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Cytogenetic and Gene Expression Study in Radiation Workers Occupationally Exposed to Low Levels of Ionizing Radiation

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Abstract: Background: Exposure to IR (ionizing radiation) causes damage to living cells, especially to DNA, the degree of cellular damage depends on the radiation amount. Humans are naturally exposed to IR from cosmic rays, and artificially through diagnostic procedures, medical treatments or occupationally during work shifts. Cytogenetic assay of peripheral blood lymphocytes can provide a biological estimation of the dose received in IR exposures. The CBMN (cytokinesis-blocked micronucleus) assay is a widely used, since it represents a reliable test to detect radiation-induced chromosome aberration and it is a valuable biomarker in many biomonitoring studies on human populations occupationally exposed to IR. The NDI (nuclear division index) is a cell proliferation marker in cultures which is considered a measure of general cytotoxicity, the relative frequencies of the cells may be used to define cell cycles progression of the lymphocyte after mitogenic stimulation. Nevertheless the assay is frequently employed as a useful research tool for understanding the cell cycling kinetics of the cultures. Many gene expression studies demonstrated an up-regulation of genes involved in the processes of signal transduction, control of cell cycle, DNA repair and apoptosis after exposure of IR in different mammalian cell types. Altered expression of a few genes plays specific roles in DNA repair/cell cycle control such as CDKN1A, XPC, GADD45A, DDB2 and PCNA and cell cycle regulation/proliferation such as IL16, CABLES2, TGFB2 and RHOA. Objective: The present study aims to use the MN, NDI and gene expression analysis as biomarkers for investigation of the effects of IR exposure in some radiation workers occupationally exposed to low IR in Al-Tuwaitha site, Baghdad, Iraq. Also, authors assessed the effect of IR on the expression of some gene marker such as: RHOA, CDKN1A, GADD45A and RAD52. Methods: This study was carried out on thirty Iraqi male radiation workers occupationally exposed to low levels of IR at Al-Tuwaitha site, Baghdad, non-smokers and non-alcoholic, aged 30-59 years, as well as thirty apparently healthy individuals males collected randomly from population living in Baghdad, aged 30-59 years who are non-smokers, non-alcoholic as control group. Three molecular genetic parameters were employed such as CBMN, NDI and gene expression assay. The MN and NDI assay were performed according to the description by IAEA, 2001. Total RNA was isolated from blood for radiation worker and control group. The RNA concentration was determined by measuring their absorbance that depends on the ratio A260/A280 of the wavelength, which leads to the determination of RNA purity, which ranged from 1.79-2.1 in two groups. Using RT-PCR for study gene expression, four types of specialized primer genes were selected for the mRNA genes: RHOA, CDKN1A, GADD45A and RAD52 which have a relation with IR in addition to the primers for internal control. Housekeeping gene ((β-actin) was used as a reference gene to normalize the quantity of the target genes. Results: The results of cytogenetic analysis showed that micronuclei frequencies were significantly higher (p < 0.01) in the radiation workers group in Al-Tuwaithasite, Baghdadas compared with the control group, and showed significant decrease (p > 0.01) in the NDI in radiation workers as compared with the control group. Total RNA was isolated from blood for the radiation workers and control groups mentioned. Using RT-PCR for study gene expression, four types of specialized primer genes were selected for the mRNA: genes RHOA, CDKN1A, GADD45A and RAD52 which have a relation with IR in addition to the primers for internal control (β-actin) gene. The products of replicated specialized primers for the genes concerned and the cDNA for the studied samples were electrophoretically separated in agarose gels. The banding profiles were visualized by ethidium bromide staining, as the molecular weight were 135 bp, 165 bp, 185 bp and 470 bp (nitrogen-base pair) for RHOA, CDKN1A, GADD45A and RAD52 genes, respectively. Gene expression analysis revealed statistically significant ($\Delta\Delta Ct$ comparative Ct method) transcriptional changes in three genes RHOA, GADD45A, CDKN1A up-regulated while the RAD52 gene down-regulated. Conclusions: The results indicated that there is a possibility of using the changes in the MN, NDI and genes expression such as RHOA, CDKN1A, GADD45A and RAD52 as useful biomarkers for detection of IR in radiation workers occupationally exposed to low levels of IR.

