



Assessment of DNA damage in peripheral blood lymphocytes of workers occupationally exposed to low levels of ionizing radiation in Al-Amal hospital for cancer in Baghdad.

Amel J. Muttar¹ , Wiaam A. Al-Amli² , Abulsahib K. Ali¹ , Shatha K.K¹ , Yasser A. H.¹ , Adul H. A.¹ , Naeema , H. Z¹

¹ Ministry of Science and Technology, Central Labs. Directorate, Baghdad, Iraq.

² Institute of Genetic Engineering and Biotechnology for post gradual study, University of Baghdad, Iraq.

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Abstract: The present study aims to use the cytogenetic analysis as a biomarkers of professionals occupationally exposed to ionizing radiation in Al-amal National Hospital for cancer Management in Baghdad –Iraq. This study was carried out on Thirty Iraqi radiation workers exposed to a low dose of ionizing radiation, included twelve females and eighteen males between the ages of (22-57) years, as well as Twenty apparently healthy individuals collected randomly from population living Baghdad , aged (19 - 55) years which are non-smokers non- alcoholic as control group . The cytogenetic analysis including, chromosomal aberrations in lymphocytes, as fragment, ring and dicentric chromosomes also chromatid aberration , Micronuclei MN frequency, nuclear division index and mitotic index were performed on peripheral blood lymphocytes for workers and control group. The present study showed significant increase ($p < 0.05$) in the chromosomal aberration for the worker as compared with the control group. Also there were found significant increase ($p < 0.05$) in Micronuclei MN frequency in nucleated lymphocytes and nuclear division index for the worker as compared with the control group. While it was no significant differences in nuclear division index between worker and control group. Also there were found a significant increase ($p < 0.05$) in comet tail length and tail moment values in the human lymphocyte in these radiation worker of studies as compared with the control group. The present study shows that the increase of chromosomal aberration and Micronuclei frequency, in hospital workers due to exposed to a low dose of ionizing radiation than in the controls. In conclusion, the results indicated that there is a possibility of using the changes in the chromosome aberration and micronuclei in human lymphocytes as a useful as biodosimetric markers for the detection of human exposure to ionizing radiation. Also, the results obtained confirmed usefulness of the alkaline comet assay as a sensitive additional biomarker in the regular health screening of workers occupationally exposed to low doses of ionizing radiation. The current results of unstable chromosome aberration within of normal values according of the technical report of International Atomic Energy Agency (IAEA) No. 405, 2001.

Key words: Ionizing radiation, Lymphocytes, CA, MN, Comet assay, Biological Dosimetry.

Corresponding author: should be addressed (Email: ameljm2006@yahoo.com)

Introduction:

Over the years, ionizing radiation (IR) has become a universal diagnostic and therapeutic tool, making the largest

man-made contribution to the population dose(1). Thus, medical personnel represent the group most consistently exposed to low doses of IR. High doses of IR are known to produce

deleterious consequences in humans, including, but not exclusively, cancer induction; however, the effect of such radiation at lower doses, as in occupational work settings, is less clear (2) and needs a comprehensive elucidation. The levels of exposure to ionizing radiation in hospitals have decreased in recent decades and are now far below the regulatory limit of 20 mSv/year, indeed below the detection limit of dosimeters. Some medical uses of radiation, such as nuclear medicine and interventional procedures, may expose the personnel to higher doses, and these are subjects of concern(3). With development of techniques in cytogenetic, the response of human chromosomes to ionizing radiations both, *in vivo* and *in vitro* has been investigated thoroughly. *In vitro* studies on human lymphocytes exposed to different type of radiations have shown that, the frequency of induced aberrations are same as if they were exposed *in vivo* (4,5) *In vivo* cytogenetic studies of accidental exposure of human to radiations have been carried out and are confined to groups like people exposed to Chernobyl explosion (6,7), radiation accident in Goiania-Brazil (8), assess chromosomal damage in Tunisian hospital workers occupationally exposed to low levels of IR (9), also assessment of DNA damage in peripheral blood lymphocytes of radiation workers at Al-Tuwaita Site(10).

