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DNA Methylation in Radiology Workers in some Hospitals of Baghdad

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Abstract

Background: DNA methylation, malondialdehyde (MDA) levels and comet assay are indicators of biological effects of ionizing radiation, and the Geiger-Muller counter is used to determine the annual effective dose in hospitals. Aims: To determine the effects of ionizing radiation on radiology workers by measuring the DNA methylation, malondialdehyde (MDA) levels, comet assay, and Geiger-Muller counter. Materials and Methods: eighty-one individuals were including in this study (forty-one from radiology workers who working in radiology departments in some hospitals of Baghdad, Iraq and forty from persons who not work in radiology department). Venous blood collected from the participants to determine the DNA methylation, malondialdehyde (MDA) levels and comet assay. Geiger-Muller counter was used to determine the indoor and outdoor annual effective dose obtained by placing the counter in operator and imaging room in x-ray, CT- scan and mammography imaging and catheterization section of hospitals. Results: it was fodeviceat in the radiation assessment the annual effective dose that the workers exposed for the outdoor radiation dose rate for ranged from 5.2 mSv/y to 22.1 mSv/y and the result for the indoor radiation dose rate from 20.6mSv/y to58.9mSv/y for background radiation (devise is switched off) But when the devise is switch on the indoor annual effective dose ranged from 44.2 to 106 mSv/y and the outdoor annual effective dose ranged From 12.5 to 640 mSv/y,which is exceed the permission levels of ICRP. For DNA methylation It has been found that workers have higher DNA methylation than control with p-value <0.001, methylation increases with ages for both groups, and there is no effect of gender on methylation. for malondialdehyde MDA were found that the workers recorded higher levels of MDA than non-workers with p-value<0.001, MDA increase with age for both groups, MDA levels increase as the employment duration increase for workers, MDA levels not effected by the gender with p-value 0.194.for comet assay the parameters(DNA in head, DNA in tail, tail length, tail area, comet length, comet height, tail moment) noticed that for workers have more damages (DNA fragmentation) than non-workers with p-value <0.001 unless for comet length where there is no significant effect with p-value 0.711,as the ages raised the DNA damage is increase for workers. Where the DNA in head is decrease with the ages,but for non- workers the DNA in the head Almost stay in one level with ages, the comet shows no effect of gender on DNA fragmentation with p-value ranged from0.333 to 0.884 for both groups. Conclusion: The present evaluation of the Geiger-Muller counter to count ionizing radiation in the radiology departments of hospitals shows an increase in the ionizing radiation annual effective dose that the radiology workers received more than the permission of International Commission on Radiological Protection, and this led to an increase in DNA damage for the tests (DNA methylation, Malondialdehyde MDA, Comet assay).

Keywords

Ionizing radiation, DNA Methylation, Malondialdehyde (MDA), comet assay, Geiger Muller.

Ionization occurs when high-energy radiation knocks electrons off atoms, resulting in the formation of ions. The ionizing region of the electromagnetic spectrum consists of gamma rays, X-rays, and ultraviolet light with a larger energy range. Ionization happens when an electron from an atom's electron shell is stripped (or"knocked out"), leaving the atom with a net positive charge. Exposure to ionizing radiation is thought to raise the risk of cancer because it can damage living cells and, more crucially, the DNA in those cells. Because of its high potential for biological damage,"ionizing radiation" is intentionally split from particle radiation and electromagnetic radiation. While each cell has trillions of atoms, only a small proportion of these will be ionized at low to moderate radiation levels [1]. The methylation of deoxyribonucleic acid (DNA) is essential in the life cycle, and the body is in a state of methylation equilibrium, which is an important guarantee for the proper control of gene expression and the stability of genetic material and other living activities [2][3]. DNA's biophysical properties are altered by the addition of CH3, which prevents some proteins from attaching to DNA while allowing others to do [4]. Ionizing radiation can alter the DNA's methylation patterns, including the global genome's DNA hypomethylation and gene promoter hypermethylation, which is linked to genomic instability and the activation of proto-oncogenes [2][5]. Several studies have suggested that ionizing radiation can indirectly ionize the water in cells to produce a significant amount of reactive oxygen species (ROS) such as superoxide anion radical (O_2), hydrogen peroxide (H_2O_2), hydroxyl radical (OH), nitric oxide (NO), and nitrogen dioxide (NO_2) [6] [7]. Increased DNA damage and changes in DNA methylation are linked to oxidative assault [8]. When the quantity of free radical formation exceeds the rate of elimination (or buffering) by cellular defense mechanisms, this condition is known as oxidative stress [9]. Malondialdehyde (MDA), an end product of lipid peroxidation, is one of the markers of oxidative damage [10]. ROS contributes considerably to cell damage by producing lipid peroxidation, protein modification, and DNA strand breakage, ultimately resulting in physical and chemical damage in tissues if not scavenged [11]. The comet assay is a sensitive and fast method for detecting DNA strand breaks in individual cells. Its popularity has skyrocketed in recent years [12].'The comet assay method is used to measure double-strand breaks,' for example, appears far too frequently in publications and presentations. There is a widespread belief that alkaline conditions are required to reveal single-strand (SS) breaks, and that using a pH close to neutral ensures that only double-strand (DS) breaks are detected. A period of alkaline treatment in these allows the DNA strands to separate, beginning at the sites of breaks, which can be either SS or DS. In the case of alkaline elution, the smaller the pieces of unwound DNA are, the more likely they are to pass through the filter. The alkaline unwinding method uses a high pH for a short period of time to partially unwound the DNA, with the extent of strand separation determined by the number of breaks present. After neutralization, a mixture of SS and DS DNA is formed, with the proportion of SS DNA reflecting the frequency of break. The DNA in a living cell is organized in nucleosomes, and the winding of the DNA around the histone core causes the (negative) supercoiling; the double helix is underwound, with fewer than the 10 bases per turn found in a relaxed molecule. Most histones are removed, and nucleosomes are disrupted after lysis in 2.5 M NaCl, but the DNA remains supercoiled. In comparison to the homogeneous staining of neutral comet tails, the DNA in an alkaline comet tail appears granular, as if DNA fragments are present. If two breaks occur within one strand of a loop, fragments will form (a possibility at high levels of damage) [13].

