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INFLUENCE OF RADIATION EXPOSURE ON DETOXIFICATION GSTM1 ENZYME IN AL-HUSSEIN TEACHING HOSPITAL WORK PLACE

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Abstract:

Radiation is un energy that travels in the form of waves or particles and is part of our everyday environment. People are exposed to radiation from cosmic rays, as well as to radioactive materials found in the soil, water, food, air and also inside the body.

Human-made radiation sources are widely used in medicine, industry, and research. There are two types of radiation: ionizing and non-ionizing radiation that effected on detoxification enzyme content which is necessary for proper cell to remove toxicity of this materials .The objective of this study was to evaluate the effect of Radiation on Detoxification enzyme content in workers and non- workers in al- Hussein teaching hospital.: GSTM1 quantified by gel electrophoresis in the blood of 79 workers and 521 non-workers. Blood DNA fragmentation was analyzed employing the terminal deoxynucleotidyltransferase-mediated dUTP nick-end labelling (TUNEL) assay The oxidative stress, reactive oxygen species (ROS), gender and age were significantly higher (P≤0.010) in workers than in non-workers,

High prevalence of Radiation that are associated with this phenotype, it is relevant to examine the pathway of *GSTM1* in order to determine effective treatment option. The detoxification gene, which is one of the main gene that have been linked to the detoxification GSTM1 phenotype in human. Mutation of the GSTM1 occurs by radiation, Therefore, the present study aimed to explain the influence of Radiation and the role of GSTM1 gene according to some criteria (gender, age, location and period of exposure). The current study included 600 workers at al-Hussein teaching hospital (20-60 years) with highest *GSTM1* lacking of espouse radiation (80%) compare with non-exposure 48%at the level of significant (P≤0.05). Current study showed also, delete that genes about ten times in exposure male workers compare with female exposure.. When samples are divided into old expousre and new one, the results Showed That old are subject to gene deletion more Than the new ones**.** The results of the current study showed The highly percentage of gene deletion in radiation unit x- ray (exposure cases) 49.4% compare with sonar-exposure groups 33.2% and MRI (17.4%)with significant different at (P≤0.05) Results statically analysis have been shown significant different at (P≤0.05). gene deletion increased about (4.33) four times exposure compare with others- exposure group OR=4.33,95% (CI=1.78-10.52)**.**

Keywords: Radiation / Detoxification / GSTM1 / PCR / x-ray

1.INTRODUCTION

Radiation influence is highly spread in the world. There are four major types of radiation: alpha, beta, neutrons, and electromagnetic waves such as gamma rays. They differ in mass, energy and how deeply they penetrate people and objects[2] . The last kind of radiation is electromagnetic radiation, like X-rays and gamma rays. They are probably the most familiar type of radiation because they are used widely in medical treatments. These rays are like sunlight, except they have more energy[1] . Unlike the other kinds of radiation, there is no mass or charge. The amount of energy can range from very low, like in dental x-rays, to the very high levels seen in irradiators used to sterilize medical equipment [4]

X-ray is a kind of electromagnetic waves that can create picture of the inside of body and also is a form of ionizing radiation which means they can cause natural atoms to eject electrons when they interact with matter .Different tissues absorb different amount of x-ray ,so bones look white and soft tissues look black or gray on x-ray image[3] .

