) was added to each well, except the blank well, and incubated for 30 minutes at 37°C. After final washing and blot drying, substrate A (50µl) and substrate B (50µl) were added into each well and incubated for 15 minutes at 37°C. Finally, reaction was stopped by adding stop solution (50µl) and optical density (OD) was measured at 450nm by a microplate reader (Stat Fax 4200, Awareness Technology, Inc, USA).

Serum IL-6 levels were measured using an ELISA kit (96T; Catalogue No. ELH-IL6; RayBio, USA). Briefly, diluent-A was used to make serial dilutions (pg/ml) of standard protein for standard curve. After addition of serum samples (100µl) into each well and plate, it was incubated in closure membrane for 2.5 hours at room temperature. Thereafter, incubated mixture contents were aspirated and each well was washed 4 times with 1X wash solution diluted with distilled water. The plate was blot-dried and biotinylated antibody (100µl) was added into each well and incubated for 1 hour at room temperature. Following washing, streptavidin solution (100µl) was added and incubated for 45 minutes. After final washing, tetramethylbenzidine (TMB) substrate reagent (100µl) was added and incubated for 30 minutes in the dark followed by addition of stop solution (50µl) and OD was measured 450nm by a microplate reader (Stat Fax 4200, Awareness Technology, Inc, USA).

Liver function was determined by estimating the serum levels of ALT, AST, ALP, GGT and bilirubin on automated chemistry analyser (Micro lab 300, France) using test kits (Human, Germany).

Total RNA was isolated from trizol-containing blood samples of cases and controls (PureLink RNA Mini Kit invitrogen; Catalogue No. 12183018A, USA). After normalisation of all RNA samples by nano-drop, complementary DNA (cDNA) was synthesised from 6µl of extracted RNA using high-capacity cDNA reverse transcriptase kit (Applied Biosystems, USA) in accordance with the manufacturer's instructions.

The expression of TNF-α and ALP were analysed using reverse transcription (RT) PCR (MyGo Pro®, Malaysia). The assay was performed with SYBR® Green supermix (Bio-Rad, Hercules, CA, USA). PCR primers used for TNF-α were: forward primer 5′-GAAAGCATGATCCGGGACGTG-3′ and reverse primer 5′-GATGGCAGAGAGGAGGTTGAC-3′. For ALP the primers were: forward primer 5′-ATGGGATGGGTGTCTCCAC-3′ and reverse primer 5′-CCACGAAGGGGAACTTGTC-3′. The PCR reactions were run (MyGo Pro® Real Time PCR System, Malaysia), and 40 cycles of amplification as per the following PCR thermal cycling steps: denaturation at 95°C for 5 min; amplification at 60°C for 15 sec; and extension at 65°C~72°C. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an endogenous control.

Data was analysed using SPSS 20. To observe the difference between average responses in different biochemical parameters between the groups, student t-test was used for equal variances if assumption was satisfied through Levene's test, otherwise student t-test for equal variances not assumed were used. To describe the variations around the median values of different studied groups, box plots were generated. To describe the normality of data, detrended normal Q-Q plots were made.

Results

Of the 70 subjects, 50(71.4%) were cases with a mean age of 36.98±8.07 years and 20(28.6%) were controls with a mean age of 36.80±7.78 years. Among the cases, 23(46%) subjects were working 48 hours (subgroup A), 12(24%) doing 60 hours (subgroup B)and 15(30%) were working 72 hours (subgroup C)per week. The mean employment duration of the cases was 15.50±3.64 years (range: 5-20 years).

Serum analysis indicated significant elevation in TNF-α with mean value 71.62±13.71 pg/ml in X-rays technicians compared to control group with mean values 17.85±2.81pg/ml ($p<0.0001$). Serum IL-6 mean value 6.32±0.91 was high ($p=0.02$) in the cases compared to 5.70±0.97 in the controls. Moreover, a linear positive association between elevations of serum TNF-alpha and IL-6 levels was observed in the subgroups. Subgroup C showed high serum TNF-alpha and IL-6 level compared to subgroups A and B (Table-1 Figure-1). There was a significant difference in the average behaviour of TNF-α ($p<0.0001$). Average difference with standard error was noted for all the subgroups (Table-2).

Serum ALT in the cases, although within reference range of 10-46u/l, was significantly high compared to the controls ($p<0.0001$). In contrast, serum ALP levels of the cases was not only significantly elevated than the controls ($p<0.0001$), but also higher than the reference range of 44-147u/l. There was a positive linear association of serum liver markers with respect to duration of radiation exposure ($p<0.05$).

