Association between Oxidative Stress Biomarkers and Glutathione Transferases Polymorphisms as an Indicator Susceptibility for Benzene's Effect on Workers at A Gasoline Filling Station in Baghdad

Baqer K. Hothefa[1,*], Sarmad A. Hazzaa[1], and Sumayah Mahmood[1]

[1]*Department of Community health, Technical Medical institute,MAIL, Baghdad 1Middle Technical University.*

[*]*Corresponding Author:* baqer.kahled@mtu.edu.iq

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Abstract: Oxidative stress is considered one of the most important processes that result in significant damage and adverse effects on human and animal health alike. Regular exposure to gasoline by employees of private fuel stations poses many occupational and environmental risks. The purpose of this work and this study is to compare and evaluate the health of employees at a Baghdad fuel station affiliated with the Iraqi Ministry of Oil. This study focused on how occupational exposure to gasoline affects oxidative stress indicators (MDA, GSH, CAT, and POD). The study aimed to identify any potential links between gasoline metabolism and GST gene polymorphisms. The mean oxidative stress values for MDA, GSH, CAT, and POD differed significantly between the employees and the control group ($P < 0.01$). According to genetic analysis, the fuel station employees and the control group differed significantly in their GSTM1 and GSTT1 variations. This study yielded the following allele frequencies for GSTM1-null genotypes in the worker population: 36.5%, GSTT1-null (19.42%), and CSTP1-null (7.7%), respectively. Associations were also found between GSTM1-null and several benzene exposure-related changes.

***Keywords*:** malondialdehyde, catalase, peroxidase, Glutathione, stress biomarkers

1. Introduction

Imbalance between the production of oxidants and antioxidants is referred to as oxidative stress defenses in biological systems. Initially, oxidative stress was primarily associated with pathological disease, but the field of redox biology has evolved to recognize the role of redox signaling in physiological processes (1).

Gas stations have sprouted out in both urban and rural regions as a result of countries having more automobiles, which have increased fuel [illegible], particularly in metropolitan areas. When refilling, several gasoline components that evaporate into the air will be released, causing air pollution (2). Gasoline is a complex combination of heavy-chain hydrocarbons, light-chain volatile chemicals, and short-chain organic compounds, among other hydrocarbons and additives. The source of the crude oil, the refining procedure, and the production lines employed all affect the relative concentration of the components in gasoline (3). Environmental benzene exposure is a significant concern due to its adverse health effects. In

addition to its role in hematologic cancers, benzene exposure affects the cardiovascular, respiratory, neurological, immunological, hepatic, renal, and reproductive systems. It can impair immune cell function, induce chromosomal aberrations, and disrupt neurotransmitters and enzyme activities (4).

Glutathione S-transferases (GST) Genes Polymorphisms: GST enzymes, encoded by GST genes, play a crucial role in the detoxification of environmental chemicals and naturally synthesized metabolites, thus protecting tissues from oxidative damage. GSTs, This class of phase II metabolic enzymes, formerly known as ligandins, includes both bacterial and eukaryotic enzymes. They are best known for their ability to catalyze the conversion of foreign substances to the reduced form of glutathione (GSH) for detoxification. The cytosolic, mitochondrial, and microsomal (sometimes referred to as MAPEG proteins) superfamilies make up the GST family (5). A frequent polymorphism in certain GST family members, including GSTM1 and GSTT1, is characterized by a complete gene deletion (null allele). The absence of the enzyme and its catalytic activity due to homozygous deletions (null genotypes) results in a reduced capacity for detoxification against specific genotoxic agents; these null genotypes have been linked to increased genotoxicity and are thought to be important determinants of susceptibility to xenobiotic-related diseases, including cancer (6). The tripeptide glutathione, C10H17N3O6S, has a molecular weight of 307.3235 g/mol. It is made up of the amino acids glutamate, cysteine, and glycine and functions as an antioxidant for the body (7). The transformation of glutathione from its oxidized form (GSSG) to its reduced form (GSH) is catalyzed by glutathione reductase (GR). The lack of this enzyme can enhance the erythrocyte membranes' vulnerability to hydrogen peroxide (H2O2), which can contribute to oxidative stress and the pathogenesis of a number of illnesses. This enzyme helps maintain proper amounts of cellular GSH. (8). Glutathione levels in the body are crucial, as disruption of glutathione synthesis and metabolism can impair its function as an antioxidant, leading to various diseases (9).

Catalase (CAT), also known as H_2O_2 oxidase, is composed of four poly peptide chains, each containing over 500 amino acids. CAT is present in all living organisms, ranging from unicellular prokaryotes to multicellular eukaryotes. That catalyzes the convert of H_2O_2 into water and O_2 (10). Catalase, among all antioxidant enzymes, has the highest turnover rate. Catalase breaks down H_2O_2 in a first-order process, and the amount of H_2O_2 present affects how quickly this happens. One hydrogen peroxide molecule oxidizes catalase, transferring the attached oxygen to a different substrate molecule (11). The amount of CAT activity varies substantially between tissues. The activity is found in high concentrations in the liver and kidneys and in low concentrations in

connective tissues such as blood, bone marrow, and mucous membranes (12).

