Investigating the Expression Levels of Bax and Bcl-2 Genes in Peripheral Blood Lymphocytes of Industrial Radiation Workers in the Asaluyeh Region

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ABSTRACT

Background: Industrial radiography uses gamma or X-ray radionuclide sources to investigate the safety of industrial materials. Industrial radiation workers receive the highest occupational radiation doses.

Objective: The present study investigates the relationship between Bax and Bcl-2 gene expression variables in industrial radiation workers.

Material and Methods: In this case-control study, data was collected using blood sampling from 40 workers, including two groups of non-radiation and radiation workers employed at the location. Expression levels of Bax and Bcl-2 genes were assessed in the laboratory. The environmental and absorbed doses of workers were measured using environmental and pen dosimeters.

Results: Statistical analysis showed that the radiation group's Bcl-2 gene expression level was significantly higher. Findings also demonstrated a correlation between Bcl-2 gene expression and the number of workdays. Also, the Bax gene expression did not show a significant change, and the expression ratio of Bax/Bcl-2 was insignificant in the two groups.

Conclusion: Exposure to low doses of radiation could promote an adaptive response in cells by increasing Bcl-2 gene expression.

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Keywords

Industrial Radiography; Bax Gene; Bcl-2 Gene; Peripheral Blood; Lymphocytes; Radiation Workers; Asaluyeh; Gammagraphy; Apoptosis; Real-Time Polymerase Chain Reaction

Introduction

Industrial radiography is the utilization process of gamma or X-ray radionuclide sources to investigate industrial material safety. Industrial radiography has also expanded as there are approximately 700 industrial radiography centers with 7000 active radiographers in the country [1]. In this modern era, industrial radiography, which is usually considered a non-destructive test method, plays a critical role in science and technological fields and guarantees the reliability of products by detecting surface and subsurface defects. Additionally, industrial radiography requires additional precautions and protective measures against

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radiation for workers and other individuals involved in related services, as the utilization of high-energy gamma rays is more frequent than in other fields.

In Iran, industrial radiographers are covered by Thermoluminescent Dosimeter (TLD) dosimetry services with bimestrial dosimetry courses. The doses of their entire bodies are recorded in a central dose data bank using TLD-100 dosimeters and automated Harshaw-6600 card readers [2]. Every authorized dosimetry service provider in Iran is certified by the related authorities according to ISO 17025 standards for test laboratories [3]. Industrial radiographers are among the radiation workers, who receive the highest occupational radiation doses [4]. Non-Destructive Tests (NDTs) are standard tools to investigate the integrity of the components of a compound or system without damaging its function and structure. Workers must usually utilize high-activity resources, including cobalt or iridium, for non-destructive tests.

Compared with other applications of ionizing radiation, this method increases the possibility of radiation [5]. Industrial radiography requires the utilization of high-penetrating power rays. Gamma rays can pass through thick and dense materials, such as steel. Moreover, most radiography sources must be portable to use the device at various outdoor locations [6].

Radiographers must use personal radiation monitoring devices when exposed to ionizing radiation. The radionuclides and the activity level of the radiography sources are selected with specific criteria to maintain the doses for all workers As Low As Reasonably Achievable (ALARA). Radiography is conducted when the radiation container and all necessary equipment are present and functioning optimally [7]. 'Occupational exposure' refers to the radiation levels active personnel receive while providing service. To ensure the safety of the employed radiation workers, the annual effective dose received by each worker must comply with the recommended mean by national and international organizations (20 mSv during five years, with a maximum yearly dose of 50 mSv) [7]. According to previous experiences in Iran [8], based on the number of abnormal exposures in dosimetry courses, the safety culture of the radiation field, and the number of possible correspondences carried out in a two-month dosimetry period, the Iranian authorities suggested four mSv as the review threshold for this activity and a yearly 50 mSv [8]. Exposure to such conditions and environments causes occupational danger and damage to the bodies of radiation workers. The International Agency for Research on Cancer (IARC) has categorized Industrial Radiography (IR) as a group 1 cancerous element for the human body [8].

