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Original Research Article

Effect of Different Single Doses of CT Scan on DNA Fragmentation and Oxidative Stress in the Blood of Healthy Young Adults in Baghdad, Iraq

Hiba O. Muhammed[1], Hussain S. Hasan[1], Qasim S. Al-Mayah[2]*

[1]*Department of Physiology and Medical Physics, College of Medicine, Al-Nahrain University, Baghdad, Iraq.*
[2]*Medical Research Unit, College of Medicine, Al-Nahrain University, Baghdad, Iraq*

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ABSTRACT

Ionizing radiation (IR) induces many cellular changes by direct and indirect interactions, which alters multiple cellular processes, causing DNA damage and reactive oxygen species-induced biochemical disturbances. This study aimed at investigating the effect of various doses of computed Tomography (CT) scanning on the fragmentation of DNA and oxidative stress in healthy young adults.

The DNA damages associated with different doses of CT scan was explored using alkaline comet assay. Serum concentration of malondialdehyde (MDA) in each sample was measured using enzyme-linked immunosorbent assay (ELISA). There was a significantly higher percent of DNA fragmentation in the blood samples subjected to high dose of radiation (120 kV-210 mAs-21sec) than those exposed to lower doses. Furthermore, the serum concentration of MDA was proportional to the intensity of radiation. Therefore, DNA fragmentation can be associated with high doses of CT radiation, maybe due to radiation-induced oxidative stress.

Keywords: Ionizing radiation, Oxidative stress, DNA Fragmentation, Comet assay.

Introduction

Radiation is a process that is present in everyday life, arising from natural and manufactured sources. Health-related radiation is mainly a non-ionizing type, involving ultraviolet (UV) sunrays, radio waves, and microwaves (as electromagnetic radiation). The potential of non-ionizing radiation to damage biological tissues is well known. X-ray is part of the electromagnetic radiation.[1] X-ray could be characterized as a flow of photons carrying quantities of energy. The ability of X-rays to penetrate materials has been greatly utilized, especially in medical and scientific applications.[2] X-ray radiation, including CT scan, is a type of infrared close to gamma radiation, but with low energy. It is an efficient X-ray imaging tool that offers more diagnostic information than X-rays.[3] Targeted organs are given tiny doses during the diagnosis. Generally, the increased radiation dosage resulting from such operations is not large. For instance, a total of 3 mSv/person was estimated in one year, of which 2.4 mSv/person was of normal source, while only 0.4 mSv from medical field tests.[4] Due to the small amount of radiation used for medical examination, it is difficult to be investigated using traditional methods. However, a variety of procedures, such as CT scan, might provide large total estimated doses reaching 100 mSv to the targeted tissue, making it a good reference point for studying the health risks of short-acting radiation.[5]

In the last 20 years, the use of medical diagnostic imaging has increased considerably. A large proportion of these medical diagnostic techniques including radiography, fluoroscopy, CT involves IR.[6] About 80% of medical imaged patients are exposed to IR.[7]

*Corresponding author. E mail: sciencefond2015@gmail.com
Tel: 00964 770 454 9713

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Advanced imaging techniques are increasingly playing a significant role in monitoring disease progression.

A number of articles on the dangers of CT have been published since 2000.[4,8,9]

Oxidative stress is another consequence of IR arising from the generation of reactive oxygen species.[10] Based on these facts, the objectives of this study was to analyze the impact of different doses of CT scan on DNA fragmentation and oxidative stress.

Materials and Methods

Blood Samples

A total of 30 blood samples were collected in heparinized tubes from apparently healthy adult individuals (age range 25-45 years, 16 males and 14 females) who were visiting Al-Imaimain Al-Kadhumain Medical City, Baghdad, Iraq from April to May 2019. These individuals were not exposed to IR during the last one year, and they were accompanying their diseased relatives. Individuals with known chronic pathology such as diabetes mellitus and autoimmune disease were excluded from the study. An approval of the Institutional Review Board (IRB) of the College of Medicine, Al-Nahrain (No. 2019/148) was obtained to conduct the study. Eligible participants were asked to sign an informed consent documenting their willingness to participate in the study.

