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The human 8-oxoguanine DNA glycosylase and xeroderma pigmentosum group haplotypes variation in x- ray employees

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ABSTRACT

DNA repair genes polymorphism including The human 8-oxoguanine DNA glycosylase (*hOGG*) Ser326Cys and Xeroderma Pigmentosum Group (*XPD*) Arg399Gln genes in the x-ray employees were studied in present study. PCR-SSCP technique was used to detect haplotype polymorphisms. The results found that the haplotypes distribution of both genes were significant association with cases, *XPD* have high percentage of di haplotype than control group in significant association (p=0.0019).The *hOGG* haplotype distribution in study groups shows significant association of di haplotype with cases than control group (P=0.000), both genes haplotypes were distributed according work time in cases per day to more and less than 6 hours, *XPD* showed non-significant association *XPD* (p=0.9395), uni and di haplotype more frequent in cases exposure to x-ray less than 6 hours per day. The *hOGG* appeared in significant association with work time (p=0.000) that more frequent of di haplotype in less than 6 hours category, di haplotype more frequent in case work less than 6 hours per day. The di haplotypes more frequent in worker with less than 6 hours per day in non-significant association (p=0.4132). The exposure day to x-ray classified to less and more than 4 days per week, *XPD* non-significant association with exposure day (p=0.798) about 50% of worker have uni haplotype that work less than 4 days. The *hOGG* haplotypes also non-significant association with exposure day (p=0.798), Different work periods were reported and classified to less and more than 10 years, *XPD* showed non-significant association (p=0.582) with work period, uni haplotype was frequent in same percentage in both categories. The *hOGG* also non-significant correlations with work periods (p=0.1775), di haplotype more frequent in worker with less than 10 years, the current results concluded that there were vital role of x-ray in the *hOGG* Ser326Cys and (Arg399Gln) *XPD* genes.

Key words: human 8-oxoguanine DNA glycosylase, xeroderma pigmentosum group, haplotypes, variation, x- ray employees

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INTRODUCTION

The Ionizing Radiation (IR) disrupts chemical bonds by deposits energy on molecules that lead to breaks genomic DNA covalent bonds when it passes through cells. A wide variety of DNA damage were produced, like DNA single-strand breaks, base damage, double-strand breaks and DNA-protein crosslinks, furthermore the formation of oxidative stress promoted DNA damage by irradiation [1]. The double strand break number stimulated under normoxic conditions more than under hypoxic conditions [2, 3]. Different chemical modifications were formed during exposure of IR in hypoxic conditions like 5,6-dihydrothymine [4], α-deoxyadenosine (α-dA) [5], 5',8-cyclo-dA [6], and DNA-protein cross link [7, 8]. The Xeroderma Pigmentosum group D (*XPD*) gene, is found in chromosome 19q13.3. The *XPD* protein is important in the nucleotide excision repair pathway, with other functions, like the site of DNA lesions enzyme uncoiling the double helix and transcription[9], other studies have observed that variant alleles of *XPD* Lys751Gln (rs13181) polymorphism associated with DNA adduct levels increments [10,11,12], and DNA repair capacity reduction [13].

The Human oxoguanine glycosylase 1 or called (*hOGG* 1) is one of the DNA repair enzyme, that has a vital roles in the base excision repair pathway. Several studies reported a common polymorphism Ser326Cys (rs1052133) in *hOGG* 1 in different disease [14], the *hOGG* 1 enzyme is a major member of base-excision repairing for damage of oxidative DNA [15]. It encoded by the *hOGG* 1 gene can directly remove 8-hydroxyguanine (8-OH-G), one of the major constituents in DNA damage [16,17, 18].

METHODOLOGY

Samples and study subjects

A case control study were implemented on the x-ray technicians that employees in Al-Sadder medical city, about 20 cases and 30 individuals as a control group, blood samples were collected according to ethical approval of ministry of health and environment in Iraq.

DNA extraction and target genes amplifications

DNA isolated by favour gene kits with 40 µl of proteinase K

(20 µg/µl). The *hOGG* Ser326Cys and *XPD* Arg399Gln genes amplified using primers that mention in previous study [18].

