

Glutathione S and M Transferase Gene Detection in Gasoline Station Workers

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ABSTRACT

The present investigation aims to detection antioxidant enzyme genes state in worker exposure to gasoline during occupation; a case-control study included 30 workers exposure to gasoline and 25 healthy individuals, DNA extracted then Glutathione S-transferase (GST), and GSTM were detected using multiplex PCR, the results show significant differences for all genes states between workers and control groups, There was a high percentage of deletion observed in GSTT and GSTM 83.33% in workers while deletion both genes were observed in 86.66% in comparison with control groups which have a deletion in GSTM 52% and 12% for GSTT and both genes, the present study concluded that the work in occupation should be under health laws, Workers should undergo periodic medical examinations in addition to a good diet, taking into account the working hours and exposure to gasoline.

Keywords: Gasoline, GSTT, GSTM multiplex PCR, Workers.

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INTRODUCTION

In the last decades, health awareness and workers' health laws in some institutions have been poor, leading to appeared problems in health; investigations were implementing about genes related to working in some foundations having environmental contamination like gasoline station in The absence of health care laws for workers.

In gasoline stations, the nature of petrol that volatilized in the atmosphere contains toxic materials that cause adverse health effects in individuals like toluene, benzene, and xylenes that consist of crude oil and fuel vapors. Moreover, some volatile hydrocarbons Also direct touch with foul for a long time can be effective in worker health.^{1,2}

Different studies focused on the effect of gasoline and its derivatives in organs like Organs, lungs, heart, skin, and kidneys that improved its causes diseases and various toxic effects like genotoxic, immunotoxic, carcinogenic mutagenic, and neurotoxic aspects.³⁻⁶

Furthermore, it causes disturbance in the oxidative stress balance in the body; a study shows that inhalation of gasoline vapor causes elevated antioxidant enzyme activation like glutathione-S-transferase, glutathione reductase in addition of glutathione peroxidase.⁷ Another study concluded that the attendants to gasoline stations suffer from higher levels of oxidative stress, that they should be antioxidants taken to lower the effects of oxidative stress.⁸

MATERIALS AND METHODS

Study subjects: The study included 30 gasoline station workers 33 to 46 years old and 25 from different gasoline station healthy individuals. Blood samples were collected due to the ethical approval of the ministry of environment and health in addition to the written consent of subscribers in the present study. DNA was extracted according to manufacture protocol, glutathione S and M transferase were detected using multiplex PCR using the following primers GSTM1: forward 5'-GAACTCCCTGAAAAGCTAAAGC-3', revers 5'-GTTGGGCTCAAATATACGGTGG -3' amplified 215 bp and GSTT1: F 5'-TTCCTTACTGGTCCTCACATCTC-3', R 5'-TCCCAGGTCACCGGATCAT-3' amplified 312 bp (Moasser *et al.*, 2014). the amplification condition was 5 minutes at 94°C, 35 cycles consist of 60 sec 94°C, 60 sec 58°C, 72°C 30 sec) 10 minutes at 72°C, then PCR products visualized by agarose gel, for statically analysis by odd ratio at CI 95% and (p-value <0.05).

RESULTS AND DISCUSSION

The results of the research show that DNA was extracted from workers and control group were good purity and concentrations in spite of high heavy metals levels in gasoline worker subgroups which included Fuel provider 72.41%, Maintenance workers 13.7%, and Administrative staff 13.7% (data not shown) and amplifications were visualized using

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electrophoresis technique which shows 312 bp for GSTT and 215 bp for GSTM in multiplex amplification (Figure 1). The gene state shows high percentages of deletion in GSTM and GSTT in the workers compare control group (Table 1, Figure 2). There was a high percentage of deletion observed in GSTT and GSTM 83.33% in workers (while deletion of both genes was observed in 86.66% compared with control groups with a deletion in GSTM 52% and 12% for GSTT and both genes.