Cytogenetic studies on individuals occupationally exposed to radiation have been carried out extensively. High frequency of chromosomal aberration (CA) such as dicentrics, rings and acentrics in the peripheral blood lymphocytes of personnel handling

diagnostic X-ray machines has been reported by Kasuba *et al.*, 1997 and Gadhia *et al.*, 2004 (11,12). However, many cytogenetic studies have been conducted among hospital workers exposed to IR. (13). Observed increased rates of chromosomal aberrations in hospital workers exposed to IR. A low, but statistically significant, increase in the rate of chromosomal aberrations in circulating lymphocytes among hospital workers with thyroid nodules was also observed in workers occupationally exposed to radiation (14). A cytological consequence of induction of chromosome aberrations is the formation of micronuclei (MNs) 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 cytokinesis-blocked micronucleus (CBMN) 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 or environmentally exposed to IR (15). The micronucleus-centromere assay combines the MN assay with fluorescence *in situ* hybridization (FISH). It uses a pan-centromeric probe to detect micronuclei (MNs) derived from acentric chromosome fragments or whole chromosomes (16, 17). Due to its predominantly clastogenic action, IR exposure is expected to induce micronuclei than positively labeled micronuclei. There is now some

evidence that centromere identification in MNs can improve the detection of *in vivo* effects of clastogenic exposures in humans (18). The nuclear division index (NDI) is a marker of cell proliferation in cultures which is considered a measure of general cytotoxicity, the relative frequencies of the cell,s 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 (19, 20).

Single-cell gel electrophoresis (SCGE) provides a rapid, visual method for assessing DNA breakage quantitatively in single cells. DNA damage is visualized at the individual cell level as an increased migration of genetic material (“comet tail”) from the nucleus (“comet head”). It requires viable cells, but not growth, and can be applied to terminally differentiated cells(21). They have a head and a tail, with the head being the stained remains of the lymphocyte nucleus, and the tail being fractionated DNA which has trailed out along the gel when electrophoresis is conducted (22). The comet assay very sensitive and requires a researcher who is skilled at interpreting the results. This test is widely conducted when searching for genetic damage (23). During the last years, the single cell gel electrophoresis (SCGE) or comet assay was introduced as a useful technique for human biomonitoring studies (24-28). The objective of the present study was to

assess the role of chromosomal aberrations, cytokinesis-blocked micronucleus assay and DNA gel electrophoresis (comet) assay as biomarkers of radiation injury in individuals occupationally exposed to ionizing radiation.

Materials and Methods:

The study of professionals occupationally exposed to ionizing radiation comprised 30 individuals (18 males and 12 females), age range (22-57) years, 18 were non-smokers and 12 ionizing radiation in Al-Amal National Hospital for cancer management in Baghdad –Iraq. Cytogenetic findings of the whole group were compared to those obtained in an age-matched control group of 20 healthy blood donors without radiation history. The age of the control group was range 19–55 years. No subject had a personal medical history of cancer, genetic disease, chronic inflammatory disease, or recent infectious phase. None of them was taking known mutagenic drugs.

Assay procedure:

The chromosome aberration (dicentric) and CBMN assay were performed according to the description by IAEA , 2001 (29).The DNA gel electrophoresis (comet) assay was performed according to the description by Kassie *et al.* , 2000 (22).

Blood sampling and lymphocyte cultures:

Samples of 4 ml whole blood were obtained using heparinized vacutainer tubes. The cultures were set up by

adding 0.5 ml of heparinized blood to 4.5 ml RPMI 1640 medium (sigma), supplemented with 20% fetal calf serum (sigma), and 0.1 mg/ml streptomycin (Sigma-Aldrich). Phytohemagglutinin (PHA) at a concentration of 10 μ l / ml was used to stimulate lymphocyte proliferation. Blood cultures were incubated at 37°C for 56 and 72 hours for CA and MN respectively. Cytochalasin B (Sigma) was added 44 h after PHA stimulation at a concentration of 4.5 μ g /ml to block cells at cytokinesis. Colchicine was added to a final concentration of 0.5 μ g /ml before 2 h the end of the CA incubation.