Material and Method

The present study is a retrospective cohort that was conducted in the Department of Physiology and Medical Physics at the College of Medicine/Al-Nahrain University and was comprised of 81 individuals (41 radiology workers and 40 people who did not work in the radiation field).Samples were collected from May 2022 to July 2022.This study reviewed data on workers in two hospitals of the Medical City Hospital in Baghdad (Baghdad Teaching Hospital,oncology teaching hospital)and AL-Imamian AL-Kadhimiyain medical city who have a history of occupational exposure to radiation, with information taken including (gender, age,type of device they working with).The 41 samples were collected from radiation workers at X-ray rooms, CT-scan rooms, catheterization rooms and Multiroom. The samples of control were collected randomly and

excepted the radiology departments of 40 individuals, who were matched by age and gender with radiology workers.

Radiation Measurement:

The radiation has been measured in various places, namely the operator's room, the radiation chamber and at the door of the radiation chamber during the operation of the device using the Geiger Muller counter radiation detector.

Methylation measurements

The determination of the relative prevalence of a particular pattern of methylated CpG dinucleotides in vertebrates is of particular interest in epigenetics research. MethyLight assays, probe-based real-time PCR for methylation analysis, are often used for sensitive quantification of the methylation pattern. depending on the level used for sequence discrimination.

The 2x EpiTect MethyLight Master Mix (w/o ROX), 50x ROX Dye Solution, primer and probe solutions, RNase-free water, and converted DNA.the solutions were Mixed individually(For the reaction, 2x EpiTect MethyLight Master Mix (w/o ROX) (25 L) was mixed, 50x ROX Dye Solution is 1 µL,10x primer-probe was (5 µL) with 0.4 µM forward primer, 0.4 µM reverse primer and 0.2 µM probe, RNase-free water is variable, Template of DNA is variable that was entered into PCR, Cycling conditions for quantitative methylation PCR analysis.

Malondialdehyde (MDA)

The NWK-MDA01 assay is based on the reaction of MDA with thiobarbituric acid (TBA); forming an MDA-TBA2 adduct that absorbs strongly at 532 nm. Ten microliter of BHT Reagent were added to microcentrifuge vial, Added 250 µL Acid Reagent to vial, Added 250 µL TBA Reagent to vial, then Vortex vigorously (5-count) then Incubated 60 minutes at 60°C,Centrifuge it at 10,000 x g for 2-3 minutes, then transferred the reaction mixture to well plate 99, Finally, the samples were added to the mixture in a well plate before inserting it into the GlowMax reader and calibrating the device's wavelength, which ranges from 400 to 700 nm. The result shown by the device is the MDA concentration in mmol/L.

Comet Assay Method

In the first step, 5 mL of diluted PBS were added to 500 µL of blood sample, then the solution was separated in a centrifuge for 10 minutes (2500 rpm), after that a part of the solution was thrown away and a small amount was left at the end of the tube. Again, 5 mL of diluted PBS were added to the rest of the amount in the tube. It was then put in an incubator at 37°C for 5 minutes, and the cells were put in a centrifuge (2500 rpm) for 10 minutes. A part of the solution was thrown away, and a small amount was left at the end of the tube. The diluted R-lysis solution (5 mL) was then added to the cells, where the cells then left for 5 minutes and centrifuged at 2500 rpm for 10 minutes. After that, the cells were left for 24 hours. LMAgarose was melted in a beaker of boiling water for 5 minutes with the cap loosened. The bottle was placed in a 37 °C water bath for at least 20 minutes to cool. one hundred Microliter from LMAgarose and 100 µL from cells were taken and mixed in a microtube. They were mixed gently by pipetting once or twice, and the mixture was speared onto a slide. Then 75µL aliquots were transferred onto each sample area as required. The slides were placed in a deep freezer to cool (-20 °C) for 20 minutes. A 0.5-mm clear ring appears at the edge of the slide area. The slides were immersed in prechilled Lysis Solution for two hours. The excess buffer was Taped off from slide and immersed in freshly prepared Alkaline Unwinding Solution, pH>13. The slides were left in an alkali unwinding solution at room temperature for 20 to 60 minutes. The slides then were removed from alkaline solution and taped gently the slides from the excess buffer and washed by immersing in TBE buffer for 5 minutes. The slides were transferred from the TBE buffer to a horizontal electrophoresis apparatus at a level that immersed the slides in the container of the electrophoresis apparatus, and then the voltage was calibrated to 75 V and the current to 300 mA for 30 minutes for each of the four slides. The slides were then dipped in 70% ethanol for 5 minutes. Air dry samples. Drying brings all the cells in a single plane to facilitate observation. Samples may be stored at room temperature. Diluted ethidium bromide (EtBr) was placed into each dried samples and stained for 30 minutes at room temperature. Intensity of staining can be visualized under the microscope using 10X objective, and reaction stopped when comets are easily visible.

