Association of the Variant Alleles of the X-Ray Cross Complementing Gene (XRCC1) Gene with Levels of DNA Adduct in Iraqi Workers

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Abstract

Background. Today, the world is witnessing a significant increase in the number of vehicles which have been carcinogenic emissions of PAHs, The carcinogenic pathway of PAHs. The inter-individuals differences in xenobiotics metabolizing enzymes and DNA repair capacities (XRCC1 gene) that may reduced or increase the effects of the exposure to these carcinogenic compounds. **Objectives.** Assessment of a single nucleotide polymorphism of the XRCC1 (codon Arg399Gln) gene and its association with levels of benzo[a]pyrene-7,8-diol-9,10-epoxide-DNA adduct in sera of car repairers and control group.**Subjects and Methods.** The current study included (60) participants, (20) of the iraqi car repairers, (20) sellers of spare part, and (20) healthy (controll), with the same age range. benzo[a]pyrene-7,8-diol-9,10-epoxide-DNA (BPDE-DNA) adduct was determined by using ELISA technique. Alleles frequency of singl nucleotide polymorphisms of XRCC1 gene was determined by Restriction fragment length polymorphisms (PCR-RFLP). **Results** The results of current study indicated that there is a highly significant increase of BPDE-DNA adduct level (p= 0.0001) in the repairers group compared to the groups of spare part sellers and control together. The genotype frequencies studies of XRCC1 gene of car repairers group and controls. The effect of rs25487 genotypes on exposure to PAHs include higher levels of PAHs and PAH-DNA adduct in TT genotype followed by CT genotype and CC showed lower levels.**Conclusions** As results of prolonged exposure to the higher levels of PAHs that Iraqi car repaiers suffer from, they have highe levels PAH-DNA adduct. especially those who have the TT genotypes, as results of the inefficiency of thier DNA repair system. Compared to the other genotypes.

Kywords: polycyclic aromatic hydrocarbons (PAHs), XRCC1 gene, car repairers, spar part seller.

1. Introduction

Occupational health interest in health and safety issues in workplace and and it mainly focuses on the hazards preventation. The workers health has virouse determinants, among them risk factors at work environment leading to cancer. Millions of workers in variouse occupational precautions have potential to be exposed to hazardous materials, which can exist in occupational environment in many form such as gases, vapour, fumes, and particles. Garage workers are exposed to complex mixture of PAHs, becacuse of thier occupation, Also automobile mechanics are at an increased risk skin, lung, urinary tract cancer [1]. several recent studies indicate that car repair shops are considered as anthropological sources of PAHs and heavy metals in some cities of the world [2]. With regad to iraq, specifically in city of hilla, the distribution of the automobile repair repair workshops across the city is random and irregular. Among the negative practices that occure in workshops, most of the auto-lubricants are spill onto soil, that contain PAHs, (that are classified as probable carcinogens), as well as lack of fresh air indor.usually, workers responses to exogenous exposures varies, due to variable of metabolic rate, processes of DNA repair, and other factors [3]. Benzo[a]Pyrene, among all individuals of the PAHs, represent one of the global studied pollutants and its found in Car exhaust fumes. Numerouse studies have confirmed that its a potent immunosuppressive, proinfammatory and carcinogenic agent [3,4]. Carcinogencity of B[a]P is begins after phase I biotransformation by ibducible P450 superfamily (CYP1). And later the reactive spesices like (Benzo[a]pyrene-7,8-diol-9,10-epoxides) (BPDE) are formed and mediated by the CYP2E1. (BPDE) is distinguished by have a high affinty for DNA and thus it attacks the DNA to generate BPDE-DNA adduct [1,5]. BPDE-DNA adducts is incorporated into many hydrolysis reactions that are catltzed by phase II conjucation to protect cell genome via eliminate PAHs metabolites. There are two main pathways through which PAHs exert their carcinogenic, mutagenic, genotoxic properties: first mechanism involve generating of specific PAH-DNA adducts with replication errors and subsequent mutation. And the another mechanism involves induction of oxidative stress. The interactiona between envirnmental and gentics factors play an effective role in the devlopment of most cancers in humans [6]. Bulky PAHs-DNA adducts are repaired by two

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pathwayes, the first is base exsision reapir pathway (BER) and second is single strand breaks (SSB), where the X-ray repair cross-compleminting gene (XRCC1) is implcated in both pathwayes, and it is play vitol role in the base exsision reapir pathway (BER) [7]. Protien XRCC1 acts as a scaffolding protein in BER, via interaction with the ADP-ribose polymerase, DNA polymerase β, and DNA ligase III.14 A polymorphism of the XRCC1 gene at codon results frome substitution of amino acidarginine in the place of glutamine, resulting in an ineffective repair pathway [8]. We conducted the present study to investigate the relationship between biomarker of DNA adducts in car repaires, and to assess the role DNA repairing gene (*XRCC1*) polymorphism in detecting workers at risk. Especially, the reports on occupational exposure and related health risks are almost non-existent, and this reflects paucity of availability of survey data and criteria for estimating whether unsafe exposure has occurred.