Exposing Samples

Each blood sample was divided into four aliquots. The first 30 samples were considered as control and were not exposed to any radiation. The other samples were exposed to different dose of radiation from CT scan machine (Philips Medical Systems, Eindhoven, The Netherlands) in the radiology department of AL-Imamain AL-Kadhumain Medical City. The second, third and fourth groups (each with 30 samples) were exposed to a low dose of radiation (the dose of the sinuses, 120 kV- 80 mAs- 12.12sec), a medium dose of radiation (the dose of the chest, 120 kV- 170 mAs- 18.57 sec), and a high dose of radiation (the dose of the abdomen, 120 kV-210 mAs – 21sec), respectively. After radiation exposure, all samples were cooled

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at 4°C and transferred to the laboratories of Medical Research Unit, College of Medicine, Al-Nahrain University.

Alkaline Comet Assay

To measure DNA damage after irradiation, the comet assay was used under alkaline conditions. Reagents were prepared immediately before use. These include; 1X PBS, lysis solution with dimethyl sulfoxide (DMSO, low melting agarose, alkaline unwinded solution (pH >13, per 50 mL of alkaline solution combined with NaOH pellets (0.4 g), 200 mM EDTA (250 mL) and dH_2O (49.75 mL). Slides were placed in a dark place at 4°C for 10 min. The slides were immersed in an alkaline solution for 20 min at room temperature and then were applied to the comet assay electrophoresis (ES-unit) ~850 mL at 4°C alkaline electrophoresis solution. Electrophoresis solution was gently drained by immersing in dH_2O twice for 5 min, and then for 5 min in 70% ethanol. Samples were dried at 37ºC for 10-15 min. One hundred mL of diluted SYBR Green stain were applied to agarose which was dried and stained for 30 min (at room temperature) in the dark. The slides were gently tapped to take off unwanted SYBR green solution and thereafter washed with water. These slides were fully dried at 37°C. Slides were viewed under fluorescent microscope (of SYBR Green) with a high range emission of 496 nm/522 nm, and sufficient fluorescent filter.

Comet Analysis

Image analysis software (Comet Score) was used to calculate the DNA fragmentation. Three parameters were used to calculate the DNA fragmentation, the length of the tail (the dimension from the middle of the head to the end of the tail), the mean of tail moment (measure via product of tail DNA/total DNA by tail center of gravity), and tail DNA percentage = 100* Tail DNA intensity / cell DNA intensity.[10]
The Length of the tail was calculated from the midpoint of the comet to the ending of the tail. The percent of DNA in the tail **(**DNA percent due to comet tail was determined by the rate of the total intensity of the tail to the total intensity of comet multiplied by 100**,** and the tail moment was determined as the percent of the DNA in the tail multiplied by the length of the tail.[11]

Malondialdehyde Concentration (MDA) measurement

A ready commercial kit (MyBioSource, USA) was used to measure the serum concentration of MDA using enzyme-linked immunosorbent assay (ELISA). The manufacturer's protocol was followed.

Statistical Analysis

The statistical package for social sciences (SPSS) software (version 24) was used for all statistical analyses. Data were subjected to normality test using Shapiro Wilk test. Results were expressed as mean ± standard deviation (SD) and all statistical comparisons were carried out by means of independent t-test or analysis of variance (ANOVA) test as required. Pearson's correlation test was used to explore the possible correlation between different parameters. A p-value of ≤ 0.05 was considered significant.