X-rays are essential to medicine because of their ability to use light rays to produce images of the human bone. In 1895, German physicist Wilhelm Rontgen first discovered the X-ray by experimenting with cathode rays. The radiological density of these solids determines whether the electron beams pass through the material or not; therefore, the light rays pass through skin tissue (less dense) and are absorbed in the bones (very dense). [6]
. The principle concern includes excess radiation exposure; intense radiation can cause cell mutations. This is especially dangerous for pregnant women, whose fetus is still developing. In the case of dental X-rays, the possibility of a pituitary or thyroid link in low birth weight infants owing to maternal exposures to low levels of dental X-rays justifies the scrutiny of exposures in maxillofacial imaging. By understanding the risks of radiation exposure, it is possible to understand methods used to prevent it. [5]
Radar and Sonar Although they rely on two fundamentally different types of wave transmission, Radio Detection and Ranging (Radar) and Sound Navigation and Ranging (Sonar) both are remote sensing systems with important military, scientific and commercial applications**[8]** . Radar sends out electromagnetic waves, while active Sonar transmits acoustic (i.e., sound) waves. In both systems, these waves return echoes from certain features or targets that allow the determination of important properties and attributes of the target (i.e., shape, size, speed, distance, etc.) **[7]** . Because electromagnetic waves are strongly attenuated (diminished) in water , Radar signals are mostly used for ground or atmospheric observations. Because Sonar signals easily penetrate water, they are ideal for navigation and measurement under water **[8;9]**.
The highly radiation does not protect the DNA from oxidative stress and other internal or external factors [11] and does not protect the genetic material during transfer through the male and female reproductive tracts (reviewed by [13]. A defect in DNA bulding and disulphide bridge formation due to inadequate oxidation of thiol groups will negatively affect [12] . Oxidative stress is caused by an imbalance between the production of ROS and antioxidant capacity of the cell [2 and 1].
Oxidative stress appears to be the major cause of DNA damage in the male germ line [14;15]. Furthermore, many studies have indicated a significant correlation between DNA damage and high levels of ROS in infertile patients [11;16].
. The exact causes of this DNA damage are still unclear but the major candidatesare oxidative stress and aberrant apoptosis [14] . The precise etiology of DNA fragmentation is still poorly understood, but a relationship between Radiation expousre and increased DNA damage in workers compared with non-workers has been demonstrated[13] .
Several investigators have now found a link between oxidative stress and sperm DNA damage (reviewed by [4].
X_Ray has been associated with significantly increased levels of ROS, which cause oxidative stress [5] . X_Ray may induce alterations of the sperm plasma membrane and cause a high degree of DNA fragmentation[7] . Recent studies have shown that the Influence of Radiation on workers have significantly higher levels of DNA fragmentation than those of non-workers
The purposes of the present study were to determine the effect of Radiation on Detoxification enzyme by directly quantifying Radiation concentrations in workers and non- workers ,and to evaluate the relationship between GSTM1 and oxidative stress.
Magnetic resonance imaging (MRI) is a medical imaging technique used in radiology to form pictures of the anatomy and the physiological processes inside the body. MRI scanners use strong magnetic fields, magnetic field gradients, and radio waves to generate images of the organs in the body. MRI does not involve X-rays or the use of ionizing_radiation, which distinguishes it from computed tomography (CT) and positron emission tomography (PET) scans. MRI is a medical application of nuclear magnetic resonance (NMR) which can also be used for imaging in other NMR_applications, such as NMR_spectroscopy.

MRI is widely used in hospitals and clinics for medical_diagnosis, staging and follow-up of disease. Compared to CT, MRI provides better contrast in images of soft tissues, e.g. in the brain or abdomen.

2.MATERIALS AND METHODS

2.1.Subjects

A total of 600 blood sample from workers partners of couples facing radiation who attending assisted laboratory at the AL- hussein hospital teaching, radiation unit were included in this study. The samples included 521 non-workers79 workers. Information regarding gender, exposure periods, smoking, age and occupational exposures was obtained from a questionnaire.... This stringent selection was done to exclude as many known co-existing factors as possible from the study groups, since we aimed to study the impact of radiation affection on specific aspects of detoxification gstm1 characteristics

2.2.Blood collection and preparation

Only one sample per workers was included in the study.. Samples were collected in sterile EDTA-GEL tube under -20 according to World Health Organization[13] Briefly, DNA samples were examined for PCR technique

2.3.Assessment of Radiation Expousre

by chromomycin A3 (CMA3) staining as previously described by[8]). Fluorochrome was examined using a Zeiss photomicroscope III using acombination of exciter dichromic barrier filter of BP 436/10: FT 580:LP 470.

2.4.DNA fragmentation analysis DNA fragmentation was assessed using the terminal deoxyribonucleotidyltransferase-mediated dUTP nick-end labelling (TUNEL) assay as previously described [8]. The TUNELassay was performed using the in situ Cell Death Detection Kit following the manufacturer's guidelines (Roche Diagnostics GmbH, Mannheim, Germany).

