

Health Assessment Approach for Evaluating Hematologic and Immune Toxicity of Prolonged Gasoline Inhalation in Fuel Station Workers at Mansoura City, Egypt

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ABSTRACT

Fuel station workers are more susceptible to gasoline toxicity. The current study aimed to evaluate harmful effects of gasoline vapors in 25 male subjects (25-40) years worked at fuel stations for at least one year compared to 25 matched unexposed ones, randomly recruited from Mansoura City. Results indicated that gasoline exposure induced significant reduction in different hematological parameters including; RBCs, Hb, HCT, MCV, MCH, total and differential WBCs, except for neutrophils. Meanwhile, levels of H₂O₂ and malondialdehyde (MDA) were significantly increased, while total antioxidant capacity (TAC), superoxide dismutase (SOD), catalase (CAT) levels and glutathione (GSH) content were decreased. This goes with marked immunologic changes presented by decreases in T- cell surface markers; CD₄ and CD₈, as well as immunoglobulins; IgA and IgG, along with increases in levels of IgM and the inflammatory marker CRP. Hence, the study inferred that prolonged gasoline inhalation can lead to hematologic and immune disorders, probably through potentiating oxidative stress and inflammation pathways.

KEY WORDS: Gasoline, Fuel stations, Oxidative stress, Hematotoxicity, Immunotoxicity.

1. INTRODUCTION

Mansoura City, is one of the largest Egyptian cities inhabited by great number of populations with increasing demand of using cars. In turn, the need for more petrol stations will be increased and occupational exposure to gasoline vapors could be inevitably enhanced. Gasoline is a complex petroleum product consists mainly of a mixture of aliphatic and aromatic chemicals, which are easily smelt by human due to their high volatility. The bulk of aromatic component consists mainly of hydrocarbons including; benzene, xylene and toluene [1,2]. Several authors reported that toxicity of gasoline comes mainly from benzene metabolites [3,4]. Once gasoline is inhaled, benzene vapors enter the lung, then passed to the blood stream from which it goes to the liver, where three main phenolic metabolites of benzene are released including; transient phenol and accumulated hydroquinone and catechol in relatively high concentrations [5]. Benzene is a lipophilic agent, so its metabolites go directly to fatty tissues such as, bone marrow where actual toxic species are generated [6].

Exposure to gasoline for long periods may lead to permanent suppression of bone marrow functioning, accompanied by reduction in the formation of new blood cells in a condition known as aplastic anemia [7]. Evidence is provided also for wide toxic effects of benzene metabolites with prolonged exposure including; pancytopenia (decrease in all blood contents counts) and leucopenia (decrease in number of leucocytes) [8] and other blood disorders as leukemia [9]. Steinmaus and Smith, [10] described a link between living in proximity to gasoline stations and incidence of leukemia [3]. Meanwhile, occupational exposure to crude oil containing benzene is closely related to immune dysfunction comprising; a reduction in serum immunoglobulins [11] and lowering in total leucocytes, in addition to changes in lymphocyte subpopulations which are vital to the immune system function and considered as the most sensitive biomarker among several measured blood parameters [12].

In view of the fact that blood cells profile is an important indicator for the body health, the present study was carried out to assess induced hematologic and immune changes among fuel station workers with continuous exposure to gasoline for at least one year. Also, to evaluate underlying mechanisms, which may be useful in biomonitoring studies of populations chronically exposed to gasoline.

2. MATERIALS AND METHODS

2.1. Subjects

A total number of 50 male volunteers aged (25-40) years; 25 worked at least for one year at gasoline stations and other 25 participants worked away from any source of gasoline exposure, randomly recruited from Mansoura City, Egypt were included in this study. Personal data of each participant were collected through

short interviews using questionnaires mainly based on multiple-choice questions focusing on health status, smoking and getting any medications. The study was approved by Faculty of Science authorities and research ethics committee.

2.2. Blood sampling

About 5 ml of venous blood was obtained from each participant of both groups and immediately divided into three portions; the first was collected on EDTA anticoagulant for complete blood count (CBC) and T-lymphocytes surface markers (CD₄ and CD₈) determinations, while the second portion of blood sample was received on heparin anticoagulant for detection of antioxidant enzymes and oxidative stress markers in lysed RBCs. The third part was put into centrifuge tube without any anticoagulant, then centrifuged at 3000 rpm for 10 min. at room temp. for separating serum that kept at -20 °C until use.