Treatment with hypotonic solution and fixation:

After 56 h of incubation, the CA cultures were harvested by centrifugation, suspended in hypotonic solution (0.075 mol/L KCl), incubated for 20 minutes at 37°C and fixed in three changes of methanol: acetic acid (3:1). Cell suspensions were dropped on wet, cold slides and dried. Then the slides were routinely stained with Giemsa (29). After a 72h incubation period, the MN cells were collected by centrifugation (1000 rpm, for 5 min), hypotonically treated with cold 0.01M 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 washed with four further exchanges of fixative solution, After fixation, the cells were gently resuspended, dropped onto wet clean glass slides and allowed to dry. Slides were stained in 4% Giemsa solution for 8 min .After fixation, the cells were gently resuspended, dropped onto wet clean glass slides and allowed to dry.

Slides were stained in 4% Giemsa solution for 20 min.

Microscopic examination:

The coded slides were scored and analyzed at amagnification of 1,000 X. A total of 200 cells / individual were screened in the control group and in the exposed group, and different types of aberrations were classified. Stained samples for MN were evaluated using microscope; a total of 500 binucleated cells was evaluated for the frequency of MN using 400 x magnification for surveying the slides while 1000 \times magnification was used to confirm the presence or absence of MN in the cells. A total of 1000 living interphasic cells were used for assessment of mono-, bi-, and poly-nucleated cells and calculation of NDI by using the formula (29):

$$NDI = (M1 + 2 \times M2 + 3 \times M3 + 4 \times M4) / N.$$
Where M1 to M4 represent the number of cells with one to four nuclei and N is the total number of viable cells scored. The mitotic indexes were calculated (MI = number of cells in division/number of analyzed cells) for both groups.

To quantify the DNA damage, the following comet parameters were evaluated: tail length (TL), and tail moment (TM). Tail length (length of DNA migration) is related directly to the DNA fragment size and presented in micrometers.

Data Analysis and Statistics:

The data of this study were compiled into the computerized data file and frequency, distribution and statistical description (Mean, SE) were divided using SPSS statistical software. We used statistical analysis of variance (ANOVA) test and least significantly

difference (LDS) test by probability of less than 0.05 ($p < 0.05$) according to (30).

Result and Discussion:

The chromosomal aberration, micronuclei and comet assay was performed in 30 workers exposed to a low dose of ionizing radiation and in 20 matched controls. Chromosomal aberrations scored in metaphase chromosomes were identified by Giemsa staining (Figure 1). The data on frequencies of chromosomal aberrations in lymphocytes among radiation workers and control groups are shown in Table 1. Results revealed a significant increase in fragment ($P < 0.05$) and chromatid type aberration gaps and break ($p < 0.05$) among the chromosomes of the radiation workers when compared to controls. The increase frequencies of chromosomal aberration in radiation workers indicate the cumulative effect of low-level chronic exposure to ionizing radiation

(11, 12). There are evidences of association between occupational exposure, cytogenetic alterations and the increase in cancer rates (31). It is known that the probability of carcinogenesis is greater in populations exposed to radiation, since ionizing radiation can raise the frequency of CA and spontaneous mutations.

Cytogenetic monitoring of hospital workers exposed to low doses of ionizing radiation has been carried out by means of analyzing the frequencies of chromosomal aberration (5) or centromere-positive micronuclei (17), comparing the results with those of a control group. A biologically significant result was obtained a greater frequency of acentric chromosomes in workers occupationally exposed to radiation for radiological diagnoses, when compared with control individuals (1). The present observations support the findings of other investigators and agree with many cytogenetic studies carried out in workers exposed to chronic low doses of ionizing radiation (11,13,32).

