Effect of the Expousre to Low Dose of Ionizing radiation on KAU Hospital Medical Stuff by Using Early Response of Biological Dosimetry

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Abstract: Cytogenetic and Comet analysis were performed in forty volunteer students and hospital workers who were chronically exposed to Low ionizing radiation from king Abdulaziz University Hospital (KAUH), Radiology Department and Faculty of Applied Medical Sciences, were enrolled and divided into three groups. Peripheral blood samples were collected by venipuncture in heparinized and EDTA tubes (BD vacationer, Becton Dickinson, NJ, USA) on 6 different times during a period of 3 months. Accumulated absorbed doses calculated for the radiation workers ranged from 9.5 to 209.4 mSv. The mean of chromosomal aberration (CA) frequencies demonstrated statistically significant differences between the mean frequencies of CA between staff, intern students and second year students. Dicentric chromosome was only found in one technician from workers group while the other two groups have shown no dicentric chromosomes at all. Mean values (± standard deviation of the mean) of comet tail moment were 7.44±2.35 for the staff worker group and 3.51±2.1 for the intern students group and 3.01±1.33 for second year students (control group). Difference between mean tail moments were statistically significant when comparison between the worker stuff group and second year student group (P<0.01, ANOVA) and also significant between staff group and intern students (p<0.01, ANOVA) while there is no significance between intern and second year student groups (p>0.05, ANOVA). The range of tail moment in exposed worker stuff was 5.21- 12.53 and for the intern students was 2.99-5.31 and for the control second year student was 2.00-4.37. These results also indicate that occupation and occupation periods significantly contributed to the level of primary DNA damage as recorded by mean of alkaline comet assay and the relevance of conducting cytogenetic analysis in parallel to physical dosimetry in routine clinical setting

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1. Introduction

Application of ionizing radiation in many different fields is continuously increasing, including the use for medical purposes. Many assays use molecular endpoints that measure DNA breakage, changes in the regulation of some sentinel genes or the presence of protein biomarkers that may be detected within cells or in blood plasma/serum. This is an area of rapidly emerging technologies with a number of assays at differing stages in development and verification. The range of biological dosimetry options now available have led to proposals for a multi-parametric approach to investigating an overexposed person [1] and having a variety of assays available may be particularly useful if a laboratory has to deal with an event involving many casualties.

Genomic abnormal changes can be analyzed by using cytogenetic parameters such as chromosomal aberrations (CA), sister chromatid exchanges (SCE), and micronuclei (MN), which are considered to be the biomarkers of carcinogenic effects [2]. Chromosomal aberrations have been correlated with genetic changes that can trigger the development of cancer. Therefore, a biological dosimeter based on CA frequencies makes possible to estimate the cancer risk [3], and this method has been applied to monitor hospital workers in order to estimate the absorbed radiation doses during the period of employment in the hospital [4-7]. It has been shown that workers engaged in operational radiology [8] and Nuclear Medicine [9] is chronically exposed to low-level ionizing radiation.

The lymphocytes are the major and most important cells that are used as the bio indicators for the effect of ionizing radiation. Two main types of lymphocytes can be distinguished, i.e. T and B cells. Both types originate from immunologically incompetent stem cells in the yolk sac and

eventually settle in the bone marrow. These undifferentiated stem cells migrate into the thymus and other primary lymphoid organs, multiply there, undergoing somatic mutations and give rise to a pool of long lived lymphocytes that circulate. On the basis of their surface markers, T and B cells comprise a mixture of naïve and memory cells with differing life spans and differing roles in the immunological processes [10]. It is the T cells, mostly of the CD4+ and CD8+ subtypes, which are stimulated *in vitro* by phyto-haemagglutinin (PHA) and are used for biological dosimetry.

