Evaluation of Sister Chromatid Exchanges and Hypoxanthine guanine phosphoribosyl transferase Gene Mutation Assay in Peripheral Lymphocytes of Workers Exposed to Radiation at Al-Tuwaitha Site

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Summary

The present study aims at using the biological techniques in a genotoxicity assessment of low doses of radiation in samples of workers in Al-Tuwaitha site due to decommissioning to radioactive contamination as a result of work. This study included 50 male blood samples, aged 35 - 63 years as well as 35 blood samples from non-smokers and non-alcoholic as control group which included 25 male and 10 female aged 25 -57 years. The endpoints used were sister chromatid exchange (SCE) and hypoxanthine guanine phosphoribosyl transferase (HPRT) mutation assay. The sister chromatid exchange in the human lymphocyte for radiation worker was significantly ($P<0.05$) higher than in the control group. While the results of mutation for HPRT were non-significant when compared with the control group. In conclusion, the results indicated the possibility of using the changes in sister chromatid exchange as useful biomarkers for the detection of human exposure to ionizing radiation. In conclusion, the increase frequencies of sister chromatid exchange in radiation workers indicate the cumulative effect of low-level chronic exposure to ionizing radiation.

Keywords: Ionizing radiation, Human lymphocyte, Sister chromatid exchange, Hypoxanthine guanine phosphoribosyl transferase.

Introduction

Exposure to ionizing radiation causes damage to living cells, especially to DNA in the cell nucleus. Sister chromatid exchange (SCEs) are induced by UV-irradiation and low dose of ionizing radiation (1). The SCEs are probably initiated by unrepaired DNA double strand breaks (DSBs) formed during the G1 phase of the cell cycle. It is also plausible that unrepaired DNA adducts induce DSBs during DNA replication when the replication machinery gets stuck in adduct in the S phase of the cell cycle. DSBs are then repaired during the S phase by homologous recombine -ation repair which leads to the formation of SCEs. It has been proposed that homologous recombination may reactivate stalled replication forks (2). Thus, SCEs may actually be an indirect measure of successful DNA repair. 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 (3). 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. An increased mutation frequency with increasing age in normal healthy people is generally observed (4 and 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 known and unknown environmental mutagens (6 and 7). The aim of the present study is to identify the susceptibility mutational of low ionizing radiation doses using sister chromatid exchange and investigate the HPRT gene mutation in human blood lymphocyte in order to detect the effect of radiological hazard in local human samples of Al-Tuwaitha worker.

Materials and Methods

The present study included collected 50 male blood samples, aged 35-63 years old from workers in the nuclear center destroyed at Al-Tuwaitha site due to decommissioning to radioactive contamination as a result of work, for the test of sister chromatid exchange and HPRT mutation assay. As well 35 blood samples from non-smokers and non-alcoholic as control group 25 male and 10 female aged 25-57 years were used. They were asked to fill in an extensive information concerning (age,

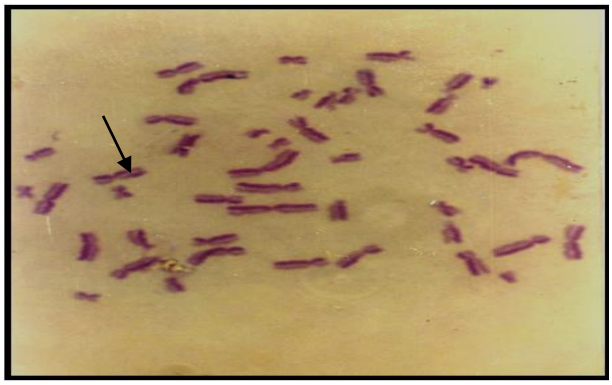
sex, smoking drink alcohol, X-ray, abortion, time of living in these area, kind of their work). Five ml of human peripheral blood from all selected subjects were collected and placed into sterile plain tube that contained lithium heparin. The blood was placed in a cool-box under aseptic conditions and transfer to the laboratory. The sister chromatid exchange (SCE) was performed according to the description by (8), the whole blood samples of were collected from each subject. The cell cultures were incubated in RPMI-1640 medium contain bromodeoxy-uridine with 20% calf serum and stimulated with phytohaem -agglutinin. The HPRT gene mutation assay was performed according to the method descriped by (9), two sets of media cultures were prepared, each set of culture contained 0.5 ml heparinized blood and 4.5 ml RPMI 1640 with 20% fetal calf serum and 0.2 mg/ml PHA-M. One set of culture was added with 0.2 mM 6-thioguanine (Sigma). At 33 hr. of incubation, cytochalasin B (the final concentration, 4.5 mg/ml) was added into two sets of cultures. At 72 hr. of incubation, the lymphocytes were harvested by centrifugation and fixed with methanol: acetic acid (3:1). The slides were prepared and stained with Giemsa stain solution. The slides of SCE were prepared, as (8). A hundred cell spread in metaphases were analyzed for each subject. The slides of HPRT gene was calculated binucleated, trinucleated and quadrinucleated lymphocyte cell per 1000 lymphocytes in two sets of cultures scored under light microscopy (magnification 1000X). Mutant frequency of HPRT gene (Mf- HPRT) was calculated with the following formula (9):