Peroxidase (POD), a heme-containing enzyme that uses H_2O_2 to oxidize a variety of substrates, avoids an excessive buildup of H_2O_2 produced by normal metabolism or under stressful circumstances. (13). Peroxidase is extremely effective in metabolizing H_2O_2. The degree of peroxidase activity is used as a gauge for measuring the severity of stress since it is regarded as a "stress enzyme" (14).

Malondialdehyde (MDA) This chemical is one of the byproducts of polyunsaturated fatty acid peroxidation. This makes it a valuable marker for assessing oxidative stress and the extent of cellular membrane lipid damage caused by reactive oxygen species (ROS) (15). MDA can come from two different sources: the lipid peroxidation processes occurring in our tissues and the food we consume

. Numerous internal and external factors affect MDA production as well as the extent and pace of lipid oxidation in living things. MDA is distinctive among the byproducts of lipid peroxidation due to its cytotoxic, mutagenic, and carcinogenic characteristics. Additionally, it has the ability to block enzymes that help protect cells from oxidative damage. But the body has several defenses against the impacts of free radicals that can capture and destroy them (16).

2. Methodology (Experimental Procedure)

The study group included 30 control individuals and 52 workers; all of them were from the city of Baghdad. The workers were specifically gasoline filling station workers aged between 20 and 56 years old with a mean age of 40.5 ± 9.3 years, whereas the control group consisted of individuals engaged in different occupations, aged between 20 and 50 years old with a mean age of 39 ± 9.2 years. Using venous punctures, about 10 ml of peripheral blood was extracted from each participant. The blood was separated into two sections: The first step was to do a complete blood count (CBC) immediately; the second step involved carefully pushing the blood into disposable tubes with separating gel, letting it clot in the room for 30 minutes, and then centrifuging it for 15 minutes at 3000 rpm. Before being utilized for biochemical testing, the serum was isolated and kept at -20°C.

Glutathione (GSH) concentration, Malondialdehyde, Catalase Activity and Peroxidase activity were determined by Ellman method, thiobarbituric acid (TBA) reactivity, Colorimetric analysis, all using Spectrophotometer UV-VIS (Cecil, CE10N)

DNA extracted using FavorPrep™ Blood / Cultured Cell Genomic DNA Extraction Mini Kit then DNA amplification done by PCR using primers

Table (1): primers sequence used in PCR.

Gene GSTM1

Forward Primer	5′-GAACTCCCTGAAAAGCTAAGC-3′
Reverse Primer	5′GTT GGG CTC AAA TAT ACG GTG G 3′
Gene GSTT	
Forward Primer	5′-TTC CTT ACT GGT CCT CACATC TC-3′
Reverse Primer	5′-TCACCG GAT CAT GGC CAG CA-3
Gene GSTP1	
Forward Primer	5′-ACC CCA GGG CTC TAT GGG AA-3′
Reverse Primer	5′-TGA GGG CAC AAG AAG CCC CT-3′

Statistical Analysis

The impact of various elements on the research parameters was examined using the Statistical Analysis System SPSS v26 software. To significantly compare the parameter means across groups, the independent t-test and one-way ANOVA were employed. A test was used for genetic analysis. P values were deemed significant if they were less than 0.05 and highly significant if they were less than 0.01.

3. Result and Discussion

The oxidative stress indicators in the research groups, such as malondialdehyde (MDA), glutathione (GSH), peroxidase (POD), and catalase (CAT), are shown in Table (2). According to the data, there was a significant statistical difference (p-value < 0.01) in the levels of MDA, GSH, and POD among employees of gas stations, whereas the level of CAT was lower than in the control group.

The study demonstrates a significant MDA levels was increase to gasoline filling station work compared to the normal group ($p<0.01^{**}$). The study also revealed an increase in the level of the GSH enzyme among gasoline filling station work compared to the control group ($p<0.01^{**}$)

The results indicate a significantly higher level of peroxidase activity in all gasoline filling workers compared to the control group ($p<0.01^{**}$). This increase may be attributed to the exposure to benzene vapors, which contain phenol. Peroxidase functions in the dehydrogenation of phenols.

In contrast, the study showed a significant decrease in CAT levels among gasoline filling station work compared to the control group ($p<0.01^{**}$) the result is support by Asghar Ghahri a b, Pouria Seydi c, other 2024 (17).

Table (2): Effect of benzene on oxidative stress.

Groups	Control	Exposure workers	P values
Oxidative stress biomarkers			
MDA µmol/ml	0.84±0.248	1.05±0.290	0.01^{**}
CAT (U/L)	5.13±1.702	3.16±1.239	0.001^{**}

Glutathione(GSH) (µmol/L)	1021±432.5	1857±1144	0.001**
Peroxidase (POD) (U/L))	0.26±0.124	0.42±0.184	0.001**

* (P≤0.05), **(P≤0.01), NS: Non-Significant.