Data Analysis

In healthy cells, the DNA is confined to the nucleoid: undamaged DNA is supercoiled and thus does not migrate very far under the influence of an electric current. In cells that have accrued damage to the DNA, the alkali treatment unwinds the DNA, releasing fragments that migrate from the nucleoid when subjected to an electric field. The negatively charged DNA migrates toward the anode and the extrusion length reflects increasing relaxation of supercoiling which is indicative of damage.When using alkaline electrophoresis conditions, the distribution of DNA between the tail and the head of the comet should be used to evaluate the degree of DNA damage. The characteristics of the comet tail including.Length, width, and DNA content may also be useful in assessing qualitative differences in the type of DNA damage.

Results and discussion

Results

Case study

Table 1: Baseline demographic characteristics of the study's sample (n=81)

Study groups of the collected dataset (Age and Gender)				
	Female Workers (n=25)	Male Workers (n=15)	Female Non-workers (n=27)	Male Non-workers (n=13)
Mean± SD	35.38±6.98	37±7.67	35.23±7.02	36.89±7.74
Range (Min-Max)	30 (22-52)	31 (25-56)	30 (22-52)	31 (25-56)
P-Value	0.267		0.606	

Comet assay

Table 2 indicates that there is a significant difference exists among workers and non-workers with p-value less than 0.05 except the p-value of comet length shows no significant difference exists.

Table 2: Two independent sample T-test between workers and non-workers for comet assay test.

	Study groups (Mean± SD)		P-Value
	Workers (n=41)	Non- workers (n=40)	
Comet Length	40.6±14.3	41.1±6.5	0.851
Comet Height	27.36±6.2	33.58±5.9	<0.001
DNA in Head	73.68±12.78	90.93±12.11	<0.001
Tail Length	8.51±5.54	1.97±3.14	<0.001
Tail Area	193.29±146.17	44.3±75.8	<0.001
DNA in Tail	26.31±12.78	9.06±12.11	<0.001
Comet Area	845.53±432.21	1074.1±226.2	<0.001
Tail Moment	2.57±2.32	0.433±1.087	<0.001

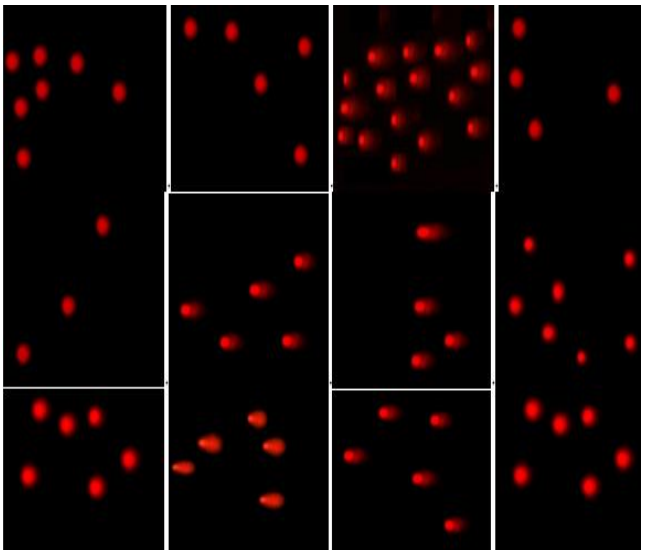

Figure 1: comet score images for all samples (controls and workers)

From figure (1) comet pictures of workers and non-workers shows the contrast between the two groups.

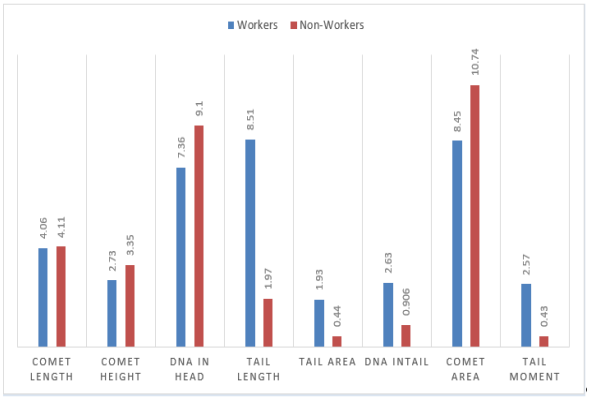

Figure 2: Normalized mean values of study groups between workers and non-workers.