Genetic polymorphisms of DNA-repairing enzymes can play critical roles in expressing the consequences of exposure to low-dose IR [9]. Ionizing radiation can damage DNA, as an apoptosis cause in specific cells, including human lymphocytes [10, 11]. Chromosomal abnormalities in individuals who received diagnostic X-ray doses are lower than 0.3 and 2 rad [12]. Approximately 77.0% of dicentrics were found in the lymphocytes of radiation workers exposed to cumulative doses of 10-25 mSv during working shifts [13]. Chromosomal aberrations, including dicentrics, were demonstrated in three groups of hospital workers exposed to very low doses of gamma or X-rays [14]. A biomonitoring study on industrial radiographers also revealed a significant increase in chromosomal aberrations among exposed radiation workers [15]. Since reports indicated a significant positive relationship between long durations of exposure to low-dose radiation and leukemia in radiation workers [16], apoptosis is maintained by phosphatidyl-inositol-serine transfer from the internal to external cell membrane and integration with the membrane. At the last apoptosis stages, integration with the cell membrane stops, and the time of cell death or apoptosis is

estimated between several hours and days, depending on the cell type [17, 18]. Programmed cell death depends on the function and reaction of several gene products that activate or inhibit apoptosis. Two significant gene families, including caspases and Bcl-2, play roles in the apoptosis pathway [19]. The Bcl-2 family consists of two protein groups: apoptosis promoters and apoptosis inhibitors. The mean ratio of these proteins determines the fate of the cell. The Bcl-2 protein is localized in the nucleus and mitochondrial membrane and functions through attachment to Bax [20]. Radio Adaptive Response (RAR) describes a phenomenon, in which small priming doses of Ionizing Radiation (IR) reduce the detrimental effects of subsequent higher doses [21].

Most previous research [9-16] investigated chromosomal and genetic damage. Also, there was no gene-expression investigation of individuals. Considering the sensitivity of Bax and Bcl-2 genes to radiation and since industrial radiation workers deal with high-energy radiation, we considered it necessary to measure the dose received by these individuals in this area. Moreover, there has been no scientific investigation into absorbed doses by radiation workers in Asaluyeh, Iran. Accordingly, the current study aimed to assess the Expression Levels of Bax and Bcl-2 Genes in Peripheral Blood Lymphocytes of Industrial Radiation Workers in the Asaluyeh Region.

Material and Methods

In this case-control study, the relationship between Bax and Bcl-2 gene expression was investigated in industrial radiographers and company workers in Asaluyeh Region.

Blood collection

The sampling was done using the convenience sampling method at company clinics. Blood samples were obtained from 40 workers (20 radiation and 20 non-radiation workers). Blood samples (2 ml of peripheral blood) were collected using K2EDTA anticoagulant tubes. The expression level of Bax and Bcl-2 genes was determined using a real-time reverse transcriptase polymerase chain reaction.

Lymphocyte isolation

Based on the standard protocol, lymphocyte isolation was conducted using a Ficoll-Lymphodex Innotrain kit made in Germany. Blood samples were washed with equal volumes of Phosphate-Buffered Saline (PBS). Then, the four-milliliter solution (blood and PBS) was added to 2 ml of Ficoll and centrifuged at 3000 rpm for 20 minutes. The isolated lymphocytes were centrifuged at 1400 rpm for 10 minutes after washing in PBS three times. The supernatant was removed, and the extracted lymphocytes were mixed in 500 µl of PBS.

RNA extraction

After isolating the lymphocytes from the peripheral blood samples, RNA was extracted via an RNX Plus kit (SinaClon BioScience) and stored at -70 ℃. Chloroform and isopropanol were used for RNA extraction, and ethanol (70%) was used to wash the RNA. The concentration and quality of the RNA were measured using a nanodrop spectrophotometer, and the 260/280 ratio was obtained for all samples.

cDNA synthesis

According to the protocol, an additive biokit made in Korea was used for cDNA synthesis, and the product was stored at -20 ℃. The designed primers were used, as shown in Table 1. The B2M gene was used as the reference gene.

Polymerase chain reaction verification and primer design

To confirm cDNA synthesis, a PCR reaction was performed using an Ampliqon 2X Master Mix Red kit made in Iran. Using the synthesized cDNA samples and 5 µl of PCR product loaded into each well of the 2% agarose gel (electrophoresis at 70 volts for 80 minutes),

Table 1: Sequences of reverse and forward primers

Primer name	Primer Sequence (5' to 3')	Product size (base pair)
Bax-forward	GCTTCAGGGTTTCATCCAG	169
Bax-reverse	GGCGGCAATCATCCTCTG	
Bcl-2-forward	TACTTAAAAAATACAACATCACAG	153
Bcl-2-reverse	GGAACACTTGATTCTGGTG	
B2M-forward	GTATGCCTGCCGTGTGAAC	87
B2M-reverse	AACCTCCATGATGCTGCTTAC	

the Bcl-2, Bax, and B2M primers (with band sizes of 169, 153, and 87 base pairs, respectively) were verified. The determined band sizes of each gene primer were observed with colored bands.

