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RESEARCH ARTICLE

HAEMATOTOXICITY IN FUEL STATION WORKERS EXPOSED TO PETROLEUM AIR POLLUTANTS: INFLUENCE OF NQO1 GENE POLYMORPHISM

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ABSTRACT

Fuel station workers are chronically exposed to petroleum derivatives air pollutants, mainly benzene, primarily through inhalation during vehicle refueling. The adverse health effects of benzene exposure may be primarily related to impairment of haemopoietic system with bone marrow depression. We studied haematological changes among fuel station workers with different NQO1 genotypes in Sudan. The study included 100 fuel station workers, chronically exposed to variable concentration of petroleum derivatives air pollutants during their work on the fuel filling station, there blood cell count (determined by Sysmex KX-21N) and NQO1 genotypes (determined by PCR-RFLP) were determined and compared with 50 normal (non-exposed) subjects as control. Mean TWBC was significantly lower, with lower neutrophils count, among fuel station workers than controls (P value=0.043 and 0.004 respectively). Mean platelet count was also lower among cases than controls (P value=0.001), further reduction in the platelets count was observed among workers with mutant NQO1 genotypes. Significant correlation was observed between work duration and RBC count (P value=0.004), Hb level (P value =0.024) and PCV (P value=0.016). In conclusion, we have demonstrated robust changes in TWBC count, neutrophils count and platelets count among fuel station workers which could be attributed to benzene-containing gasoline exposure at workplace. Lower platelets count among workers with mutant NQO1 genotypes highlighted an evidence of increased susceptibility to benzene-containing air pollutants haemotoxicity among such workers.

Key words: Haematotoxicity, fuel station workers, NQO1, Sudan.

INTRODUCTION:

Gasoline is a volatile and flammable solvent produced from petroleum in the refining process (1). The main components are paraffinic, isoparaffinic, olefinic, naphthenic, and aromatic compounds. Of the latter, benzene, toluene, and xylenes are the most dangerous ones (2,3)

Benzene constitutes approximately 1% of gasoline by weight in the United States and Western Europe and more in other nations (4, 5). Exposure is elevated in areas of heavy motor vehicle traffic and around gasoline filling stations (4). The main route of exposure is inhalation (1), although dermal absorption is also possible (2). Experimental studies indicate that approximately 50% of inhaled benzene is absorbed into the body (6). Workers in areas where gasoline loading and unloading takes place, such as high-volume storage terminals, delivery stations, car repair stations, and gasoline stations, have the highest potential for exposure (3).

A characteristic effect of chronic benzene exposure can cause blood disorders including aplastic anemia and acute myelogenous leukemia , significant decrease in the number of white blood cells and platelets have been reported in workers exposed to benzene (7). Although these toxic effects are related to metabolism of benzene in the liver , the particular metabolite (s) that damage bone marrow cells and the mode of toxic action are subject of debate (8,9).

There are numerous enzymes system that are involved in the metabolism of benzene such as Microsomal epoxide hydrolase(EPHX), various glutathione S- transferase (GSTS) and NAD(P)H quinone oxidoreductase NQO1. It has been speculated that polymorphic gene of the above enzymes predispose some individuals to benzene toxicity through this metabolism (8,10-12).

(NQO1) protein, is an enzyme that has attracted considerable attention because of its ability to detoxify a

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number of natural and synthetic compounds and, conversely, to activate certain anticancer agents (13,14).

It plays necessary role in the protection of benzene workers against benzene toxicity by catalyzing two and four electron reduction of benzoquinone. NQO1 enzyme is controled by *NQO1*gene which is located in the long arm of chromosome 16 (16q22.1), it expands approximately 20 kb with 5 entrons and 6 exons. The polymorphism at position 609 in exon6 (C- T) in the human NQO1 gene results in a proline to serine substitution at position 187 in the amino acid structure of the NQO1 protein, resulting in loss of enzyme activity (15). Failure to induce functional NQO1enzyme (mutant alleles) makes the cells more susceptible to the effects of benzene metabolites which may lead to increased risk of benzene poising. NQO1 enzyme activity is normal in individuals with 2 wild-type alleles (NQO1 609CC). It is variably reduced in individuals who are heterozygotes for the polymorphism (NQO1 609CT) (16). NQO1 enzyme activity is absent in those who are homozygous for the point mutation (NQO1 609TT) (17). Sudanese fuel station workers are heavily exposed to benzene-including air pollutants (about 72 to 84 work hours per week) with high risk of benzene toxicity, without policy of protection. This study aimed to determine the effect of chronic exposure to benzene-containing air pollutants on the haematological indices among gasoline filling workers with different NQO1 genotypes in different fuel stations in Khartoum city, Sudan.