Key words: IR, radiation workers, occupational exposure, MN, NDI, gene expression.

1. Introduction

Exposure to IR (ionizing radiation) causes damage to living cells, especially to DNA, the degree of cellular damage depends on the amount of radiation administered. Humans are naturally exposed to IR from cosmic rays, and artificially through diagnostic procedures, medical treatments or occupationally during work shifts. Cytogenetic assay of peripheral blood lymphocytes can provide a biological estimation of the dose received in exposures to IR. CAs (Chromosomalaberrations) especially dicentrics induced by IR in human lymphocytes offer useful means to assess radiation exposure [1]. A cytological consequence of induction of CAs is the formation of MNs (micronuclei) that are observed in interphase cells. A micronucleus is formed during cell division when the nuclear envelope is reconstituted around chromosome fragments lacking a centromere (acentric fragments) or a lagging whole chromosome that is not incorporated into the main daughter nucleus, or both. This gives rise to a separate smaller nucleus in addition to the main daughter nucleus. The CBMN (cytokines blocked micronucleus) assay is widely used, since it represents a reliable test to assess radiation-induced chromosome damage and it is a valuable biomarker in many biomonitoring studies on human populations occupationally exposed to IR [2]. The NDI (nuclear division index) is a marker of cell proliferation in cultures which is considered a measure of general cytotoxicity, the relative frequencies of the cells may be used to define cell cycles progression of the lymphocyte after mitogenic stimulation and how this has been affected by the exposure, the index is in itself not sufficiently robust for direct application as a biodosimeter. Nevertheless the assay is frequently employed as a useful research tool for understanding the cell cycling kinetics of the cultures. It will indicate perturbations that may be caused by exposure to a mutagen such as radiation [3, 4].

Radiation is considered carcinogenic; exposure to IR produces several forms of cellular DNA damage, including single-strand breaks, alkali-labile sites, double-strand breaks, DNA-protein cross-links and damage to purine and pyrimidine bases [5, 6]. Several steps in the gene expression process may be modulated, including the transcription step, translation step and the post-translational modification of a protein. Gene regulation gives the cell control over structure, function, and is the basis for cellular differentiation, morphogenesis, the versatility and adapta-bility of any organism [7]. Many studies have also shown that gene expression, including expression of many cell cycle-regulated genes, is markedly affected by IR the trans-criptional regulation of cell cycle-regulated genes that may be closely related to checkpoint functions upon DNA damage. Changes in gene expression may be a mechanism for initiation of cell cycle arrest or a consequence of cell synchronization [8, 9]. Many gene expression studies demonstrated an up-regulation of genes involved in the processes of signal transduction, cell cycle control, DNA repair and apoptosis after IR exposure in different mammalian cell types [10, 11].

Several of these pathways have been found to be responsive to IR exposure as determined by microarray analysis of gene expression. Genes commonly associated with radiation response belong to multiple processes of DNA repair, cell cycle/proliferation, stress response and signaling transduction. Altered expression of a few genes play specific roles in DNA repair/cell cycle control such as *CDKN1A*, *DDB2*, *XPC*, *GADD45A* and *PCNA* and cell cycle regulation/proliferation such as *RHOA, CABLES2, TGFB2*and*IL16* [11-14]. The present study aims to use the MN, NDI and gene expression analysis as biomarkers for investigation of the effects of IR exposure in some radiation workers

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occupationally exposed to low IR in Al-Tuwaitha site, Baghdad, Iraq. Also, authors assess the effect of IR on the expression of some marker genes such as: *RHOA*, *CDKN1A*, *GADD45A* and *RAD52*.

2. Materials and Method

2.1 Subjects and Sample Collection

During the period March 2016 till September 2017, this study was carried out on sixty volunteer blood donors: thirty Iraqi male radiation workers occupationally exposed to low levels of IR Al-Tuwaitha site, Baghdad, Iraq, non-smokers and non-alcoholic, aged 30-59 years, as well as thirty apparently healthy individuals males collected randomly from population living Baghdad, aged 30-59 years who are non-smokers, non-alcoholic as control group. Three molecular genetic parameters were employed, such as CBMN, NDI and gene expression assay. Five milliliters (5 mL) of human peripheral blood from all selected subjects were collected and placed into sterile lithium heparin tubes.