Validation of Bax and Bcl-2 primers

The PCR product was loaded onto a 2% agarose gel with and without study primers to verify the ordered primers. Real-time PCR was conducted using the SYBR Green kit, and the relative expression of Bax and Bcl-2 genes was analyzed. Figure 1 presents the image obtained from the Gel Doc device. In this image, sample C indicates the PCR product that lacks cDNA (conducted with an equal amount of DEPC). Sample C1 and P2 indicate the PCR product of the control sample and the target sample, respectively.

The three obtained PCR products with specific primers (Bax, Bcl-2, and B2M) were loaded on a 2% agarose gel and imaged (Figure 2), using a gel doc to verify the band sizes of the ordered primers. The positive samples indicate products with primer, and the negative samples indicate products without primer (replaced with equal volumes of DEPC).

Q-PCR

Iran-made SYBR Green (Amplicon) was used for real-time PCR. The heat cycle included 2 minutes at 95 ℃ for primary denaturation, 40 denaturation cycles every 30 seconds at 95 ℃, renaturation for 40 seconds at 57 ℃, and polymerization at 72 ℃ for 30 seconds.

Data analysis method

In this study, statistical data analysis was conducted using SPSS and GraphPad Prism (version 8). The data for all groups indicated a non-normal distribution (Shapiro-Wilk test). The Mann-Whitney test determined the relationship between the radiation doses received and the gene expression level in the control and target groups. Spearman's test was used to investigate the correlation between the data variables of the history of radiation workers, the number of doses received in one month, the training history of radiation workers, and the amount of monthly work of radiation workers in terms of days. *P*-values lower than 0.05 were considered statistically significant.

Results

Dosimetry results

The obtained results from the investigation of the primary information of the control and target groups indicated that the mean age of participants of the target group in the study was 33.6±7.49 years. All study participants were male, were high school graduates, or had higher educations. Moreover, all individuals in the target group had passed 2 to 8 hours of training on radiation protection rules during their employment period.

The mean age of participants in the control

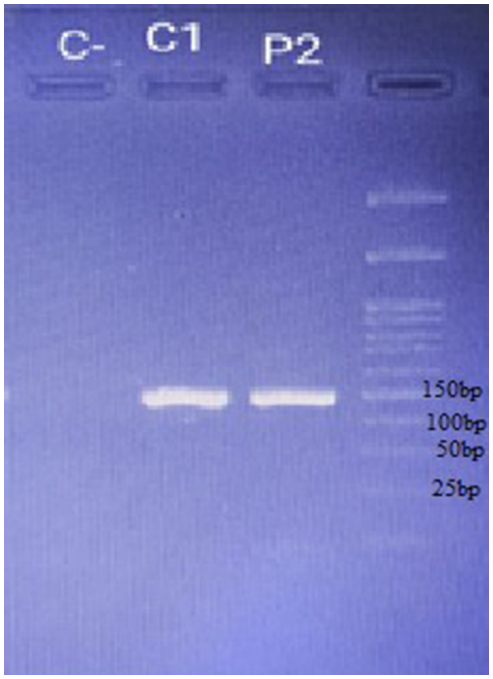

Figure 1: The results of the cDNA synthesis confirmation test by loading the PCR (Polymerase Chain Reaction) product with the Bax gene on a 2% agarose gel