From figure (2) comet height, DNA in head and comet area in workers lower than the same parameters of non – workers but tail length, tail area, DNA in tail and tail moment in workers higher than the same parameters in non-workers, but comet length shows approximately the same results.

The effect of gender on comet results

Table 3: ANOVA test among (female workers, male workers, non-female workers, and non-male workers) of the study's sample (n=81)

	Female Workers (n=26)	Male Workers (n=15)	Female Non-workers (n=27)	Male Non-workers (n=13)	P-Value
Comet Length	39.36±14.36	40.86±13.38	42.77±8.28	39.61±6.04	0.711
Comet Height	26.36±6.47	28.13±4.92	33.81±5.13	33.69±7.59	<0.001
DNA in Head	74.76±13.79	72.04±11.61	90.31±13.17	90.7±10.95	<0.001
Tail Length	8.32±5.9	8.6±5.2	2.55±3.82	1.53±2.66	<0.001
Tail Area	168.98±125.56	208.6±149.9	65.48±126.47	40.92±75.73	<0.001
DNA in Tail	25.23±13.79	27.95±11.61	9.68±13.17	9.29±10.95	<0.001
Comet Area	816.4±445.05	832.86±363.65	1104.14±251.45	1064.8±250.35	<0.001
Tail Moment	2.5±2.47	2.62±2.2	0.59±1.29	0.32±0.88	<0.001
Malondialdehyde	2.95±0.54	3.16±0.55	0.71±0.39	0.7±0.11	<0.001

Table (3) demonstrates that the ANOVA test has indicated that a significant difference exists among the variances of the groups (female workers, male workers, non-female workers, and non-male workers) where p-value is<0.001 except for comet length is (0.711) that shows no significant difference exists.Thus, Post hoc test is required to perform on all possible comparisons, as demonstrated in Table 4.

Table 4: Post hoc test to determine a comparison among (female workers, male workers, non-female workers, and non-male workers) and malondialdehyede

	P-value		
	Female Workers, Male Workers	Female Workers, Female Non-workers	Male Workers, Male Non-workers
Comet Height	0.368	<0.001	<0.001
DNA in Head	0.517	<0.001	<0.001
Tail Length	0.856	<0.001	<0.001

Tail Area	0.333	<0.001	<0.001
DNA in Tail	0.517	<0.001	<0.001
Comet Area	0.884	0.004	0.038
Tail Moment	0.850	<0.001	<0.001
Malondialdehyde	0.142	<0.001	<0.001

Table (4) (post hoc) indicates that there is no significant difference exists among the variances of the groups of female and male (workers) and there is no significant difference exists among the variances of the groups of females (workers and non- workers), there is no significant difference exists among the variances of the groups of males (workers and non-workers).

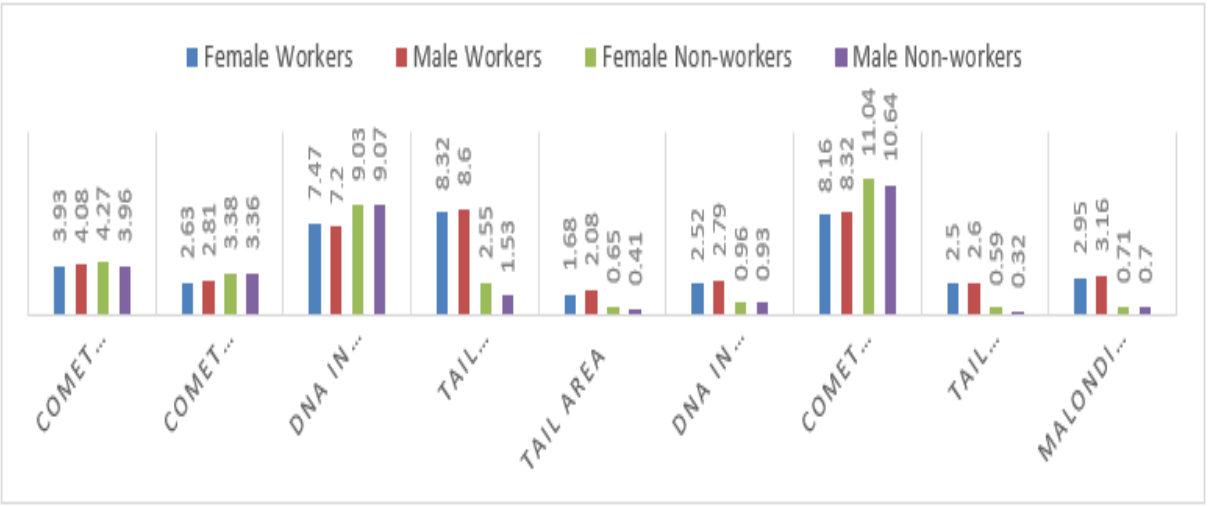

Figure 3: Normalized mean values of (female workers, male workers, non-female workers, and non-male workers) groups