Chromosomal aberrations have been widely accepted for many years as a biological marker of exposure for ionizing radiation. Also the risk of cancer increase by increasing of chromosomal aberration. The past two decade have seen significant improvement in the ability to identify and quantify chromosomal damage. One of the most effective methods is fluorescence in situ hyperdization (FISH) with whole chromosome paints. FISH painting can identify translocation (with one centromere, dicentric or acentric fragment). The ability to identify translocation with light accuracy and efficiency is significant because translocation have substantially greater persistence through cell division than dicentrics [11].The using of chromosomal painting to detect the translocation has significant advantage when it is compared by any other techniques. The use of translocation for bio-dosimetery has increased recently and some of commercial probes are become available. There are some observations prove that translocations may be the most relevant cytogenetic and point for assessing cancer risks.

Prior development of single cell gel electrophoresis methods in 1980, measurement of the effect of radiation on the DNA strand breaks in the individual cell was limited to conventional methods such as classical karyotyping and also micronuclei test. Development of comet assay which relies on single-cell gel electrophoresis that depends on migration of DNA fragments has shown a rapid and sensitive method of quantifying the level of DNA damage either in single or double strand breaks from single cells through movement of damaged DNA (tail fraction) away from the distinct head fraction representing the intact DNA. The distance between the means of head and tail defines the tail moment parameter during analysis which represents the percentage of fluorescently labeled damaged DNA by the increase in the tail moment. The degree of DNA migrated from the head is proportional to the amount of damaged DNA per cell [12]. Applying such methodology in clinical practice will eliminate the need to use radio labeled cells and Furthermore, methods to detect the migration of DNA from single cell permitted the direction of initial radiation induced DNA in single cells. Therefore, using comet assay has eliminated the use of radio labeled cells and this gives a new opportunity for analysis of radiation induced DNA damage at any tissue provided a single cell suspension.

In this study assessment of lymphocytes by using fluorescence in situ hybridization (FISH) and single cell gel electrophoresis technique (Comet) for detection of single DNA strand breaks mediated by quantifying the DNA damage in tail moment was carried out as a mode of comparing between the two methods interims of sensitivity, cost and duration of analysis which enables further selection of the best suited technology as an indicator of DNA damage among exposed hospital workers to radiation. The results of this study may provide further insights towards the possible implementation of such method in routine clinical setting. Also, it will enable further establishment of biological dosimetry data base at KAUH radiology department by evaluating radiation risks to various groups of exposed personnel, and conveying recommend criteria for development and construction for a Saudi Standard Bio-dosimetry Laboratory.

2. Material and Methods

Subjects

The sampled groups consisted of forty volunteer students and hospital workers from king Abdulaziz University Hospital (KAUH), Radiology department and Faculty of Applied Medical Sciences were enrolled and divided into three groups. The first group (A) included 15 staff from X-rays, radiotherapy and nuclear medicine departments who had been occupationally exposed to low-level ionizing radiation during their work for a period of time ranging from 10 to 32 years (20 ± 5). The second group (B) covered 15 students who were spending the whole year in the radiology department (intern students) at KAUH. The third group (C) was consisted of 10 second year students who were taking a practical experience of working with X ray machine for only few months (control).

The study was performed in accordance with high standards of ethics (approved by ethical committee at faculty of Applied Medical Sciences). Informed consent was obtained from all participants prior to the start of the study. All participants were also informed about the aim and the experimental details of the study and they were free to withdraw from the study at any time. All of them were healthy and did not complain of any health issues. However, no adverse effects occurred, and the data of all participants were available for analysis. All exposed

subjects completed a standardized questionnaire that covers personal data, working activities, type and duration of occupational exposure to X ray radiation at the time of the study, and information on exposure to possible confounding factors (smoking habits, intake of medications ,contraception, viral diseases, recent vaccinations, presence of known inherited genetic disorders, chronic disease, family history of cancer, sunlight exposure, and radio diagnostic examinations) was recorded followed by blood samples collection from all participants.

Sample collection and preparation

The requirements of samples collection and preparation for comet and FISH assays were fulfilled such as avoidance of hemolyzed samples, the use of sodium heparin as an anticoagulant of choice for FISH studies and collected samples were maintained at room temperature during processing.

