Detection of XRCC1 Expression and (8-OHdG) Levels as a marker of Oxidative DNA Damage in Individuals Exposed to Low Dose of Gamma Rays

Rafed Abbas Kadhum*1, Wathiq Abbas Aldrghi2
1,2 Institute of Genetic Engineering And Biotechnology for Post Graduate Studies, University of Baghdad, Baghdad, Iraq.
* rafedstudent@gmail.com

Abstract. To study ionizing radiation (IR) effects on biological systems there several techniques are used to identify molecular features of IR exposure, urinary detection of 8-ohdG by ELISA as a marker of DNA oxidative stress is one of the techniques was used in this research, when IR absorbs in a cell so that causing DNA damage and releases 8-ohdG through correction damage. The detection of XRCC1 expression by QRT-PCR is else technique in this study. The genotoxic of IR can be observed even at a low doses 5cGy, According to RPC (Radiation Protection Center) limited place in Baghdad as a source of low-dose IR (Europium 152) before it is removed, some of workers and slums are exposed to low dose of γ-rays (5cGy to 10 cGy). The study groups included the (G1)fifty workers in metal melting are working closely with (IR source), (G2)twenty five individuals from slums near the IR source, and(G3) twenty five volunteers individuals of male in different age, didn’t exposure to diagnostic x-ray examination or other radiotherapy. Peripheral blood are collected and urine from all individuals. The results indicate that the level of 8-OHdG factor in workers higher than slums individuals and negatively related with XRCC1 expression, also the expression of XRCC1 decreased in individuals exposed for more than 5cGy, and increasing in slums exposed to 5cGy. The low dose of γ-rays causing genotoxic effects due to a combination of DNA-damaging effects and reduced capacity of DNA repair.

1. Introduction:
Radiation can be classified two main categories: ionizing (IR) and others non-ionizing(NIR). The effect of IR with bimolecular more than NIR because of the ability of ionizing to stimulate atom ionization. Radioisotopes (radio nuclides) are unstable that be main source of IR, these radioisotopes emit particles have high energy to displace atomic electrons, also to easy happen the chain reaction. There are main types of IR include particles as (alpha , beta ),rays(X-rays, and gamma - γ-rays), the particles(α,β) stopped at barriers of an aluminum or sheet of paper, whereas the most penetrating are the rays (X,γ), environmental exposure to particles (α,β) less than the rays (X,γ). Therefore; consider these rays the main reason of biological damage with greater degree than the particles(α,β), these types used for therapeutic purposes successfully and also lead to cellular damage [1]. In this study the Europium 152 was the source of low dose gamma rays (5cGy equivalent to 50 mSv, to 10 cGy equivalent to 100 mSv). The non-IR formed largely type of Environmental radiation, included
all region the spectrum such (UV) rays. The effect of non-IR like UV rays on biological molecular is now well-studied [2-3], ionizing radiation most widely employed in research centers and therapies are from sources of man-made like as Co-60 , Cs-137 and Europium 152 . this study focused on the interactions of ionizing radiation with biological molecules [4] , radioactive waste as result of the using radioisotopes in industry, science and medicine . This waste needs to be managed in a way to keep the environment safe and people over long periods of time, as well known that the Ionizing radiation may be occur either naturally or artificially in our environment , these types have same the effects on healthy human. In 1902 were recorded first case of cancers induced by Radiation [5] other cases registered in (1917-1926) included painters deal with radium element [6] as response to some environmental factors or stress such as chemical reagent or physical factors like gamma rays , the cell activated mechanisms of DNA repair , single strand break repair (SSBR) , base expression repair (BER) and other mechanisms as apoptosis and cell cycle control all the body parts effected by ionizing radiation but the response depending on the dose and period of exposure. The tissue , such as blood affected by IR and causes DNA damage , cells of mammalian have several types of DNA repair systems that it has main role to correct different types of DNA damage . The gamma rays lead to damage of DNA either directly or indirectly and causes usually DNA base damage that is repaired by base excision repair (BER) [7]. X-ray repair cross complementing group 1 (XRCC1) and others such as (XRCC2 ,XRCC3) are necessary proteins in the BER pathway. The location of XRCC1 gene on chromosome 19q13.2–13.3. XRCC1 protein coordinate with other proteins that play a essential role as scaffold for the DNA repair complex [8]. The primary role for this gene XRCC1 assemble proteins of SSBR in position of DNA damage in order to support the efficiently repair .This operation have controlled by PARP1-XRCC1 scaffold , the mechanisms of repair include the temporary assembly complexes of multi-protein the assembly of which rely upon the kind of the initiating lesion, for more information about the chemistry mediate in mechanisms of repair and post-translational modifications of the constitutive pathway members see Svilar et al.[9-10].