Gene expression analysis of TNF-alpha and ALP revealed down-regulation of TNF-α and ALP genes with respect to

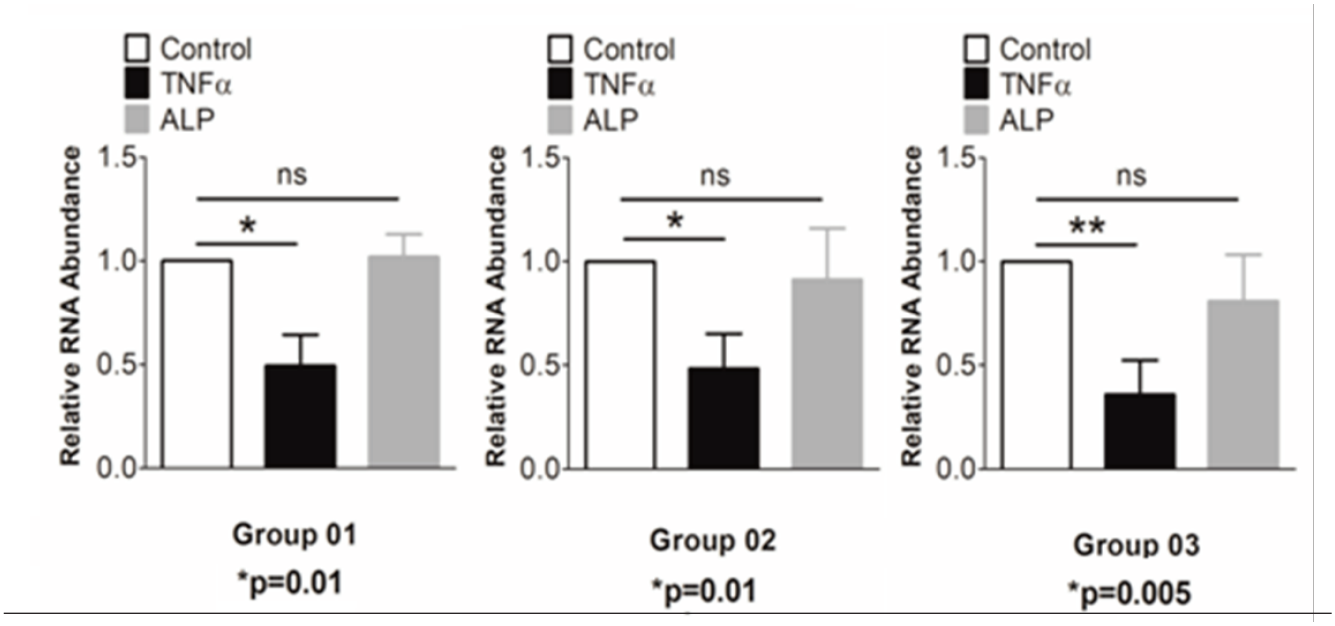

Figure-1: Relative gene expression analysis of alkaline phosphatise (AP) and tumour necrosis factor-alpha (TNF-α) genes in three subgroups of X-rays workers.

increase in duration of radiation ($p<0.05$).

Compared to subgroup A, subgroup C showed low blood mRNA levels of TNF-α and ALP, and, compared to the controls, blood mRNA levels of TNF-α and ALP genes of all the three subgroups were reduced (Figure-2).

Discussion

The use of medical imaging procedures has drastically increased exposure to IR. Over the past 10 years, imaging-based diagnosis in medical practice has over-burdened radiology technicians that has resulted in repeated occupational Ld-IR exposure which makes them highly susceptible towards numerous adverse health issues.[30] These occupationally exposed radio-technicians with inappropriate radiation protection protocol are considered the most suitable candidates for the detection of LD-IR-induced biological markers.[31] Several studies have reported that body releases various biological mediators in response to IR, which are produced by radiation-sensitive body

Table-1: Comparison of TNF-α, IL-6 and LFT profile of X-rays technicians on their work basis and control group.

