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Ionizing Radiation Effect and DNA damage in the workers of Al-Tuwaitha Nuclear Site

LinaS.Al-Jameel[1*], Rasha S.Ahmed[1], Haidar A.Shamran[2]
[1]Department of Physiology, Medical physics, School of Medicine, Al-Nahrain University, Baghdad, Iraq
[2]Dept of MedicalResearch Unit, School of Medicine, Al-Nahrain University, Baghdad, Iraq.

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Abstract

Single cell gel electrophoresis (SCGE), also called comet assay, is a rapid and sensitive technique used to analyse DNA Fragmentation Index(DFI). This study aimed to evaluate DNA damage in lymphocytes due to ionizing radiation in workers of Al-Tuwaitha nuclear site, which has been used for nuclear activities and contains a potentially significant amount of radioactive waste.The workers in this site are vulnerable to pollution due to a highly polluted environment of ionizing radiation. Blood samples were collected from 36 workerswho were divided into two groups;8 workers without protection and 28workers with protection, in addition to 30 control subjects.Alkaline comet assay was applied for analysis and the results indicated significantly higher DNA damage in the workers without protection as compared with the workers with protection and control groups.The result also showed significant differences between the workers with protection and control groups.

Keywords: DNA Fragmentation Index, Comet assay, DNA damage, Ionizing radiation , Al-Tuwaitha nuclear site.

تأثير الاشعاعات المؤينة وتلف الحمض النووي في عمال موقع التويثة النووي

لينا سرمد الجميل*[1]، رشا صبيح احمد[1] ، حيدر احمد شمران[2]
[1]قسم الفسلجه ، الفيزياء الطبية، كلية الطب، جامعة النهرين ، بغداد، العراق
[2]قسم وحدة البحوث الطبية، كلية الطب، جامعة النهرين ، بغداد، العراق

الخلاصة

الترحيل الكهربائي لخلية منفردة ويسمى ايضا تحليل المذنب هي تقنية سريعة وحساسة تستخدم لتحليل مؤشر تجزئة الحمض النووي .تهدف هذه الدراسة الى تقييم تلف الحض النووي في الخلايا اللمفاوية بسبب الاشعاع المؤين لدى العاملين في موقع التويثه النووي, وقد استخدم الموقع للأنشطة النووية ويحتوي على كمية كبيرة من النفايات المشعة ,والعاملين في هذا الموقع عرضة للتلوث بسبب تلوث البيئة للإشعاع المؤين. تم جمع عينات الدم من 36 عاملا, وتم تقسيم العمال الى مجموعتين: 8 عاملا دون حمايه و28 عاملا مع حماية, و30 مجموعة السيطرة , وتم تطبيق اختبار المذنب القلوي للتحليل . اوضحت النتائج حدوث تلف كبير في الحمض النووي في العمال دون حماية مقارنة بالعمال مع الحماية ومجموعة السيطرة, كما اظهرت النتائج وجود فرق ذات دلالة احصائية بين العمال ذوي الحماية مع مجموعة السيطرة .

*Email:kitty.lina.93@mail.ru

Introduction

Ionizing radiation is considered as the most hazardous kind of environmental pollution, since it cannot be smelled, tested, or seen, butcan only be detected by special equipments and instruments [1,2]. Ionizing radiation is a kind of high energy radiation that has kinetic energy that is sufficient to knock out electrons from atoms and molecules such as DNA, protein, and water, thus creating ions [3].

There are several factors affecting the outcomes of the human body'sexposure to radiation, such as the age of the person and the part or the organ of the body exposed[4].

Ionizing radiation can cause direct and indirect DNA damage. The direct effect is caused bythe energy absorbed by the photoelectric effect and Compton interaction as well as the predominanceof high linear energy transfer (high LET) radiation. Direct effect can cause single-strand and double-strand DNA breaks.Single-strand break can be repaired by the cells whereas double-strand break can cause cell death. In the indirect effect, the free radicals are formed via energy transfer from the radiation that interacts with DNA and causes molecular damage [5-7].

Ionizing radiation produces DNA damage through various mechanisms,including double-strand breaks, single-strand breaks, damage to purine and pyrimidine bases, and loss of bases [8-10].

The alkaline comet assay is a technique used to detect various lesions in the DNA molecule, such as crosslinks, alkali labile sites, and single strand breaks. Cells that are usually estimated, with this assay, in humans are blood lymphocytes [11,12].

In this study, we measured DNA fragmentation in lymphocytes from Al-Tuwaitha nuclear site's workers exposed to ionizing radiation, by using the alkaline comet assay. The comet assay is a rapid, sensitive,and simple technique that is used to evaluate DNA damage within individual cells [13].