2.5.Extraction of DNA blood sample by(DNAExtraction Kit,Gene aid,Korea and PCR by[17]

2.6. ROS measurement

The concentration of ROS was measured by a colourimetric assay for the quantitative determination of peroxides in EDTA–plasma, serum and other biological fluids using Enzyme linker immunosorbant assay (ELISA) kit (Oxy Stat; Cat. No. BI-5007 Biomedica Medicine product GmbH & Co KG, Wien, Austria) as previously described by [6]

3.STATISTICAL ANALYSIS

Data from 79 workers and 521 non- workers were expressed as mean+SD and statistically analyzed using SPSS v. 17.0 for Windows Software Package (SPSS Inc., Chicago, IL, USA). The Kolmogorov–Smirnov test was used to verify a normal or non-normal distribution of values. The non-parametric Mann–Whitney U-test was used to examine differences between samples from workers and non- workers, and Spearman's test was used to calculate the correlations. The probability value of P , 0.050 was considered significant .

4. RESULTS

4.1 .Radiation Distribution. Prevalence of Radiation among hospital workers in AL-hussien Teaching hospital was 600, age between 20-60 years, female number was 288. While male was 312 ,prevalence of exposure cases was higher in male (14.2%) compare with female (11.8) as show in table 1.

Table 1:Prevalence of radiation exposure among hospital workers in al-Hussein teaching hospital according to gender

Gender	No. hospital workers	No. of non-exposure cases	No. of exposure cases	Prevalence ratio
Female	288	254	34	%11.8
male	312	257	45	%14.2
Total	(%100) 600	521	79	%26.0

4.2 .Radiation Distribution : Data analyses has been shown that significant between workers and control groupe,gstm1 deletion about ten times (OR=10) highest *GSTM1* lacking of espouse radiation (80%) compare with non-exposure 48%at the level of significant (P≤0.05)

Table 2: Genotypes Distribution of *GSTM1* gene samples hospital exposure case to workers comparison with control

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Genotypes GSTM1	control	Exposure workers	OR	95%CI*
Present(31)	16 (20 %)	56(52. %)	1.0	_____
Absent(48)	63(80%)	23(48.0%)	4.33*	1.87-10.52

4.3.Radiation exposure prevalence

The results of the current study showed The highly percentage of gene deletion in radiation unit (exposure cases)x-ray 58.3% compare with sonar-exposure groups 25.0% and MRI (17.4%)with significant different at (P≤0.05) Results statically analysis have been shown significant different at (P≤0.05). gene deletion increased about (4.33) four times in x-ray exposure compare with others- exposure group OR=4.33,95% (CI=1.78-10.52)**.** as show in Table 3. There is a significant different at (P≤0.05).

Table 3: Genotypes Distribution of *GSTM1* gene samples hospital exposure cases according to occupation (Exposure kinds).

Genotypes GSTM1	x-ray-exposure	Sonar-expousre	MRI exposure	OR	95%CI*
Present(31)	8 (25.6%)	14(45.3%)	9(29.1%)	1.0	_____
Absent(48)	28(58.3%)	12(25.0%)	8(17.4%)	4.33*	1.87-10.52

Magnetic resonance imaging= (MRI)

CI= 95% Confidence Interval %95 OR =Odd ratio

4.4. Gender

When compare between male and female, the gene absent twice time in female as shown in Table 6.

Table 4: Genotypes Distribution of *GSTM1* gene samples hospital expousre cases according to exposer genders.

Genotypes GSTM1	male-exposure	female exposure	OR	95% CI*
present	14 (43.7%)	18 (40%)	1.0	_____
Absent	20 (58.8%)	27 (60%)	2.30*	2.67-9.44

CI= 95% Confidence Interval %95 OR =Odd ratio

4.5. exposure period.

A correlation between the genotypes of the GSTM1 gene and the periods of radiation for radiation units (exposure cases) that have exposure more than 10 years compare with that have exposure less than 10 years, as the results show significant difference (OR=10,95% CI=2.501-0.02) between two different periods, gene deletion increased about 10 times as shown in table5.

Table 5: Genotypes Distribution of *GSTM1* gene samples hospital expousre cases according to exposer periods.