As accordingly, the oxidative stresses play the main effect on certain pathologies, especially in carcinogenesis. The reactive oxygen species (ROS) can be induced deferent of damaged and contamination to DNA, included oxidative bases then be repaired and release in urine the 8-ohdG, can be measured the oxidized in urine or blood with various methods. 8-ohdG urinary measurement usually used in many studies period subjects with occupational exposure. Indeed the factors of chemical and physical can be increased the oxidative stress [11]. The repaired single strand breaks or base excision repair by XRCC1 proteins that be associated with produce 8-ohdG to determined the level of DNA damage used firstly 8-ohdG as marker. The objective of this research is to identify the association between DNA repair gene expression(XRCC1) and low dose of gamma ray then analysis the association of the XRCC1 and level of 8-ohdG in urine with DNA damage from exposed individuals and control group also study the correlation among the age ,smokers and urinary 8-ohdG levels .

2.Materials and Methods

2.1.Blood and Urine sample collection:

Blood samples were obtained from 100 male individuals before removed the source of ionizing radiation, and distributed into three groups 50 from workers, 25 from slums who living near the gamma rays source and 25 from healthy donors with no history of radiotherapy , 30- 65 years old with no alcohol or medicine consumption . Blood samples were drawn in tubes.

| Age group          | No  | %     |
|--------------------|-----|-------|
| Less than 40       | 39  | 37.50 |
| 40-50              | 44  | 42.31 |
| More than 50       | 21  | 20.19 |
| P-value            | --- | 0.0083** |

Table 1. Distribution of samples study according to age groups.
For the gene expression study, transformed 250 µl blood directly to tube containing Trizol reagent to protect the RNA from analysis. Whole blood from each tube were processed immediately. Urine samples were obtained from same individuals and drawn in free heparin tubes for the 8-ohdG ELISA test. In this study used urinary 8-ohdG test because the level of 8-ohdG in urine more sensitive than monocytes that exposure to same gamma dose. The 8-ohdG levels in monocytes were not increased at doses lower than 10,000 cGy [12].

2.2. Detection of Urinary 8-hydroxydeoxyguanosine (8-ohdG) Levels by ELISA:
To measured the Level of 8-hydroxydeoxyguanosine (8-ohdG) in the urine as the indicator of oxidative stress can be used enzyme-linked immunesorbent assay (ELISA), this marker lead to know the level of oxidative stress of each person then make other test for the same individuals that appear high result in level of 8-ohdG [13] Kasai et al. show that the DNA damaged by oxidative stress can be seen by the measured concentration of 8 hydroxydeoxyguanosine (8-ohdG)as biomarker for cellular oxidative stress during carcinogenesis[14]. Briefly, Urinary 8-ohdG level was assessment by an ELISA according to the manufacturer’s instructions (Germany), fresh urine samples centrifuged at 2,000 x g for 10 minute and was stored at -20°C immediately after collected and before used. The level of 8-ohdG in urine were measured by using a competitive enzyme linked with immune sorbent assay kit, 50µL from standard and samples was prepared then added in triplicate to appropriate plate well that precoated with antibody specific for 8-ohdG and incubated at 37 °C the plate was Covered at room temperature and incubated for 1 hour then the plate washed four time by 1x wash Buffer, and the plate was covered and developed in the dark for 30 minutes at room temperature.