Table (1): Chromosomal aberrations (Mean \pm SD) among radiation workers in Al-Amal National Hospital for cancer Management in Baghdad and control groups

Study groups	No. of samples	No. of metaphases score	Chromosomes aberration			Chromatid type aberration		Total chromosomal aberration
			Fragment chromosome	Ring chromosome	Dicentric chromosome	Break	Gap	
Radiation workers	30	6000	0.0189 * \pm 0.007	0.00066* \pm 0.0022	0.0096* \pm 0.007	0.0150* \pm 0.002	0.012* \pm 0.003	0.0578* \pm 0.019
Control	20	4000	0.0077 \pm 0.002	0.000 \pm 0.00	0.0008 \pm 0.002	0.005 \pm 0.002	0.005 \pm 0.002	0.018 \pm 0.004

*Significant at ($P < 0.05$) as compared to control.

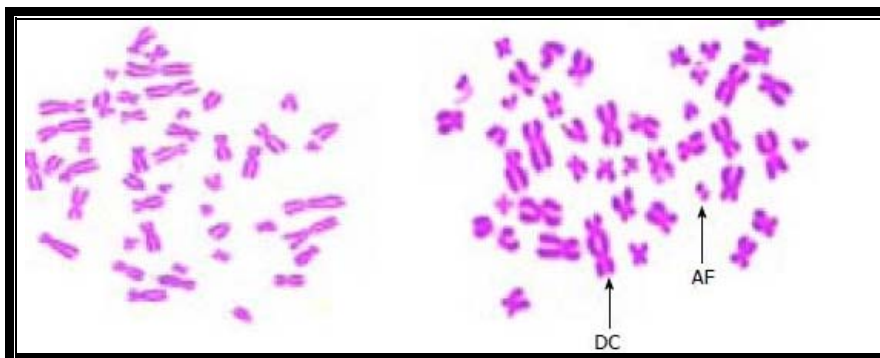


Figure (1): Chromosome aberrations type dicentric and fragment in metaphase cell(1000X).AF: Fragment , DC: Dicentric).

The frequencies of MN frequency, nuclear division index and mitotic index were performed on peripheral blood lymphocytes which were obtained from 30 individuals of workers exposed to a low dose of ionizing radiation, then compared with 20 individuals control living in Baghdad. Micronuclei (MN) are formed from lagging chromosomal fragments or whole chromosomes at anaphase which are not included in the nuclei of daughter cells (Fig. 2). They are therefore seen as distinctly separate small spherical objects that have the same morphology and staining properties of nuclei, within the cytoplasm of the daughter cells (33).

The results of the frequency of MN measured in binucleated lymphocyte

cell in radiation workers and control group are summarized in Table 2. The MN frequency was significantly higher ($p < 0.05$) in the radiation workers (0.0285 ± 0.0034 MN/cell.), compared to the controls (0.0074 ± 0.0013 MN/cell.), as shown in Figure 2 and table 2. Results on the MN frequency, NDI and MI study have been compiled in Table 2. However MN frequency was statistically significant ($P < 0.01$) among radiation workers as compared to control individuals. No significant change in either mean mitotic index of radiation workers as well as controls was noticed (Table 2). To assess the origin of the observed MNs induced as a result of an aneugenic or a clastogenic effect of a low dose of IR.

Table (2): frequency of MN in peripheral lymphocytes among radiation workers in Al-Amal National Hospital for cancer Management in Baghdad –Iraq and control group

Study groups	No. of samples	Micronuclei frequency			Nuclear Division Index (Mean \pm SD)	Mitotic index (1,000 cells/individual) (Mean \pm SD)
		No. of BN cells	Total of MN	MN/cells (Mean \pm SD)		
Radiation workers	30	15000	428	$0.0285^* \pm 0.0034$	$1.150^* \pm 0.007$	2.78 ± 0.23
Control	20	10000	74	0.0074 ± 0.0013	1.312 ± 0.0114	3.10 ± 0.21

*Significant at ($P < 0.05$) as compared to control.

Micronuclei in mitotically active cells arise from structural chromosomal aberrations or disturbed function of mitotic spindle. That is why some authors consider that the follow up of MN frequencies in peripheral blood lymphocytes in the samples of human individuals could be a very effective test

to estimate the effects of biological, physical and chemical agents (34). According to our results, the MN background level is 8- 35 which is in agreement with IAEA manual reporting the background MN values to range 2 to 40 per 1000 BN cells (25) and other studies (12, 17, 35, 36).

