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Study the Effects of (8-oxo-Guanine DNA Glycosylase 1 rs1052133) Polymorphism on Gene Expression as a Predisposing Factor for DNA Mutation in Midland Refineries Company-Dura Refinery Workers

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Abstract

Background Refineries are exposed to different types of air pollutants, among them, polycyclic aromatic hydrocarbons (PAHs), they are a group of over hundred different kinds of hazardous organic chemicals (pollutants), which are formed primarily during the incomplete burning of oil, gas and coal. PAHs are metabolized in different organs of human body especially in the liver leading to generation of mutagenic metabolites, of them, the reactive oxygen species, which damage DNA by binding with guanine base leading to formation of 8-hydroxyguanine. The 8-oxo-guanine DNA glycosylase (hOGG1 rs1052133) gene, is a regulatory gene binds with 8-oxo-DG in the DNA to initiate base excision repair (BER) pathway.

Objective To investigate the role of (hOGG1 rs1052133) gene polymorphism in single nucleotide polymorphism repair.

Methods One hundred sixty-eight (168) subjects were participated in this study. They were they divided into three groups (country side, office workers and field workers). PAHs were analyzed in all participant by Gas Chromatography Coupled with a Mass Spectrophotometry GC/MS. SNP for hOGG1 and gene expression were detecting by real time polymerase chain reaction and quantitative polymerase chain reaction for each subject respectively.

Results PAH were detected in blood serum of refineries workers and not detected in country side participants. The level of hOGG1 gene expression was higher in GG genotype than GC and CC genotype.

Conclusion Refineries worker are at high risk of developing malignant transformation and serious disease due to high level of PAH compared to other population. hOGG1 plays an important role in DNA repair following DNA mutation.

Keywords Poly cyclic aromatic hydrocarbons, HOGGI, gene expression

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List of abbreviations: BER = Base excision repair, ELISA = Enzyme-linked immunosorbent assay, GC/MS = Gas chromatography coupled with mass spectrometry, hOGG1 = 8-oxo-guanine DNA glycosylase, oh8Gua = 8-hydroxyguanine, PAHs = Polycyclic aromatic hydrocarbon, ROS = Reactive oxygen species, SNP= single nucleotide polymorphism

Introduction

Air pollution is the major environmental hazard all over the world, as well as urban areas, associated with increased

morbidity and mortality rates. Refineries worker are exposed to different types of air pollutants, among them polycyclic aromatic hydrocarbons (PAHs) are the common. PAHs are a class of organic chemical substances that contain 2 to 7 fused aromatic rings. Several PAHs are very well known as mutagens, carcinogens and teratogens, and thus pose a serious threat to the global health environment. The physicochemical properties of PAHs make them highly mobile in the environment, allowing them to distribute across air, soil, and water [1]. PAHs are carcinogenic environmental pollutants resulting from incomplete combustion that are commonly found in tobacco smoke, ambient and indoor air, charbroiled foods and oil refinery. After exposure, these compounds are metabolized in human body (liver, kidney and lungs) [2,3]. Reactive metabolites (e.g., epoxides and dihydrodiols) of some PAHs have become one of the major health concerns because of their potential to bind to cellular proteins and DNA with toxic effects, even more toxic than PAHs itself [4]. The resulting biochemical disruption and cell damage can lead to mutations, developmental malformations, tumors, and cancer. Evidence indicates that mixtures of PAHs may be more carcinogenic to humans than individual PAHs. The evidence comes primarily from occupational studies of workers exposed to mixtures of PAHs. According to the U.S. Environmental Protection Agency, seven PAH compounds have been classified as probable human carcinogens: benz(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(k) fluoranthene, chrysene, dibenz(ah)anthracene, and indeno(1,2,3-cd) pyrene [1].

In vitro and in vivo, 8-hydroxyguanine (oh8Gua), (a main form of oxidative DNA damaged caused by reactive free radicals that generate from PAHs metabolism, is extremely mutagenic) [5]. The occurrence of oh8Gua in DNA results in a G:C to T: A transversion because oh8Gua controls the incorporation of cytosine and adenine nucleotides in the opposite direction of the lesion [6]. The base excision repair (BER) of the oh8Gua lesion in human DNA is 8-oxo-guanine DNA glycosylase (hOGG1) that initiate the first step of the repair pathway. hOGG1 has been extensively studied since its initial cloning and characterization in 1997 to better understand its catalytic mechanism and the roles of specific amino acid residues in this process. So far, it has been discovered that a few key residues are required to varying degrees for the enzyme's functionality in both substrate recognition and the subsequent catalytic process [7,8].