Subjects materals and methods

The current cross-sectional study included 60 male volunteers (n=60) with age range (25-45 years). Volunteers were divided according to their exposure to vehicle exhaust emissions into three groups: (i) (no=20) car repairers (as highly exposed group), (no=20) spare part sellers, and (iii) (no=20) non-exposed volunteers. All exposed subjects were matched in age, smoking status with unexposed group.

blood samples collection

1ml of venous blood sample was taken from was drawn from each volunteers and placed into disposable EDTA contaning tubes and stored at -20 °C unitl it was used in the determination of BPDE-DNA adduct and genotyping study. Quantity and quality of extracted DNA was determined by nano-drop, UK, using the scanning power of the diode assembly, within the wavelength 200-320nm. the Quantity and quality of extracted DNA was determined by calculating the (260/280) and (260/230) ratios. where samples that were (260/280) ratio less than 1.8 and /or (260/280) ratio 2, were re-extracted. The integrity and molecular weight of extracted DNA was determined by agarose gel electrophoresis according Samboork and Russell.

PCR-RFLP Analysis Design

According to [11], The genotyping technique was selected, while the primers are designed according to protocol of [12] briefly as follows:The primers were designed by the aid of NCBI-primer BLAST online software (http://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi?LINK_LOC=BlastHome),
AR87f TAAGCAGGCTTCACAGAGCC
AR87r TGGCATCTTCACTTCTGCCC.
and the produced primers were checked for specificity of their target sequence by performing a BLAST against the human genome, then the primers pair was selected according to the demand criteria such as: product length , the similarity of melting temperature , primers length , specificity , etc. Then the mutations was interred according to the design demands. The primer ability to form secondary structure was checked by the aid of Oligo Calc online software (http://www.basic.northwestern.edu/biotools/oligocalc.html), the primer would be rejected if it had 5 bases or more able to form self-dimerization and/or it had 4 bases able to form hairpin . Each primers pair was checked for dimer formation by the aid of "Multiple Primer Analyzer" online software from Thermo Fisher Scientific Inc.© , the sensitivity of the software was adjusted to the value 2 , the primer pair would be rejected if it made any dimers in this degree of sensitivity.

Restriction Enzyme Selection

The selection of the suitable restriction enzyme (AsuC2I CC^SGG sib) was performed by the aid of WatCut online software (http://watcut.uwaterloo.ca/template), we selected the restriction enzyme according to several criteria such as: the lesser primer mutations needed , the distance of mutation from the variant, compatibility of the produced primers , cost and availability.

Optimization PCR condition of Arg399Gln (rs25487)

The mixture shown in table 1. Was used as a preliminary mixture in the PCR reaction.

table 1: optimized reaction mixture for PCR.

No	Composition	concentreation	Volum
1	Master mix	2.5X	8 µl
2	Forward primer	10pmol/µl	1 µl
3	Revers primer	10pmol/µl	1 µl
4	DNA sample	10-20ng/µl	2 µl
5	Nucleases free water		7.5 µl
6	$MgCl_2$	25mM	0.5 µl
	Total volume		20 µl

Then, different annealing temperatures were used to obtain a specialized and efficient product. The temperatures and optimized PCR condition used are shown in table 2.

table 2: Optimized PCR condition of Arg399Gln (rs25487).

Stage	step	Temperature oC	Time	No.of cycles
1	Initial denaturation	94	5 min	1
2	DNA denaturation	94	30 sec	35
	Primer annealing	55-67	30 sec	
	Extension	72	30 sec	
3	Final Extension	72	5 min	1

Arg399Gln (rs25487) genotyping

Genotyping of XRCC1 (rs25487) polymorphisms was conducted by PCR-RFLP technique. And the restaraction digestion of amplicon was digested by (AsuC2 I), and the reaction mixture whose components were used: one unit of enzyme 0.25µl, 5µl of PCR product, 1.5 µl of buffer, and volum was completed to 15 µl by molecular graded water. the reaction mixture accubated in 37 °C overnight. Then the reaction product resolved in 2% of agarose gel.