The effect of age on comet results

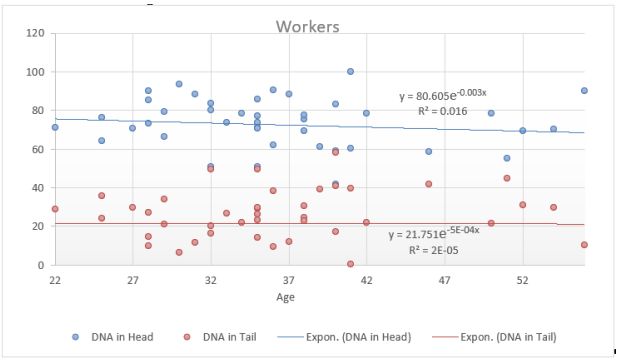

Figure 4: Scatter plot of DNA in head and DNA in tail related to ages of workers.

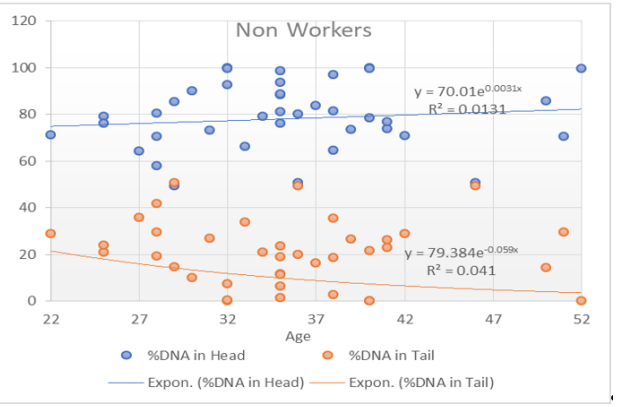

Figure 5: Scatter plot of DNA in head and DNA in tail related to ages of non- workers.

The figures (4) are shows that the DNA in tail is shows no significant difference exists but DNA in head shows decrease as the ages is increase for workers.

Figure (5) shows that the DNA in tail shows decrease as the ages is increase but the DNA in head shows increase as the ages is increase for non- workers.

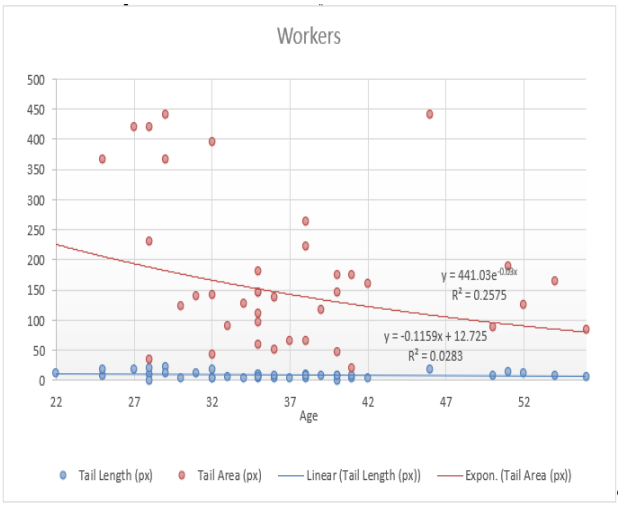

Figure 6: Scatter plot of tail length and tail area related to ages of workers.

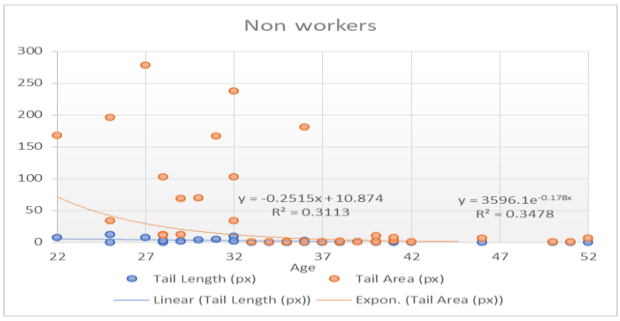

Figure 7: Scatter plot of tail length and tail area related to ages of non- workers.

Figure(6)is shows that the tail area is decrease as the ages are increase but the tail length shows no significant difference exists for workers, Figure(7)shows that the tail area is decrease as the ages is increase but the tail length shows no significant difference exists for non-workers.

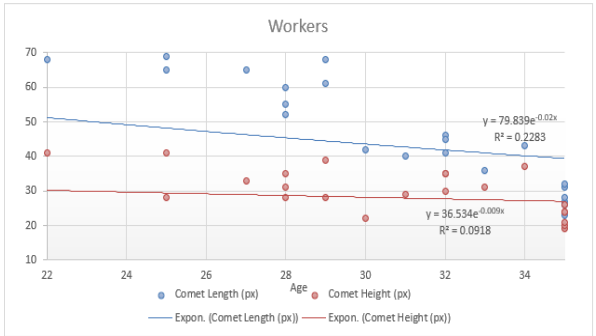

Figure 8: Scatter plot of comet length and comet height in workers.