1- The stop solution (100 µL) was added to each well. The absorbance was measured of each well on the reader of plate at 450 nm.
2- Then Plot the standard curve then determine the concentrations of sample. The result was expressed as 8-ohdG ng/ml [13].

RNA isolation and reverse in to cDNA RNA was extraction according to recommendation of kits. The kits allowed an average yield of up to 30 µg of total mRNA. Total RNA was reversely transcribed to complementary DNA(cDNA) using WizScript™ RT FDmix (Hexamer) Kit. The procedure was carried out in a reaction volume of 20 µl according to the manufacturer's The total RNA volume to be reversely transcribed was (15µl). A volume of 15 µl of total RNA was added into a tube containing RT FDmix (Hexamer) and pipetted up and down two times to mix. The tube was briefly centrifuged to spin down the contents and to eliminate any air bubbles. The tube was placed on ice, the thermal cycler was programmed using the conditions shown in Table 2.

Table 2. Show thermal cycler program.

| Temperature | Step 1 | Step 2 | Step 3 | Step 4 |
|-------------|--------|--------|--------|--------|
| Time        | 10 min | 30 min | 5 min  | ∞      |

2.3. Analysis of XRCC1 mRNA expression by Quantitative real time polymerase chain reaction (QRT-PCR):
Primer sequence for XRCC1 were 5-`GAGGATGAGGAGGCGCAACTC-3` Forword and 5-`TCACTCAGGACCCAGTGTGTC-3` Reverse, primers sequences for genes were prepared according to[15] synthesized by Alpha DNA Ltd (Canada), the final volume of PCR reaction was 20 µl, 10µl from SYBR green, 1µl forward primer, 1µl revers primer, 2µl cDNA and completed to 20 µl by the free nucleus water.
The samples were amplified for 40 cycles at 95 °C for 5 min., 95 °C for 30 sec. and the annealing 62°C for 60 sec, then analysis the Ct value for XRCC1 to calculate the folding times of expression for each group, the expression levels of XRCC1 were normalized to housekeeping expression.

2.4. Statistical Analysis
The Statistical Analysis System- SAS (2012) program was used affect of difference factors in study parameters. Least significant difference –LSD test (ANOVA) was used to significant compare between means Estimate of correlation coefficient between variables in this study [16].

3. Results

3.1. 8-OHDG Levels in Urine
Levels of 8-ohdG in IR-exposed workers and slums individuals in urine samples are presente in Table 3. The levels of 8-ohdG in both workers and slums were in the different ranged. There was significantly difference in the levels of 8-OHdG among three groups. The level of urine 8-OHdG was different between the two groups (workers and slums). It found that different in urinary 8-OHdG/creatinine excretion between the exposure individuals participated in present study and their matched controls. Exposure to ionizing radiation more than 500 cGy did not increase the levels of 8-ohdG in urine or blood.

Table 3. levels of 8-ohdG in gamma-exposed workers and slums individuals in urine samples

| Group       | Mean ± SE 8-ohdG ng/ml | LSD-value | P-value | " Means having with the different letters in same column differed significantly" |
|-------------|------------------------|-----------|---------|--------------------------------------------------------------------------------|
| G1: Workers | 48.81 ± 0.67 a         | 2.324 **  | 0.0001  |                                                                                 |
| G2: Slums   | 36.27 ± 0.82 b         |           |         |                                                                                 |
| G3: Control | 14.03 ± 0.83 c         |           |         |                                                                                 |
| LSD-value   | 2.324 **               |           |         |                                                                                 |
| P-value     | 0.0001                 |           |         |                                                                                 |
| " ** (P<0.01).                                                                                 |

3.2. Effect of smoking in 8-ohdg level
In other hand, it observed that significant increased in 8-ohdg levels in smokers control group and significant results in smokers individuals in groups (worker, slums) Table 4.