2.2 Micronuclei and NDI Assay Procedure

The MN and NDI assays were performed according to the description by IAEA [15] in 2001.

2.3 Lymphocyte Cultures

Lymphocyte cultures were established with heparinized blood collected from a male donor. For each culture, 0.5 mL of whole heparinized blood was added to 4.5 mL of RPMI-1640 medium (Sigma) supplemented with 10% heat-inactivated fetal calf serum (sigma), phytohemagglutinin (PHA) at a concentration of 10 μg/mL was used to stimulate lymphocyte proliferation, antibiotics (100 IU/mL penicillin and 0.1 mg/mL streptomycin) (Sigma-Aldrich). Blood cultures were incubated at 37 °C for 72 h. In order to obtain cytokinesis-blocked cells, 44 h after PHA stimulation cytochalasin B (Sigma) dissolved in DMSO was added to a final concentration of 3 μg/mL [16].

2.4 Treatment with Hypotonic Solution and Fixation

After a 72-h incubation period, the MN cells were collected by centrifugation (1,000 rpm, for 5 min), hypotonically treaded with cold 0.01 M KCl for 3 min to lyse red blood cells. After removal of the supernatant, the pellet was fixed with a fixative solution containing methanol: acetic acid (3:1). The cells were gently fixed four times, after fixation, the cells were gently resuspended, dropped onto clean glass slides and allowed to dry. Slides were stained in giemsa stain solution for 20 min.

2.5 Microscopic Examination

Five hundred binucleated cells were evaluated for the frequency of MN using 400× magnifications for surveying the slides while 1,000× magnification was used to confirm the presence or absence of MN in the cells. The micronuclei were detected according to the criteria described by Fenech [16] in 2000. A total of 1,000 living interphase cells were used for assessment of mono-, bi-, and poly-nucleated cells and calculation of NDI by using the formula by IAEA [15]:

$$NDI = (M1 + 2 \times M2 + 3 \times M3 + 4 \times M4) / N$$

where M1 to M4 represent the number of cells with one to four nuclei and N is the total number of viable cells scored.

2.6 Gene Expression Assay by Real-Time Polymerase Chain Reaction (RT-PCR)

Three milliliters (3 mL) of human peripheral blood from radiation worker subjects were collected by venipuncture using a disposable 5 mL syringe, and it is drawn into lithium heparin tube. The same amount of blood was collected from control group subjects. Fresh human bloods were used for genomic RNA isolation directly after collection, genomic RNA was extracted using the Trizol method (Invitrogen, USA) according to the manufacturer's instructions [17]. The RNA concentration and purity were determined spectrophotometrically by measuring their absorbance at 260 (A_{260}) and 280 (A_{280}) by nano spectrophotometer. RNA integrity was evaluated by agarose gel electrophoresis. RNA sediments were dissolved in 20 µL Diethyl pyrocarbonate (DEPC)-treated RNase-free water and were assessed by electrophoresis on agarose gel. The cDNA was synthesized from 1 µg of total RNA with the TransSriptFirst-Strand cDNA Synthesis Super Mix according to the manufacturer's instructions (Promega, USA) in the same way as was described in our previous study [18]. Gene expression assessments were performed by real-time PCR system (Applied Biosystems) using SYBR® Premix Ex Taq™ (Promega-USA) as described previously [17]. Relative quantitative real-time PCR method was employed to assess *RHOA*, *CDKN1A*, *GADD45A* and *RAD52* which have a relation with IR in addition to the primers for internal control Housekeeping gene (*β-actin*) was used as a reference gene to normalize the quantity of the target genes. The sequences of primers are listed in Table 1.

The total volume of PCR reactions was 25 µL containing 2.5 µL 10× Taq buffer (Promega), 1.25 U Taq DNA polymerase (Promega), 5 mM dNTPs (Promega), 5 mM dNTPs (Promega), 50 pmol of each primer and 100 ng of template DNA. PCR conditions were: 94 °C, 5 min; 38 cycles of 30 s at 94 °C, 30 s at each T_m as appropriate and many seconds as appropriate (60 s/kb) at 72 °C; and 72 °C for 10 min. The PCR product was analyzed by 1.5% agarose gel electrophoresis and one band was obtained. The QRT-PCR amplification conditions were: 95 °C, 3 min; 95 °C, 30 s, 60 °C, 63 °C as appropriate, 30 s and 72 °C, 15 s for 40 cycles. Melt curves were obtained by increasing the temperature from 56 °C to 95 °C, then cooling at 25 °C for 30 s. Each primer set amplified a single product as indicated by a single peak present for each gene during melting curve analyses.