Sample processing and investigation

The following tests were performed in each sample form each subject: Complete blood count (CBC), Comet, Karyotyping and FISH techniques for the following chromosomes 1, 4, and 18 using whole chromosome painting (WPC) technique. Peripheral blood samples were collected by venipuncture in EDTA and Heparinized tubes (BD vacationer, Becton Dickinson, NJ, USA) on 6 different times during a period of 3 months. Blood samples from the exposed subjects were always collected in the morning hours, between 9 am and 10 am of the last day of working week. Blood samples from the age- and sex matched control group were taken at the same time during the study (a balanced collection design was used). Samples from both exposed and non-exposed individuals were handled in the same manner. After collection, all blood samples were randomly coded, refrigerated at 4 ^{0}C, transported to the laboratory and processed immediately (usually within 2 hours after blood sampling).

Alkaline Comet Assay

Comet assay was carried out under alkaline conditions [5, 14]. 1% of normal melting point (NMP) agarose (Sigma) was added to fully frosted slides. Following solidification, all slides were scraped off the gel followed by coating the slides with 0.6% NMP agarose. Then a second layer containing the whole blood sample (2 mls) mixed with 0.5% low melting point (LMP) agarose (Sigma) was placed on the slides. Following this, slides were covered with 0.5% LMP agarose after 10 minutes of solidification on ice. Afterwards the slides were immersed for 1 hour in ice-cold freshly prepared lysis solution (2.5MNaCl, 100mM Na_2EDTA, 10mM Tris–HCl, 1% Na-sarcosinate (Sigma), pH 10) with 1% Triton X-100 (Sigma) and 10% of dimethyl sulfoxide (Kemika) to lyse cells and allow DNA unfolding. Then random placing of slides side by side was taken place in the horizontal electrophoresis tank, facing the anode. The unit was filled with freshly prepared electrophoresis buffer (300mM NaOH, 1mM Na2EDTA, pH 13.0) and the slides were set in this alkaline buffer for 20 minutes to allow DNA unwinding and expression of alkali-labile sites. Denaturation and electrophoresis were performed at 4 ^{0}C under dim light. Electrophoresis was carried out for the next 20 minutes at 25V (300 mA). After that, the slides were washed gently three times for 5 minutes with a neutralizing buffer (0.4M Tris–HCl, pH 7.5) to remove excess alkali and detergents. Each slide was stained with ethidium bromide (20 mg/ml) and covered with a cover slip. Slides were stored at 4 ^{0}C in humidified sealed containers until analysis. To prevent additional DNA damage, handling of blood samples and all steps included in the preparation of slides for the comet analysis were conducted under yellow light or in the dark. Moreover, two parallel replicate slides were prepared per sample in order to avoid possible position effect during electrophoresis. Each replicate slide was processed in a different electrophoretic run.

Comet Capture and Analysis

Each slide was examined at 250-fold magnification with a fluorescence microscope (Zeiss, Germany), equipped with an excitation filter of 515–560 nm and a barrier filter of 590 nm. One hundred comets per subject were scored (50 from each of two replicate slides). Comets Random capturing of the comets was carried out at a constant depth of the gel, avoiding the edges of the gel, occasional dead cells and superimposed comets. The microscope was connected to a black and white camera and to a computer-based image analysis system (Comet Assay II, Perceptive Instruments Ltd., UK). This system acquires images, computes the integrated intensity profiles for each cell, estimates the comet cell components, and then evaluates the range of derived parameters. To avoid potential observer variability, one well-trained personnel performed all scorings of the comets. Tail length (µm) was calculated from the centre of the head and served as measure of DNA damage.