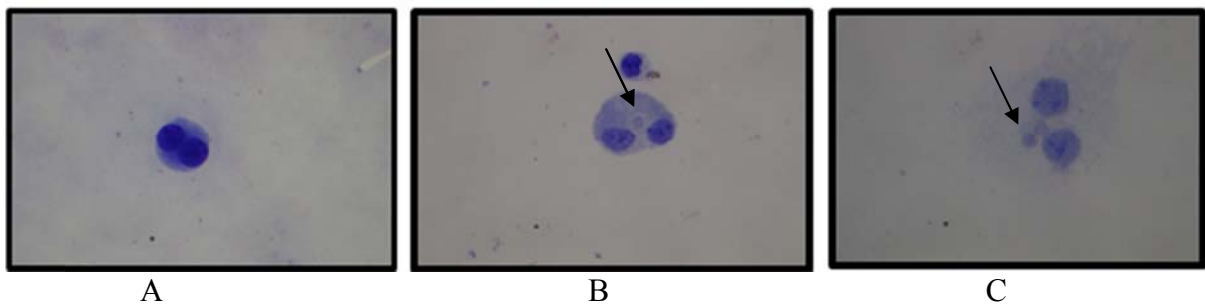


Figure (2): Binucleated lymphocyte (A) without micronuclei (B) cell with one micronuclei (C) cell with two micronuclei (400X).

These results suggest that the frequency of MN can be used as a potential biomarker for assessing a specific environmental risk. Furthermore, studies of mutations at MN frequency have provided insights into several aspects of somatic mutations *in vivo*, including molecular mechanisms of mutagenesis, the relationship between DNA damage and mutation, as well as individual susceptibility factors such as DNA repair capacity (15, 37).

The NDI assay was performed according to the description by (35), the NDI was calculated binucleated, trinucleated and quadrinucleated

lymphocyte cell per 1000 lymphocytes (Fig.3). The average of NDI (Mean \pm SD) for exposed groups were 1.150 ± 0.0071 when compared with the control 1.312 ± 0.0114 .

A significant decrease ($P > 0.05$) in NDI was observed in the exposed group, compared to the controls (Table 2). The nuclear division index as biomarker 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 (19,20).

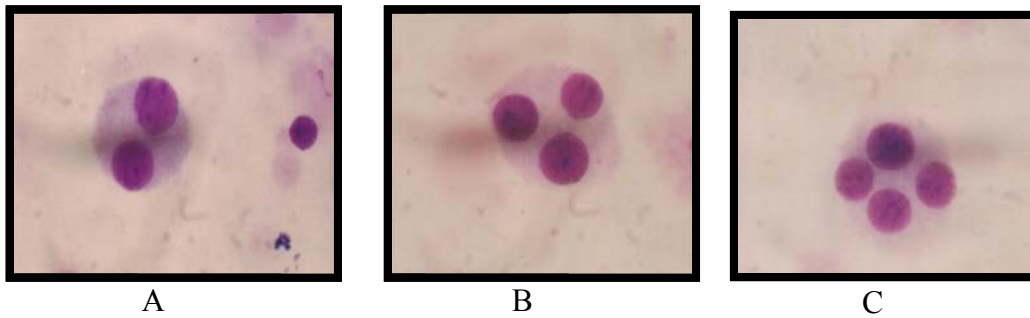


Figure (3): Cytokinesis blocked human lymphocyte cell ,(A) : Binucleated lymphocyte cell, (B): Trinucleated lymphocyte cell and (C): Quadrinucleated lymphocyte cell (1000X).

The analysis of mitotic index (MI) did not indicate significant differences ($p < 0.05$) between the group of individuals exposed to ionizing radiation and the control group (table 2). It is normally measured and it can detect the increase or decrease in cell proliferation rate (38). In the present study, the mitotic index of the control group was not statistically different from that of the exposed group, indicating that there were no cytotoxic effects from ionizing radiation on the lymphocytes from the studied dentists.