3. Data Analysis and Statistics

The data of these studies were compiled into the computerized data file and frequency, distribution and statistical description (mean, SE) was divided using SPSS Statistical software. We used statistical analysis of variance (ANOVA) test and least significantly difference (LDS) test by probability of less than 0.01 ($p < 0.01$) according to Ref. [16]. The relative quantitative gene expression level was evaluated using the $\Delta\Delta Ct$ comparative Ct method. Fold inductions were calculated using the formula $2^{\wedge}(\Delta\Delta Ct)$, ΔCt = cycle of threshold, $\Delta Ct = Ct$(housekeeping gene) - Ct(target gene), $\Delta\Delta Ct = \Delta Ct$(treated) - ΔCt(control). *T*-test was used to statistically analyze the difference of the derived expression ratios of radiation worker and control groups [19].

Table 1 Primers sequence and molecular weight used for qPCR validation and additional expression profiling.

Gene symbol	Primer sequence (5′-3′)	Target size (bp)	T_m (°C)
RHOA	Forward: 5′-CGC TTT TGG GTA CAT GGA GT-3′ Reverse: 5′-CAA GAC AAG GCA CCC AGA TT-3′	135	60
GADD45A	Forward: 5′-TCAACGTCGACCCCGATAA-3′ Reverse: 5′-GATGTTGATGTCGTTCTCGCA-3′	185	60
CDKN1A	Forward: 5′-GACAGCAGAGGAAGACCATGT-3′ Reverse: 5′-GGCGTTTGGAGTGGTAGAAATC-3′	165	63
RAD52	Forward: 5′-GGG AAA CCT GAT CTC GAC AA-3′ Revere: 5′-AAT TCG GAG CTG TGT CTG CT-3′	470	60
Housekeeping gene (β-actin)*	Forward: 5′-GAT GAG ATT GGC ATG GCT TT-3′ Reverse: 5′-ATT GTG AAC TTT GGG GGA TG-3′	100	60

* β-actin was used as loading internal control.

4. Results and Discussion

The frequencies of MN assay were performed on peripheral blood lymphocytes which were obtained from 30 male blood samples, aged 30-59 years and duration of employment more than 5 years from radiation worker at Al-Tuwaitha site, as well as 30 male blood samples as control group whose ages ranged 30-59 years. The MN frequency was significantly higher ($p < 0.01$) in the radiation workers occupationally exposed to low levels of IR in Al-Tuwaitha site, compared to the controls (Table 2). To assess the origin of the observed MNs induced as a result of clastogenic effect of a low dose of IR. Micronuclei in mitotically active cells arise from chromosomal aberrations or disturbed function of mitotic spindle [16]. According to our results of the present study, the MN background level is 8-35MN/1,000 BN cells, which is in agreement with IAEA manual reporting the background MN values to range from 2 to 40 per 1,000 BN cells [8]. Table 3 shows distribution of MN (mean ± SE) in peripheral lymphocytes for some radiation workers occupationally exposed to low levels of IR and control group's cells lymphocytes having one or more micronuclei are rendered evident to all radiation workers and some control group subjects according to the criteria reported by Fenech et al. [20]. The increase frequencies of MN indicate the cumulative effect of low-level chronic exposure to IR due to occupationally exposed to low levels of IR.

The frequency of NDI in peripheral lymphocytes among radiation workers at Al-Tuwaitha site and control group was shown in Table 1. A significant decrease ($p > 0.01$) in NDI was observed in radiation workers in Al-Tuwaitha site, compared to the controls (Tables 2 and 4). The NDI as biomarker of cell proliferation in cultures is considered a measure of general cytotoxicity and the relative frequencies of the cells may be used to define cell cycles progression of the lymphocyte after mitogenic material like IR exposure and how this has been affected by the exposure [16, 21, 22]. Therefore, this type of study may become an indicator for the need for greater control and protection against the harmful effects of IR over occupationally exposed professionals [21, 23, 24].