Table 4. Effect of smoking in 8-OHDG level.

| Smoking | Mean ± SE 8-ohdG ng/ml | LSD-value | P-value | " Means having with the different letters in same column differed significantly" |
|---------|------------------------|-----------|---------|--------------------------------------------------------------------------------|
| Yes     | 45.16 ± 1.55 a         | 6.491 **  | 0.0021  |                                                                                 |
| No      | 34.81 ± 1.93 b         |           |         |                                                                                 |
| LSD-value |                  |           |         |                                                                                 |
| P-value  |                       |           |         |                                                                                 |
| " ** (P<0.01) , NS: Non-Significant ".                                                                                 |

3.3. Effect of age in 8-OHDG level
Also, it observed that no significant increases in 8-ohdg levels for age less than 40 when compared with (40-50) age group and more than 50, the increased was found but non significant, and significant results in 40-50 age group individuals when compared with age group more than 50 (worker, slums and control) Table 5.
Table 5. Effect of age in 8-OHDG level.

| Age Group (year) | Mean ± SE |
|------------------|-----------|
| Less than 40     | 38.84 ± 2.59 ab |
| 40-50            | 34.65 ± 2.41 b |
| More than 50     | 43.13 ± 2.72 a |
| LSD-value        | 7.518 * |
| P-value          | 0.0497 |

* (P<0.05) , NS: Non-Significant.
" Means having with the different letters in same column differed significantly "

Urinary 8-ohdG a highly sensitive oxidative stress, also smoking [17-18] was reported high affect on 8-ohdG levels. Though experimental study of animals showed that urinary 8-OHdG levels not appear any affect caused by content of nucleic acid diet [19].

3.4. Influence LD ionizing radiation of XRCC1 DNA repair Gene:

The expression of XRCC1 gene was determined in whole blood that isolated from different exposure groups. The results show that decreased XRCC1 mRNA expression levels in workers groups(Gamma rays doses higher than slums ) and increased of XRCC1 mRNA expression levels in slums( low gamma rays doses ) as presented in Table 6, significantly the decreased of XRCC1 gene expression has been observed at 20 cGy (po0.05) [20]. The expression levels of XRCC1 were negatively correlated with levels of 8-ohdG concentration level.

Table 6. Fold of XRCC1 expression depending on $2^{-\Delta\Delta Ct}$ Method

| Groups | Means Ct of XRCC1 | Mean of GAPDH | $\Delta$Ct (Means Ct of XRCC1 - Mean Ct of GAPDH) | $2^{-\Delta Ct}$ experimental group/Control group | Fold of gene expression |
|--------|------------------|--------------|-----------------------------------------------|-----------------------------------------------|------------------------|
| Group1 | 25.35            | 21.12        | 4.23                                          | 0.0532                                        | 4.2                    |
|        | Group2           | 24.81        | 21.15                                         | 3.66                                          | 0.0791                 | 6.32                   |
| Control| 27.33            | 21.02        | 6.32                                          | 0.0125                                        | 0.0125                 |

