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Study the Effect of Ionizing Radiation on a Sample of Workers Using Three Genetic End-Points

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Abstract: The present study aims to use the molecular biological techniques in a genotoxicity investigation of low radiation doses in samples of workers in Al-Tuwaitha site, this study including 30 male blood samples, aged (32 - 59 year), as well as 20 male blood samples, aged (30 -57 year) which are non- smokers and non-alcoholics as control group . Three genetic parameter were studied by using hypoxanthine guanine phosphoribosyl transferase (*HPRT*) mutation assay, micronuclei in cytoplasm of reticulocytes cells and comet assay .The statistical analysis showed that there was significant difference ($p < 0.01$) the *HPRT* gene mutation assay between the radiation workers and controls group. The present study showed significant increasde ($p<0.01$) in micronuclei of reticulocytes cells for the radiation worker as compared with the control group. Also there were found a significant increase ($p<0.01$) in comet tail length and tail moment values in the human lymphocyte in these radiation worker of this study as compared with the control group. In conclusion, the obtained results confirmed the usefulness of the mutation frequency for *HPRT* assay, micronuclei in cytoplasm of reticulocytes and alkaline comet assay and as a sensitive additional biomarker in the regular health screening of workers occupationally exposed to low doses of ionizing radiation.

Keywords: Ionizing radiation , *HPRT* gene , MN in reticulocytes , Comet assay .

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Introduction:

Exposure to ionizing radiation causes damage to living cells, especially to DNA, the degree of cellular damage depends on the amount of radiation administered. Humans are naturally exposed to ionizing radiation from cosmic rays, and artificially through diagnostic procedures, medical treatments or occupationally during work shifts. It is well known that ionizing radiation (IR) produces DNA damage through different mechanisms: by loss of bases, single-strand breaks, double strand breaks, and damage to purine and pyrimidine bases. This early damage may lead to chromosomal aberrations and thus to increased risk of mutagenesis and carcinogenesis (1). Hypoxanthine guanine phosphoribosyl transferase (*HPRT*) is a purine salvage enzyme that catalyzes the conversion of the purine bases hypoxanthine and guanine to the respective nucleotides inosine 5- monophosphate and guanosine 5- monophosphate (2). Several studies of *HPRT* gene mutations in human cultured cells and lymphocytes *in vivo* have provided evidence for age, exposure and genetics to influence mutation frequency(3). An increased mutation frequency with increasing age in normal healthy people is generally observed (4,5). The approach for somatic mutation analysis in human has been widely used to determine *in vivo* background as well as

acquired somatic cell mutation frequencies in pediatric and adult populations exposed to know and unknown environmental mutagens(6,7). The micronucleus assay using acridine orange fluorescence staining is commonly used as a genotoxic marker for chromosomal aberrations. This assay involves the analysis of peripheral blood differentially stained to visually identify damaged cells under fluorescent microscopy. With this assay, the investigator can evaluate DNA damage, bone marrow stem cell frequency, and immune cell production.

After telophase, this fragment is excluded from the nucleus of daughter cells because of its lack of spindle attachment regions (kinetochore or centromere) and is visualized as a nuclear body in the cytoplasm of the cell (8). 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(9). 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 (10). 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 (11). During the last years, the single cell gel electrophoresis (SCGE) or comet assay was introduced as a useful technique for human biomonitoring studies (12-15). The objective of the present study was to assess the role of single cell gel electrophoresis (comet) assay as biomarkers of radiation injury in individuals occupationally exposed to ionizing radiation. Also, use of the *HPRT* gene mutation and micronuclei in the reticulocytes to evaluate genotoxic effects in radiation worker staff exposed to low-dose ionizing radiation in comparison with a selected control group.

Materials and Methods:

Population Studies:

The present study including blood sampling from 30 male, aged 32 - 59 years who were occupationally exposed to low level of radiation with and more than 5 years duration of employment during their works from workers in Al-Tuwaitha site ; in addition to 20 male as apparently health control aged 30 -57 years . They were asked to fill in extensive questionnaire which included the following criteria (age, sex, smoking drink alcohol, X-ray, abortion, time of living in these area, kind of their work). Three genetic parameter were studied by using the *HPRT* mutation assay ,micronuclei MN in cytoplasm of reticulocytes cells and comet assay.

Sampling:

Five ml of human peripheral blood from all select subjects were collected and placed into sterile plain tube that contained lithium heparin. The blood was placed in a cool - box under aseptic conditions and transfer to the laboratory.