Quantification of Benzo[a]pyrene-7,8-diol-9,10-epoxides (BPDE-DNA adduct)

The samples of extracted DNA were next diluted in 1X TE buffer to a concentreation of 4µg/ml. And by using sandwich-based immunoassay OxiSelect BPDE-DNA ELISA Kit, MyBioSource, Inc, USA. The reduced DNA standare was used as an absorbance blank, to reduced background signals from unadducted DNA. the calibration curve (consisting of eight points) was included in each measurement.

2. Statistical analysis

The statistical calculations included in this study were carried out using SPSS software (IBM Corp. Released 2012. IBM SPSS statistics for windows, Version 21.0. Armonk, NY: IBM. Crop. USA) and microsoft Excel (2010 microsoft Crop. USA). The results expressed as mean ± SEM, and p <0.05 was considered statistically significant. To evaluate the presence of significant differences, ONE WAY ANOVA, and unpaired-sample T-Test were employed. Also regression analysis used to asses presence of correlations, and the logistic regression was performed to adjust odd ratio.

3. Results and discussion

Annealing temperatures range (55-67oC) were used in optimization PCR conditions of Arg399Gln (rs25487) to obtain efficient product, The results obtained are shown in figure 1. The best PCR product was obtained at a tempreature of 63oC .

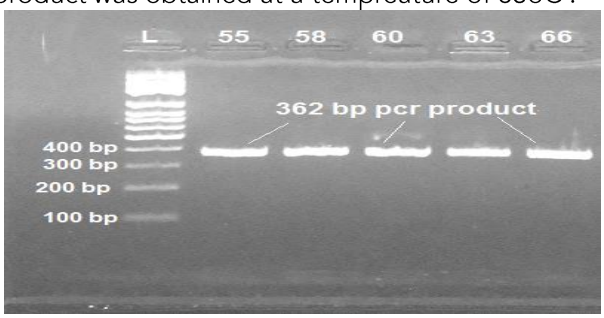

Figure 1: PCR-reaction products at different annealing temperatures (55, 58, 60, 63, 66 oC), under same optimized PCR conditions of Arg399Gln (rs25487).

The results obtained from PCR-RFLP of (RS 25487) genotyping shown in the figure 2, where the CC allele revealed 221bp and 141bp fragments, whilest TT allele was not digested, and visulazed at 362bp single product. CT (hetrozyget) allele revelaed three bands of 362bp, 221bp, and 141bp.

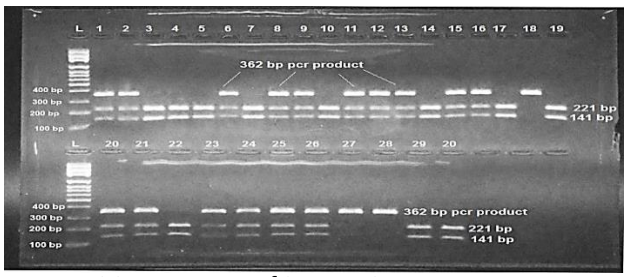

Figure (2): PCR-RFLP of (rs25487) genotyping; Lane L= 100bp DNA ladder; Lane(362bp) = TT genotype; Lanes (221bp+141bp)= CC genotype; and Lanes (362bp+221bp+141bp)= TT genotype.

Estimation of BPDE-DNA adduct levels

The levels of BPDE-DNA adduct was estimated in the three groups of volunteers, whereas, the results showed that the highest level of BPDE-DNA adduct was in the car repairers (1.695±0.32ng/ml), followed by the group of spare part sellers (1.24ng/ml), and in the end, the lowest level of adduct was in control group (1.14 ng/ml) as observed in both table (3) and figure (1). And a significant difference among studied groups was (P<0.0001). Where no significant difference between control group and spare part sellers group, also significant difference was found between both of these groups (b) and car repaires group (a).

table 3: comparison of BPDE-DNA adduct levels among studied groups based on ANOVA test.

group	Control mean±SD	Car repaires mean±SD	spare part sellers mean±SD	P-value
BPDE-DNA adduct	1.14±0.21b	1.695±0.32a	1.24±0.26b	<0.0001

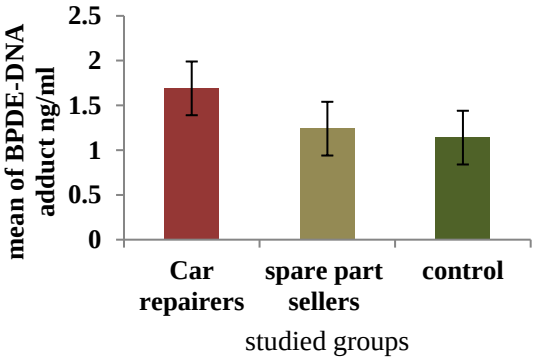

Figure 1: comparison of BPDE-DNA adduct levels in studied groups (control, car repairers and spare part sellers).

