Radioadaptive response in peripheral blood leukocytes of occupationally exposed medical staff with investigation of DNA damage by the use of neutral comet assay

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ABSTRACT

Background: ˝Radioadaptive Response˝ is well-documented phenomenon appeared in low dose ionizing radiation received *in vitro* and *in vivo*. Occupational exposure has always been a great concern for radiation workers; therefore this study was performed to study radioadaptive response in terms of residual DNA double strand breaks as an endpoint in peripheral blood leukocytes of occupationally exposed persons. ***Materials and Methods:*** Peripheral blood leukocytes of medical staff as well as control subjects were obtained, separate cultures were set up and irradiation was performed at a challenge dose of 4 Gy of gamma rays. After 48-hour incubation, the neutral comet assay was performed according to the standard method. After single cell gel electrophoresis, cells were stained with ethidium bromide and observed by the fluorescence microscope for DNA damage. ***Results:*** Although there was no statistical difference between baseline DNA damages in two examined exposed and control groups, radiation induced adaptive response was observed in occupational exposure significantly ($p<0.001$). ***Conclusion:*** The obtained results indicate that adaptive response in occupational exposures could be induced in doses lower than detection limit of personal dosimeters leading to enhanced repair mechanism of DNA double strand breaks after irradiation.

Keywords: *DNA damage, radioadaptive response, occupational exposure, neutral comet assay.*

INTRODUCTION

A huge bulk of evidence has pointed to the fact of difficulty in measuring low dose effects comparing to high doses due to lack of sufficient statistical power in health risks assessment [(1)]. Irradiation in the workplace; namely occupational exposure has been regarded as a major concern and a considerable amount of experimental data has proved the association between occupational exposure and increased level of cellular or molecular damages [(2-6)]. Therefore, having a protective attitude has been considered as a top priority for medical radiation staff.

Regarding the adverse impact of ionizing radiation, various models of risk assessment has been introduced but the linear no-threshold (LNT) hypothesis was the model totally accepted as a basis of radiation protection by international commission on radiological protection (ICRP).

The LNT approach indicated that low level of radiation (bellow 100msv) could be estimated by extrapolation of data obtained at high doses [7]. However, notable progress in radiation biology during past decades has challenged the LNT hypothesis and the validity of this model might be in doubt by an abundance of findings [8-10]. Accumulating evidences are available considering the dose response as a dynamic process in which a biological system as an active entity could respond to genotoxic damages with organized series of repair process [11]. This proposed issue has arisen controversy in which low doses irradiation could result in special effects, emphasizing on different respond of organism to chronic exposure comparing to acute one and making less detrimental effects as a consequence [12]. One of the well-documented phenomenon's in low dose level is ″Radioadaptive Response″ which is defined as decreased deleterious effects in a way that primary low dose exposure could lead to an elevated radioresistance to subsequent challenge dose of ionizing radiation exposure [9]. First definition of radioadaptive response was proposed by Olivieri *et al.* in 1984 [9]. This phenomenon was later shown by the use of various endpoints including cellular damage, DNA damage, chromosomal aberration, micronucleus formation, neoplastic transformations or apoptosis induction [9,13-16]. Despite the general notion indicating obvious existence of adaptive response, the specific mechanism underlying this protective reaction particularly molecular basis has been obscured [16] and there has been a considerable controversy in this regard due to cell type, tissue variations and distinct experimental conditions [17].

It has been widely accepted that the most important defense mechanisms against radiation constituted protection against induced reactive oxygen species (ROS), DNA repair, in particular double strand breaks (DSB) repair, and elimination of genomically damaged cells via immune systems and apoptosis activation. However, radioadaptive response as a protective reaction might be associated with these mechanistic approaches as well [10].

Additionally, radioadaptive response as a well-known consequence of low dose exposure has been assessed in the field of occupational exposure. While some studies reported a decreased level of cellular damages such as micronucleus formation after applying an acute dose [16,18], some other reported inter-individual variations or even no observation of radioadaptive response [13,19-21]. There are reports of effective radioadaptive response induced by high natural background radiation comparing to occupational exposure [22].

The present study was aimed to investigate the radioadaptive response in occupationally exposed individuals by the use of single cell gel electrophoresis (SCGE), so-called "comet assay". Comet assay has been widely utilized as a genotoxicity testing, a reliable biomarker for human biomonitoring and valuable tool in risk assessment [23]. Examining single strand breaks (SSBs), double strand breaks (DSBs), alkali-labeli sites along with DNA fragmentations associated with apoptosis could be achieved by distinct modifications of this assay [24]. This study was conducted to examine the radioadaptive response by measuring the extent of residual DSB in leukocytes of healthy control and occupationally exposed individuals before and after irradiation with a challenge dose of gamma rays by the use of neutral comet assay.