Parameters	Exposed Group (n=50)			Control Group (n=20)	General Status of Liver Biomarkers	Percentage change	p-value
	Radiation Work Durations (Hours per week)						
	48 hours/week (n=23)	60 hours/week (n=12)	72 hours/ week (n=15)				
TNF-α (pg/ ml)	59.39±5.16	73.75±4.99 71.62±13.71	88.5± 6.09	17.85±2.81	Higher	301.23	<0.0001
IL-6 (pg/ ml)	5.78±0.73	6.50±0.79 6.32±0.91	7.0±0.75	5.70±0.97	Within range	10.87	0.02
ALT (10 to 46 *u/l*)	29.54±1.80	33.5±1.38 32.34±3.29	36.06±1.33	24.10±1.33	Within range	34.19	<0.0001
ALP (44 to 147 *u/l*)	181.60±3.55	198.75±4.75 194.94 ±13.88	212.20 ±4.22	90.35±4.79	Higher	115.76	<0.0001
AST (10-40 *u/l*)	23.65±1.07	24.08±1.24 24.02±1.16	24.53±1.12	23.15±1.13	Within range	3.75	0.003
GGT (10-40 *u/l*)	22.82±1.19	23.25±1.13 23.26±1.24	23.93±1.16	22.7±1.17	Within range	2.46	0.04
Bilirubin (0.2 to 1.2 mg/dL)	0.62 ±0.09	0.69±0.10 0.70±0.13	0.84 ±0.09	0.64±0.10	Within range	9.37	0.02

TNF-α: tumour necrosis factor-alpha; IL-6: Interleukin-6; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatise; GGT: Gamma-glutamyl transferase.