The hOGG1 rs1052133 gene, located on human chromosome 3p26.2 [9], is a regulatory gene binds with high affinity to 8-oxo-DG especially at guanine-rich promoter regions because the oxidation potential of guanine is the lowest among the four DNA bases in the DNA double-stranded to initiate BER pathway. As well as, hOGG1 has variable signal transduction functions, interacts with 8-oxo-DG in gene regulatory regions, and facilitates gene expression [8,10]. Because of the characteristics of single nucleotide polymorphism (SNP), the interaction of gene mutation and environment may result in DNA repair defects and, as a result, an increase in the happening of certain tumors. hOGG1 genetic polymorphism has been detected in various populations [11].

This study aimed to investigate the role of hOGG1 rs1052133 gene polymorphism in SNP repair.

Methods

Subjects and study groups

One hundred sixty-eight (168) male subjects of Midland Refineries Company - Daura Refinery workers with age between (25-60 years) with healthy (mentally and physically) were participated in this study, a case control study, anywhere carcinoma and subjects with autoimmune disease were excluded, the samples we collected from February to October 2020: these study populations are subdivided into three groups.

Group one: control (lives in country sides (rural)) away from pollution (n=56).

Group two: office workers (who work far of the refinery field (around field)) (n=56).
Group three: field workers (close to the pollution) (n=56).

Specimens' collection

About 10 ml of venous blood was drown from each participant. It was divided to:

A. Seven ml for serum sample (for PAHs determination).
B. Three ml for whole blood sample for:
    - Real-time polymerase chain reaction (RT-PCR) for SNP of hOGG1 gene.
    - RNA extraction (100 µl in 300 µl TRI Reagent®) for hOGG1 expression.

Methods

Determination of PAHs Concentration in Serum by Gas Chromatography Coupled with a Mass Spectrophotometry (GC/MS)

Liquid-liquid extraction technique used for PAHs separation in serum, a volume of (40 ml) n-hexane and (10 ml) dichloromethane (DCM) was used for extraction. The extractant (10 ml) and plasma samples (2 ml) were added in the vials.

The vials were capped and vortexed for 20 sec at 300 rpm. The organic layer was sucked out using pipette attached with pipette filler into clean and thermally treated amber bottles. The extracts were cleaned up in a column (1 cm x 15 cm, internal diameter and length) with slurry of silica gel as stationary phase, preconditioned by distilled water and hexane/DCM before the samples were eluted, collected and concentrated in a stream of nitrogen gas [12].

After extraction, the PAH was analyzed and detecting by GC/MS. A Shimadzu QP 2010 plus GC/MS equipped with an auto-injector AOC-20i have been used for this purpose. The samples were introduced in the split less mode with an injection temperature of 280°C. The transfer line and ion source temperatures were 300°C and 200°C. The column temperature was initially held at 60°C for 5 min, and then raised to 180°C at the rate of 25°C per minutes, then to 220°C at the rate of 10°C per minutes, and finally to 300°C at the rate of 5°C per minutes, held at final temperature for 15 min. Detector temperature was kept at 320°C. The carrier gas was helium, which was used at a constant flow rate of 1 mL per minutes.

Molecular analysis

The Quick-gDNA™ Blood MiniPrep is a simple procedure for the rapid isolation of total DNA (e.g., genomic, mitochondrial, viral) from a variety of biological sample sources. This Procedure has been optimized for maximal recovery of ultra-pure DNA without RNA contamination and is compatible with whole blood (fresh or stored), serum, and plasma. For processing, simply the specially formulated Genomic Lysis Buffer was added to a sample, vortex, and transfer the mixture to the supplied Zymo-Spin™ Column. There is no need for organic denaturants or proteinase K digestion because of the unique chemistries featured in the kit. Instead, the product features Fast-Spin technology to yield high-quality, purified DNA in just minutes. PCR inhibitors are effectively removed during the purification process. DNA purified using the QuickgDNA™ Blood MiniPrep is suitable for PCR, nucleotide blotting, DNA sequencing, restriction endonuclease digestion, bi sulfite conversion/methylation analysis, and other downstream applications.

1- SNP Real Time Taq Man PCR

Applied Biosystems™ TaqMan® SNP Genotyping Assays use TaqMan® 5´ nuclease chemistry for amplifying and detecting specific polymorphisms in purified genomic DNA samples. Each assay allows genotyping of individuals for SNP.