Results and Discussion

Effect of Different Single Dose of CT Scan on DNA Fragmentation

The results from the alkaline comet assay showed that blood samples with no radiation exposure had the highest percentage of intact DNA (44.47 ± 3.21%) which differed significantly from other groups. Blood samples exposed to low dose radiation had 38.65 ± 1.52% of intact DNA which did not differ significantly from that of medium exposure (36.76 ± 2.1%). However, both groups were significantly higher than the high exposure group (34.89 ± 1.57%) as shown in Figure 1.
The percentage of DNA with low fragmentation almost took a similar pattern. However, there were no notable variations among the control group and the group of low dosage, which were significantly higher than either medium dose or high dose group. High dose group showed the highest percentage of lymphocyte with DNA fragmentation (14.93 ± 1.63%) and differed significantly from the control group (6.82 ± 1.04%) and low dose group (10.65 ± 1.24%) but not from medium dose group (13.4 ± 1.5%) as illustrated in Figure 2.
Such findings are consistent with a number of previous studies. Wang *et al.* used comet assays to test DNA DSBs in lymphocytes isolated from mice and exposed to specific doses of IR (1-6 Gy). There was an increase in the level of DNA damage in exposed subjects versus controls, and the authors recommended a regular monitoring of DNA risk to medical staff exposed to IR.[12]
In a somewhat similar study to the present study, Singh *et al*. exposed human lymphocytes to different doses of IR; however, they used micro-gel electrophoresis to detect the DNA fragmentation. A large increase in DNA SSBs was observed when the radiation dose was elevated to 6.4 rad. Furthermore, there was a significant correlation between radiation dose and DNA immigration.[13]
Therefore, it is reasonable to assume that CT diagnostic dose can increase the risk of many DNA fragmentation-related diseases. Therefore, regular monitoring of IR effect is of paramount importance to avoid the possible harmful effect of such radiation.
Through the comet assay, three parameters of DNA fragmentation were calculated in low, medium and high dose groups: these were the tail of the length, the percent of DNA in the tail with the tail moment. Mean tail lengths in low, medium and high dose groups were 2.2 ± 1.74 μm, 11.23 ± 3.88 μm and 19.0 ± 5.5 μm, respectively with highly significant differences between the three groups. Likewise, the high dose group showed the highest percent of the DNA inside the tail for the affected lymphocyte (16.97 ± 7.49%) and differed significantly from either medium dose group (4.92 ± 1.74%) or low dose group (1.34 ± 0.68). Finally, the tail moment was significantly higher in high dose group (2.47 ± 0.98) than medium dose group (1.54 ± 0.57) or low dose group (0.29 ± 0.16) as shown in Table 1.
Almost all previous studies in this regard emphasized the impact of IR on parameters of DNA fragmentation. In one study, the tail DNA percentage, tail length, and tail moment were found to be increased proportionally with a dose of IR, which indicates a dose-response relationship.[12] The percent of DNA in tail and tail moment can vary significantly based on the conditions of the assay, and they are considered very important to record DNA damage.[14,15]

The Effect of Different Single Dose of CT on Serum MDA Level

In the control group, mean serum level of MDA was 6.24 ± 1.0 nmol/mL with no significant difference from that of low dose group (9.29 ± 1.51 nmol/mL). High dose group portrayed the highest level of MDA (12.62 ± 1.27 nmol/mL) which differed significantly from the control and low dose groups, but not from the medium dose group (11.5 ± 1.57 nmol/mL).
Such results are consistent with previous studies which suggest that MDA is high in person's exposed to IR. For example, Malikrad *et al*. found that the levels of MDA were elevated in the blood samples of workers in the hospital, particularly radiologists, because of the chronic exposure to low-dosage of IR.[16] Furthermore, Arterbery *et al*. found that after the full radiation, lipid peroxidation is maximized between animals and humans.[17]
During the time interval between IR exposure and measuring the MDA, the level of MDA undergoes dramatic changes, because there are a variety of factors that affect lipid peroxidation (LP). In addition, LP may be a consequence of the fractional lowering of oxygen (H_2O_2 and OH^-) intermediate, active auto-oxidation of lipids, nitric oxide metabolism intermediates, and improvements in lipid membrane surface structure.[18-20] Furthermore, MDA could be produced from prostaglandin biosynthesis regardless of oxidative stress. Thus, most of these studies do not reflect the actual effect of IR on MDA production.
Ionizing radiation is known to induce ROS, which causes LP. The end result of LP is the production of MDA. The MDA itself acts as ROS, forming DNA adducts which are mutagenic,[21] although LP may induce biochemical reactions on the cell surface, leading to the production of second messengers, such as ceramide, which activates apoptosis or plays a role in the cell's adaptive response to radiation.[22]