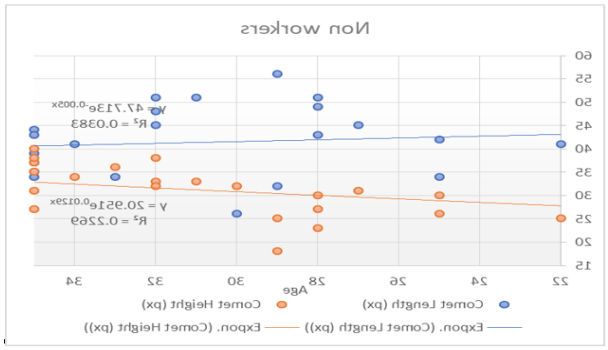

Figure 9: Scatter plot of comet length and comet height in non- workers.

Figure (8) shows that the comet length is decrease as the ages are increase and the comet height is decrease as the ages is increase.

Figure (9) shows that the comet length decrease as the ages is increase but the comet height is increase as the ages is increase.

MDA

The scatter plot shows a positive correlation between the MDA and the employment period.

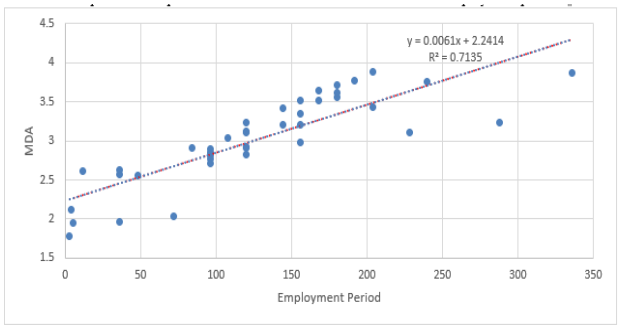

Figure 10: Scatter plot of MDA (mmol/ L) to the employment period (months), showing mean of MDA as the dotted red line.

From Figure (10) there is a significant difference exists, where MDA is increase as the employment period is increase.

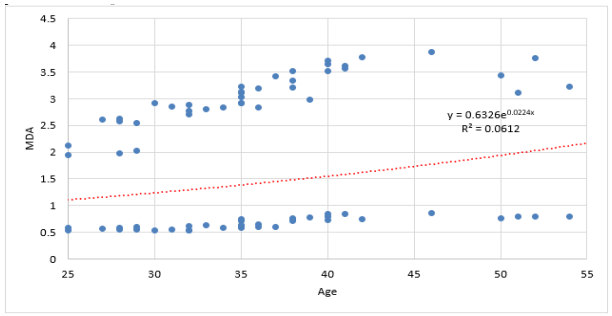

Figure 11: Scatter plot of MDA (mmol/ L) to the age (years), showing mean of MDA as the dotted red line.

Figure 11 shows that MDA increase as the ages of

workers and non-workers is increase.

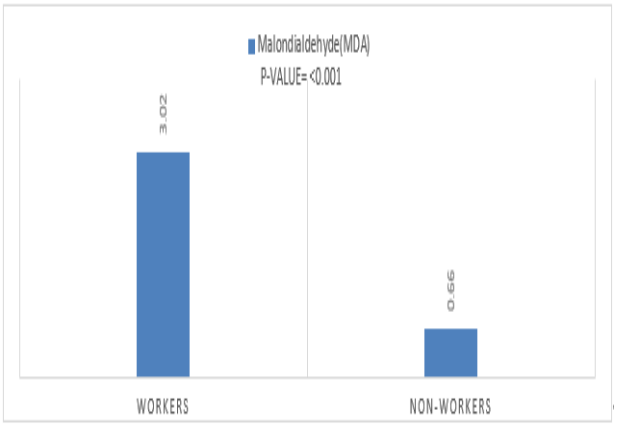

Figure 12: mean values of malondialdehyde between workers and non-workers

From figure (4-12) MDA in workers is higher than non-workers with p-value <0.001.

Table 5: Two independent sample T-test of malondialdehyde (MDA) between female workers and male workers

G	Mean	P-Value
Female worker (n=26)	2.93±0.53	0.194
Male worker (n=15)	3.16±0.55	

Table 6: the result of OAED and AIED that the workers are received annually and the hospitals measured in.

switched on		switched off			
IAED in mSv/y	OAED in mSv/y	IAED in mSv/y	OAED in mSv/y	Type of device	Hospitals
44.2	46.4	20.6	5.2	Ct scan	Al Imamain Al-Kadimain Medical City
111.84	67.7	41.2	10.3	X-ray (room 1)	Al Imamain Al-Kadimain Medical City
594.6	148.6	47.1	10.3	X-ray (room2)	Al Imamain Al-Kadimain Medical City
50	12.5			mammogram	Al Imamain Al-Kadimain Medical City
44.2	44.9	29.4	11.8	Ct-scan	Baghdad Teaching Hospital
88.3	44.2	58.9	17.7	x-ray (room1)	Baghdad Teaching Hospital
58.9	19.1	58.9	22.1	x-ray (room2)	Baghdad Teaching Hospital
94.2	32.4	29.4	11.8	Ct-scan	Baghdad Teaching Hospital(emergency)
58.9	66.2	58.9	14.7	x-ray	Baghdad Teaching Hospital(emergency)
106	674.0	44.2	13.2	Ct-scan	Teaching oncology hospital