Table (3): statistical evaluation of the research individuals' GSTM, GSTT, and GSTP states Distribution of GSTM1 and GSTT1 Genotype Frequencies.

Genotype	Control, 30 (100)	Exposed, 52 (100)
GSTM		
Null	12(40)	19(36.5)
Non-null	18(60)	33(63.5)
GSTT1		
Null	12(40)	10(19.2)
Non-null	18(60)	42(80.8)
GSTP		
Null	2(6.7)	4(7.7)
Non-null	28(93.3)	48(92.3)
GSTM1 non null+GSTT1 non null	13(43.3)	26(50)
GSTM1 non null+GSTT1 null	7(23.3)	7(13.5)
GSTM1 null+GSTT1 non null	5(16.7)	15(28.8)
GSTM1 null+GSTT1 null	5(16.7)	3(4)
GSTM1 null+GSTT1 null+ nullGSTP1	1(3.3)	2(5.76)

Table (3) presents the distribution of GSTM1 and GSTT1 variants in the study group. In the gasoline filling station workers, the frequency of GSTM-null, GSTT-null, and GSTP-null genotypes was found to be 36.5%, 19.3%, and 7.7%, respectively. While the frequencies of GSTM1-non-null, GSTT1-non-null, and GSTP-non-null genotypes were 63.5%, 80.8%, and 92.3%, respectively, based on odds ratios and frequencies of GSTM1-null genotypes, a high percentage of null genotypes (36.5% for GSTM) was observed in filling station workers. In contrast, a low percentage of null genotypes GSTT1 (19.2%) and GSTP (7.69%) were observed in workers. Additionally, a null genotype for both genes was observed in 4% of filling station workers compared to 40% for GSTM, 40% for GSTT, and 6.7% for both genes in the control group.

Table (4) showed the relationship between oxidative stress indicators and GST genotypes among employees of gas stations. The results revealed that workers with a null GSTM1 genotype had significantly higher mean values of CAT (catalase) and POD (peroxidase) compared to workers with a non-GSTM1 genotype (p = 0.008 and p = 0.014, respectively). Additionally, the mean value of GSH (glutathione) was significantly higher in workers with a null GSTP1 genotype compared to those with a non-null GSTP1 genotype (p = 0.030). However, no statistically significant association was observed between GST genotypes and the mean value of MDA (malondialdehyde), another oxidative stress biomarker.

Table(4): Distribution of Oxidative Stress Biomarkers in Filling Station Workers by GSTM, GSTT, and GSTP Genotypes.

Genotypes Parameters	CAT	POD	MDA	GSH
***GSTM1*(null)**	3.77±0.26	0.34±0.02	0.96±0.06	1658±216.8
GSTM1- (non null)	2.83±0.21	0.46±0.04	1.09±0.05	1972±216.7
p value	**0.008****	**0.014***	0.139	0.346
GSTT1-null	3.00±0.34	0.48±0.05	0.95±0.10	2131±358
GSTT1-non null	3.21±0.20	0.41±0.03	1.07±0.05	1792±177
p value	0.634	0.312	0.267	0.405
GSTP- null	4.11±0.46	0.39±0.09	1.01±0.06	3039±253
GSTP-non null	3.09±0.18	0.42±0.03	1.05±0.04	1759±163
p value	0.121	0.684	0.782	**0.030***

The association between GST genotypes and oxidative stress biomarkers among gasoline filling station workers was presented in Table (4). The results revealed that workers with a null GSTM1 genotype had significantly higher mean values of CAT (catalase) and POD (peroxidase) compared to workers with a non-GSTM1 genotype ($p = 0.008$ and $p = 0.014$, respectively). Additionally, the mean value of GSH (glutathione) was significantly higher in workers with a null GSTP1 genotype compared to those with a non-null GSTP1 genotype ($p = 0.030$). However, no statistically significant association was observed between GST genotypes and the mean value of MDA (malondialdehyde), another oxidative stress biomarker the result support by Shahsavari, F. et. al. (18).

4. Conclusion

Working in gas stations is one of the jobs that is widely spread due to the increasing need for petroleum derivatives, especially gasoline, and this work is considered a risk indicator for human health in the long term of work, so we recommend that workers take caution and take safety measures for the purpose of reducing the symptoms and risks of these substances.

5. Recommendations

1. Fuel station workers must be careful when working with fuels that contain toxic vapors.

2. Workers must be regularly checked by relevant authorities to ensure their safety.
3. Further tests must be conducted by incoming researchers to further ensure the health of workers.

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Data availability statement:

https://drive.google.com/file/d/165Q1Qt85JS9GiWc29i-OJAhoGAy4AzPf/view?usp=sharing.

Conflict of Interest Statement:

The authors declare that there is no conflict of interest regarding the publication of this paper.

Ethical Approval

Not applicable.

Informed Consent

Informed Consent: Written informed consent was obtained from all individual participants included in the study.

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