2.3. Preparation of lysed RBCs

The collected heparinized blood samples were centrifuged at 4000 rpm for 10 min., then plasma was aspirated off and packed RBCs were firstly washed by cold saline 1:4 (v/v), followed by ice-cold deionized water in the same proportion 1:4 (v/v) for three times. Next, the washed samples were centrifuged at 4000 rpm for 15 min. and the collected supernatants (erythrocyte lysate) were stored at -80 °C for further analysis [1].

2.4. Tested parameters

2.4.1. Complete blood count (CBC)

Immediately after blood collection, EDTA samples were analyzed using the fully hematological analyzer (Sysmax XE-2100, Japan) for estimating CBC including; red blood cells (RBCs), hemoglobin (Hb) content, haematocrite (HCT)%, mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH), in addition to total and differential counts of white blood cells (WBCs) [13].

2.4.2. Antioxidants and oxidative stress markers

Reduced glutathione (GSH), total antioxidant capacity (TAC), hydrogen peroxide (H₂O₂) and malodialdehyde (MDA), as index of lipid peroxidation were assessed using kits from Biodiagnostic Co., Giza, Egypt based on the methods of Beutler *et al.* [14], Koracevic *et al.* [15], Aebi, [16] and Ohkawa *et al.* [17], respectively. Activities of superoxide dismutase (SOD) and catalase (CAT) were determined according to Nishikimi *et al.* [18] and Aebi, [16].

2.4.3. Immunoglobulins

Serum immunoglobulins, IgA, IgG and IgM were estimated using ELISA kit from Sigma-Aldrich (USA), depending on the method of Khamashta and Hughes, [19].

2.4.4. T-lymphocyte surface markers (CD₄ and CD₈)

EDTA samples were also used in the same day for isolating lymphocytes, then cells were stained with antibodies against fluorescein isothiocyanate (FITC-CD₄) and phycoertherin (PE-CD₈) (BD-Bioscience, USA) and the histogram was derived according to program of Dean and Jett, [20].

2.4.5. C-reactive protein (CRP)

Serum levels of CRP were determined, using Sigma Diagnostic, EU kit, as described by Pepys, [21].

2.5. Statistical analysis

Data analysis were achieved using the GraphPad prism software (V 5.04 Graph Pad Software Inc., La Jolla, CA) in which Student *t*-test was used for comparing gasoline exposed group with the control, where *p* < 0.05 was considered significant.

3. RESULTS

3.1. Complete blood count (CBC)

Obtained data showed significant decreases in all blood indices including; RBCs, Hb, HCT, MCV and MCH, as well as total and differential WBCs counts, except neutrophils, which were significantly increased in gasoline exposed workers compared to unexposed subjects (Table 1).

3.2. Antioxidants and oxidative stress markers

Significant decreases in the antioxidants (GSH, SOD, CAT), as well as TAC were observed in lysed RBCs of gasoline exposed group compared to the control one. However, oxidative stress markers (MDA and H₂O₂) were elevated significantly with exposure to gasoline as shown in Table 2.

3.3. Immunoglobulins, T-lymphocytes surface markers (CD₄, CD₈) and CRP

Prolonged exposure to gasoline vapors induced significantly lowered levels of IgA and IgG, with higher levels of IgM and CRP. Results also indicated significantly decreased T-cell surface markers; CD₄ and CD₈ in gasoline exposed workers compared to the unexposed subjects (Table 3).

Table 1. Complete blood count in control and gasoline exposed subjects.

Parameters	Control subjects	Exposed subjects
RBCs($10^6/\mu\text{L}$)	5.22 \pm 0.18	4.08 ^a \pm 0.16
Hb (g/dl)	15.19 \pm 0.25	10.72 ^a \pm 0.26
HCT(%)	44.47 \pm 1.10	31.21 ^a \pm 0.95
MCV(fl)	83.98 \pm 2.72	77.40 ^a \pm 2.08
MCH(pg)	29.33 \pm 0.93	26.64 ^a \pm 0.45
WBCs($10^3/\mu\text{L}$)	8.59 \pm 0.31	5.59 ^a \pm 0.22
Neutrophils(%)	40.86 \pm 1.40	57.04 ^a \pm 1.21
Eosinophils (%)	3.80 \pm 0.38	1.70 ^a \pm 0.10
Lymphocytes(%)	49.38 \pm 1.91	37.68 ^a \pm 1.55
Monocytes(%)	5.96 \pm 0.34	2.89 ^a \pm 0.14

Results are presented as means \pm SE (n=25 for each group).

a: significant change when compared to unexposed control group ($p < 0.05$).