Material and methods

Study subjects and design

Two directorates where workers are occupationally exposed to ionizing radiation were studied; all of the workers assigned to these fields accepted to participate. A total of 36 workers were included, consisted of 18 workers from the decommissioning directorate and 18 workers from the directorate of the management and treatment of radioactive waste. The workers were divided into two groups: 8 workers without protection and 28 workers with protection (Table-1).

The control group consisted of 30 individuals who have never worked in Al-Tuwaitha Nuclear site (Table-1).

All participant answered a detailed questionnaire on years of their work and general characteristics including age, smoking, and comorbidities. Workers who live near Al-Tuwaitha Nuclear site were excluded from the study.

Table 1-Demographic characteristics of the workers and control group

Parameter	Workers	Control
N	36	30
Age (years) 9.04	47.69 ±	40.1±6.91
Gender , n(%) Female Male	 5(14%) 31(86%)	5(17%) 25(83%)
Duration of exposure "(years)	22.36± 8.65	_____
Nature of job, n(%) Decommissioning directorate	 18(50%)	_____
Directorate of the management and treatment of radioactive waste	18(50%)	_____ 11(36.66%)

School employees	_____	4(13.33%)
University employees	_____	3(10%)
Court employees	_____	12(40%)
Unemployed	_____	
Smoking, n(%)		32(76%)
Smokers never	25(72%)	7(23%)
Current smokers	10(27%)	
Comorbidities		3(10%)
Yeas	1(3%)	27(90%)
No	35(97%)	

Blood Sampling

Venous blood was obtained from each individual in EDTA tubes for lymphocytes isolation.

DNA Fragmentation Index Analysis

DNA fragmentation of lymphocytes was performed by The alkaline comet assay as described by Singh et al [14, 15]. Briefly, lymphocytes were embedded in a low melting point agarose gel (LM agarose, 250 µl/cell, in10 µl 1xPBS) on microscope slide.After gel solidificationthe slides were immersed in a lysis solution, and for added convenience or sensitivity they were incubated overnight at 4C°. The slides were then treated with freshly alkaline unwinding solution, PH> 13.After 30 min, the slides were washed by distillate water and placed in an electrophoresis slide tray (label of the slide adjacent to the black cathode), and the power supply was set to 400mA and 40v for 60 min.

Staining and scoring

Dry slides were stained with SYBR Green(5 µl) before screening under a fluorescence microscope. The DNA Fragmentation Index (DFI) was established by measuringmore than 100 cells per slides. Images were taken by fluorescence microscope (Olympus, Japan) at a magnification of 10x andanalyzed with TriTek Comet Score™ freeware v 1.5. The parameters taken for the comet were tail length,% DNA in the tail, and tail moment. The comet can be classified into five arbitrary damage levels according to the tail appearance (Figure-1); level 0: no damage, level 1: low damage, level 2: medium damage, and level 3: high damage. Levels 3 and 4 were considered as a damage in the cells [16].

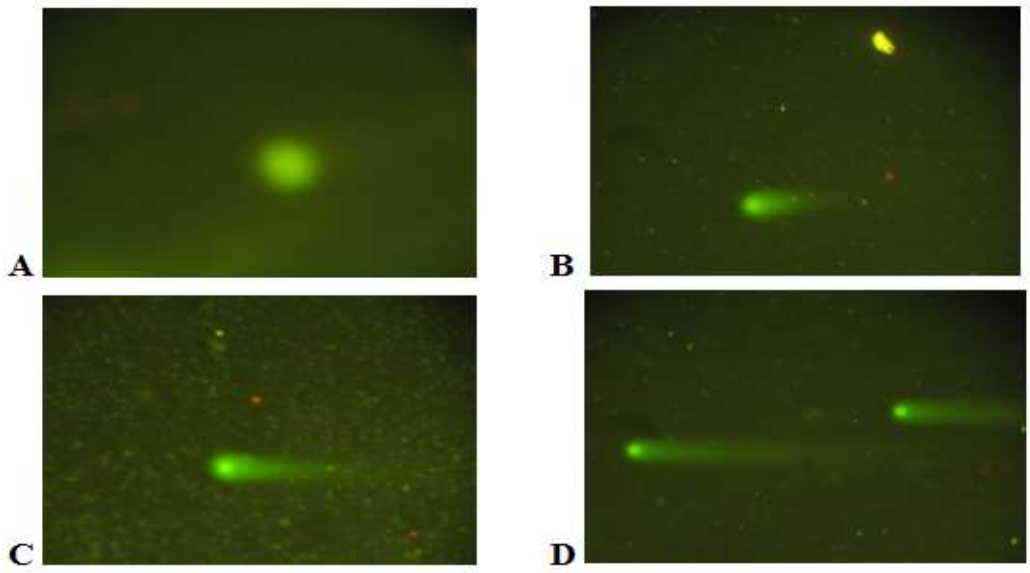

Figure 1-Classification of comet tail level visualized by SYBR Green staining.
(Imaging in fluorescent microscope at magnification 10x).
A:No damage ,B: Low damage ,C: Medium damage ,D: High damage

Statistical Analysis

DNA Fragmentation Index(DFI) was calculated by using one way analysis of variance (ANOVA-test). The comet parameters were calculated by using Independent t-test with SPSS (V.23.0) for Windowsand the data were presented as mean± SD. The P value was set at 0.05 for statistical significance.