Haplotypes detected using SSCP technique according to the mixture consist of 40% of acrylamide-bis acrylamide 8 ml, glycerol 2.8 ml, TBE (5X) 8 ml and dH_2O 20.8 ml, ammonium-per sulfate 400 µl (0.1 gm/ml) and TEMD (40 µl) to gel casting, PCR products were mixed with loading dye (formamid, xylene cyanol, bromo- phenol blue with EDTA) in same ratio, then it incubate at 95°C to 7 min, after that its chilled in ice for 2 min, next samples were loading in vertical casting and electrophoresis running at 100V, to 40 min, after electrophoresis finished gel was stained by ethidum bromide and visualized under UV light.

Data analysis

Haplotype was represented as percentage, significant detected by chi square at p-value less than 0.05.

RESULTS

The results found that the age of cases was (36.1 years ± 10.5 years), the exposure time to x-ray was (4.09 years ± 2.99 years), and the work duration was (9.9 years ± 8.8 years). The present outputs of present study that deal with (Arg399Gln) in *XPD* and Ser326Cys in *hOGG* gene in the x-ray tecnitions that work in hospital, results of *hOGG* haplotypes found three haplotypes (uni, di and tri) in control and two haplotype (uni and di) in workers, haplotypes were visulized using PCR-SSCP technique (Figure 1A-C), the *XPD* have two haplotypes in both cases and control group (di and tri) (Figure 1D and E).

The haplotypes distribution and significant association were clarified in Figure 1, *XPD* have high percentage of di haplotype than control group in significant association (X2 9.55152, p=0.0019), while uni haplotype is low frequent in cases than control group.

The *hOGG* haplotype distribution in study groups show significant association of di haplotype with cases than control in addition to disappear of uni haplotype in cases (X2 28.8245, P0.000) (Figure 2).

The genes haplotypes were distributed according work time in cases per day to more and less than 6 hours, *XPD* showed non-significant association *XPD* (X2= 0.00576, p=0.9395), uni and di haplotype more frequent in cases exposure to x-ray less than 6 hours per day (Figure 2). The *hOGG* appeared in significant association with work time (X2 8.14815, p 0.000) that more frequent of di haplotype in less than 6 hours category (Figure 3).

The work time of technicians classified to more and less than 6 hours per day, the haplotypes distribution clarified in Figure 4, *XPD* non-significant association with work time (X2=0.6694, p=0.4132), di haplotype more frequent in case work less than 6 hours per day. The di haplotypes more frequent in worker with less than 6 hours per day in non-significant association (X2=0.6694, p= 0.4132) (Figure 4).

The exposure day to x-ray classified to less and more than 4 days per week, *XPD* non-significant association with exposure day (X2 0.0653, p=0.798) about 50% of worker have uni haplotype that work less than 4 days. The *hOGG* haplotypes also non-significant association with exposure day (X2 0.0653, p=0.798) in spite of 70% of workers have di haplotype (Figure 5).

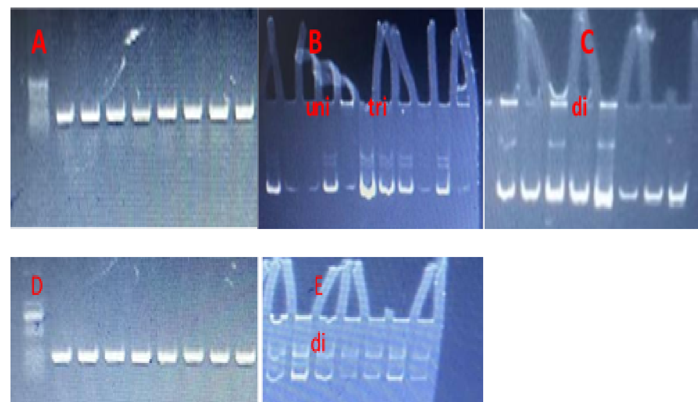

Fig. 1. Electrophoresis patterns of DNA repair genes, (A and D) XPD and hOGG amplifications products using agaros gell (1% agaros, 100 V for 30 min), (B and C) hOGG electrophoresis using PCR-SSCP technique with three haplotypes, (E) electrophoresis using PCR-SSCP technique of XPD with di haplotype