Exposure period	*Absent GSTM1*	*Present GSTM1*	OR	CI %95
(1-5)	11(22%)	12(41.3%)	1.0	_____
(6-10)	17(34 %)	8(28.5%)	1.07	0.07-.11.99
(11-15)	22(44 %)	9(32.2%)	10. 303	0. 02-2.501

4.6.Correlations between Radiation and GSTM1 activity

The mean x-ray levels were not found to be significantly (P . 0.050) correlated with absent GSTM1 and DNA fragmentation (TUNEL positive) (r ¼ 20.168, r ¼ 20.137, respectively). In addition, sonar levels showed a negative correlation with absent GSTM1 (r ¼ 20.209, P , 0.050) but DNA fragmentation was not correlated (r ¼ 20.036, P . 0.050). X-RAY/SONAR ratios showed a significant positive (P , 0.050) correlation with absent GSTM1 (r ¼ 0.183), but correlation with DNA fragmentation (Table 6). Significant positive correlations (P , 0.01) were also observed between X-RAY and sonar (r ¼ 0.623)

Table 6; Correlation of radiation and DNA fragmentation with oxidative stress parameters of workers samples (n 5 116).

Parameters	Exposure workers	Non-Exposure workers	Gstm1 phenotype	P-value AND CORRELATION
gender	-0.168 0.075	-0.209* 0.026	0.181 0.051	*r* *p*
DNA fragmentation (positive TUNEL) (%)	-0.137 0.147	.-0.036 0.712	0.063 0.509	*r* *p*
Exposure periods	-0.035 0.788	-0.188 0.138	0.346** 0.007	*r* *p*
Reactive oxygen species (ROS) (mmol/l)	-0.123 0.343	-0.298* 0.018	0.369** 0.004	. *r* *p*
age	-0.048 0.7023	-0.269* 0.035	0.412** 0.002	*r* *p*
Exposure workers	-0.162 0.207	-0.302* 0.018	0.375** 0.004	*r* *p*
Non-exposure workers	1.000	0.624** 0.0001	0.106 0.262	*r* *p*
Gstm1 phenotype	0.624 0.001	1.000	-0.639** 0.000	*r* *p*

r =correlation and *p*=*p*-value p=0.010 was considered highly significant (**)

Table 7 gstm1 and radiation risk factor parameters of non-worker and workers

Parameters	All workers	Non- workers	workers	P-value
	464	264	212	

radiation	416.31+101.71	420.3+95.9	411.09+108. 81	0.750
Gstm1	363.61+114.08	388.9+107.3	343.8+117.8	0.030
x-ray	1.23+0.37	1.12+0.21	1.35+0.47	0.000
gender	7.83+1.78	6.52+1.61	9.02+1.47	0.000
Reactive oxygen species (ROS) (mmol/l)	103.86+5.69	65.75+30.17	138.48+41.86	0.000
Expousre periods	53.43+67.34	2.69+2.37	99.54+64.59	0.000
DNA fragmentation (positive TUNEL) (%)	14.3+5.7	11.4+4.3	17.5+5.4	0.000

P , 0.050 was considered significant and P , 0.010 was considered highly significant

These results support Oxidative stress occurs in human body with increased levels of ROS [2] and with increases in the concentrations of cadmium and lead[8] and with decreases in the concentration of ascorbic acid and the activity of other components of the antioxidant defense mechanism [2;4]. workers men who has radiation exposure injury have higher levels of oxidative stress indicators than workers non-exposure [2] . Oxidative stress has been shown to be a major cause of tumors; ROS causes oxidative damage to normal DNA, proteins and lipids, In the present study, the finding that expousre periods were significantly higher for exposure than for non-exposure. Oxidative stress (ROS) may cause several forms of sperm DNA damage such as chromatin cross-linking, chromosome deletion, mutations, DNA strand breaks, base oxidation and other lethal genetic effects [1,3] . ROS may also induce apoptosis through cytochrome c release from mitochondria and caspases 9 and 3 activation, which result in high frequenciesof single- and double-stranded DNA breaks DNA damage in sperm may arise from several sources: firstly, improper packaging and ligation during sperm maturation[4]; secondly, oxidative stress [1] and the increased levels of specific forms of oxidative damage and thirdly, induction of DNA fragmentation due to apoptosis [4] .