MATERIALS AND METHODS

Blood sampling

Peripheral blood samples were taken via venopuncture from 20 medical staff including 10 males and 10 females with mean age of 33.8± 1.17 working in radiology and CT scan departments and 20 corresponding control individuals including 10 males and 10 females with mean age of 32.75 ± 1.01. Sampling was in accordance with ethical committee of Shahid Beheshti University of Medical Sciences (Tehran). It is crucial to consider that physical dosimetry records (film badges reading) of all exposed subjects were controlled over a one-year period in case the effective dose did

not exceed the annual dose limit (20 mSv). Moreover, all donors were non smokers, not suffering from special malignant or infectious disease and had no history of antibiotics consumption or medical radiation exposure at least during the last month prior to sampling. After collecting fresh blood samples from individuals, separate cultures were set up in a micro tube (Eppendrof) under a laminar flow hood, containing 200µl blood in 0.5 ml RPMI1640 medium supplemented with 10% fetal bovine serum (FBS) (Gibco BRL).

Gamma irradiation

Irradiation was carried out at a challenge dose of 4 Gy of gamma rays generated from a Co-60 source (Theratron II, 780C, Canada) with a source surface distance (SSD) of 80cm, fixed field size of 20×20 cm^2 and at room temperature (23±2 °C). After γ-irradiation, the exposed samples as well as control ones were incubated for 48 hours at 37 °C.

Neutral comet assay

The assay was fulfilled based on the procedure of neutral comet [25] and it was crucial to perform a meticulously planned schedule in particular considering time and pH in order to achieve a resounding success in evaluation of intended biomarker. In brief, after 48-hour incubation, the blood samples were centrifuged at 2400 rpm for 5 min, and then the supernatant was discarded. At the second place, 200µl of low melting point (LMP) agarose (Fermentas) which was prepared at 0.75% concentration in distilled water was mixed with the remainder and 50µl of this suspension was poured over each window of comet slides. It is necessary to state that special two-window slides were used in comet assay, precoated with a supporting layer of 1% normal melting point (NMP) agarose (Fermentas). The slide windows were covered with cover slips and kept in 4°C for about 5 min in a dark to allow solidification of agarose gel.

Lysis condition and electrophoresis

By initiation of lysis stage, the cover slips were removed then the slides were placed in horizontal dish, containing fresh lysis solution made up 2.5M NaCl,10mM Tris Base, 0.1M Na_2EDTA, 10% dimethyl sulphoxide (DMSO)(all from Merck), 1% N-lauryl sarcosine (Sigma) and 1%triton x-100 (Sigma) at a final pH of 10. It was crucial to leave slides in lysis solution at 4°C in a dark condition for about 30min in order to destroy all cell contents except nucleus. The slides were then washed 3 times by using electrophoresis buffer, comprising of 90mM Tris base, 90mM Boric acid and 2.5mM Na_2EDTA at a final pH of 8.2-8.4. The slides were put in a membrane horizontal electrophoresis chamber filled with fresh electrophoresis buffer at 30 volts (0.8 V/cm), 8mA and around 10 minute which caused DNA migration, forming a comet appearance. The slides were washed with distilled water for about 5 minute and then dried at room temperature.

Staining and microscope analysis

The air-dried slides were stained with ethidium bromide solution (Merck, 20µg/ml) and covered with cover slips. The slides were analyzed in a two-sided blind manner under a fluorescent microscope (ZEISS) equipped with video camera (Sony). The frequency of DNA damaged cells were evaluated by counting a total number of 1000 cells per slide and determining DNA damage (DD %). To calculate DD%, various extent of migration of DNA; which formed the tail of comet, were scored qualitatively as distinct categories of n_0, n_1, n_2, n_3 and n_4; from absolute normal cell (scored as 0) to the most severe damaged one (scored as 4). DD% was then assessed using the formula 1.

$$DD\ \% = (0n_0 + 1n_1 + 2n_2 + 3n_3 + 4n_4)/(\Sigma/100) \quad (1)$$

Where; DD% is defined as DNA damage, n_1-n_4 as total counted comets of n_1-n_4 and Σ as total counted comets including normal cells [26]. Figure 1 shows typical normal and DNA damaged cells analyzed under fluorescent microscope.