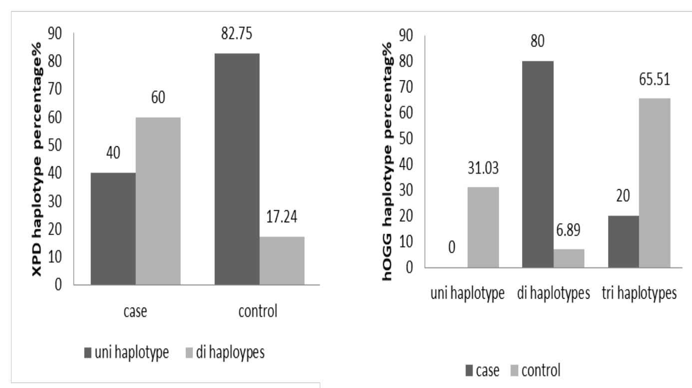

Fig. 2. The haplotypes percentage distribution of XPD (X2 9.55152, p 0.0019) and hOGG gene in study groups (X2 28.8245, P0.000)

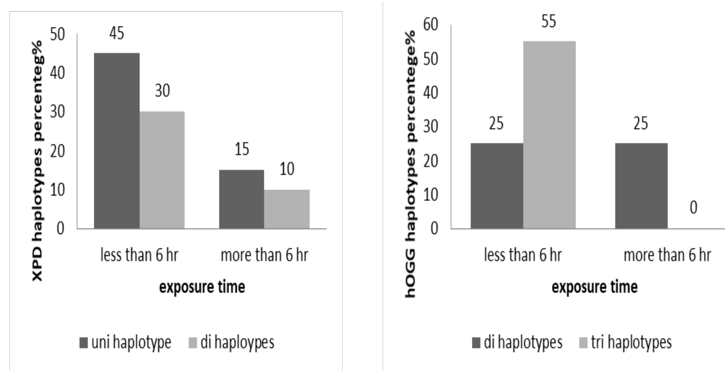

Fig. 3. The haplotypes percentage distribution of XPD(X2= 0.00576, p=0.9395) and hOGG (X2 8.14815, p 0.000) according to exposure time (less and more than 6 hours)

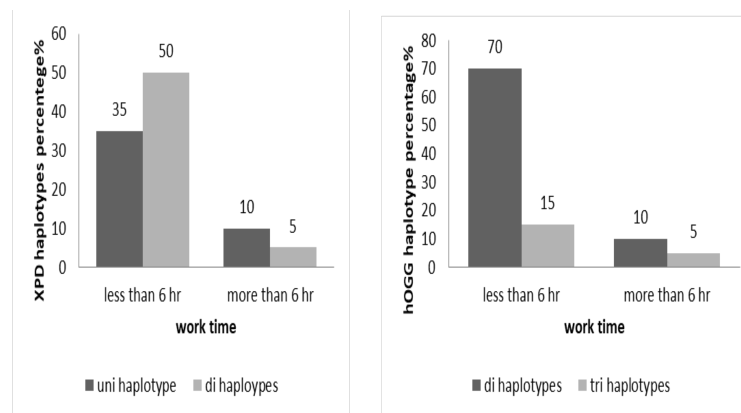

Fig. 4. The haplotypes percentage distribution of XPD(X2= 0.6694, p 0.4132) and hOGG (X2 0.392, p 0.5312) according to work time per day (less and more than 6 hours)

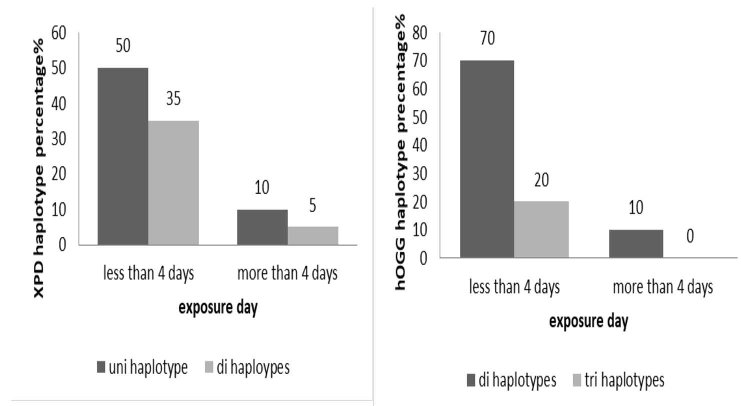

Fig.5. The haplotypes percentage distribution of XPD (X2 0.0653, p 0.798) and hOGG (X2 0.5555, p 0.4561) according to exposure day number (less and more than 4 days)