In Iraq, there were some working problems related to worker health laws and environmental pollution processes. Thus several diseases were observed in the absence of health awareness; one of this problem is oxidative stress unbalanced due to exposure to petroleum derivative⁹ how found increased in oxidative stress and antioxidant enzyme activity in gasoline stations worker in Basra city, thus study antioxidant enzymes gene polymorphism in people exposed to a high level of environmental pollution in their work for a long time is very important to assess health state for decreased adverse health effects and avoiding disease, the high percentages of gene deletion that recorded in the present study in GSST and GSTM may be influenced by extend exposure to petroleum derivatives in polluted environments.^{10,11} Another study found

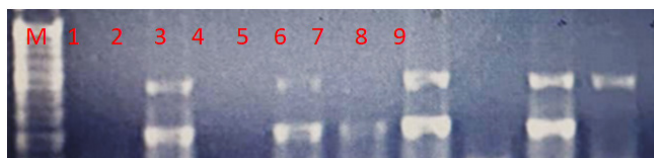


Figure 1: Electrophoresis pattern of GSTT and GSTM gene state in study subjects M DNA marker (100 bp) lanes 1 and 7 gene null, lane 2,4,6 and 8 normal genes, lane 5 GSTT gen and lane 9 GSTM gene. (1% agars, 70 V 40 min).

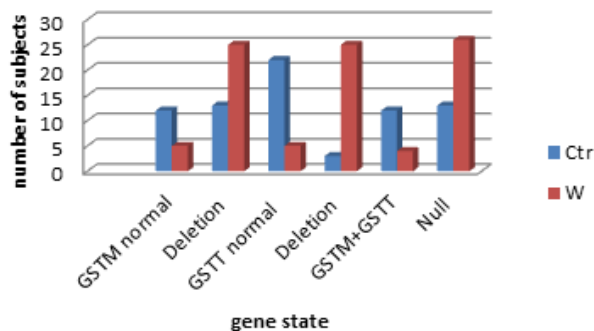


Figure 2: Number of GSTT and GSTM gene state amplification in study subjects.

Table 1: Statically analysis of GSTT and GSTM state in study subjects

Gene	Ctrl%	W %	Odd ratio	p- value
GSTM normal	48%	16.6	4.6154	0.0156*
Deletion	52%	83.33	1.3356 - 15.9491	
GSTT normal	88%	16.6	36.6667	< 0.0001*
Deletion	12%	83.33	7.8465 - 171.342	
GSTM+GSTT	88%	13.33	6.0000	0.0075*
Null	12%	86.66	1.6142 - 22.30	

a direct relation between GST gene and weights of infants that their mother exposure to polluted air during pregnancy.¹² The deletion of GST gene may depend on the type of pollutants and population in addition to other factors like genetic predisposition, DNA repair system activity, detoxification ability of the body, and period of exposure.¹³ The toxic effect of gasoline occurs due to the formation of DNA adducts and the production of reactive oxygen species (ROS) that affect DNA and causes mutation, deletion, or DNA strand break.

Studies exhibit that workers exposed to petroleum products via inhalation during their occupation were exposed to some gases like Nitro aromatics, CO₂, CO, NO₂, Benzopyrene, Hydrocarbons, and Benzene have a chance to mutagenesis in the workshop of engine repair workers exposure to polycyclic aromatic hydrocarbons (PAH).^{14,15}

Navasumrit P, *et al.*, and Khisroon M, *et al.*,^{16,17} and exhibited significant damage in DNA of individuals exposure to gasoline derivative using comet assay compared with control group¹⁸ found chromosome deletion in workers' lymphocytes exposed to petrol.

Research by¹⁹ found that elevated heavy metals levels affected in DNA state and increased genotoxic effects correlated with duration of exposure.

The direct teach with petrol during work by wash hands causes inhale petrol gases which contain genotoxic materials,²⁰ which enter the blood circulation led to genotoxic and cytotoxic effects that detected by Karahalil B, *et al.*,²¹ by chromosome alteration test and micronucleus assay for lymphocytes cells, they found the significant high value of Micronuclei and chromosome aberration.

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