Procedure Assay:

The *HPRT* gene mutation assay was performed according to the description by Coa *el al* (15). The

acridine orange micronucleus assay was performed according to the description by (8). Comet assay was performed according to the description by Moller *el al* (16). Mf-*HPRT* (%) = [(binucleated and multinucleated cells per1000 lymphocytes when cultured with 6-TG) ÷ (binucleated and multinucleated cells per 1000 lymphocytes when cultured without 6-TG)] ×1000.

Microscopic examination:

The binucleated and multinucleated cells per 1000 lymphocytes in two sets of cultures were scored under light microscopy (magnification 1000X). Mutant frequency of *HPRT* gene (Mf-*HPRT)* was calculated according to(17). A total of 50 randomly captured comets (50 from each slide) were examined using fluorescent microscope connected through camera to an image analysis system. 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 these studies 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(18).

Results and Discussions:

The mutant frequencies of *HPRT* gene was performed on peripheral blood lymphocytes obtained from 30 individuals of radiation workers at Al-Tuwaitha site, then compared with 20 individuals control living in Baghdad. Mutant frequency of *HPRT* gene was calculated binucleated, trinucleated and quadrinucleated lymphocyte cell per 1000 lymphocytes in tissue culture with and without 6-thioguanine were identified by Giemsa staining. The result of Mfs-*HPRT* gene mutation for workers and controls are shown in (Table 1). The average Mf-*HPRT* (Mean ± SE) for radiation workers were 0.932 ± 0.0085%,when compared with the control 0.886 ± 0.0092 % (Table 1).

The results of *HPRT* assay in our experiment indicated the significant difference ($P < 0.01$) between the exposed group and control group .So the frequency of *HPRT* assay have been used to detect the genetic hazard of workers occupationally exposed to IR in the present investigation.

Table (1): Mean ± SE of mutant frequency- *HPRT* gene mutation in peripheral lymphocytes among radiation workers at Al-Tuwaitha site and control group.

Studies groups	No. of samples	Mutant Frequency-*HPRT* (Mean ± SE)
Radiation Workers	30	0.932 ± 0.0085^{a}
Control	20	0.886 ± 0.0092^{b}
LSD Value	0.0349	
P Value	0.01	

- **Least Significant Difference $_{(HPRT\ 0.05,\ 0.01)}$ = 0.0259, 0.0362**
- **Latter in a column (for comparison between studies groups) mean there is no significant difference ($p < 0.05$).**

In the present study, radiation workers chronically exposed to ionizing radiation were studied cytogenetically to evaluate the frequencies of *HPRT* gene mutation, in comparison with control individuals. It is well known that the exposure of mammalian cells to ionizing radiation produces a variety of DNA lesions, including base alterations, DNA protein cross links, and single and double strand breaks (19). However, it has been reported that human populations exposed to ionizing radiation also present increased frequencies of *HPRT* mutant frequencies of lymphocytes (20,21). After having been validated as an *in vivo* biomonitor in several subject studies, the *HPRT* gene assay has been applied for large scale biomonitoring of occupationally exposed radiation workers, e.g. nuclear power plant and hospital staff, radioactive waste treatment (7,22). This result of the current study has been compatible with other study that low doses of ionizing radiation increase *HPRT* mutant frequencies of lymphocytes (20,21).

The micronucleus assay using acridine orange fluorescence staining is commonly used as a genotoxic marker for chromosomal aberrations. This assay involves the analysis of peripheral blood differentially stained to visually identify damaged cells under fluorescent microscopy. With this assay, the investigator can evaluate DNA damage, bone marrow stem cell frequency, and immune cell production(8). For each human, one thousand polychromatic erythrocytes (PCEs) and one thousand normochromatic erythrocytes (NCEs) were examined. However, micronuclei MN were scored only in PCEs. PCEs were identified by red fluorescing reticulum in the cytoplasm. The micronucleus assay is a widely accepted method for evaluation of clastogens and aneugens. In the current study, acridine orange supravital staining to assess micronucleated cells in peripheral erythrocyte. The result of acridine orange micronucleus assay in human peripheral erythrocyte blood cell of workers in nuclear facilities destroyed at Al-Tuwaitha site due to decommissioning to radioactive contamination are shown in (Table 2). The average (Mean±SE) of micronucleus-polychromatic erythro-cytes for workers in nuclear facilities destroyed at Al-Tuwaitha were 1.80 ± 1.79 %, when compared with the control 2.30 ± 1.41 %.