Therefore, this type of study may become an indicator of the need for greater control and protection against the harmful effects of radiation over occupationally exposed professionals (39). The results of the present study revealed that in this group of professionals, whose work environments were not regularly inspected, dentists exposed to several years of ionizing radiation presented MI levels similar to exposed and control individuals, which indicates the

adoption of safety measures during occupational exposure.

The alkaline comet assay was selected as a biomarker of exposure to evaluate the ongoing exposure to ionizing radiation of 30 radiation workers occupationally exposed to ionizing radiation and 20 controls. The results of the alkaline comet assay are summarized in table 3, which gives the mean values of tail length and tail moment for the radiation workers and control groups (Figure 4). The average of comet tail lengths (Mean \pm SE) in radiation workers were $18.10 \pm 0.30 \mu\text{m}$, when compared with the control were $14.23 \pm 0.15 \mu\text{m}$. According to the results obtained, the radiation workers were highly significant ($P < 0.05$) compared with control groups (Table 3). The average of comet tail moment (Mean \pm SE) in radiation workers were 14.77 ± 0.24 , when compared with the control groups were 11.44 ± 0.20 . The observed values differed significantly ($P < 0.05$) from the tail moment values measured in control (Table 3).

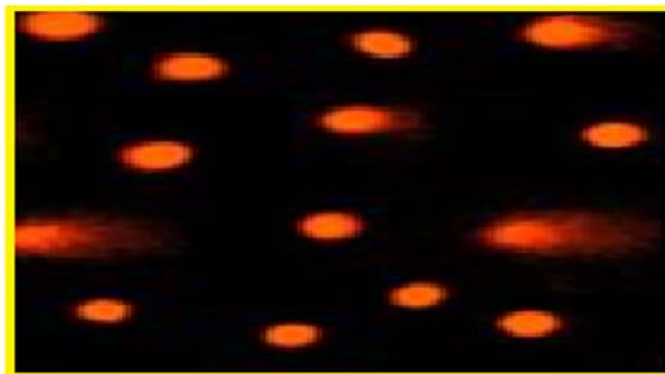


Figure (4): Microphotographs of comet in human blood cell by fluorescent microscope (400X).

Table (3): The individual results of the alkaline comet assay on peripheral blood leukocytes of radiation workers at Al-Tuwaitha site and control group as mean values of the measurements of 100 comets per subject.

Studies groups	No. of Samples	Comet parameters evaluated (Mean \pm SE)	
		Tail length (μm)	Tail moment
Radiation workers	30	18.10 \pm 0.30*	14.77 \pm 0.24*
Control	20	14.23 \pm 0.15	11.44 \pm 0.19

*Significant at ($P < 0.05$) as compared to control.

Lesions induced by ionizing radiation in DNA can be detected by the alkaline single cell gel electrophoresis or Comet assay (20, 40). The same method was evaluated in the present study on occupationally exposed radiation personnel. The comet assay is an easy, quick and accurate test that has been widely applied to measure both *in vitro* DNA damage and repair following exposure to various genotoxic agents and for human biomonitoring (21, 41, 42).

Conclusion:

The present study shows that chromosomal damage leading to the formation of micronucleated lymphocytes is more frequent in hospital workers exposed to a low dose of ionizing radiation than in the

controls. This increase can be attributed to preferential clastogenic mechanisms. Therefore, other studies involving an increased number of individuals are necessary in order to obtain more reliable conclusions about the cytogenetic impact of the chronic exposure of dentists to low levels of ionizing radiation. In conclusion, the results indicated that there is a possibility of using the changes in the chromosome aberration and micronuclei in human lymphocytes as biosimetric markers for the detection of human exposure to ionizing radiation. Also, the results obtained confirmed usefulness of the alkaline comet assay as a sensitive additional biomarker in the regular health screening of workers occupationally exposed to low doses of ionizing radiation. The current results of unstable

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