Statistical analysis

Statistical analysis was carried out using SPSS software (version 13.0). Student's *t-test* along with repeated measurement ANOVA were

used to evaluate differences between exposed and control populations, either male or female. P value of less than 0.05 was regarded as a significant level.

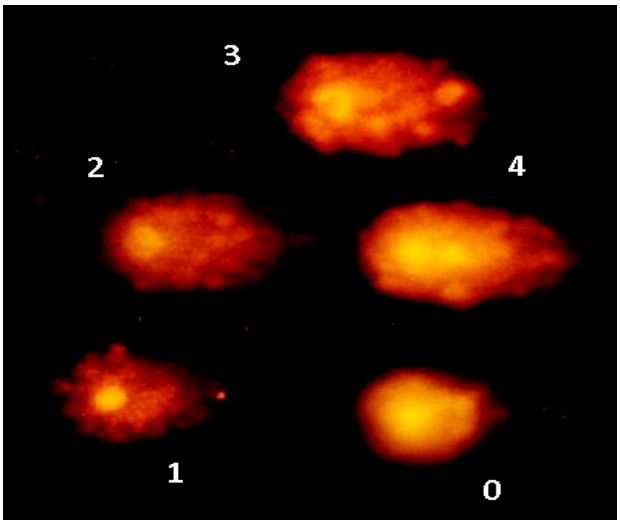

Figure 1. Typical photomicrographs of comets: normal cell (0) and different grades of DNA migration as DNA damaged cells (1-4). Magnification x400.

RESULTS

The results of DNA damage (DD) either baseline or induced along with Net DNA damage which is calculated by subtraction of baseline DNA damage (D_0) from induced DNA damage (D1) are summarized in table 1. Regarding baseline DNA damage there was no significant difference between studied groups ($p > 0.05$). There was also no gender effect was observed significantly.

After irradiation of cells with 4Gy gamma ray; as shown in figure 2, extent of DNA damage was high in both groups but there was a significant reduction of Net DNA damage ($DD_1 - DD_0$) in samples from occupationally exposed individuals ($p < 0.05$). Also, there was a significant difference in net DNA damage in male donors ($p < 0.05$), whereas the statistical difference was not significant for female subjects ($p > 0.05$).

As seen in figure 3, overall DNA damage induced in samples from radiation workers was considerably lower compared to samples from healthy control individuals after 4 Gy gamma irradiation while the baseline DNA damage for both groups was similar.

DISCUSSION

The obtained results indicate that there was no significant difference in background DNA damage between medical radiation workers exposed to low level of ionizing radiation compared to control donors. However, several previous studies have shown variation in the extent of baseline DNA damages within exposed populations [2-6]. There are probably a number of reasons behind the different results reported and the results of this study. One of the main of them might be the inter-individual variations in DNA damage and distinct genomic susceptibilities, the difference which was confirmed with repeated measurement ANOVA analysis in the survey ($p=0$) which is in agreement with the report of Kopjar and Garaj-Vrhovac [27]. In addition the very low number of double strand breaks (DSBs) induced at low doses is worth mentioning. Obviously, neutral comet assay via

Table 1. Baseline as well as induced values of DD% following γ- irradiation in leukocytes of healthy control and occupationally exposed individuals.

Studied groups		No. of cells Scored	*DD_0 (Mean ± SE)	**DD_1 (Mean ± SE)	Net DD (DD_1-DD_0) (Mean ± *SE)
Control Subjects	Female [10]	7,780	25.44 ± 3.43	94.08 ± 11.02	68.64± 7.30
	Male [10]	5,850	28.18 ± 3.46	95.05 ± 17.17	66.87± 15.06
	Total [20]	13,630	26.81 ± 3.42	94.57 ± 6.14	67.76±8.15
Occupationally exposed subjects	Female [10]	8,100	28.59 ± 5.47	86.87 ± 10.15	58.28±7.37
	Male [10]	7,600	22.29 ± 3.83	43.53 ± 6.58	21.31± 6.74
	Total [20]	15,700	25.44 ± 4.86#	65.23± 6.05	39.79 ±5.41@