MATERALS AND METHODS

One hundred and fifty subjects were enrolled in this cross-sectional study: 100 fuel station workers, worked 72 - 84 hours per week, that were selected randomly from different fuel stations in Khartoum state, Sudan; and age and sex matched 50 individuals as controls. Subjects with gross anemia, known history of diabetes mellitus, cardiopulmonary disease, acute or chronic infection, autoimmune disease, malignancy and subjects with current or previous history of tobacco addictions were excluded. Ethical approval was obtaind from the ethical committee, Facutly of Medical laboratory Sciences, Alneelain University, Khartoum, Sudan. Informed consent was obtained from each subject before enrollment in the study.

Two ml of EDTA anticoagulated blood was collected from each subject for haematological and molecular analysis. Laboratory investigations were performed at the department of haematology, faculty of medical laboratory sciences, Alneelain University, Sudan. Blood cell count was performed by automated cell counter (Sysmex KX-21N). DNA was extracted by sodium chloride method, (300 µl of whole blood was lysed using red cell lysing buffer containing (8.3gm NH4CL, 1gmKHCO3, 1.8ml 5% EDTA and 1liter of distilled water.), the pallet was lysed by white blood cell lysing buffer containing (1.576gm, Tris-HCL, 1.088gm, EDTA, 0.292gm Nacl, 2% SDS, and 100ml distilled water). High molecular concentration of sodium chloride was added (35gm to 1liter of distilled water) to separate the protein fraction. Finally, ice cold ethanol was added to get the DNA fibre which were separated and re-suspended in TE buffer (2.42 Tris base, 0.57ml acetic acid, 50µlEDTA (.01M), and 100ml distilled water) and stored until used. The quality of genomic DNA was determined by agarose gel electrophoresis. NQO1 fragment Was Amplified using the forward primer: 5`-AGTGGCATTCTGCATTTCTGTG-3` and reverse primer: 5`-GATGGACTTGCCCAAGTGATG-3`. The amplification was carried out in thermo-cycler (Techne) with initial denaturation step for 8 minutes at 95°C followed by 35 Cycles consisting of 3 steps: Denaturing step at 94 °C for 30 second, Annealing step at 56 °C For 1 minute and extension steps at72 °C for 40 minute with final Extension step at 72 °C for 10 Minutes.

The PCR reactions was performed in a final volume of 20 µl containing (4 µl premixed ready to use 5x FIREPol master mix (Solis BioDyne, Russian), 12.0µl DNAase free DW, 3 µl genomic DNA and 0.5 µl from each primer). The amplified fragment was digested with 10 U Hinf1 endonuclease (New England Bio lab, UK) over night and was visualized on agarose gel electrophoresis.

Statistical analysis was performed using statistical package for social science (SPSS) software. Evaluation of patient data was performed using the t-test and Pearson correlation test. Results with p value <0.05 were considered statistically significant.

RESULT

The median age of fuel station workers was 38 years, with minimum age of 21 and maximum of 65 years. All workers were male. The median work duration was 6 years with minimum work duration of 1 and maximum of 30 years. All subjects were tested for blood count and NQO1 polymorphisms. The results of blood count for the fuel station workers were as follows: Mean red blood cell (RBC) count 5.2±0.74 $\times10^{12}$/L; mean total white blood cell (TWBC) count 5.6±1.5$\times10^{9}$/L; mean platelets (PLT) count 200.9±77.9 $\times10^{9}$/L; mean hemoglobin (Hb) concentration 14.9±2 g/dl; mean packed cell volume (PCV) 45±6%; mean lymphocytes count 2.3±0.7$\times10^{9}$/L; mean neutrophils count 2.5±1.0$\times10^{9}$/L. While for the control group: mean RBC count 5.3±0.5 $\times10^{12}$/L; mean TWBC count 6.8± × 10^{9}/L; mean PLT count 234±52.1$\times10^{9}$/L; mean Hb l4.7±1.2g/dl; mean PCV 52.5±5.6%; mean lymphocytes count 2.3±0.7$\times10^{9}$/L; and mean neutrophils count 3.1±1.4$\times10^{9}$/L.