4. Discussion

In this study, the determination of urinary 8-ohdG stimulated with ionizing radiation have been performed in both the workers and slums individuals exposure to different doses ranging from 5 cGy to 17 cGy. There significantly changed of the 8-ohdG levels in two groups samples . 8-ohdG levels in workers with (5cGy to 10 cGy) are (48.81 ± 0.67 ng /ml ) in concentration and slums with (less than 5 cGy) was (36.27 ± 0.82 ng /ml ). The low doses of ionizing radiation were significantly increasing 8-ohdG levels above the standard , because of the fact that ionizing radiation induced single and double strand breaks as a result generation of (ROS) [21], free radicals or (ROS) involved as share DNA mutation lead to cancer, diabetes, and neurodegenerative diseases [22]. The endogenous ROS are generated by influenced of various metabolic and other biochemical reactions. The exposure to low dose -LET radiation due to the death cases of 1% from exposure individuals to period 10 years after exposure [23]. The damaging bases was occurred even less than 5 cGy , 8-ohdG as biomarker for BER and SSBR was released during the reaper, that is mean increasing the level of 8-ohdG with increase of gamma rays doses so the level of 8-ohdG in workers higher than slums , but exposure to doses more than 500 cGy of gamma radiation did not significantly increase the levels of
8-ohdG [20]. The range level of 8-ohdG in workers and slums effects with many other factors such as age, gender and smoking [17], chemical reagent and metabolic rate. Most these factors were observed in workers may be lead to increase the 8-ohdag in urine. In this study, found that damaged DNA oxidative, can be expressed by levels of urinary 8-ohdG/creatinine, the increasing in smoking individuals (45.16 ± 1.55) and (34.81 ± 1.93) in non-smoking exposure individuals the urinary 8-OHdG/creatinine levels significant observably in different age. Less than 40 years about (38.84 ± 2.59 ng/ml) non significantly increased when compared with age group between 40-50 years (34.65 ± 2.41 ng/ml) because of the ages are close, more than 50 years were significantly increased urinary 8-ohdG level (43.13 ± 2.72 ng/ml) when compared with the first and second age as result of the aging, smoking and they were working more hours than all age groups above besides affect with the low dose of gamma rays. The levels of urinary 8-ohdG was reported by Tagesson and et al. found that high in patients treated by irradiation for lymphoma patients for bone marrow transplantation of leukemia [24] Plummer reported that measurement level of 8-ohdG may be limited use for dosimetry of radiation when used with urine DNA, because it the measurement only at doses higher than occupational exposures[25].

The mRNA expression levels of XRCC1 gene, that shown decreased as response to IR. A significantly decreased expression level of XRCC1 gene was observed in workers exposure to 10 cGy, the ct values of XRCC1 shown in Figure 1, The decreases of XRCC1 expression prove that ionizing radiation not only lead to DNA damage but also influence on the expression. Monika and et.al reported that the level of XRCC1 gene expression in exposure individuals were less than expression in individuals exposure to low dose of IR[26]. Some of researchers found that level XRCC1 protein expression lower in patients treated by radiotherapy vs control group [27]. These results lead to explain the significant increasing XRCC1 expression in slums (5cGy) as comparison with higher doses of gamma rays in workers (5cGy to 10 cGy). In other studies was the exposed to high dose lead to increased the XRCC1 expression level,Figure 1. XRCC1 amplification plots by QPCR. Samples included workers groups. Ct values ranged from 22.16 to 27.9. The photograph was taken directly from QPCR machine.

inverse the slums individuals the mRNA expression levels of XRCC1 repair gene, was found to be increased in response to gamma radiation, the Ct values as shown in Figure 2. A significantly increased expression level of XRCC1 gene was observed in individuals exposure to lower than 5 cGy. The observed increasing in XRCC1 expression proved that low IR don’t effect clearly to the normal expression levels of XRCC1 repair gene. Table 6.
Figure 2. XRCC1 amplification plots by QPCR. Samples included slums individuals group. Ct values ranged from 22.75 to 28.64. The photograph was taken directly from QPCR machine.

The results indicated is a possibility that there of using the 8-OHDG and gene expression as molecular assay for the assessment of DNA damage in the human peripheral blood and urine of population exposed to low dose of ionizing radiation.

**5. Conclusion**

In present study, the γ-ray at a dose as low as 5 cGy for a long time can cause DNA strand breaks then increased 8-ohdG level in urine. This finding is significant since the genotoxic effects of γ-rays can be observed even at a low dose between 5 cGy – 10 cGy and less than 5 cGy, levels of XRCC1 gene expression were significantly decreased in workers by a dose of 10 cGy of γ-rays compared to those from lower doses less than 5 cGy. These findings suggest that the genotoxic effects of γ-ray may be due to a combination of DNA damage and reduced DNA repair capacity. It is well known that the biological effects of IR do not follow a linear dose–response but a quadratic dose–response pattern, therefore; The results provide that used 8-OHDG as a marker to DNA damaged and XRCC1 gene also good indication for effecting by γ-ray and some of chemical materials, Mutation of the XRCC1 gene causes defective DNA repair.