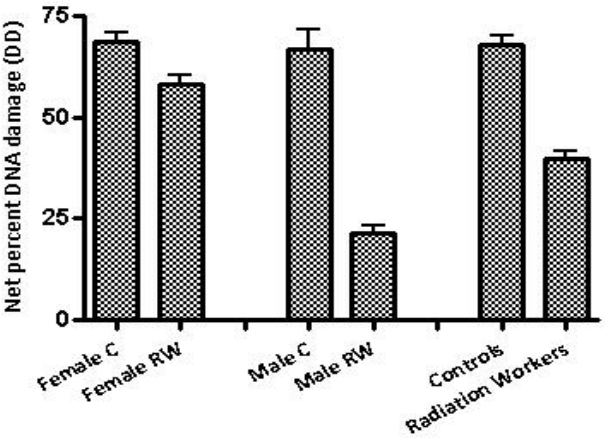

Figure 2. Net percent of DNA damage (DD%) of studied subjects which is deduction of radiation induced DD% from background DD%. C, denotes control and RW, denotes radiation workers. Error bars indicate standard error (SE) of mean values.

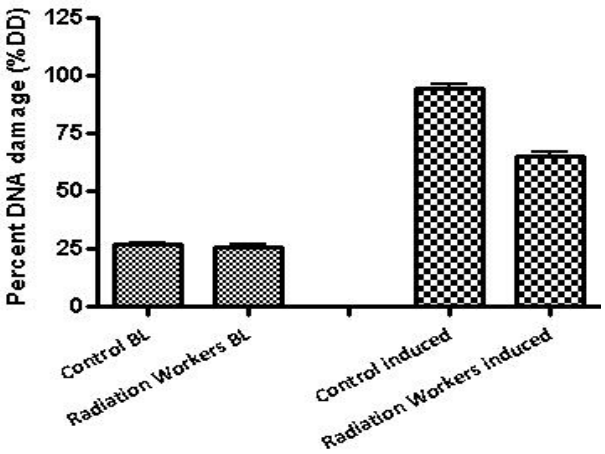

Figure 3. Comparison of baseline (BL) and radiation induced DNA damage in studied groups. Error bars indicate standard error (SE) of mean values.

using non denaturing-pH electrophoresis buffer and different lysis condition was previously identified as a modified version of comet assay, estimating double strand breaks [28]. Moreover, it might seem crucial to emphasize on the fact that high dose of 1 or 2 Gy, namely D_0 or D_{37}, could induce only the number of 40 DSBs in comparison with 1000 SSBs, which is nearly 25 times less numerous than SSBs [28]. Hence, the number of DSBs could be subsequently scanty at low dose levels. Therefore, taking these points into considerations, vast quantities of SSBs or more clearly, insignificant frequencies of DSBs in low dose ionizing radiations could be regarded as a probable explanation for no statistical differences in baseline DNA damage between radiation workers and healthy controls.

The main outcome of this study was the observation of radio adaptive response in leukocytes of occupationally exposed individuals compared to control subjects ($p < 0.05$) despite no significant differences in background DNA damage. The presence of adaptive phenomenon in occupational exposure is in line with the observations of Gourabi and Mozdarani [16] and also of Georgieva *et al.* [18].

The radioadaptive effect could be operated within a specific dose range in mammalian cells, indicating the upper and lower thresholds between 1 and 100 mGy for a single low dose exposure [29]. In this study, evaluation of dosimetry records of medical workers highlighted the fact that effective dose levels of occupationally exposed donors were lower than 0.05 mSv, suggesting that the induced radioadaptive response was observed in dose levels which were lower than detection threshold of film badges (0.05 mSv).

DNA DSB repair is considered as the key mechanistic approach in adaptive response. Activated natural protection (ANP) against genomic instability has been regarded as a mechanistic approach in low dose protection [10]. Based on the study of Scov *et al.*, SSBs' repair deficient hamster cells were capable of showing adaptive response whereas those of DSB's repair deficient showed no radioresistance [30]. Furthermore, it has been shown previously that the induced radioadaptive effect could be due to increased capability of double strand breaks' repair [31].

It is worth noting that the presence of unrepaired or persistent DSBs in low doses of ionizing radiation shown by Rothkamm and Lobrich by using the gamma H_2AX foci assay [32] could be a supportive evidence of the results obtained in this study (table 1 and figures 1 and 2). The findings are consistent with persisting

DSB as an unrepaired defect of long-term occupational exposure which could trigger protective mechanism [1] leading to radio-adaptive response.

In conclusion, low dose ionizing radiation as much as below detection limit of personal dosimeters effectively induce radio-adaptive response probably via trigging DNA repair mechanisms.

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