2- Gene expression of hOGG1

Gene expression is the process by which information from a gene is used in the synthesis of a functional gene product that enables it to produce end products, protein or non-coding RNA, and ultimately affect a phenotype, as the final effect. These products are often proteins, but in non-protein-coding genes such as transfer RNA (tRNA) and small

nuclear RNA (snRNA), the product is a functional non-coding RNA.
RNA extraction kit supplied by Direct-zol™ RNA MiniPrep, R2051, ZYMO RESEARCH / USA. RNA was extracted from whole blood sample. A fragment 89 bp of hOGG1 was amplified using a forward and reverse primers were supplied, by IDT (Integrated DNA Technologies company, Canada) table (1).

Table 1. Specific primer for hOGG1gene

Primer	Sequence	Tm (°C)	GC (%)	Products size
Forward	5'-GAAATAGGGAAGGTTGTTAAATAGTAT-'3	51.9	32.1	89
Reverse	5'-AAACTAAAATACGATACCCCATAC -'3	52.8	36.0	

Prime Script TMRT reagent Kit was prepared to carry out the reverse transcription optimized for RT-PCR. It uses Prime Script TM RTase, which has excellent extendibility and allows for efficient, fast cDNA template synthesis for RT PCR. The step experimental procedure is straightforward and well-suited to high-throughput analysis. This kit can be used in combination with Real Time PCR reagent, SYBR (sybr green) Premix. The gene expression level for the sample have been determined using the following equation (obtained from the kit leaflet of Direct-zol™ RNA MiniPrep, R2051, ZYMO RESEARCH / USA).:

ΔCt of patient=Ct target – Ct reference
ΔCt of control=Ct target – Ct reference
ΔΔCt= ΔCt of patient- ΔCt of control

$$\text{Folding}=2^{-(\Delta\Delta Ct)}$$

Results

The participant ages shown in table (2); age of participants ranged from (25-59) yr, they were divided into 4 groups at a range of every ten years, there is no significant difference in age among studied groups

Table 2. The age of participants

Age group	Control	Field	Office	p value
25-35	30	30	29	0.071
36-45	6	5	4	
46-55	10	9	11	
>56	10	12	12	
Total	56	56	56	

PAHs were detected in the blood of field and office groups and not detected in the blood of control group; the level of PAH was significantly higher in field group compared with office group.
HOGG1 gene expression levels were significantly higher in field group than in office group and control group respectively as shown in table (3).
The genotype GG was higher in control groups than office and field groups respectively, where the CC genotype was higher in field than office and control groups respectively. As shown in tables (4 and 5).

Table 3. Results of PAHs and hOGG1 expression among the studied groups

Parameter	Group	Mean±SD	p value
PAH (ppm)	Control	-	0.001
	Office	2.66±0.15	
	Field	6.78±0.27	
HOGG gene expression(fold)	Control	1.1±0.23	0.001
	Office	1.69±0.31	
	Field	4.1±0.46	

Table 4. Frequencies association between OGG1 rs1052133 genotypes in samples of the field and control

		Control n = 56 n (%)	**Field n = 56 n (%)**	**P value**	**Odds Ratio**	**95% CI**
Genotype	GG	17 (8.9%)	5 (5.3%)	0.008	0.22	0.1
	GC	19 (33.9%)	18 (32.1%)	0.922	0.92	0.42 to 2.01
	CC	20 (57.1%)	33 (65.5%)	0.02	2.5	1.21 to 5.50
Allele	G	53 (25.9%)	28 (21.4%)	0.05	0.73	0.42 to 1.44
	C	59 (74.1%)	84(78.5%)			

Table 5. Frequencies association between OGG1 rs1052133 genotypes in samples office and control

		Control n = 56 n (%)	**Field n = 56 n (%)**	**P value**	**Odds Ratio**	**95% CI**
Genotype	GG	17 (30.5%)	10 (17.8%)	0.267	0.45	0.71 to 6.90
	GC	19 (33.9%)	25 (44.6%)	0.333	0.85	0.74 to 3.35
	CC	20 (35.9%)	21 (37.5%)	0.050	2.2	0.21 to 0.95
Allele	G	53 (25.9%)	45 (40.2%)	0.033	1.92	1.09 to 3.38
	C	59 (74.1%)	67 (59.8%)			

The effect of genotype on the level of hOGG1 gene expression are illustrated in figure (1). The results showed higher level of gene expression for the infield samples are showed by the GG genotype (4.7) followed by the CC genotype (4.101) and then GC genotype (3.842). The expression level around the office subjects is showed higher level by the GC genotype (2.368) followed by the CC genotype (1.472) then by GG genotype (0.193).