FISH Protocol

Analysis of chromosomes aberration was performed according to current IPCH and IAEA guidelines [14,15]. Whole blood cultures were established by adding 0.5 ml heparinized whole blood into 5ml of RPMI 1640 medium (Chromosome kit P, Euroclone) containing 10% foetal bovine serum, phyto-haemagglutinin, heparin,

glutamine, growth factors, and gentamycin. Duplicate cultures per subject were set up and incubated at 37 ^{0}C for 48 hours. Metaphase arrest of dividing cells was done using colchicine (0.004%) for 2 hours prior to the harvest. Cultures were centrifuged at 1000 rpm for 10 minutes, the supernatant was carefully removed, and the cells were re-suspended in a hypotonic solution (0.075MKCl) at 37 ^{0}C. After centrifugation for 5 minutes at 1000 rpm, the cells were fixed with a freshly prepared fixative of ice-cold methanol/glacial acetic acid (3:1, v/v). Fixation and centrifugation were repeated several times until the supernatants were clear. Cells were pelleted and re-suspended in a minimal amount of fresh fixative to obtain a homogeneous suspension. The cell suspension was dropped onto microscope slides and left to air-dry. Whole chromosome labeled probes for chromosomes 1, 4 and 18 were added to the slides for specific hybridization process (Metasystm Probe, Germany). 10 µl of probe mixture was applied to the slide and covered with a cover slip 22x22 mm^2 and sealed with rubber cement. The slides were heated at hot plate at 75^{0}C for 2 minutes to allow co-denaturation process of template DNA with labeled probes of single stranded DNA. Following this, the slides were incubated in humidifier chamber overnight at 37 ^{0}C for probes hybridization with targeted region of chromosomes indicated by emission of fluorescence. Post-hybridization washing step was applied to the slides by firstly removing the cover slips and all traces of glue carefully followed by washing the slide with 0.4x of SCC (pH 7.0) at 72^{0}C for 2 minutes. The slides were then drained and washed in 2x SSC, 0.05% Tween-20 (pH 7.0) at room temperature for 30 seconds. Following this, the slides were rinsed briefly in distilled water to avoid crystal formation and subjected to air drying. All slides were counter stained with DAPI/anti-fade of 10 µl and covered by 24x32 mm^2 cover slip for 10 minutes to allow the anti-fade to penetrate chromosomes followed by analysis using fluorescent microscope. Metaphase analysis was conducted by a well trained and experienced cytogeneticist. Two hundred metaphase cells per subjects (100 metaphases from each parallel culture) were counted and analyzed for chromosomal aberrations (CA). Structural CAs were classified based on the number of sister chromatids and breakage events involved. Total numbers and types of aberrations, as well as the percentage of aberrant cells per subject were evaluated.

Statistical Analysis

Statistical analyses were carried out using microstat software (StatSoft, Tulsa, USA). Each subject was characterized for the extent of DNA damage by considering the mean (±standard deviation of the mean), median, range and dispersion coefficient (H) for the comet tail moment. It was calculated as the ratio of the sample variance to the sample mean in order to determine the effect of exposure on the distribution of comet tail moment within each subject [14]. Multiple comparisons between groups were done by means of multifactor ANOVA on transformed data in order to normalize distribution and to equalize the variances. Post analysis of differences was done by Scheffe´ test. The level of statistical significance was set at $p \geq 0.05$. The correlations between confounding factors and the studied parameters were also determined using Pearson's correlation matrices

3. Results

Alkaline Comet Assay

Characteristics of the study subjects (gender, occupation, and mean frequency of the comet) and groups mean values are reported in Table (1). Also, the comparison of the groups mean values was carried out between the exposed radiology department staff, intern students and second year students (control group). Mean values (± standard deviation of the mean) of comet tail moment were 7.44±2.35 for the staff worker group and 3.51±2.1 for the intern students group and 3.01±1.33 for second year students (control group). The difference in mean tail moments were statistically significant between the staff group and second year students group (P<0.01, ANOVA) and also significant between staff group and intern students (P<0.01, ANOVA) while there is no significant difference between intern and second year student groups (P>0.05, ANOVA). The range of tail moment was 5.21- 12.53 in exposed worker staff and 2.99-5.31 for the intern students and 2.00-4.37. for the control second year students.