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Correlations between Parameters

The test of Pearson's correlation was utilized for discovering the possible correlation among the different parameters. In the low dose group, only DNA fragmentation indices were positively and significantly correlated with each other. In the medium dose group, the percentage of lymphocyte with high DNA fragmentation showed significant positive correlation with MDA ($r = 0.405$, $p = 0.06$) and age ($r = 0.572$, $p < 0.01$). MDA also had a positive significant correlation with the percentage of DNA along the tail ($r = 0.415$, $p = 0.023$). However, in the high dose group, the percentage of lymphocyte with high DNA fragmentation showed a significant positive correlation with MDA ($r = 0.379$, $p = 0.039$) and tail length ($r = 0.387$, $p = 0.034$). MDA also correlated positively with the length of the tail ($r = 0.428$, $p = 0.022$) and with the percentage of the DNA in the tail for the affected lymphocyte ($r = 0.473$, $p = 0.008$).

In accordance with these results is a Croatian study, in which there was no significant impact of age on DNA fragmentation in neither exposed nor unexposed subjects.[23] As fragmentation parameters and MDA arise from the same cause which is IR, it is reasonable to assume a positive correlation between these parameters, because the originated ROS from IR initiate breaks in DNA strand accompanied by lipid peroxidation and formation of MDA.

The Effect of Gender

The results showed that gender have no significant effect on IR-related DNA fragmentation. For low dose exposure group, all parameters were comparable between the two genders with no significant differences. Although the tail length of affected lymphocyte was slightly higher in females than in males (2.57 ± 2.93 µm vs. 1.87 ± 2.63 µm), the difference was insignificant. Similarly, in the medium dose exposure group, all parameters were almost similar in the males and females with no significant difference. Although MDA concentration was slightly higher in male 12.72 ± 1.26 nmol/mL than in females (12.32 ± 1.39 nmol/mL), the difference was insignificant. The situation was not different in the high dose exposure group; all parameters were comparable between males and females with no significant difference. It is noteworthy that, among females, there was a marked increase in the percentage of DNA in the tail of affected lymphocyte compared with males (19.43 ± 6.46 vs. 14.81 ± 4.4). However, the difference did not reach the acceptable level of significance as shown in Table 2.

The results found by Nadia *et al.* are in agreement with the results that we found in this present study. They indicated that there is a lack of difference in the rates of health risks between men and women exposed to IR.[24] In contrast, Anna *et al*., they found that sex-specific response to DNA damage in some organs was more pronounced in females than in males.[25]

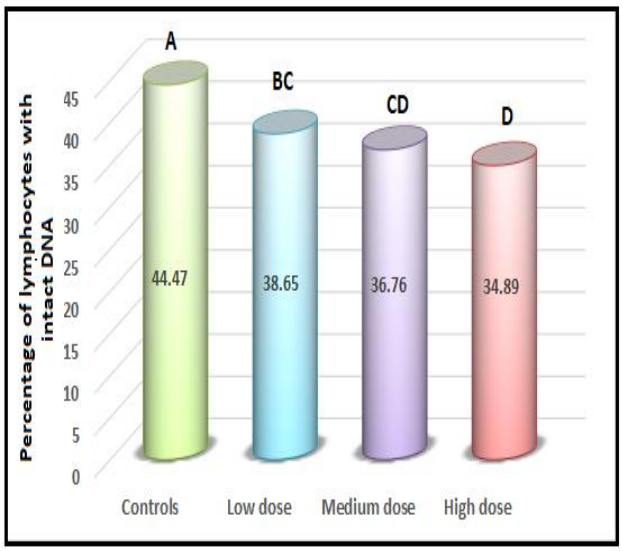

Figure 1: Percentage of lymphocytes with intact DNA in different groups.