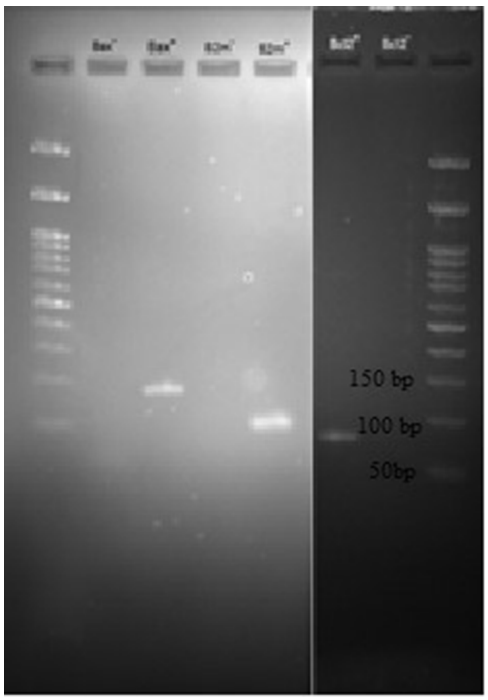

Figure 2: The obtained results of the primer size verification test using PCR (Polymerase Chain Reaction) products on a 2% agarose gel

group was 32.7±6.37 years. All participants were male, were high school graduates, or had higher educations. There was no record of genetic disease history in the control group. None of the individuals had passed radiation protection courses.

Given that the half-life of an Iridium-192 source (15 Ci) is 74 days and that 14 days had passed since the source change, the activity level of the source was estimated 13.15 Ci. The background radiation at various distances was between 15 and 17 µSv/h. Table 2 shows the amount of radiation of the silent source at distances 0, 1, 2, and 4 meters.

The mean work days of radiation workers during one working month were 19.95±1.53 days. The individuals had to take a mean mandatory leave of 11.05±1.53 days per working month. Table 3 presents the results of industrial radiation workers' daily, monthly, and yearly dosimetry.

Table 4 presents the radiation doses received at 7- and 10-meter distances, with and without using a collimator, to conduct an average of 22 radiographs (10-35) with a mean 6.375 duration per radiograph and a mean 10.55-second duration for turning on and off the source switch. The collimator reduces the workers' exposure to radiation approximately 8 to 10 times.

Gene expression results

Analysis of Bax gene expression alterations

Analysis of the findings of the present study

Table 2: Measured dose in an off-mode source, Ir-192, 15 Ci/h (background dose not included)

Distance (m)	Dose µSv/h- Back-ground
0 (On the source)	199±5.03
1	101±3.51
2	63±3.5
4	7±5.06

Table 3: Mean doses of radiation workers measured by pen dosimeters

	Industrial radiation worker (n)	Mean standard deviation
Radiation dose (mSv) in one workday	20	0.04±0.007
Radiation dose (mSv) in 19.95±1.53 workdays	20	0.8±0.14
Radiation dose (mSv) in one year	20	10.42±1.72

Table 4: Radiation received at 7- and 10-meter distances

Collimator	Distance (m)	Total dose (mSv)
-	7	49.5
+	7	5.3
-	10	30.2
+	10	3.5

indicated that the difference between the mean Bax gene expression level of the target group and the control group was insignificant (*P*-value=0.8287>0.05). In other words, the mean CT value of Bax gene expression in the target group was 29.96±1.19, and the change fold method makes it 7.15±13.87 [22]. In the control group, the mean value of CT was 29.35±1.43, and the change fold number was 3.79±2.51. Since the mean differences were not statistically significant, the Interquartile Range (IQR) and medians were applied. Considering the analysis results, the median and IQR of the numbers using the change fold method for the target groups were 0.68 and 8.91, respectively, and for the control group, they were 1.44 and 2.26, respectively. As to the median value, no statistically significant relationship (*P*-value>0.05) was found between the Bax gene expression levels of the target and control groups. However, as to the IQR value, the alterations were directed toward increased gene expression in the target group. In other words, the cells were probably preparing for apoptosis. Figure 3 presents the Bax gene expression in the target and control groups. The signs on each error bar indicate