Table 2 Characterization of the samples (control and radiation worker populations), frequencies of micronuclei (MN), and NDI.

Sample characteristics		Populations	
		Control	Radiation worker
Number of individuals		30	30
Exposure time (years) (X ± SE)		0	21.39 ± 8.20
Sex (No., %)	Male	30 (100%)	30 (100%)
	Female	0	0
Age (years) (Range)		30-59	30-59
Smoking status (No., %)	Smokers	0	0
	Non-smokers	30 (100%)	30 (100%)
Duration of employment (year) (Range)		-	5-10
Radiation burden over the last 5-10 years (Rang mSv/year)		-	0.702-1.330 m Sv/years
Micronuclei frequency	No. of BN cells	15,000	15,000
	Total of MN	207	344
	MN/cells (Mean ± SE)	0.0138 ± 0.0009 [a]	0.0230 ± 0.0010 [b]
NDI	No. of cells scored	30,000	30,000
	NDI/100 cell (Mean ± SE)	1.352 ± 0.0310 [a]	0.0261[b]

Similar latter in a column means there is no significant difference ($p < 0.01$).

Table 3 Distribution of micronuclei (MN) (mean ± SE) in peripheral lymphocytes for radiation workers at Al-Tuwaitha nuclear site and control group.

Study groups	No. of samples	Micronuclei frequency							
		No. of BN cells	Total of MN	MN/cell (Mean ± SE)	MN distribution in BN cells				Cells with MN
					0	1	2	3	
Radiation workers	30	15,000	344	0.0230 ± .0010 *	14,683	294	19	4	317
Control	30	15,000	207	0.0138 ± 0.0009	14,808	177	15	0	192

*Significance of differences with control ($p < 0.01$)

Table 4 Frequency NDI in peripheral lymphocytes for radiation workers at Al-Tuwaitha nuclear site and control group.

Study groups	No. of samples	NDI = (M1 +2M2 +3M3 +4M4) / N(Mean ± SE)				NDI/100 cell (Mean ± SE)
		M1	M2	M3	M4	
Radiation workers	30	799.32 ± 7.95	170.80 ± 7.90	18.78±1.24	10.98 ± 0.52	1.235 ± 0.0261*
Control	30	696.90 ± 12.88	263.32 ± 11.28	25.78±1.61	5.74±0.63	1.352 ± 0.0310

*Significance of differences with control ($p < 0.01$)

Total RNA was extracted from the peripheral blood from each radiation worker and control group. The RNA concentration and purity were determined by measuring their absorbance at 260 (A_{260}) and 280 (A_{280}) by nano spectrophotometer. The concentration of total RNA ranged from 47 to 199 ng/μL with a mean ± SE of 123.2 ± 7.85 ng/μL in radiation group. For control group it ranged from 52 to 197 ng/μL with a mean ± SE of 112.2 ± 7.70 ng/μL. The yield of RNA from peripheral blood leukocytes acutely depends on the physiological state of the human, reflecting the dynamic shift in circulating leukocytes fraction with subsequent wide-ranging variability on RNA constituents and their yields [25, 26]. In the present study, the RNA has been isolated from whole blood with a purity ratio ranging from 1.75 to 2.1 for both radiation worker staff and control groups. If there is contamination with protein or phenol, this ratio will be significantly less than the values given above, and accurate quantitation of the amount of RNA will not be possible.

The PCR products were analyzed on 1.5% agars gel electrophoresis to detect the absence or presence of band patterns. The amplified cDNA with β-actin gene was 100 bp in length of all human blood samples in this study. These results also showed that there was a single DNA band which was clearly visible in each sample, which indicates no primer-dimer formation. The presence of *RHOA*, *CDKN1A*, *GADD45A* and *RAD52* primer genes identified the molecular weight was 135 bp, 165 bp, 185 bp and 470 bp, respectively. These results also showed that there was a single DNA band which was clearly visible in each sample, which indicates no primer-dimer formation (Fig. 1).