To determine the effect of X ray radiation on the distribution of the tail moment within each tested individual the dispersion coefficient (H) for the row data was also calculated. H was defined as the ratio of sample variance to the sample mean. The mean value of (H) was 4.7 in the staff, 0.71 in the intern students and 0.21 in the second year control students. This indicates that the increase in DNA damage was due to the increase in the percentage of damaged cells with a high extent of damage. According to the study results, the distribution of tail moment among the staffs was highly adverse with shift towards higher values. On the other hand, the distribution of tail moment among intern and second year students were

homogenous with majority of tail moments showing base line DNA damage level.

These results also indicate that occupation and occupation periods significantly contributed to the level of primary DNA damage as recorded by the mean of alkaline comet assay. However, there was a significant difference in the level of DNA damage represented by tail moment between the three groups as shown in table 1. The DNA damage decreased gradually among

The three groups depending on the level of exposure hospital where radiology staff showed higher level of DNA damage as compared to other two groups. On the other hand, the level of DNA damage between intern and second year students showed no significant difference. Furthermore, the gender in this study showed no effect on the DNA damage when exposed to x ray radiation as there was no significant difference between male and female subjects (P>0.05 ANOVA).

The distribution of tail moment (tail length µm x amount of DNA in the tail) measured in peripheral blood leukocytes of exposed staff, intern radiology students and second year students with regards to their occupations is illustrated in Figure 1.

Chromosomal Aberration (CA)

The mean frequencies of CA is summarized in Table (2) as recorded for radiology staff, intern and second year students from the faculty of applied medical science.

Table 1: Demonstrates the characteristics of the study population including the median, minimum and maximum of DNA breakage using the comet techniques.

			Comet Tail Moment				
Subject	Gender	# of Subject	Mean Frequency (M±SD)	Median	Min	Max	H
Radiology staffs group (A	Male	10	7.11 ± 1.73	6.71	4.66	14.50	6.33
	Female	5	7.45 ± 2.61	6.8	6.17	11.27	3.07
	(Total) Mean	15	7.44 ± 2.35	6.72	5.21	12.53	4.7
Group (B	Male	8	3.50 ± 2.6	2.90	3.11	5.5	1.25
	Female	7	3.41 ± 2.0	3.84	1.73	4.75	0.17
	(Total) Mean	15	3.51 ± 2.1	3.25	2.99	5.13	0.71
Group (C)	Male	5	3.26 ± 0.90	3.05	1.69	4.11	0.22
	Female	5	2.75 ± 1.51	2.75	2.17	5.21	0.20
	(Total) Mean	10	3.01 ± 1.33	2.96	2.0	4.37	0.21

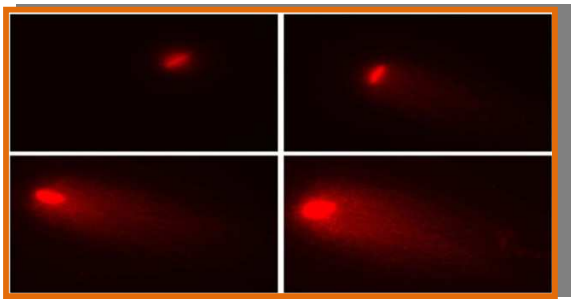

Figure 1: Demonstration of the comet assay results showing the normal control at the upper and the lower right sides. The left side image represents the DNA damage for exposed radiology staff.

Table 2: Demonstrates the results of the three tested groups exploring the mean of chromosomal aberrations and its percentage among males and females (Mean±SD).