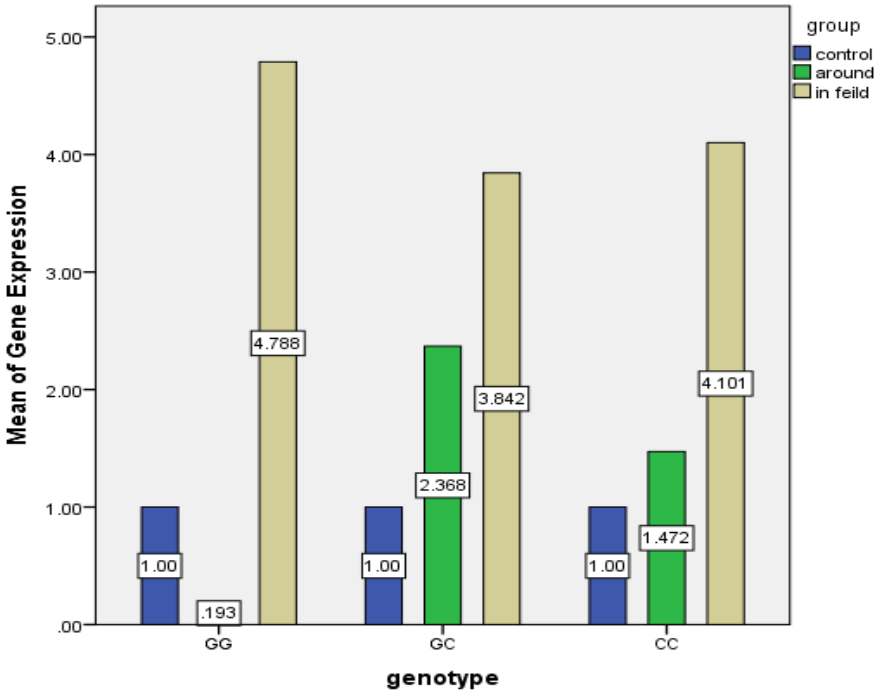

Figure 1. Effect of rs1052133 genotype on hOGG1 gene expression level

Discussion

PAH were not detected in the blood of control group, and their levels were significantly higher in field workers compared with office workers. hOGG1 gene expressions are increased in refineries workers (around field and in field) as compared with control group with significant increase in field workers compared to office workers. Most PAHs are not genotoxic by themselves and must be metabolized to their diol epoxides diene and/or hydroxyl, which then react with DNA to induce genotoxic damage. Genotoxicity plays an important role in the carcinogenicity process and may be in some forms of developmental toxicity [13].The level of hOGG1 was higher in participant with GG genotype than those CC and GC genotype this indicate that GG genotype is a protective genotype in refineries workers against 8-oxo DG accumulation that generate from PAH metabolites in human and CC genotype group the least susceptibility to increase hOGG1 gene expression and the BER pathway eliminates many varieties caused by ROS generate from PAH metabolism. Our results are in line with Hassan results who proposed that GG genotype were significantly correlated with refineries worker's. This indicates that hOGG1 rs1052133 genotypes (CC and CG) promoted the increase 8-OXO-Dg level, which is in contrast to the GG genotype. This suggests that CC genotypes are significantly correlated with the risk of gene variation and mutation. These findings indicate that specific genotypes within a single repair pathway are factors that affect the risk of mutation. Because SNP rs1052133 significantly interacts with numerous variants of proteins with a slightly accelerated or reduced useful activity [14]. These results are in line with Alanazi et al. suggested in his study that that hOGG1 rs1052133 genotypes (CC and CG) promoted the development of leukemia, which is in contrast to the GG genotype. CC genotypes are significantly correlated with the risk of leukemia. These findings indicate that specific genotypes within a single repair pathway are factors that affect the risk of leukemia [15].

In conclusion, refineries workers are at high risk of developing malignant transformation

and serious disease due to high level of PAH compared to other population. SNP in the gene involved in DNA base excision repair hOGG1 rs1052133h might play a crucial role in DNA repair, GG genotype has the most defensive effect Against DNA mutation.

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Author contribution

Al-Ani went to Midland Refineries Company, AL-Daura Refinery and collected blood samples from office and field groups. Blood samples of control group (lives in country sides (rural)) away from Baghdad city) were collected by Shukur.

Al-Ani and Dr. Al-Wasiti took the collected blood samples to Wahj Al-Dna laboratory and Environmental Research Center, Ministry of Science and Technology to observed the process of obtaining the results. All authors collected articles with similar topics.

Conflict of interest

Authors declare they have no conflict of interest with others.

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