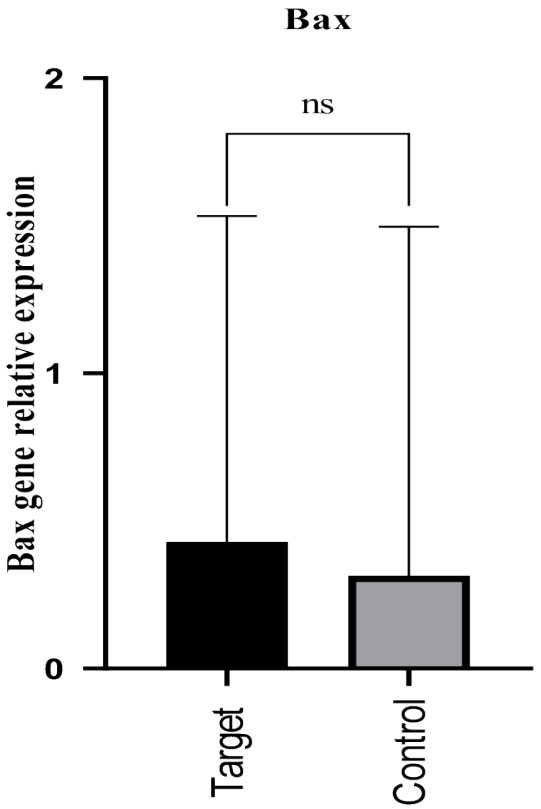

Figure 3: Bax gene expression in the target and control groups

the difference from the control group. Significant differences in various groups (if present) are marked with stars.

Analysis of Bcl-2 gene expression alterations

According to the analysis conducted on the findings, the Bcl-2 gene expression of the target and control groups had a significant relationship (*P*-value<0.05). Bcl-2 gene expression in the target group was higher than in the control group. Results demonstrated

that considering the obtained values, the mean Bcl-2 gene expression in the target group was significant (compared with the control group) (*P*-value=0.0123<0.05). The mean CT value of the Bcl-2 gene in the target group was 27.29±4.97, and the change fold value was 5.23±5.59. The mean CT value of this gene in the control group was 33.39±3.7, and the obtained value by the change fold method was 3.25±6.37. Figure 4 presents the Bcl-2 gene expression in the target and control groups. The signs on each error bar indicate the difference from the control group. Significant differences in various groups (if present) are marked with stars.

Investigation of the alterations in the Bax/Bcl-2 expression ratio

The study results indicated no significant relationship between the expression ratios of the two genes (*P*-value=0.1081>0.05). Since the Bcl-2 family consists of two protein groups (i.e., apoptosis promoter and apoptosis inhibitor), the mean ratio of these proteins determines the cell fate [20]. The mean ratios of the target and control groups were 3.3±6.27 and 15.33±55.56, respectively. IQR and data medians were used since mean differences were not statistically significant. According to the analysis, the median and IQR values with the change-fold method for the target group were 0.31 and 3.91, respectively. The control group's median and IQR values with the change fold method were 1.97 and 5.76, respectively. Figure 5 presents the control and target groups' Bax/Bcl-2 expression ratios. The signs on each error bar indicate the difference from the control group. Significant differences in various groups (if present) are marked with stars.

Correlation of radiation history, radiation dose in one month, and monthly workdays with Bax and Bcl-2 gene expression levels