Subject	Gender	Mean Frequency	Chromatids	Chromosome	Acentric	Dicentric	% of aberration
Radiology staffs group (A)	Male	3.35 ± 0.69	2.24 ± 0.43	0.28 ± 0.10	0.85 ± 0.18	0.005	1.50
	Female	2.90 ± 0.29	2.1 ± 0.27	0.22 ± 0.02	0.60 ± 0.12		1.42
	Mean	3.14 ± 0.45	2.17 ± 0.35	0.25 ± 0.05	0.75 ± 0.15	0.005	1.45
Group (B)	Male	1.43 ± 0.10	0.75 ± 0.11	0.08 ± 0.02	0.57 ± 0.12		0.44
	Female	1.18 ± 0.07	0.91 ± 0.10	0.03 ± 0.01	0.34 ± 0.09		0.29
	Mean	1.25 ± 015	0.83 ± 0.10	0.05 ± 0.02	0.42 ± 0.08		0.35
Group (C)	Male	1.36 ± 0.05	0.75 ± 0.77	0.08 ± 0.02	0.53 ± 0.05		0.33
	Female	0.95 ± 0.03	0.75 ± 0.02	0.03 ± 0.02	0.19 ± 0.08		0.41
	Mean	1.10 ± 0.42	0.75 ± 0.05	0.05 ± 0.02	0.32 ± 0.06		0.35.

There was a significant differences between the mean frequencies of CA between staff (3.41± 0.45 CA per 200 cells), intern students (1.25± 0.15) CA per 200 cells) and second year students (1.10 ± 0.42) (P<0.001, ANOVA). The percentage of aberrant cells was also significantly higher in the staff (1.45±0.08) as compared to the other two groups where the percentage was (0.35±0.04). Among the exposed group, a remarkable inter individual variation in aberration types were observed. The control and intern students on the other hand, have a more homogenous distribution of CA in their peripheral blood. The mean frequencies of chromatids breakage was 2.17±0.35 per 200 cells among staff, while the mean frequencies was 0.83 ±0.10 and 0.75± 0.05 per 200 cells among the intern and second year students respectively. The mean frequencies of chromosome breaks were 0.25±0.05 per 200 cells in radiology staff and 0.05 ±0.02 in both intern and second year students. The mean yield of acentric fragments was 0.75±0.15 in the exposed workers and 0.42±0.08 in the intern student subjects and 0.32± 0.0.06 in the control group of second year students. Dicentric chromosome with two centromeres representing an abnormal structural change was only found in one technician from staff group while the other two groups have shown no dicentric chromosomes at all.

The results also implied that the observed chromosomal aberrations have no significant interaction between the aberration type, gender, and age. Also, it has been noticed that there is no significant difference between the two students groups and some of the results showed similar finding in both groups. Ring chromosome was only observed in one case involving chromosome one from the hospital worker while other chromosomes (4 and 18) showed no changes in all studied subjects following FISH technique using whole chromosome painting method as shown in Figure 2 (B).