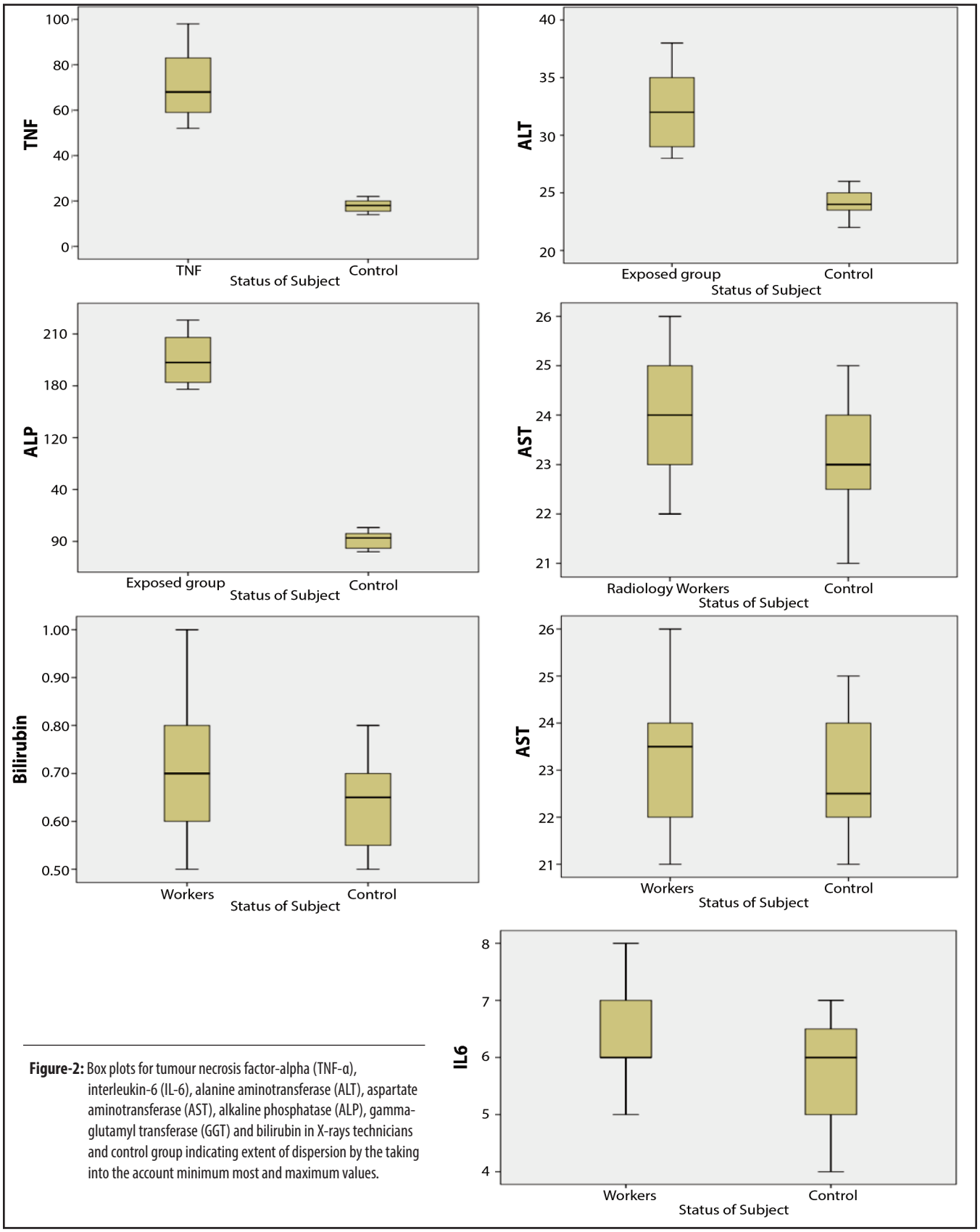

Figure-2: Box plots for tumour necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT) and bilirubin in X-rays technicians and control group indicating extent of dispersion by the taking into the account minimum most and maximum values.

Table-2: Tukey honestly significant difference (HSD) test analysis of variance (ANOVA) indicating the maximum and minimum average differences among TNF-α, IL-6, ALT, AST, ALP, GGT and Bilirubin with respect to different exposure durations (48, 60 and 72 hours) of X-rays technicians and control group.

Independent Variable	(I) No. of hours in working	(J) No. of hours in working	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Lower Bound
ALT	Control	48 hour	-5.20435*	.42678	.000	-6.3292	-4.0795
		60 hour	-9.40000*	.50971	.000	-10.7434	-8.0566
		72 hour	-11.96667*	.47679	.000	-13.2233	-10.7100
	48 hour	Control	5.20435*	.42678	.000	4.0795	6.3292
		60 hour	-4.19565*	.49709	.000	-5.5058	-2.8855
		72 hour	-6.76232*	.46327	.000	-7.9834	-5.5413
	60 hour	Control	9.40000*	.50971	.000	8.0566	10.7434
		48 hour	4.19565*	.49709	.000	2.8855	5.5058
		72 hour	-2.56667*	.54063	.000	-3.9916	-1.1417
	72 hour	Control	11.96667*	.47679	.000	10.7100	13.2233
		48 hour	6.76232*	.46327	.000	5.5413	7.9834
		60 hour	2.56667*	.54063	.000	1.1417	3.9916
ALP	control	48 hours	-91.34565*	1.30915	.000	-94.7962	-87.8951
		60 hours	-108.40000*	1.56352	.000	-112.5210	-104.2790
		72 hours	-121.85000*	1.46254	.000	-125.7048	-117.9952
	48 hours	control	91.34565*	1.30915	.000	87.8951	94.7962
		60 hours	-17.05435*	1.52480	.000	-21.0733	-13.0354
		72 hours	-30.50435*	1.42107	.000	-34.2499	-26.7588
	60 hours	control	108.40000*	1.56352	.000	104.2790	112.5210
		48 hours	17.05435*	1.52480	.000	13.0354	21.0733
		72 hours	-13.45000*	1.65836	.000	-17.8210	-9.0790
	72 hours	control	121.85000*	1.46254	.000	117.9952	125.7048
		48 hours	30.50435*	1.42107	.000	26.7588	34.2499
		60 hours	13.45000*	1.65836	.000	9.0790	17.8210
Bilirubin	Control	48 hour	-.16283*	.03667	.000	-.2595	-.0662
		60 hour	-.32333*	.04379	.000	-.4388	-.2079
		72 hour	-.46833*	.04097	.000	-.5763	-.3604
	48 hour	Control	.16283*	.03667	.000	.0662	.2595
		60 hour	-.16051*	.04271	.002	-.2731	-.0479
		72 hour	-.30551*	.03980	.000	-.4104	-.2006
	60 hour	Control	.32333*	.04379	.000	.2079	.4388
		48 hour	.16051*	.04271	.002	.0479	.2731
		72 hour	-.14500*	.04645	.014	-.2674	-.0226
	72 hour	Control	.46833*	.04097	.000	.3604	.5763
		48 hour	.30551*	.03980	.000	.2006	.4104
		60 hour	.14500*	.04645	.014	.0226	.2674
AST	Control	48 hours	-.50217	.34578	.472	-1.4136	.4092
		60 hour	-.93333	.41297	.118	-2.0218	.1551
		72 hour	-1.38333*	.38629	.004	-2.4015	-.3652
	48 hours	Control	.50217	.34578	.472	-.4092	1.4136
		60 hour	-.43116	.40274	.709	-1.4927	.6303
		72 hour	-.88116	.37534	.098	-1.8705	.1081
	60 hour	Control	.93333	.41297	.118	-.1551	2.0218
		48 hours	.43116	.40274	.709	-.6303	1.4927
		72 hour	-.45000	.43802	.734	-1.6045	.7045
	72 hour	Control	1.38333*	.38629	.004	.3652	2.4015
		48 hours	.88116	.37534	.098	-.1081	1.8705
		60 hour	.45000	.43802	.734	-.7045	1.6045
GGT	Control	48 Hour	-.12609	.35839	.985	-1.0707	.8185
		60 hour	-.55000	.42803	.576	-1.6782	.5782
		72 hour	-1.23333*	.40038	.016	-2.2886	-.1780