Relative quantitative gene expression levels for four *ROHA*, *CDKN1A*, *GADD45A* and *RAD52* genes in peripheral lymphocytes for radiation worker employed and radiation worker occupationally exposed to low levels of IR at Al-Tuwathia site, regarding their occupation using ΔCt method in the present study are shown in Table 5. These genes showed significant differences between radiation worker and control groups ($p < 0.01$). The range of *Ct* value for *ROHA* gene in the radiation worker group was 21.62-15.30 with average *Ct* value for *ROHA* gene a mean ± SE 19.848 ± 0.681. In the control group it ranged from 20.28-21.54 with *Ct* value for *β-actin* a mean ± SE 21.123 ± 0.919 (Table 5). As shown in Fig. 2, gene expression levels displayed by *ROHA* gene were up-regulated in peripheral lymphocytes for radiation workers group compared with control group.

The up-regulation occurs when a cell is deficient in some kind of receptor. In this case, more receptor

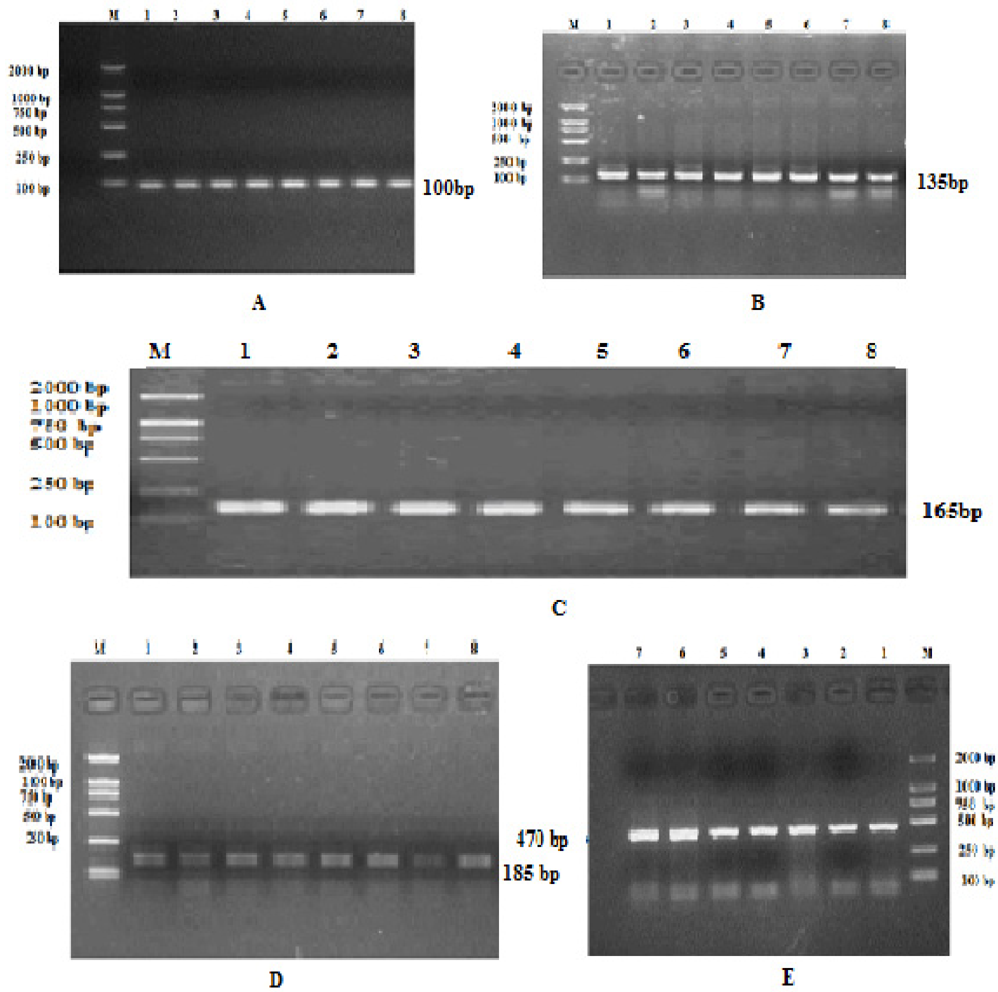

Fig. 1 RT-PCR detection of housekeeping (β-actin) (A), *ROHA* (B), *CDKN1A* (C), *GADD45A* (D) *and RAD52* (E) genes, the lance 1-8 represents partial blood samples. m: marker, DNA ladder 2,000 bp, agarose 1.5% in 1× TBE using 6 v/cm for 30 min.

protein is synthesized and transported to the membrane of the cell [27]. The expression analysis showed an up-regulation of *XPA* and *LIG3*, and a down-regulation of *DUSP22*, *ERCC5*, and *RHOA*.