**References**

[1] Lawrence T, and Rosenberg S 2008 (Eds.)." Cancer: Principles and Practice of Oncology. Philadelphia, (PA: Lippincott Williams and Wilkins)"

[2] Karagas M R, Stannard VA, Mott LA, Slattery M J, Spencer S K, and Weinstock M A J.Natl Cancer Inst. 94 224–226

[3] Zhang M, Qureshi A A, Geller AC, Frazier L, Hunter DJ, and Han J. 2012 J. ClinOncol 30 1588–1593

[4] " Reisz J A, Bansal N, Qian J, Zhao W, and Furdui C M 2014 J.Antio. Red. Sign. 21(2) 260–292 "

[5] Frieben A, Cancroid des rechten handrückens 1902 Deutsche Medicinische Wochenschrift 28 p335

[6] Gunderman RB, Gonda AS and girls R 2015 J.Radio. 274 314–318

[7] Sak A, Fegers I, Groneberg M and Stuschke M 2008 J.Lines. Cell Prolif. 41 660–670.

[8] Hoeijmakers J H 2001Nature 411 366–374

[9] Svilar D, Goellner EM, Almeida KH and Sobol RW 2011J.Antio. Red. Sign. ) 14 2491–2507.

[10] Almeida K H and Sobol RW, 2007 J.DNA Repair 6 695–711

[11] Sajous L, Botta A and MinodierI S 2008 J.Ann Biol Clin (Paris) 66 19-29

[12] Pouget J P, Ravanat J L Douki, T, Richard M J, CadetJ 1999Int. J. Radiat.Biol. 75 51–58

[13] Zhang XH, Xuan Z, Xu CW, Li FJ, Zhang PY, Cai XJ, Qing C, Xiu BR, Jian ZC, Qiang W, Yi MZ. Chronic 2011J.Heath 11:224

[14] Kasai H, Hayaami H, Yamaizumi Z, Saito H, Nishimura S. 1984 Detection and identification of 12 p2127–36

[15] William R, Langer E, Davies S and Robison L 2004J.Cancer 100 411-417.
[16] SAS 2012 Statistical Analysis System, User's Guide. Statistical. Version 9.1\textsuperscript{th} ed. SAS. Inst. Inc. Cary, N.C. USA
[17] Loft S, Vistisen K, Ewertz M, Tjonneland A, Overvad K and Poulsen H E 1992 Carcinogenesis \textbf{13} 2241-2247.
[18] Halliwell B 1996 \textit{Free Radie. Res.} \textbf{25} 57-74
[19] Shigenaga M K, Gimeno C J, Ames BN, 1989 \textit{Proc. Nati. Acad. Sci.} USA \textbf{86} p.9697-9701"
[20] Wanwisa Sudprasert, Panida Navasumrit, Mathuros Ruchirawat 2006 \textit{Int. J. Hyg. Environ.-Health} \textbf{209} 503–511
[21] Hall EJ, 2000 \textit{In: Radiobiology for the Radiologist, fifth ed.}\ (Philadelphia :Lippincott Williams &Wilkins) p. 5–16
[22] Wu L L, Chiou C C, Chang PY and Wu JT 2004 \textit{Clin. Chim. Acta} \textbf{339} 1–9.
[23] CRP 2012 ICRP Statement on Tissue Reactions and Early and Late Effects of Radiation in Normal Tissues and Organs-threshold Doses for Tissue Reactions in a Radiation Protection Context, Publication \textbf{118}, \textit{Ann. ICRP} 41 (1/2) 1–322
[24] Tagesson C, Kallberg M, Klintenberg C, Starkhammar, H 1995 \textit{Eur. J. Cancer} \textbf{31} A 934-940
[25] Plummer S M, Pheasant A E, Johnson R, Faux, S P, Chipman, J K and Hulten, M A 1994 \textit{Hereditas} \textbf{121} 139–145
[26] Hanova M, Stetina R, VodickovaL, Vaclavikova R, Hlavac P and Smerhovsky Z 2010 \textit{J.Toxic. and Appl. Pharm.} \textbf{248} 194–200
[27] Batar B, Guven G, Eroz S, Bese N S, Guven M. 2016 \textit{J.Gene} 5pages