Continued on next page

Table-2: *Continued from previous page.*

Independent Variable	(I) No. of hours in working	(J) No. of hours in working	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Lower Bound
GGT	48 Hour	Control	.12609	.35839	.985	-.8185	1.0707
		60 hour	-.42391	.41743	.741	-1.5241	.6763
		72 hour	-1.10725*	.38903	.029	-2.1326	-.0819
	60 hour	Control	.55000	.42803	.576	-.5782	1.6782
		48 Hour	.42391	.41743	.741	-.6763	1.5241
		72 hour	-.68333	.45399	.440	-1.8799	.5133
	72 hour	Control	1.23333*	.40038	.016	.1780	2.2886
		48 Hour	1.10725*	.38903	.029	.0819	2.1326
		60 hour	.68333	.45399	.440	-.5133	1.8799
IL-6	Control	48 hour	-.083	.253	.988	-.75	.58
		60 hour	-.800*	.302	.048	-1.60	.00
		72 hour	-1.300*	.282	.000	-2.04	-.56
	48 hour	Control	.083	.253	.988	-.58	.75
		60 hour	-.717	.294	.080	-1.49	.06
		72 hour	-1.217*	.274	.000	-1.94	-.49
	60 hour	Control	.800*	.302	.048	.00	1.60
		48 hour	.717	.294	.080	-.06	1.49
		72 hour	-.500	.320	.407	-1.34	.34
	72 hour	Control	1.300*	.282	.000	.56	2.04
		48 hour	1.217*	.274	.000		1.94
		60 hour	.500	.320	.407	-.34	1.34
TNF-α	Control	48 Hour	-41.54130*	1.46513	.000	-45.4030	-37.6796
		60 hour	-55.90000*	1.74981	.000	-60.5120	-51.2880
		72 hour	-70.81667*	1.63680	.000	-75.1308	-66.5025
	48 Hour	Control	41.54130*	1.46513	.000	37.6796	45.4030
		60 hour	-14.35870*	1.70648	.000	-18.8565	-9.8609
		72 hour	-29.27536*	1.59039	.000	-33.4672	-25.0835
	60 hour	Control	55.90000*	1.74981	.000	51.2880	60.5120
		48 Hour	14.35870*	1.70648	.000	9.8609	18.8565
		72 hour	-14.91667*	1.85596	.000	-19.8084	-10.0249
	72 hour	Control	70.81667*	1.63680	.000	66.5025	75.1308
		48 Hour	29.27536*	1.59039	.000	25.0835	33.4672
		60 hour	14.91667*	1.85596	.000	10.0249	19.8084

TNF-α: Tumour necrosis factor-alpha; IL-6: Interleukin-6; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatise; GGT: Gamma-glutamyl transferase

organs, such as liver, bone marrow, blood etc. These mediators are not only involved in the initiation of inflammatory reactions in the body, but also promote radiation-induced toxicity.[32] With all this background information, the current study intended to investigate biomarkers related to inflammatory process, like TNF-α and IL-6, in X-radiation technicians. Moreover, liver function tests related to ALT, AST, ALP, GGT and bilirubin were also performed to assess the X-radiation-induced liver damage. The evaluation was performed through proteo-genomic analysis of serum.

Findings suggested that TNF-α levels in the exposed group were significantly elevated compared to the control group. A positive linear association of serum TNF-α levels in radiology workers group with respect to duration of their exposure time was observed. These findings support previous studies on animals in which TNF-α protein was estimated in X-ray-irradiated rats and reported a significant elevation of serum TNF-α protein.[33] Such elevation in serum TNF-α level in response to LD-IR exposures was also in agreement with previous findings.[34]

Studies on animal models have reported that long-term radiation exposure is associated with changes in liver metabolism. Radiation-induced up-regulation of the pro-inflammatory chemokines genes occurs in the liver by the action of locally synthesised pro-inflammatory cytokines.[35,36] Similar findings have been reported in an earlier study.[37]

Generally, high serum ALT/AST levels are associated with liver injury and inflammation, but sometime high level of serum AST also occurs due to skeletal or heart muscle damage. The ALP enzyme is present in all tissues, but is mostly found in liver cells than bile ducts, kidney and bone.