They trigger specific signals that lead to uncontrolled cell growth, enhanced angiogenesis, apoptosis inhibition, and genetic instability resulting in tumor development [28]. The results of the current study are different from those mentioned by Ana Lucia et al. [10] in which there is a decrease of *RHOA* gene expression of radiation responsive genes in human blood lymphocytes of radiation worker occupationally exposed to low levels of IR. The average *Ct* value of *CDKN1A* gene used in the present study is shown in Table 5.

The range of *Ct* value for *CDKN1A* gene in the radiation worker group was 22.08-25.03 with average *Ct* value for *CDKN1A* gene a mean ± SE (23.620 ± 0.270). In the control group it ranged from 21.79-22.14 with *Ct* value for *β-actin* a mean ± SE (21.123 ± 0.150). As shown in Fig. 2, the expression of the *CDKN1A* gene was found to be up-regulated in peripheral lymphocytes for radiation worker occupationally exposed to low levels of IR, compared with control group. The up-regulation of numerous cell cycle genes suggests that IR has a major effect on cell proliferation in radiation workers. However, the up-regulation *CDKN1A* may indicate that the preferential target is the phase checkpoint of G1/S and not the G2/M phase checkpoint [28]. The expression of

Table 5 Relative quantitative gene expression levels for four genes in peripheral lymphocytes for radiation worker employed and radiation worker occupationally exposed to low levels of IR at Al-Tuwathia site to their occupation using Δ*Ct* method.

Gene	Radiation worker group (n = 30)		Control group (n = 30)		LSD value	*p*-value
	Mean ± SE of *Ct* value	Range	Mean ± SE of *Ct* value	Range		
ROHA	19.848 ± 0.681	21.62-15.30	21.123 ± 0.919	20.28-21.54	0.26*	0.017
CDKN1A	23.620 ± 0.270	22.08-25.03	21.123 ± 0.150	21.79-22.14	0.31*	0.010
GAD45A	24.46 ± 0.140	23.78-25.28	23.94 ± 0.257	23.08-25.00	0.29*	0.011
RAD52	23.85 ± 0.648	21.24-30.73	21.74 ± 0.358	20.89-22.64	0.36*	0.010

*Significance of differences with control (p < 0.01)