Proteins are one of the prime targets for oxidative damage [5] , and cysteine residues are particularly sensitive to oxidation because the thiol group (2SH) in cysteine can be oxidized [4] . high levels of oxidative stress components may affect the formation of inter- and intramolecular disulfide bonds, resulting in less compaction of the sperm chromatin and a higher incidence of DNA damage.

5.DISSCISION

Radiation has become a real public health problem as it carries a risk of pathological consequences which may sometimes be life-threatening. Radiation is a significantly associated with exposed to x-ray , sonar and MRI[6].it cause a great loss in quality of life and a lot of suffering ,not for the exposure individual, but also their families. In the eastern world 20-60 year of hospital workers and 20-30% of exposure are exposures [12] . In the present study, the prevalence of radiation exposure among hospital workers of the was 25.9% within 11.6% in female and 14.3% in male.

The wide differences in results can be explained by the differences in age of studied sample (male and female) also the use different definition of radiation exposures weather based on radiation dosage percentiles. Present study gender was shown to be associated with radiation and GSTM1 gene polymorphisms were significantly associated with gender. It was also revealed the GSTM1 had an effected on radiation exposures stress or toxicity via induction or detoxification this toxicity of many adduct materials such as radiation(x-ray, sonr and MRI radiation) .GSTM1 also activate transduction cascade. In radiation responsive *cell, with one involving CAMP. I*n female radiation exposures increases intracellular camp which activate protein kinase A and closes k channels mediated by methylation, therefore female have defense more than male. Detoxification is multifactorial as it is based on genetics, behavior and environmental factors ,various genetic disorder can cause Toxicity in in syndromic form. Environmental play key role in shaping an individual habits and life style, behavioral problem also relates to increase in meal quantity at home and when dining out, because radiation is found in many places and foods Toxicity results from an imbalance between radiation intake and stress antioxidant in human body resulting in excessive accumulation of the radiation dose in organs tissue, liver, muscles and other organs involved in metabolisms. Before the discovery the association of detoxification GSTM1 gene in human, there are two theories existed regulating way in the body can regulating body toxicity*;* toxic regulation way where maintenance of a basal body toxicity through toxification expenditure influences by regulate the balance between oxidant and anti-oxidant. And the second one by Making the X-Ray Safer via best way to decrease risks of radiation is to limit the dose and control the levels of radiation, The three basic ways to external exposure to radiation include: Minimize time, Maximize distance and Use shielding., the X-rays ability to examine the human body from an internal perspective is revolutionary, as it has spurred the discovery of imaging human bones. Ultimately, the machine has proven to be an essential medical diagnostic tool both in past times and today (9) GSTM1 function as a peripheral signal

in a negative feedback loop system to control body toxicity. Women have higher GSTM1 level than men because of an increase in GSTM1 expression in subcutaneous liver tissue and stimulate of GSTM1 synthesis by estrogen. GSTM1.

MRI is less effect because is made of lead garment can be used as a form of shielding because it helps reduce the radiation dose that the body is exposed to. As a human bone is dense, the thick lead garment helps absorb the beam of electrons and therefore limits the risk of over exposure. Additionally, doctors recommend that pregnant women use an alternative form of medical imaging, such as an ultrasound.

However, the exact effect of x-ray on male fertility remains controversial. Toxic metabolites which constitute during disease, such as stress ,heating and others have been demonstrated to be negatively associated with the normal development of male and female gametes and embryos [14;15] .

In the present study, it was found that radiation had apparent effect on gstm1 detoxifcatin enzyme activity. These metabolites may cause deficiencies in detoxification and reductions in enzyme activity, so lead to tumor's cases[12].

5.CONCLUSION.

According to these results, we can conclude that Radiation is a prevalent disorder among hospital workers in AL Hussein teaching hospital radiation unit, the overall prevalence was 25.9%, the prevalence was higher male 14.3% than among females 11.6%. Risk factors such as exposure periods and gender have been appeared differentiation relationship with the *gstm1* gene deletion, where the highest percentage was found in exposure period more than 15 years. *Gstm1* was play important play in the control of toxicity balance and detoxification enzyme which remove material toxicity action in humans ,as evidence by the fact that *gstm1* deficiency lead to morbid toxicity so as to necrosis conclusions: The current study evaluate the effect of radiation on detoxification. Enzyme activity**..**

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