Elevated serum ALP levels indicate liver inflammation or cirrhosis, biliary obstruction or bone disorders, like osteomalacia and osteoporosis. Bilirubin, synthesized from haemoglobin (Hb) passes into the bile ducts, and if bile is blocked, it leads to elevation in serum bilirubin levels which give yellowish appearance to humans. Bilirubin also acts as an antioxidant and has cyto-protective effects.[38] The IR-induced severe hepatic ailment is reported in the form of liver cancer. Recent studies have also established a correlation between exposure to IR doses with liver cancer mortalities. The Energy Employees Occupational Illness Compensation Programme (EEOICP) has listed and specified liver cancer in response to radiations. Furthermore, the evidence of radiation-induced liver cancers has also been provided by the National Research Council's Biological Effects of Ionizing Radiation (BEIR-V) committee. A cohort study on Mayak nuclear production facility reported 75 deaths of Russian radiation-exposed workmen from liver cancer and 30 from bone cancer.[39]

In the current study, the status of liver health was assessed by estimating serum ALT level which was significantly high in the cases compared to the controls, but the values were within the reference range. No significant difference in serum AST, GGT and bilirubin levels were observed between the groups.

Serum ALP was significantly elevated than the normal range and the control group. Such elevations in serum levels of liver enzymes have also been reported in rats after exposing their whole body to radiations and serum AST and ALT levels were high ($p<0.05$), whereas no significant difference in serum bilirubin concentrations was observed compared to the unexposed group.[39] In contrast, few studies have reported non-significant difference in liver function parameters of radiation-exposed group compared to the control group.[40]

Another study on radiation-exposed mice liver also observed that low to moderate doses of radiations can obstruct the glycolysis pathway and pyruvate dehydrogenase in the liver. This can lead to prominent elevation of liver inflammation and changes in lipid metabolic profile.[41]

Studies have explained that radiation-induced liver diseases (RILD) are of two types, i.e. classic and non-classic. Individuals with classic RILD have two-fold increase in ALP levels than the normal range, whereas transaminase and bilirubin levels remain normal.[42] In the current study, the possible reason of elevated serum ALP levels is that X-ray technicians might have been suffering from classic RILD due to lack of appropriate radiation protection protocol.

Research on LD-IR transcriptional and biological responses in vitro, ex vivo and in vivo settings is associated with the alterations of gene expression.[43] It is reported that IR-induced RNA-based gene expression alterations are the main focus in radiation biology nowadays, and studied through DNA microarray and quantitative real-time RT-PCR.[44] Several studies examined X-ray-induced gene expression changes in human peripheral blood though quantitative PCR (qPCR) assay and found significant expression changes in mRNA levels of 62 genes after exposure to low, medium, and high doses of X-radiation. Among these 62 genes, TNF was down-regulated at all doses.[16] Similar finding was observed in the present study. In contrast, elevated TNF-α gene expression in X-ray-irradiated human monocytic cells was reported in a study in which alpha particle radiation and X-rays differential effects was investigated on apoptosis and associated changes in gene expression.[45] In the current study, blood mRNA levels of ALP gene in the exposed group was also significantly decreased compared to the controls, indicating down-regulation of ALP gene in response to X-radiation. This down-regulation of TNF-α and ALP genes increased as the radiation work duration increased from 48 to 72 hours per week. These findings are supported by earlier findings.[46]

It is clear from our findings that long-term exposure to LD-IR caused significant increase in serum TNF-α and ALP proteins, indicating acute inflammatory conditions. The possible reason for elevated serum TNF-α and ALP protein levels and down-regulation of their genes in the cases could be due to increased protein's half-life, and, hence, reduction in protein degradation rate. Or, alternatively, mRNA may preferentially be more translated during the condition in which analysis was performed. We recommend that X-ray technicians must adopt preventive measures such as lead protective apparel, lead goggles, and thyroid shield. The workers must use protective equipments to prevent themselves from the hazards of LD-IR. It is also suggested that X-ray technicians must undergo periodic medical tests to identify the more susceptible workers.

Conclusion

Longer exposure to LD-IR can be associated with a low degree of disease severity, which in clinical terms may be correlated with acute/chronic inflammation and classic RILD.

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