Table 2. RBCs antioxidants and oxidative stress markers in control and gasoline exposed subjects.

Parameter	Control subjects	Exposed subjects
GSH(mmol/g Hb)	3.10 \pm 0.18	2.02 ^a \pm 0.13
SOD(U/gHb)	82.60 \pm 3.39	70.10 ^a \pm 2.37
CAT(U/gHb)	131.6 \pm 3.36	100.9 ^a \pm 3.52
TAC(mM/gHb)	1.61 \pm 0.06	1.06 ^a \pm 0.04
MDA(nmol/gHb)	42.33 \pm 2.18	53.15 ^a \pm 2.33
H ₂ O ₂ (mM/gHb)	1.74 \pm 0.08	2.58 ^a \pm 0.09

Results are presented as means \pm SE (n=25 for each group).

a: significant change when compared to unexposed control group ($p < 0.05$).

Table 3. Serum immunoglobulins, T-lymphocytes (CD₄ and CD₈) and CRP in control and gasoline exposed subjects.

Parameters	Control subjects	Exposed subjects
IgA(mg/dl)	96.41 \pm 4.87	74.62 ^a \pm 3.18
IgG(mg/dl)	823.8 \pm 27.5	609.6 ^a \pm 29.5
IgM(mg/dl)	93.59 \pm 5.15	137.2 ^a \pm 5.01
CD ₄ (%)	31.51 \pm 1.80	16.30 ^a \pm 1.02
CD ₈ (%)	41.33 \pm 1.55	27.52 ^a \pm 1.58
CRP(mg/L)	6.69 \pm 0.52	12.16 ^a \pm 0.60

Results are presented as means \pm SE (n=25 for each group).

a: significant change when compared to unexposed control group ($p < 0.05$).

4. DISCUSSION

Monitoring occupational exposure to chemical matters is seriously needed for evaluating health hazards and providing suitable strategies for making safe work environment. Among the most toxic chemicals is gasoline that is strongly and causally related to wide spread of health problems in human [22]. Risk of gasoline exposure is mostly related to four main components; benzene, toluene, ethylene and xylene that known as BTEX [1]. However, benzene was found to be the most hazardous component due to mutagenic and carcinogenic effects of its metabolites [23].

Spotting on fuel station workers who are chronically exposed to gasoline seemed necessary, as properly achieved in the present study. Workers were mostly suffering neurological symptoms including; headache, tiredness, irritability and disturbed sleeping, as recorded in the utilized questionnaires and remarked by Abou-ElWafa *et al.* [24].

For Further evaluation, the present study was interested in assessment of hematologic and immune changes that are easily used for detecting damaging health effects with prolonged exposure to xenobiotics [25]. Gasoline as one of these xenobiotics was reported to enhance generation of reactive oxygen species (ROS) such as; O_2^- and H_2O_2 , with more toxic and reactive hydroxyl radicals (OH^\cdot) [26]. In this concern, several authors illustrated that gasoline inhalation may lead to oxidative stress as a result of imbalance between production of free radicals and antioxidant capacity [27,28]. Total antioxidant capacity (TAC) is a dynamic equilibrium affected by interactions between each serum antioxidative constituent, where antioxidants collaboration supplies human body with greater protection against free radicals than any antioxidant lonely [15]. Malondialdehyde (MDA) is often used as index of oxidative stress [29]. Wright and Welbourne, [30] revealed that MDA increases from the reaction between (OH^\cdot) radicals and cellular polyunsaturated fatty acids, resulting in loss of cell integrity. Increased oxidative stress, as well as lipid peroxidation represented by elevated levels of MDA gives an indication of cellular damage that is usually accompanied by reduction of TAC, SOD, CAT activities and GSH content [31]. GSH helps in detoxification and protecting cells from ROS [32], while both SOD and CAT enzymes are supplementary in function. They are the primary antioxidant defense components that catalyze dismutation of superoxide radicals (O_2^-) to H_2O_2 which is then converted to H_2O by CAT. As such, the present data showing increased accumulation of H_2O_2 and MDA, with depletion of TAC, SOD, CAT and GSH in the lysed RBCs may indicate increased risk of RBCs oxidative damage with prolonged gasoline exposure.