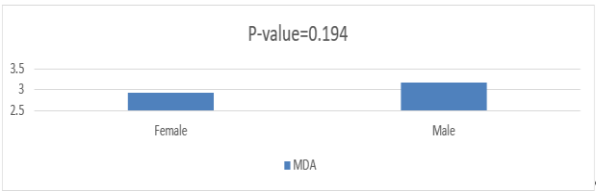

Figure 13: MDA (mmol/ L) for male and female (workers)

Table (5) shows no significant difference exists among female and male (workers) with p-value more (0.194) which is more than 0.05.

For figure (13) even not significant difference among females and males (workers) and these difference shows in this figure due to the convergent scales.

Geiger Muller-counter

Table 7: Baghdad Teaching Hospital (catheterization), the OAED and IAED that the workers received annually.

Type of mode	OAED in msv/y	IAED in msv/y	IAED in msv/y
fluoroscopic	0.5	5	72.8
Cine mode	1.8	6.2	855.1

Where IAED is the indoor annual effective dose and OAED is the outdoor annul effective dose.

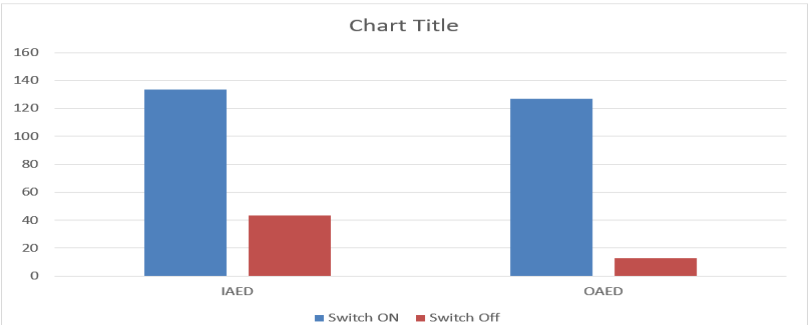

Figure14: mean values of IAED and OAED in (Switched ON and Switched off) states (when the CT-scan, x-ray, mammogram, catheterization machines is energized or not)

The recommended dose limit s by international commission on radiological protection (ICRP) for the occupationally exposed workers annually of effective dose is 20mSv and the average over defined periods of 5 years with no single year >50 mSv.

Methylation

Table 8: Two independent samples T-test of malondialdehyde and methylation between workers and non-workers

Study groups (Mean± SD)			P-Value
	Workers (n=41)	Non- workers (n=40)	
Malondialdehyde	3.02±0.544	0.66±0.106	<0.001
Methylation	52.2±26.36	13.25±11.3	<0.001

Table 8 shows that there is a significant difference exists among workers and non-workers for MDA and methylation with p-value less than 0.05.

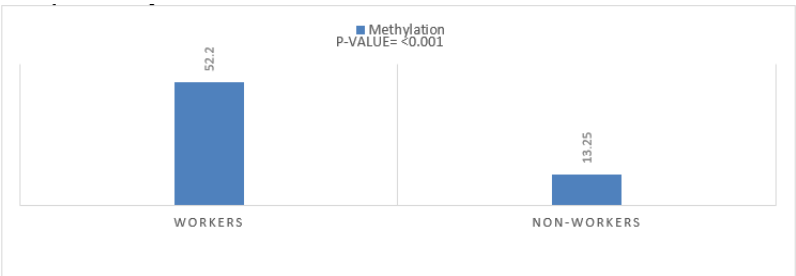

Figure 15: mean values of methylation between workers and non-workers.

Figure (15) shows that there is a significant difference exists among workers and non-workers for methylation with p-value less than 0.05.

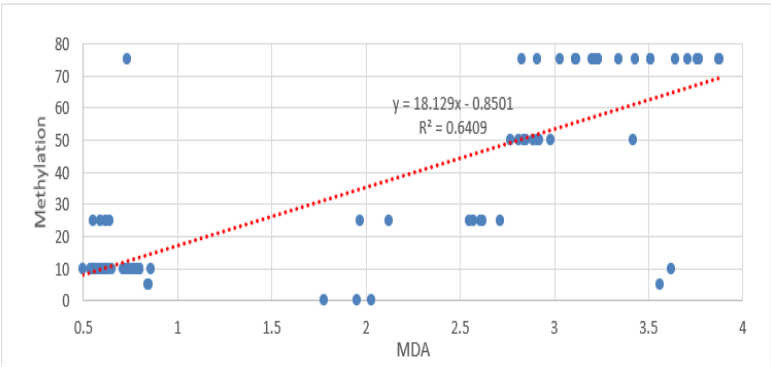

Figure 16: Scatter plot of methylation and MDA (mmol/ L). showing the meaning of MDA as the dotted red line.