B[a]P is considered the first chemical carcinogen that was discovered mong all the PAHs individuals, and it was observed in the car exhaust fume [13]. Its carcinogenic pathway depends on the enzyme metabolize it, starting from the first step and ending with the mutagenic metabolite benzo[a]pyrene-7,8-diol-9,10-epoxide (BPDE) [14-15]. BPDE is know for its higher reactivity and have affinity toward macromolecules like proteins, lipids and DNA, to form BPDE-DNA adducts. DNA adducts may lead to presistent mutations (If not repaired), that lead to cell transformation and eventual tumor progression [16],

particularly the BPDE-DNA adduct have been associated to an increased risk of lung cancer [17-18], Also automobile mechanics are at an increased risk of skin and lung cancers as a result of exposure to PAHs [19]. The results obtained in the current study support the above finidings; A significantly higher (P<0.000) levels of BPDE-DNA adducts were presented by car repairers group relative to spare part sellers and control subjects (1.695 vs 1.24 and 1.14 ng/ml) table (3). Where the levels of BPDE-DNA adducts in the car repairers group is 1.5-folds greater than in the spare part sellers group. this means an increased risk of genotoxic effects associated with the exposure to PAHs. In other occupational groups, The BPDE-DNA adducts were detected in 85% of the sample of exposed traffic workers, in general the adjested mean levels of BPDE-DNA adducts tended to be higher in the group of traffic exposed workers (≈1.24 folds) than in non-exposed subjects (1.37 vs 1.10 ng/ml), where the although it did not reach statistical significance (P=0.10) in this atudy [20]. This indicates that the levels of BPDE-DNA adduct level in car repariers is 1.2 times higher than the its levels in traffic workers.

In reagred other heavy exposed accupational categories such as coke oven workers, as know those workers show heavy exposed to PAHs based on the urinary 1-OHP as biomarker, The mean of PAHs-DNA adduts was significantly higher in group of italian coke oven workers (0.54 ng/ml) than in the group of non-coke oven workers (≈1.62 folds) (0.32 ng/ml) [21]. While the mean was equal to 3.3ng/ml for egyptian coal tar workers (Shehata et al, 2020), meaning the level of exposure to PAHs was 1.8 times greater than what it was in iraqi car repairers (mean=1.65 ng/ml).

The results of RFLP-PCR for SNP (rs25487) genotyping of XRCC1 gene are listed in table (3) for the car repaires, spar part sellers and control subjects. Where the results of genotype frequencies of car repaires specimens revealed that CC genotype were higher in sample of auto repaiers (45.94%) compared to the control group (16.21%), this is showed a significant difference (<0.0001) with an odd ratio equal to (17.8). the heterozygous genotype CT had lower frequncy in samples of car repaires (29.72%) than controls (32.34%) and this cobained with non-significant (p=0.08) and low odd ratio (0.421). the frequency of TT genotype was higher in control samples (51.35%) compared to the samples of car repaires (24.32%) with odd ratio (P 0.13) differances was signifcant (P=0.001).

Table (3) frequencies association between genotyping of XRCC1 gene of car repaiers group samples and controls.

genotype	Control (n=37)	Car repairers (n=37)	P-value	Odds ratio	95%C.I.
CC Wild	4 (10.81%)	25 (67.56%)	<0.0001	17.18	4.49 to 9.702
CT Heterozygous	16 (43.24%)	9 (24.32%)	0.088	0.421	0.156 to 1.139
TT Mutant	17 (45.94%)	3 (8.10%)	0.001	0.103	0.027 to 0.398
Allele frequency%					
Allele	Control (n=37)	Car repairers (n=37)	P-value	Odds ratio	95%C.I.
C	24	59	<0.0001	8.1944	3.88 to 17.29

gentic polymorphism of spare part sellers samples

The results of genotypes of XRCC1 gene of are listed in table (3-8) for the spare part sellers and control subjects. Where the results of genotype frequencies of spare part sellers specimens revealed that CC genotype were higher in spare part sellers (45.94%) compared to the control group (16.21%), this is showed a significant differences (p<0.0001) with an odd ratio equal to (17.8). the heterozygous genotype CT had lower frequncy in samples of spare part sellers (29.72%) than controls (32.34%) and this cobained with non-significant (p=0.08) and low odd ratio (0.421). the frequency of TT genotype was higher in control samples (51.35%) compared to the samples of spare part sellers (24.32%) with odd ratio (P= 0.13) differances was signifcant (P=0.001).