Figure 6 is drawn based on Spearman

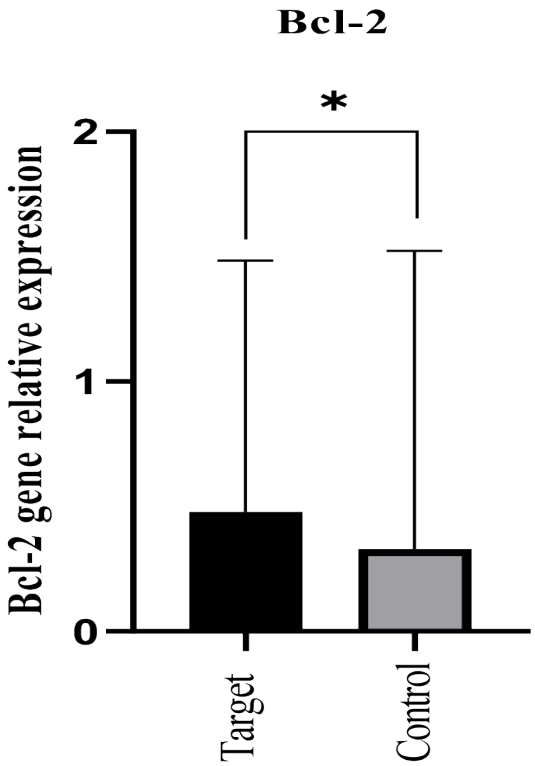

Figure 4: Bcl-2 gene expression in the target and control groups

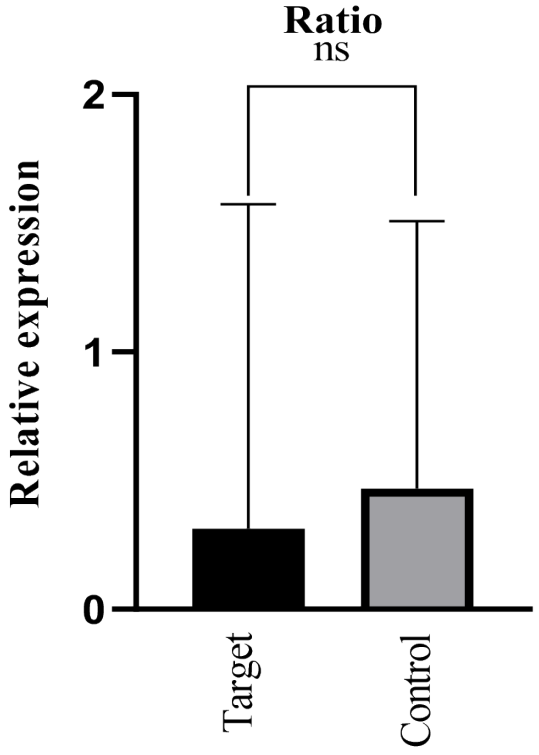

Figure 5: Bax/Bcl-2 expression ratios

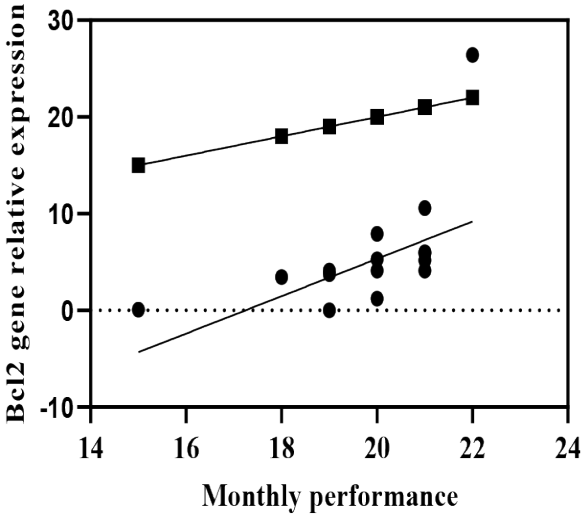

Figure 6: The relationship between monthly workdays and Bcl-2 gene expression

correlation analysis to normalize the data. A positive correlation existed between monthly workdays and Bcl-2 gene expression (P-value=0.0002<0.05). The correlation results of each variable in the history of radiographers-radiation doses in one month, education history of the radiographs, and monthly workdays of the radiographers were compared with Bax gene expression, and none was significant.

Discussion

The present study aimed to investigate the relationship between the radiation doses received by industrial radiographers and Bax and Bcl-2 gene expression. The application of radiography has increased in Iran in recent decades with industrial development. Previous studies investigated the damage and consequences of working with radiation on the body cells. This study investigated the radiation doses received and Bax and Bcl-2 gene expression levels in industrial radiation workers. As Table 2 presents, the utilized source was of the iridium-192 type, and dosimetry was conducted at 0, 1, 2, and 4-meter distances. Since the radiographers' doses when the source is off should be approximately 500 µSv/h at a 5-cm distance from the source and 100 µSv/h at a 1-meter distance [23], it appears that the utilized source by the radiation workers in the present study was within the standard range. Table 4 presents the results of two dosimetry at 7- and 10-meter distances and the collimator reduced the radiation doses received by these individuals by approximately 8 to 10 times. Accordingly, personnel education, radiation safety rules, and training are critical.

Considering the obtained results from the present study, the mean radiation dose of industrial radiographers in one year was 10.42 mSv, which was similar to other studies that demonstrated that radiation doses of industrial radiographers in one year were lower than 20 mSv (in some cases, doses higher than 20 mSv were observed) [23]. Another study revealed that radiation doses received in one year were between 0.1 and 9.4 mSv [24]. Moreover, previous studies [25] reported the cumulative radiation dose of radiation workers between 5.31 and 54.43 mSv per year, which is consistent with the results of the present study [25]. In line with the mentioned studies, another study reported a cumulative radiation dose of approximately 67.2±49.8 in five years [4]. A study on selected participants with a 5-year dosimetry history of over 20 mSv reported chromosome damage [26]. Since the previous studies reported minimum detectable doses for transportation, Dicentrics (DC), and Micronuclei (MN) as 0.15 Gy, 0.5 Gy, and 0.25 Gy, respectively [27], determining the cell alteration capability of doses within the range of radiation doses that industrial workers received required further investigation.