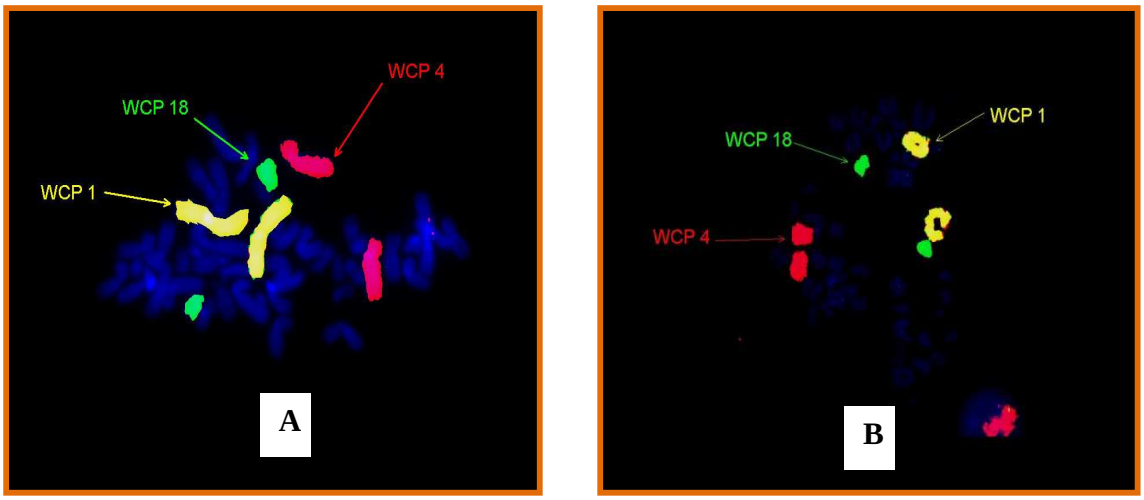

Figure 2: Whole chromosomal painting results of metaphase stage using FISH technique. Image (A) represents different fluorescents of specific chromosomes showing red, green and yellow signals (paints) in chromosomes 4, 18 and 1 respectively of normal control sample. Image (B) represents the chromosome structural change reflected by the ring chromosome on one copy as indicated by yellow arrow in image B.

4. Discussion

In this study, two different methods were used to evaluate the effect of ionizing radiation (x ray) on the students from faculty of applied medical sciences, radiology department and the technician workers at KAU hospital with different periods of exposure to the radiation. The comet and FISH assays were applied to detect unrepaired and erroneously repaired chromosomal aberrations from metaphase stage of dividing cells. Despite of some limitations with respect to FISH technique such as time of analysis, possible failure of culture, efficiency of hybridization and the resolution of chromosomal painting, the results showed possible acquired chromosomal changes resulting from chronic low dose exposure of X ray. Most common aberrations observed for all individuals were gaps, breaks, minutes, and fragments, but the frequencies of chromatid-type aberrations were higher in most of the exposed individuals, compared to the respective matched control values. Achromatic lesions or gaps were also considered to calculate the CA frequencies, since they represent discontinuity regions in the chromosomal arm [16], and they have been found to be greatly induced by ionizing radiation [17]. Most radiation-exposed individuals

presented higher CA frequencies than their matched controls, with the mean values of 3.14 and 1.10 aberrations per 200 cells for the exposed and the control groups respectively. Although the difference between those values was shown to be significant ($P < 0.01$ ANOVA), the individual CA frequencies were not correlated to the absorbed doses. Most cells showed only one aberration, and the absence of centric rings and dicentric chromosomes (which are mostly induced by high radiation doses). In fact, at this level of exposure, a very high number of scored metaphases are required in order to detect the presence of dicentric chromosomes. In the present work, the exposed group presented higher absorbed doses, which were within the range of 9.5 to 209 Gray.4

However, fluorescence detection of DNA has been used in this study that has removed the necessity of applying radiolabel substance in dividing cell as a mean of DNA damage measurement [18-21]. The alkaline comet assay has been used as bio monitoring method in multiple studies *in vitro* and *in vivo* of different cell lines as well as to the exposed population to different sources of radiation. Furthermore, occupationally exposed radiation workers, for example, exposure to radon, as well as children of Chernobyl revealed a positive correlation between DNA damage and exposure level [22]. The results of this study may imply the presence of DNA damage primarily in peripheral blood lymphocytes of hospital exposed workers in addition to the increase of chromosomal aberration frequency. Moreover, the two techniques that have been applied in this study appear to be very sensitive methods of DNA damage detection and increases therefore the need to apply such techniques in routine clinical setting as an assessment of possible biological effect particularity in chronically exposed high risk group taking into account the cost, the time of analysis, the flexibility and possible challenges of data interpretation.

It has been estimated that each dividing mammalian cell is subjected to different forms of DNA damages per day including base alterations and strand breakage during DNA replication process of mitotic cells [23-24]. However, unlike single strand breaks and base damages that can be repaired by corresponding repair mechanisms by most cells, DNA double-strand breaks and other complex DNA lesions are relatively rare and much more difficult to repair [25]. Un-rejoined double-strand breaks are likely to be lethal, and mis-rejoined double-strand breaks can cause chromosome aberrations and cell death [25-27]. In the 1980s, interest moved from detection of single strand breaks to the development of methods that could detect the DNA lesion associated with lethality and chromosome damage by ionizing radiation.