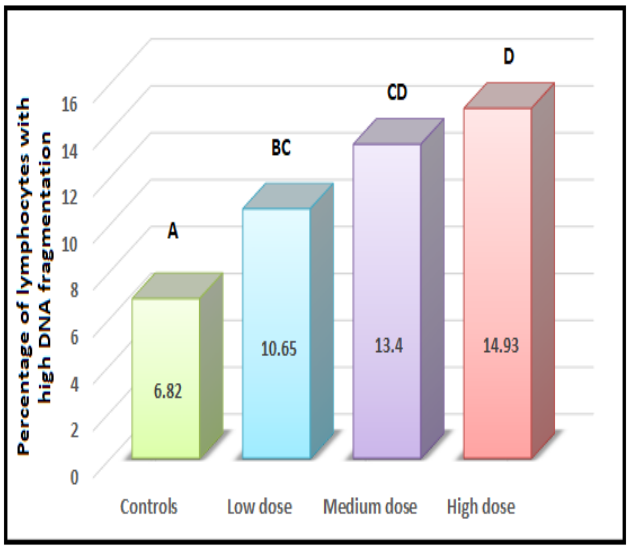

Figure 2: Percentage of lymphocytes with high DNA fragmentation in different groups.

Table 1: Quantitative analysis of tail length, DNA percent in the tail, and tail moment in samples exposed to different doses of radiation

Characteristics	Low dose (n = 30)	Medium dose (n = 30)	High dose (n = 30)	P-value
Tail length (µm)				
Mean ± SD	2.2 ± 1.74[a]	11.23 ± 3.88[b]	19.0 ± 5.5[c]	<0.001
Range	0-8.0	6.0-22.0	6.0-41	
DNA in the tail (%)				
Mean ± SD	1.34 ± 0.68[a]	4.92 ± 1.74[b]	16.97 ± 7.49[c]	<0.001
Median (range)	0-4.67	2.41-11.41	4.0-45.0	
Tail moment				
Mean ± SD	0.29 ± 0.16[a]	1.54 ± 0.57[b]	2.47 ± 0.98[c]	<0.001
Median (range)	0.-1.04	0.91-3.11	0.78-5.35	

Values are expressed as Mean ± SD: standard deviation. Different small letters indicate significant differences ($p<0.05$).

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Table 2: Parameters variation between males and females in the high dose group

Parameters	Male (n = 16)	Female (n = 14)	p-value
Percentage of lymphocyte with no fragmentation (%)	34.76 ± 1.55	35.1 ± 1.46	0.610
Percentage of lymphocyte with low fragmentation (%)	32.7 ± 2.08	32.74 ± 1.49	0.959
Percentage of lymphocyte with medium fragmentation (%)	17.58 ± 2.28	17.31 ± 2.42	0.760
Percentage of lymphocyte with high fragmentation (%)	14.96 ± 1.82	14.89 ± 1.46	0.908
Malondialdehyde (nmol/ml)	12.72 ± 1.26	12.32 ± 1.39	0.416
Tail length (μm)	18.31 ± 5.81	19.78 ± 9.24	0.601
DNA in the tail (%)	14.81 ± 4.4	19.43 ± 6.46	0.236
Tail moment	2.36 ± 0.75	2.58 ± 1.21	0.556

Conclusion

Exposure to diagnostic images including CT scan could be associated with DNA damage in a dose- and time-dependent manner. Also, oxidative stress as evidenced by MDA is proportionally associated with high doses of radiation and positively correlated with fragmentation parameters with no effect of gender on DNA fragmentation resulting from exposure to IR.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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