Further investigation of other studies on gene expression revealed that the Bax/Bcl-2 expression ratio is a potential molecular marker to predict radiation resistance [28]. Since these two genes are expressed in the early stages of apoptosis [10, 11] and also the availability of genes and their primers, we selected these genes Accordingly, in addition to

the dosimetry, the present study investigated the effect of radiation on Bax and Bcl-2 gene expression levels in the lymphocytes from peripheral blood samples from industrial radiation workers. QPCR results indicated a significant difference between the Bcl-2 gene expression of radiation workers and non-radiation workers (P-value<0.05) (Figure 4). The result of the present study was similar to that of a study on the effect of low-dose gamma radiation on the peripheral blood lymphocytes of rats, indicating decreased Bax gene expression level and a significant decrease in Bax/Bcl-2 ratio at 50 mGy doses compared with the control group and other groups that received radiation [29]. Another study on melanoma cells demonstrated that low ratios of Bax/Bcl-2 with overexpression of Bcl-2 led to increased cell radiation resistance [30]. Other studies demonstrated that radiation doses between 0.05 and 0.5 Gy increased proapoptotic and initiator genes; however, due to insufficient expression, the cell may not advance to the final stages of programmed cell death [31]. Other studies reported that 1 to 100 mGy doses caused adaptive responses [32]. Another study reported that doses between 0.05 and 0.2 Gy caused an adaptive response, consistent with the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR), which showed that 0.2 Gy doses caused an adaptive response [33].

On the other hand, another study reported that chromosome repair in individuals with received doses lower than 10.2 mSv/y was significantly higher than that in the control group [34]. Also, studies [35, 36] in areas with high background natural radiation reported that exposure to long-duration, high-level natural radiation probably stimulates the processes that decrease the frequency of chromosomal aberrations and the cancer incidence rate [37]. Another study on the difference between chronic and long-duration doses in human blood [38] indicated cell resistance at low dose. In the dose range of 1 to 45 mGy, the natural background dose of the environment caused the cells to repair and resist radiation [39]. All previous studies [34-39] were in the same line with the present study, indicating that the blood lymphocytes of industrial radiation workers try to control apoptosis when exposed to radiation and produce an adaptive response through resistance to radiation. The results of the current study also revealed that Bcl-2 gene expression significantly increased with increased working days (Figure 6; P-value<0.05), which was consistent with the results of Younghyun Lee et al.'s study. A study on the utilization of a personal dosimeter by workers reported a correlation between the recorded dose and the number of chromosome aberrations [40]. Accordingly, the working duration of radiation workers is a helpful index to demonstrate the radiation dose and control the damage to industrial radiographers.

Conclusion

Research on industrial radiographers demonstrates that radiation is a potentially damaging factor for the cells of radiation workers. On the other hand, factors such as the radiation level of the source, distance from the source, and training radiation workers on radiation protection regulations affect the extent of the damage.

In the end, the results of the current study, which investigated the expression levels of Bax and Bcl-2 genes were consistent with the research theory. The radiation recipients indicated a reduced Bax/Bcl-2 ratio, which is promising and can help improve radiation protection regulations and industrial radiation workers' training. Also, considering the diversity of the genes involved in apoptosis, further studies are recommended to investigate other apoptosis-influencing genes. Moreover, environmental pollution has challenged the lives of the residents of such regions.

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Authors' Contribution

Gh. Haddadi, R. Fardid, and M. Haghani contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Gh. Haddadi, O. Keshavarzi, M.Haghani, and R.Fardid. The first draft of the manuscript was written by M. Haghani and, O.Keshavarzi. O. Keshavarzi gathered the images and the related literature and also helped with the writing of the related works. The method implementation was carried out by O. Keshavarzi, M. Haghani, Gh. Haddadi, T. Kalantari, and A. Namdari Results and Analysis were carried out by M. Haghani, Gh. Haddadi, R. Fardid,T. Kalantari and O.Keshavarzi. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethical Approval

This article has been done with the financial support of Shiraz University of Medical Sciences, Shiraz, Iran, under the code of ethics IR.SUMS.REC.1400.875.

Informed Consent

Informed consent was obtained from all participants.

Conflict of Interest

R. Fardid as the Editorial Board Member, was not involved in the peer-review and decision-making processes for this manuscript.

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