Mean TWBC was significantly lower, with lower neutrophils count, among fuel station workers than controls (P value=0.043 and 0.004 respectively). Mean platelet count was also lower among cases than controls (P value=0.001). No significant differences were observed in the means of Hb, RBC, PCV and lymphocytes count (P value=0.605, 0.574, 0.215 and 0.163 respectively). Significant correlation was observed between work duration and RBC count (P value=0.004), Hb level (P value =0.024) and PCV (P value=0.016). No significant correlation was observed between work duration and TWBC count (P value=0.119), PLT count (P value = 0.343), lymphocytes (P value = 0.206) and neutrophils (P value=0.406).

*NQO1*C609T genotype frequencies of fuel station workers were as follows: Homozygous wild types(C/C) 69% (n 69/100), heterozygous mutant type(C/T) 21% (n 21/100) and homozygous mutant (T/T) 10% (n 10/100). Mean platelets count was significantly lower among fuel station workers with mutant type (CT and TT genotypes combined together) than the workers with wild type (CC) (P value=0.018), no significant differences were observed within the other haematological parameters between workers with mutant type (CT and TT genotypes combined together) and those with wild type (CC) (table 1)

Table 1: Comparison of haematological characteristic between CML patients with wild type and those with mutant types.

Parameter	Wild type (609CC)	Mutant type (609CT+TT)	P value
Hb mean±SD (g/dl)	14.7±1.8	15.3±2.3	0.154
RBC mean±SD (X1012/L)	5.2±0.9	5.4±0.7	0.210
PCV mean±SD (%)	44.9±5.2	46.9±7.1	0.111
TWBCmean±SD (X109/L)	5.7±1.5	5.5 ±1.4	0.345
Platelets mean±SD ($X10^9$/L)	197.9±70.5	161.6±69.2	0.018
Neutrophils mean±SD (%)	44.6±9.0	41.9±11.5	0.209
Lymphocytes mean±SD (%)	42.2±8.4	43.3±8.1	0.568

DISCUSSION

Fuel station workers are chronically exposed to petroleum derivatives air pollutants, mainly benzene, primarily through inhalation during vehicle refueling. The adverse health effects of benzene exposure may be primarily related to impairment of the haemopoietic system with bone marrow depression. We studied the haematological changes among fuel stations workers with different NQO1 genotypes in Sudan. The study included 100 fuel station workers, chronically exposed to variable concentration of petroleum derivatives air pollutants during their work on the fuel filling stations, there blood cell count and NQO1 genotypes were determined and compared with 50 normal (non-exposed) subjects as control. We found a significant reduction in the TWBC count, neutrophils count and platelets count among fuel filling workers than the comparison group in Khartoum city which could be attributed to benzene-containing gasoline exposure at workplace. No significant differences were observed in the Hb level, PCV and RBC count between cases and controls, however, a significant correlation was observed between a reduction in the Hb level, PCV and RBC count, and the duration of the exposure to the petroleum derivatives air pollutants among fuel filling workers. Our finding indicated a haematological changes affecting multiple cell lineages, several studies have also observed effects on multiple cell lineages (18-20). Platelets count was significantly lower among fuel station workers with mutant type (CT and TT genotypes combined together) than the workers with wild type, this finding highlighted an evidence of increased susceptibility to petroleum derivatives air pollutants haemotoxicity among workers with NQO1 mutant genotypes. Moran, Siegel, and Ross demonstrated that the benzene metabolite hydroquinone induces high levels of NQO1 activity in bone marrow cells, including CD34+ progenitor cells, with the wild-type (CC) genotype. Exposure to noncytotoxic doses of hydroquinone induced intermediate levels of NQO1 activity in heterozygous (CT) cells, but had no effect in cells with the homozygous mutant (TT) genotype (21). Thus, failure to induce functional NQO1 in cells with homozygous mutant alleles may make them susceptible to the toxic effects of benzene metabolites and thereby may explain the increased risk of benzene-containing air pollutants haemotoxicity in individuals with the (TT) genotype.

CONCLUSION

In conclusion, we have demonstrated robust changes in TWBC count, neutrophils count and platelets count among fuel station workers which could be attributed to benzene-containing gasoline exposure at workplace.

Lower platelets count among workers with NQO1mutant genotypes highlighted an evidence of increased susceptibility to benzene-containig air pollutants haemotoxicity among such workers.

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