$$Mf\text{ - }HPRT\ (‰) = \frac{\text{binucleated and multinucleated cells in culture with 6 - TG per 1000}}{\text{binucleated and multinucleated cells in culture without 6 - TG per 1000}} \times 1000‰$$

The data of the present study were compiled into the computerized data file and frequency, distribution and statistical description (Mean ±SE) were divided using SPSS statistical software. It 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 (10).

Results and Discussion

The sister chromatid exchange and mutant frequencies of HPRT gene were performed on peripheral blood lymphocytes obtained from 50 individuals of workers in nuclear center destroyed at Al-Tuwaitha site due to decommissioning to radioactive contamination as a result of work, then compared with 35 individuals control living in Baghdad. The SCEs were scored in metaphase chromosomes and were identified by fluorescent plus Giemsa staining (Fig. 1). The mean frequencies of SCEs per cell differed significantly (P<0.05) between individuals of workers and control, being 7.78 ± 0.45 SCE/cells and 6.28 ± 0.22 SCE/cells, respectively (Table, 1). It is well established that SCE arises during replication of a damaged DNA template and SCE assay is a sensitive method for identifying chemical and physical DNA-damaging agents (11). The increase frequencies of chromosomal aberration and sister chromatid exchange in radiation workers indicate the cumulative effect of low-level chronic exposure to ionizing radiation (12).



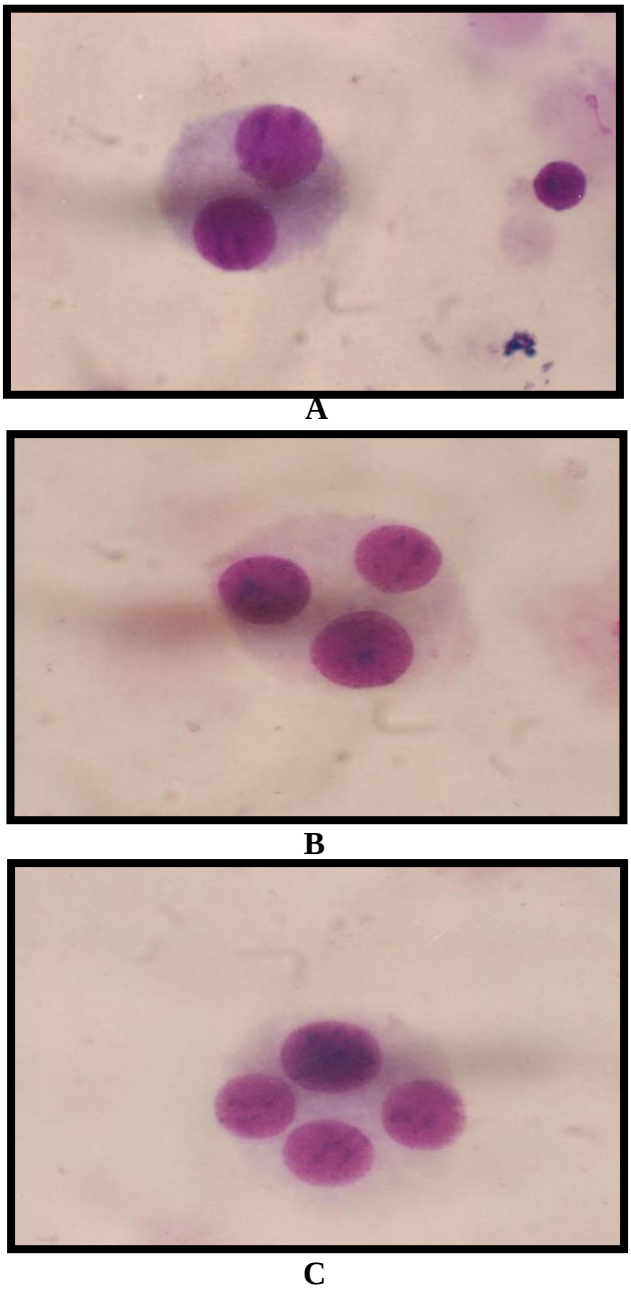
Figure, 1: Microphotographs of sister chromatid exchanges in metaphase chromosomes by fluorescent plus Giemsa staining (1000X).