Table (2): Micronucleus (MN) frequencies in human peripheral erythrocytes for the control group and workers in nuclear facilities destroyed at Al-Tuwaitha site.

Studies groups	No. of MN-PCE per 1,000 PCEs (Mean ± SE)	The ratio of CE:NCE (%).(Mean ± SE)
Radiation workers	2.30 ± 1.41 ^{a}	7.44 ^{A}
Control	1.80 ± 1.79 ^{b}	12.94 ^{B}

- **Latter in a column (for comparison between studies groups) mean there is no significant difference ($p < 0.05$).**

We found significant elevations in micronucleated polychromatic erythrocytes were observed highly significantly ($p<0.05$) between individuals of workers and control. These results support the use of acridine orange micronucleus assay to provide a rapid and sensitive indicator of

micronuclei inducers or non-inducers.as well, shown significant decrease ($p>0.05$) in the percentage of the ratio of polychromatic erythrocytes (PCE): normochromatic erythrocytes (NCE) PCE: NCE (%) in human peripheral blood of radiation worker (7.44 %) when compared with control group (12.94 %) (Table 2). Ionizing radiation is known to cause chromosomal aberrations in the form of DNA double-strand breaks. A micronucleus is a damaged fragment of a chromosome or an entire chromosome, which lags behind during anaphase of cell division. After telophase, this fragment is excluded from the nucleus of daughter cells because of its lack of spindle attachment regions and is visualized as a nuclear body in the cytoplasm of the cell(8). During analysis, differential staining with acridine orange will cause the DNA of the micronucleus to be stained bright green on an orange newly formed blood cell (8). The alkaline comet assay was selected as a biomarker of exposure to evaluate the ongoing exposure to ionizing radiation of 30 workers in nuclear facilities destroyed at Al-Tuwaitha site and 20 control. The results of the alkaline comet assay are summarized in (Tables 3) gives the mean values of tail length and tail moment for the radiation workers at Al-Tuwaitha site and control groups. The average of comet tail lengths (Mean ± SE) in radiation workers at Al-Tuwaitha site were 17.22 ± 0.21 μm, when compared with the control were 14.13 ± 0.15 μm. According to the results obtained, the radiation workers at Al-Tuwaitha site were highly significant ($P < 0.05$) comperd with control groups (Table 3). The average of comet tail moment (Mean ±SE) in radiation workers at Al-Tuwaitha site were 15.10 ±0.24 , which was differ significantly ($P < 0.05$) when compared with the control groups (11.67 ± 0.14). The observed values differed significantly ($P < 0.05$) from the tail moment values measured in control (Table 3). Lesions induced by ionizing radiation in DNA damage can be detected by the alkaline single cell gel electrophoresis (Comet assay) (23,24). The same method was evaluated in the present study to use alkaline comet assay as biomarker in assessment of DNA damage in personnel occupationally exposed to ionizing radiation.

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 ± SE)	
		Tail length (μm)	Tail moment
Radiation workers	30	17.22 ± 0.21^{A}	15.10 ± 0.24^{a}
Control	20	14.13 ± 0.15^{B}	11.67 ± 0.14^{b}

- **Latters in a column (for comparison the is significant differences between studies groups).**

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 (25,26). In the present study the alkaline comet assay revealed heterogeneity in the level of DNA breakage induced in human leukocytes by occupational exposure to ionizing radiation. Although some exposed subjects had high dosimeter

readings (up to 8548 µSv), we did not find a clear correlation between the DNA-damaging effects and the doses recorded by the dosimeters. This result is in agreement with studies mentioned previously (24,25). The increased comet values measured in peripheral blood leukocytes of exposed subjects in the present study indicate highly significant levels of primary radiation induced DNA damage compared with controls. However, the influences of the different occupational settings and doses absorbed on the levels of DNA damage assessed by use of the comet assay in the majority of subjects might be excluded. Our results indicate that the alkaline comet assay might be a useful additional complement to standard biodosimetric methods. By detection of momentary DNA damage and/or repair activity, it reflects the concurrent exposure and the actual levels of DNA damage present in peripheral blood leukocytes of the radiological workers at the moment of blood sampling.

Conclusion:

The results indicated that there is a possibility of using the changes in the micronuclei in cytoplasm of reticulocytes were select as biomarkers to evaluate the ongoing exposure to ionizing radiation.Also, The alkaline comet assay and mutation frequency for *HPRT* assay as a sensitive additional biomarker in the regular health screening of workers occupationally exposed to low doses of ionizing radiation.

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