Table 4: frequencies association between genotyping of XRCC1 gene of spare part sellers group samples and controls.

genotype	Control (n=37)	spare part sellers (n=37)	P-value	Odds ratio	95% C.I.
CC Wild	4(10.81%)	21(56.75%)	0.0001	10.828	3.181to36.849
CT Heterozygous	16(43.24%)	9(24.32%)	0.088	0.421	0.156 to 1.139
TT Mutant	17(45.94%)	7(18.91%)	0.0155	0.274	0.096 to 0.781
Allele frequency%					
Allele	Control (n=37)	Worker (n=37)	P-value	Odds ratio	95% C.I.
C	24	51	<0.0001	5.503	2.059 to 10.22
T	50	21			

In this study, which was conducted for the first time on the people of hillah city, we tride find any possible association between the three variant alleles of the

XRCC1 gene and levels of BPDE-DNA adduct. The effects of SNP (rs25487) genotyping of XRCC1 gene on BPDE-DNA adduct level are explained in the figure (3-6), however the highest level of BPDE-DNA adduct had been detected in the car repaires subjects whos carriers the TT genotype, followed by heterozygouse genotype CT, and then CC genotypes. and also the spare part sellers samples,

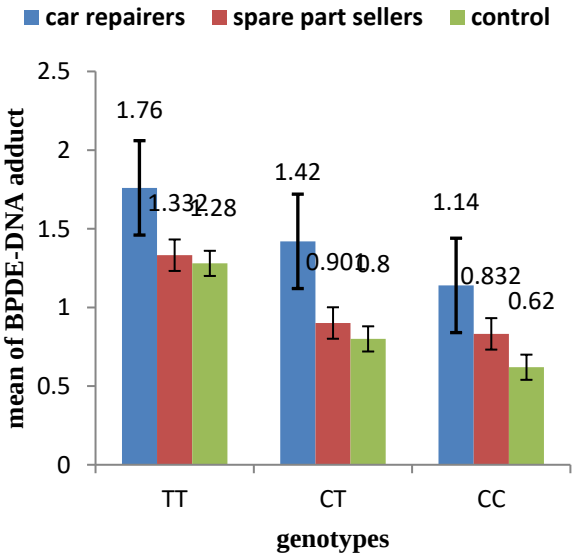

Figure (3-6) effects of SNP (rs25487) genotyping of XRCC1 gene on BPDE-DNA adduct level.

The result of our study showed the CC dominant homozygous genotype of the XRCC1 (DNA repairing gene) showed significantly lower levels of measured BPDNA-adduct comparison to the other genotypes, followed by heterozygous genotype CT, and then TT shown higher levels (figures 3-4, 3-5, and 3-6). Our results are agreement with many previouse studies, [22] found that the CC genotype has lower levels of BPDE-DNA adduct. It was also found in another study [23] that the chinese coke oven workers carrying the TT genotype had higher levels of BPDE-DNA adduct.

The variations in the levels of PAHs among the studied genotypes is due to the variation in the activity of xenobiotics metabolizing enzymes. Many previouse studies reported that the TT genotype had a decrease in activity of xenobiotics metabolizing enzymes (especially CYP2E1), as result, they have high levels of PAHs. this resulting in long-term adverse effects, promoting cytotoxicity and genotoxicity, making indivadials more susceptible to different types of cancers [24].

And considering that B[a]P is a complete carcinogen, that act as an initiator and a promoter of carcinogensis according to EPA, 2017[25]. At the same time, it was observed that the CC genotypes had low levels of BPDE-DNA adduct levels this is supports findings of matullo et al [26], who stated that the carriers of CC showed a relatively lower levels of bulky DNA adduct in lymphocytes, which may be association with reduced capacity of DNA repair system. Also In meta-analysis [27], TT where the highest level of BPDE-DNA adduct was in the spare part sellers who carriers TT genotype, followed by heterozygouse genotype CT and then CC genotypes showed the lowest level. Also the higher level of BPDE-DNA adduct detected within the genotype TT of control followed by CT of control and lower level was shown by the CC genotype of control.

genotype of the XRCC1 rs25487 (Arg399Gln) polymorphism found that was increased PAHs-DNA adducts levels and it may be association with low DNA repair capacity

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