Table, 1: The SCE and mutant frequency- HPRT gene for the control group and workers in nuclear center destroyed at Al-Tuwaitha site (Mean ±SE).

Studies groups	No. of Samples	Mutant Frequency- HPRT (Mean ± SE)	SCE /Cell (Mean ± SE)
Radiation group	50	0.913 ± 0.0083 A	7.78 ± 0.45 a
Control (Baghdad) group	35	0.864 ± 0.0090 A	6.28 ± 0.22 b

Similar latter in a column (for comparison between studies groups) mean there is no significant difference (P< 0.05).

Mutant frequency of HPRT gene gene was calculated binucleated, trinucleated and quadrinucleated lymphocyte cell per 1000 lymphocytes in tissue culture with and without 6-thioguanine identified by Giemsa staining (Fig. 2).



Figure, 2: Cytokinesis blocked human blood lymphocyte cell, A: Binucleated lymphocyte cell, B: Trinucleated lymphocyte cell and C: Qudrinucleated lymphocyte cell (1000X).

The result of HPRT gene mutation assay in workers in nuclear facilities destroyed at Al-Tuwaitha site due to decommissioning to radioactive contamination are shown in (Table, 1). The average Mf-HPRT for workers in nuclear center destroyed at Al-Tuwaitha were 0.913 ±0.0083%, when compared with the control 0.864 ±0.009%. In the present study, radiation workers chronically exposed to ionizing radiation were studied cytogenetically to evaluate the frequencies of HPRT gene mutation and SCE, 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 (13). However, it has been reported that human populations exposed to ionizing radiation also present increased frequencies of SCE and HPRT mutant frequencies of lymphocytes (14-16). In spite of many reports in the literature about SCE analysis, neither the biological effect of SCE nor the mechanisms that lead to their formation are clearly understood. The present results observed for the radiation workers indicate an increase in the SCE frequencies in parallel to higher chromosomal aberration frequencies, which can be attributed to the low level of radiation exposure.

In conclusion, using the changes in sister chromatid exchange as useful biomarkers for the detection of human exposure to ionizing radiation is possible the increase frequencies of sister chromatid exchange in radiation workers indicate the cumulative effect of low-level chronic exposure to ionizing radiation.

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تقييم فحوصات التبادل الكروماتيدي الشقيقي والطفرات لجين الهايبوزانثين فوسفورايبوسيل ترانسفيريز في الخلايا اللمفاوية للعاملين بالإشعاع في موقع التويثة

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الخلاصة

هدفت الدراسة الحالية إلى استعمال تقنيات بيولوجية في تخمين السمية الجينية للجرع الواطئة من الأشعة المؤينة على عينة من العاملين في موقع تصفية المنشأت النووية المدمرة في التويثة. تضمنت الدراسة 50 إنموذجا ذكراً للأشخاص اللذين يتعاملون مباشرة مع الإشعاع والذين تراوحت أعمارهم بين (35-63) سنة فيما يخص فحص التبادل الكروماتيدي الشقيقي وتقنية التردد الطفوري لجين الهايبوزانثين فوسفورايبوسيل ترانسفيريز، فضلاً عن 35 إنموذج دم من أشخاص غير مدخنين ولا يتعاطون الكحول كمجموعة سيطرة والتي تضمنت (25 ذكر و10 انثى) تراوحت أعمارهم بين (25 – 57) سنة. بينت الدراسة وجود زيادة معنوية ($P<0.05$) في معدل التبادل الكروماتيدي الشقيقي وفي الخلايا اللمفاوية للعاملين مقارنة مع مجموعة السيطرة، ولم تسجل أي فروقات في التردد الطفوري لجين الهايبوزانثين فوسفو رايبوسيل ترانسفيريز لدم العاملين مقارنة مع مجموعة السيطرة. نستنتج من هذه الدراسة إمكانية استعمال التغيرات في التبادل الكروماتيدي الشقيقي في الخلايا اللمفاوية للإنسان كمؤشر بايولوجي في الكشف عن تعرض الإنسان إلى الأشعة المؤينة. أنّ الزيادة في تردد التبادل الكروماتيدي الشقيقي للعاملين بالإشعاع دليل على تعرضهم لجرع واطئة من الأشعة المؤينة.

الكلمات المفتاحية: الأشعة المتأينة، الخلايا اللمفاوية للإنسان، التبادل الكروماتيدي الشقيقي، الهايبوزانثين فوسفورايبوسيل ترانسفيريز.