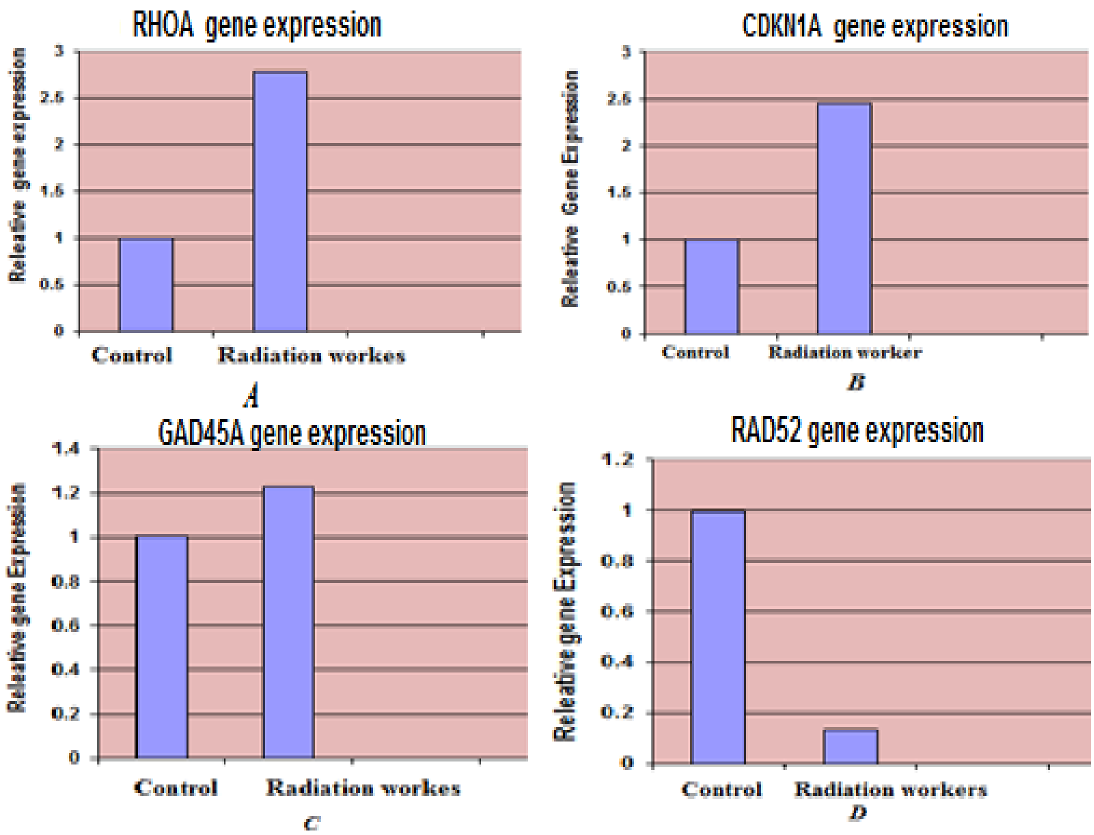

Fig. 2 RT-PCR graphs showing the relative fold expression levels for *RHOA* (A), *CDKN1A* (B), *GAD45A* (C) and *RAD52* (D) genes in peripheral lymphocytes for radiation worker and control group.

CDKN1A is controlled by the tumor suppressor protein p53, through which this protein mediates the p53-dependent cell cycle G1 phase arrest in response to a variety of stress stimuli [29].

The average *Ct* value of *GAD45A* gene used in the present study is shown in Table 5. The range of *Ct* value for *GAD45A* gene in the radiation worker group was 23.78-25.28 with average *Ct* value for *GAD45A* gene a mean ± SE (24.46 ± 0.140). In the control group it ranged from 23.08-25.00 with *Ct* value for *β-actin* a mean ± SE (23.94 ± 0.257). Also, as shown in Fig. 2, the *GADD45A* gene expression was up-regulated in peripheral lymphocytes for radiation worker in Al-Tuwaitha site, compared with control group. The up-regulation occurs when a cell is deficient in some kind of receptor. Moreover, the findings of the present study are in agreement with those reported previously by Abdul-sahib [30] who has shown GAD45A gene up-regulation after exposure to IR, while it is different from the result found in other studies, it was observed that the down-regulation of gene *GADD45A* mRNA expression [31, 32]. Very few genes *GADD45*, *ROHA*, and *CDKN1A* have been found to be consistently up-regulated by IR exposure, and interestingly, genes involved in nucleotide excision repair pathway have been characterized as IR responsive [13]. Altered expression of a few genes plays specific roles in DNA repair/cell cycle control such as *CDKN1A*, *DDB2* [33].

The average *Ct* value of *RAD52* gene used in the present study is shown in Table 5. The range of Ct value for *RAD52* gene in the radiation worker group was 21.24-30.73 with average *Ct* value for *RAD52* gene a mean ± SE (23.85 ± 0.648). In the control group it ranged from 20.89-22.64 with *Ct* value for *β-actin* a mean ± SE (21.74 ± 0.358). Also, as shown in Fig. 2, the *RAD52* gene expression was down-regulated in peripheral lymphocytes for radiation worker occupationally exposed to low levels of IR at Al-Tuwathia site, compared with control group. *RAD52* gene functions in homologous recombination repair. After radiation exposure, *RAD52* forms fociin S and G2/M phases of the cell cycle [34, 35]. In this study we found transcriptional changes in *RAD52* gene that are involved in diverse DNA repair pathways.

5. Conclusions

The results indicated that there is a possibility of using the changes in the MN and NDI as useful biomarkers for the detection of the effect of IRin peripheral blood lymphocytes for radiation workers occupationally exposed to low levels of IR. Also, this study raises the possibility of using the changes in gene expression such as *RHOA*, *CDKN1A*, *GADD45A* and *RAD52* as biomarkers for assessment of low radiation exposure in humans. Several genes involved in cell cycle regulation and DNA repair were found to be significantly induced by radiation exposed to low levels of IR.

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