Result and discussion

Table-1 summarizes the demographic characteristics of the study population for the workers and control (Table-1). Results of DFI are shown in Table-2.A higher DFI value was observed in the workers without protection(43.09+4.48) as compared with the workers with protection (16.95±1.99) and the control group (14.33±2.45).The results indicated significant differences between the workers with protection (16.95±1.99) and the control group (14.58±2.36). The damaged DNA had a tail and the undamaged DNA had an intact head without a tail (Figure-1). All parameters characterizing DNA damage(tail length, %DNA in the tail, and tail moment) showed significantly higher values in the workers without protection (TL=36.53±10.04, %DNA=7.01±3.77, TM=3.33±1.35) as compared with workers with protection (TL=13.58±6.13, %DNA= 4.64 ±1.80, TM=1.40±0.79) and the control group (TL=8.8±2.94, %DNA=1.14±0.79, TM=0.02±0.01) Tables-(3 and 4). Also, the comet parameters indicated significant differences between workers with protection and the control group (Table-5).

Table 2-Comparison of DNA fragmentation index between workers (with and without protection) and the control group.

%DFI	Workers with protection N=28	Workers without protection N=8	Control N=30	P-value
Mean± SD	16.95±1.99	43.09±4.48	14.33±2.45	<0.001

Table 3-Comparison of comet parameters between workers with protection and workers without protection

Parameter	Workers with protection Mean± SD	Workers without protection Mean± SD	P-value
Tail length	13.58±6.13	36.53±10.04	<0.001
%DNA in tail	4.64±1.80	7.01±3.77	0.006
Tail moment	1.40±0.79	3.33±1.35	0.007

Table 4-Comparison of comet parameters between workers without protection and the control groups.

Parameter	Workers without protection Mean± SD	Control Mean± SD	p-value
Tail length(px)	36.53±10.04	8.8±2.94	<0.001
%DNA in tail	7.01±3.77	1.14±0.79	0.001
Tail moment(px)	3.33±1.35	0.02±0.01	<0.001

Table 5-Comparison of comet parameters between workers with protection and control groups.

Parameter	Workers with protection Mean± SD	Control Mean± SD	P-value
Tail length(px)	13.58±6.13	8.8±2.94	0.035
%DNA in tail	4.64 ±1.80	1.14±0.79	0.001
Tail moment(px)	1.40±0.79	0.02±0.01	0.002

Ionizing radiation is a genotoxic factor for all individuals, being able to cause genetic damages even at quite low doses.Thus, it is necessary to evaluate DNA damage levels in individuals who exposed to ionizing radiation at their place of employment [17,18].In this study,DFI was measured by using alkaline comet assay and comet parameterswere analyzed by using comet score software program to assess DNA damage in lymphocytes from workers exposed to ionizing radiation in Al-Tuwaitha nuclear site.The workers were divided into two groups: workers without protection and workers with protection, for comparison with control group who had never worked in Al-Tuwaitha nuclear site. The results showed higher DNA damage in the workers without protection compared with workers with protection and the control group. The amount of ionizing radiation between the workers is different,and the workers without protection have greater ionizing radiation which was found to induced tumors and genetic defects in several tissues [19]. Ionizing radiation can cause all types of mutations. When cells are exposed to ionizing radiation the rate of mutations increases, which can change the organism's DNA[20,21. Non-synonymous mutations (often in the third nucleotide of a codon) are associated with a genetic disease;they affect protein structure and the amino acids, whilemutations may occurs in the single gene.For example, cancer is caused by mutations that occur in both oncogenes and tumor suppressor genes (TSGs) [22-24].

Exposure to ionizing radiation may cause stochastic and deterministic effects in the individual,whiel working suits can be used to protect workers from the ionizing radiation[25]. The findings of this study showed a significant decrease in DNA damage in the workers with protection. However, working suit protection from radiation should not ensure full protection against DNA damage. This may be due to handling and improper use of working suits by the workers. The quality of working suits, use, and improper storage can decrease workers protection. This may be a reason that higher DNA damage was observed in the workers with protection as compared with the control group.

Conclusion

The study indicated that the exposure to ionizing radiation increases DNA damage and suggested that the workers should carefully comply with radiation protection requirements such as wearing HazMat suit and the personal radiation detection device.

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