Although it is fortunate that double-strand breaks are produced relatively infrequently, this poses problems for their detection. Twenty to forty times fewer of double-strand breaks as compared to single-strand breaks are produced per Gy [28]. The comet assay allows detecting un-repaired initial lesions in cells. Many of these primary abrasions are successfully repaired within few minutes (4–15 minutes) [29, 30] to couple of hours (2–3 hours) [31] after exposure. Therefore, the levels of primary DNA damage in study subjects in the present study could be associated to an enhanced intracellular oxidative stress following exposure to ionizing radiation. This could give an increased steady-state DNA damage, high enough to be detected by the sensitive comet assay. If base damages are located closely together (10 bp apart) on opposite DNA strands, simultaneous excision of such modified bases can lead to the formation of DSB (double strand break), which is the supposed initial lesion in the formation of CA. Chromosome and chromatid breaks arise when DSB have been un-repaired or repaired incompletely. Double fragments can also result from repair of DSB, giving rise to polycentric chromosomes or centric ring chromosomes [32] that may be visualized on metaphase preparations. In the student group population, a high intra-individual homogeneity of DNA damage was recorded and dispersion coefficients were similar and low. However, in spite of very rigorous procedures (exactly the same conditions for all steps of the procedures and a very good reproducibility of the employed assays), the inter-individual variability of data obtained in the exposed population was considerable. Variability is a typical feature of biological systems, extensively reported by various authors when using the comet assay [20, 33-36]. It should also be pointed out that results obtained in the present comet assay study reflect DNA damage in all types of leukocytes, while the results of the CA test reflect only the response of mitogen-stimulated lymphocytes. Because peripheral blood leukocytes are a heterogeneous mixture of cells as regarding to their life-span and sensitivity, some differences may be due to the different cell populations being compared. Despite the risk of reduced sensitivity, most investigators prefer the use of whole leukocyte fractions or whole blood when studying induced or basal levels of DNA damage in the comet assay. During the process of separating the various cell types from each other it is always

possible to damage the cells, this in many cases will be hard to control [34].

Currently, the recommended radio dosage limit is 50 mSv [37]. However, with current regulation in place, it is occasional that a radiation worker exceeds this dose limit. It should be emphasized that doses recorded among the exposed subjects in this study were also below this dose. In spite of the relatively low doses that were received, the exposed worker population had significantly increased levels of primary DNA damage compared to control student populations. Other authors also discussed the difficulty of establishing relationships between radiation dose and their effect for low doses limits [38, 19]. This observation could be, at least in some cases, related to the ‘‘adaptive response’’. Chronic low-level radiation from various isotopes is known to induce the adaptive response, i.e., exposed cells become less sensitive to the chromosome breaking effects of subsequently delivered challenging X-ray doses. The magnitude of adaptive response varies among blood samples from different donors; this was observed in resting human leukocytes [22, 39]. It is possible that a similar phenomenon was also pronounced in occupationally exposed subjects.

Conclusion and Recommendations

In conclusion, this study implies the possibility of genomic structural changes of exposed workers to ionizing radiation of low doses. Therefore, carefully applying the radiation protection precautions will minimize greatly, the potential adverse effects. Furthermore, the significance of utilizing radiation dose monitoring devices is apparent which in turn provide useful information on the actual risk of radiation exposed individuals. With aspect to methods of measuring radiation effect on biological parameter, the alkaline comet assay and FISH test are both sensitive techniques that can be utilized in combination with dosimeter monitoring devices as a clinical routing surveillance of exposed workers. Also, it should be emphasized on the usefulness of comet assay in increasing awareness towards the behavior of individual cells exposed to ionized radiation as the results of his technique were consistent to those obtained by FISH assay. Also it should be focused on the simplicity and low cost of the comet assay technique when compared to the other cytogenetic techniques. So the study strongly recommends using the comet assay as a primary method for detection the effect of ionizing radiation on individual cells and also for detection of the intra-individual variation in respond to the X ray radiation. However, conducting further studies is recommended in order to aid in further exploration of the benefits of routine dose measurement combined with assessment of biological parameter as mode of decreasing the risk of biological damage.

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