Further explanation is that ROS can induce direct damage in RBCs membranes due to continuous oxidative degenerations during their life span, where biochemical, physical and structural changes occurred [33]. Thus, affecting RBCs ability in oxygen transport, which may lead to removal from circulation by reticulo-endothelial cells. [34]. Another prospect for RBCs damage may occur indirectly through benzene, as one of the most important constituents of gasoline. Benzene is mainly metabolized in bone marrow into hydroquinone, *p*-benzoquinone, catechol and muconaldehyde, which in turn aggravates destruction and cytotoxicity of bone marrow [35]. The resultant effect included marked decreases in RBCs count and the other hematologic parameters; Hb content, Hct, MCV and MCH, as in agreement with the present findings and those of previous studies Okonkwo *et al.* [7], Kamal and Malik, [36].

Gasoline hematotoxicity extended also to leucocytes, the primary protective line from infectious agents. Results of the present study showed reduced count of total WBCs and its differential cells (eosinophils, lymphocytes, monocytes), except for neutrophils which were significantly increased in gasoline exposed workers compared to control ones. Bedekar *et al.* [37] related this reduction to cytotoxic effects of gasoline constituents, especially benzene which is mostly transformed in the bone marrow into toxic metabolites, resulting in DNA destruction with lowered production of WBCs [38].

In this field, most of attention was directed toward gasoline related immunotoxicity through decreasing number of lymphocytes [39]. Several studies established that lymphocytes are involved in the cellular and humoral immunity [40] and further added that gasoline toxicity of bone marrow reflects depression and alterations in both cellular and humoral immunity with decrease in the lympho-proliferative response [41]. Cellular immunity is the immune response mediated by T-lymphocytes, while humoral immunity is mediated by antibodies known as immunoglobulins (Ig) produced by activated B-lymphocytes [42]. Different immunoglobulins (IgA, IgG, IgM) are often measured to give information about immune system homeostasis [43].

Current study recorded remarkable decreases in IgA and IgG levels with significantly elevated levels of serum IgM in fuel stations individuals compared to the unexposed ones. It was found that people with primary immunodeficiency have decreased levels of serum IgG and IgA and normal or elevated levels of IgM [44]. Uzma *et al.* [45] explained decreased levels of immunoglobulins in gasoline exposed workers to either suppression of immunoglobuline producing cells or decreased cell mediated immunity. Immunosuppression may also be related to lower number of leucocytes and reduced ability of bone marrow and lymphoid tissue to produce mature lymphocytes with decreased T cell receptor mediated immunity, such as CD4 helper cells and CD8 suppressor cytotoxic cells [35].

Subsequent explanation is that B-lymphocytes can produce IgM antibodies on their own, but they require interactive help from T- lymphocytes in order to switch production of IgG, IgA from IgM. The increase in IgM may result from a variety of genetic defects that affect this interaction between T-lymphocytes and B-lymphocytes [46]. The most common form of hyper IgM syndrome results from a defect or deficiency of protein molecules which are found on surface of activated T-lymphocytes [47]. Therefore, it seemed logically that reduction of the specific T-cell surface proteins (CD₄ and CD₈), as seen in the current trial may be the reason for the higher IgM levels arising from prolonged gasoline exposure. One clinical sequence is disruption of the immune balance with increased tendency to various infections [43]. This may explain the currently increased percentage of neutrophils being known for its ability of phagocytosis to destroy invading agents [37]. Adienbo and Nwafor, [48] related elevated neutrophils to increased migration from bone marrow or reduced migration into the tissues. Meanwhile, Owagboriaye et al. [49] associated this elevation to certain condition of hepatic inflammation and cirrhosis caused by gasoline exposure. This in turn promotes neutrophils activation and extravasation into hepatic tissue with increased release of inflammatory mediators such as CRP [50].

The present study has shown significant elevation in CRP levels in gasoline exposed workers compared to the unexposed controls. CRP is a classical acute phase protein that binds to ligands exposed to damaged tissues [51]. Evidence is provided that increased CRP production is characteristic to inflammatory, infective and tissue damage [51]. Thus, current elevation of CRP is likely due to ongoing inflammation status resulting from gasoline exposure.

6.CONCLUSION

In conclusion, profiling of gasoline exposed workers clearly showed marked reductions in almost all hematologic indices with depression in the immune system. This may be related to enhancing ability of gasoline active constituents on variety of oxidative stress and inflammatory pathways which together may account to higher risk of gasoline toxicity.

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