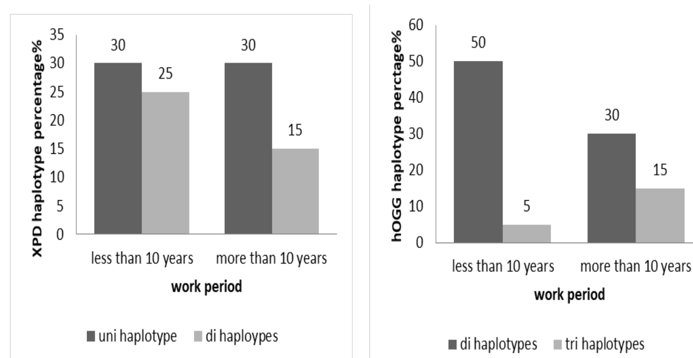

Fig. 6. The haplotypes percentage distribution of XPD(X2 0.3030, p 0.582) and hOGG (X2 1.8181, p 0.1775) according to work period (less and more than 10 years)

Different work period were reported and classified to less and more than 10 years, *XPD* showed non-significant association (X2 0.3030, p=0.582) with work period. Uni haplotype was frequent in same percentage in both categories. The *hOGG* also non-significant correlations with work periods (X2=1.8181, p=0.1775), di haplotype more frequent in worker with less than 10 years (Figure 6).

DISCUSSION

The work in medication departments in some Iraqi hospitals have been observed to be without Health standards and laws. Thus varies harmful effects may implicated in disease incidence, the current study was suggested to estimate important genes that involved in DNA repair. The effect of X-ray in the cell components has been studied. Ann Christin [19] suggested that to improve radiotherapy, radiosensitivity in S phase could be increased by combining irradiation with agents that induce secondary DSB or inhibit checkpoint signalling.

Some reports describe the X-ray irradiation effects on DNA molecules in biological conditions, especially in medical applications, DNA strand breaks, Mutations, structural changes such as gene order rearrangements and chemical modifications of bases have been identified [20,21,22]. Diffusion and Creation of radicals during X-ray exposure have been associated to modifications in molecular structure [23, 24]. The strand break may be caused by X-ray photons [25]. In organisms, the repair mechanisms found to be withstand moderate levels of radiation-induced lesions [26]. The mutation in DNA may be existed in repair genes as observed in current study that lead to absence of repair mechanisms, the accumulated of damages with increasing X-ray dose a new scan is adding to the impacts of the previous ones. When molecules with DNA alterations like oxidative and hydrolytic lesions as a result of natural decay and taphonomic mechanisms [27, 28]. The analysis of downstream genetic can be affected in cases have X-ray dose accumulation with significant levels. Particularly the hydrolytic conditions seem to have a major role as demonstrated in a report on simulated impacts of X-radiation on fragmented DNA in different conditions as well as wet, dry and frozen states, the radiation-induced DNA damage highest probability occurs in a wet state [29].

On the other hand, Wang et al., supposed that the effects of x-ray depended on the exposure dose and type of x-ray [30]. The safe dose should be very low dose of KV X-rays, the Mega-V-X-rays have been found to be dose-dependent. The higher dose than the damage threshold would be harmful, that between (1.0 - 1.5 Gray) may have some benefits like cell growth and gene transfer.

The Radiation stimulate different DNA lesions, about 10,000 bases were damaged, about 1,000 single strand breaks and 40 double strand breaking that stimulate during exposure to a gray per cell [31, 32]. These damages if didn't corrected it may be caused cell death by mitotic catastrophe and apoptosis. The double strand breaks is more harmful that can trigger cell death [33].

The association between x-ray and DNA repair genes was studied in some reports like Hu et al., found that amino acid substitution variants of *XRCC1* and *APE1* may contribute to IR hypersensitivity [34]. Toprani et al., observed that the base excision repair gene polymorphisms including (*hOGG1, APE1, XRCC1*, and *LIGASE1*) play important role in identifying donors with radio-sensitivity and Radio-adaptive response in human cells [35].

The deficiency in DNA repair mechanisms lead to different diseases and the result of present study is an important to take in consideration in medication department employees that work without health awareness about protective tools used during work to avoid harmful effects.

CONCLUSION

The exposure time, exposure day, work time and work period didn't have effects in the *hOGG* and *XPD* haplotypes but strong association with x-ray technicians. This mean that there were vital role of x-ray in human genome in addition to decline in the *hOGG* Ser326Cys and (Arg399Gln) *XPD*.

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