From figure (16) methylation is increase as the MDA is increase.

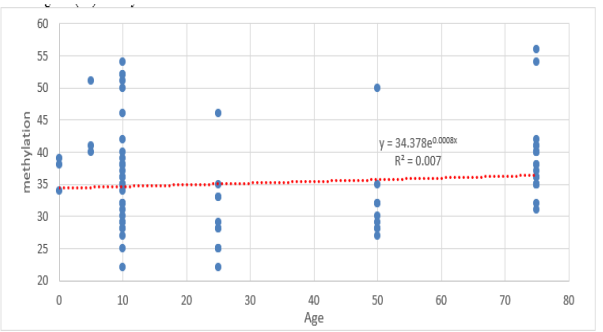

Figure (17) Scatter plot of methylation to the age (years), showing mean of methylation as the dotted red.

Figure (17) demonstrate that methylation is increase as the ages (workers and non-workers) are increase.

Table (9) Two independent sample T-test of employment period (Months) between female workers and male workers.

G	Mean	P-Value
Female worker (n=26)	112.15±65.07	0.09
Male worker (n=15)	153.60±85.84	

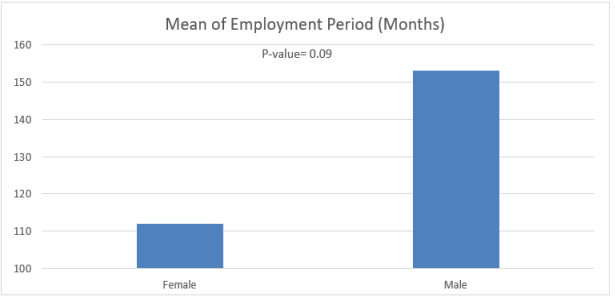

Figure (18) mean of employment period (months).

Table 9 shows no significant difference exists among workers and non-workers with p-value more than 0.05.

For figure 18 even not significant difference among females and males (workers) and these difference shows in this figure due to the convergent scales.

Discussion

The effect of ionizing radiation on radiation workers

The radiation is rising the risk of cancer of the radiation workers which that what the studies showed in South Korean radiologic technologists [14]. The dose limits preferred by the ICRP are as follows: For occupational exposure, the annual effective dose should not exceed 20 mSv, and the public should not be exposed to more than an average of 1 mSv per year [15]. in our study, the outdoor radiation dose rate ranged from 5.2 mSv/y to 22.1 mSv/y and the result for the indoor radiation dose rate from 20.6mSv/y to58.9mSv/y for background radiation (devise is switched off). The obtained results in our study were higher than those obtained in Kwali General Hospital [16] and higher than the annual average dose limited by ICRP. And when the devise is switched on the indoor annual effective dose ranged from 44.2 to106 mSv/y and the outdoor annual effective dose ranged from 12.5 to 640 mSv/y, which also exceed the permission levels of ICRP and agreed with a study performed in the Eastern Province, Saudi Arabia by Salama KF et al. [17].

DNA methylation measurement

The ionizing radiation effect on the generation of genomic instability implies the participation of epigenetic processes, such as alterations in cytosine methylation within CpG dinucleotide within the DNA [18]. In our study, methylation was higher in workers exposed to ionizing radiation than in controls, with a (p-value<0.001), indicating that the workers were affected by IR. A study approves with our study which organized in Sweden by Anna Danielsson et al. [19]. A significant correlation between methylation and MDA was displayed in our study, (MDA)an oxidant stress; in present research investigated the relationship between them and discovered that when MDA increased, methylation increased due to its role.

MDA measurements

According to the current study, MDA levels rise with age in both groups (workers and non-workers) with a p <0.001.and demonstrates that there is a significant association between employment duration and MDA, with the MDA increasing as the employment duration increases. The current study agrees with an Egyptian study in Zagazig university hospitals by Bolbol. SA et al. [20] and disagreeable with Eken in Turkey who took

samples from 40 exposed radiology staff in different hospitals in turkey and 30 control subjects.in the exposed group the MDA levels were substantially lower than in the controls [21].

Comet assay

The present study found that all parameters have a significant difference with p –value <0.001 between worker and non-workers except for comet length with p-value 0.851 and no relation between gender and DNA damage and established a link between age and DNA damage in the comet assay. And found that as workers' ages increased, so did their DNA damage. For non-workers the DNA in the head almost stay constant with ages, but in workers the DNA in head decreases due to fragmentation of DNA that accumulates in the tail, which is the main indicator of DNA damage, The more DNA in the head, the more the cell is healthier.

Conclusion

The present evaluation of the Geiger-Muller counter to count the IR in the radiology departments of hospitals shows an increase in the IR annual effective dose that the radiology workers received, and this led to an increase in DNA damage according to (DNA methylation, Comet assay) and an increase in MDA levels as compared with control for all tests.

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