Case report

Biochemical study on occupational inhalation of benzene vapours in petrol station

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ABSTRACT

inhalation of benzene vapours promote various and dangerous health problems. Fuel station workers are most susceptible to benzene inhalation toxicity. Samples were collected twice, at beginning of the study and 6 months later from 40 fuel station workers from different Egyptian governorates and 10 control healthy volunteers. Fuel station workers were subdivided into four groups according to years working in the station. All of them are exposed to benzene vapours and exhausts during their duties, their work shifts were 8 hrs./day. Results indicated that; benzene vapours exposure induced significant increasing in serum Lead and Cadmium and Myeloperoxidase (MPO) enzyme activity levels. This goes with marked immunologic changes presented by decreases in immunoglobulins; IgA and IgG, along with increases in levels of IgM and IgE. Also, Malondialdehyde (MDA) levels were significantly increased. Meanwhile, reduction in some other biochemical parameters including; Copper, Zinc and Iron micronutrients, as well as; Superoxide Dismutase (SOD), Catalase (CAT) enzyme levels and Glutathione (GSH) content. Hence, the study inferred that prolonged benzene inhalation can lead to biochemical and immune disorders, probably through potentiating oxidative stress and inflammation pathways.

1. Introduction

Air pollution was increased with urbanization and rapid increasing number of automobiles in most of the towns and cities. Numerous epidemiological studies have documented decrements in pulmonary function and various other health problems associated with long term air pollution exposure [1].

Benzene long term exposures can give rise to effects that include haematotoxicity, genotoxicity, immunological and reproductive effects as well as various cancers [2].

Benzene is a volatile compound in urban air pollution induce DNA oxidation [3]. Moreover, iron and other transition metals leaching from particles or by their presence on particle surfaces play a role in the generation of ROS in biological systems [4]. When oxidative stress is relatively low, various transcription factors, such as the nuclear factor erythroid-2 (Nrf 2), induce a series of antioxidant and detoxification enzymes (e.g., CAT, SOD, GST that counteract ROS formation protecting from adverse biological outcomes [5]).

The volatile nature of petrol makes it readily available in the atmosphere any time it is dispensed, especially at petrol filling stations and depots. People are exposed to gasoline fumes during fuelling and refuelling at gas stations, but the gas station attendants are more at risk by virtue of their occupational exposure [6]. So, spotting on fuel station workers who are chronically exposed to gasoline seemed necessary. Workers were mostly suffering neurological symptoms including; headache, tiredness, irritability and disturbed sleeping, as recorded in the utilized questionnaires and remarked by Ref. [7].

2. Materials and methods

The study was carried out on 40 fuel station workers from different Egyptian governorates and 10 control healthy volunteers after taking consent of them. Fuel station workers are exposed to benzene vapours and exhausts during their duties, their work shifts were 8 hrs./day and sub divided into four groups according to years working in the station. 

subgroup 1: Consists of 10 workers working in petrol station for 2 years. 

subgroup 2: Consists of 10 workers working in petrol station for 4 years.

subgroup 3: Contains 10 workers working in petrol station for 6 years. 

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6 years. **subgroup 4:** Compromise 10 workers working in petrol station more than 6 years. All attendants were subjected to medical and occupational history including age, marital status, respiratory and chronic diseases, history of operation, drug intake, duration of employment, location and use of protective equipment. Any individual with history of diabetes, renal or hepatic disease and atrophy or allergy was excluded.

Blood samples were collected twice, at beginning of study and 6 months later by withdrawing of ten cubic centimeter of venous blood through a vein puncture using sterile vacutainer tubes. Half of the blood samples taken in heparin tubes (green caped tubes). The other taken to plain tubes (red caped tubes) which allow to coagulate then centrifuge for 10 minutes at 400 r.p.m for getting clear and non hemolyzed sera.

The sera were subjected to estimation of the following parameters; Copper, Zinc, Iron, Cadmium, and Lead by using 5 pg atomic absorption spectra-photometry (Pye Unicam), according the methods, described by Wilse, [8] and Bauer [9]. as well as Myeloperoxidase enzyme activity (Myeloperoxidase ELISA kit, Kamiya Biomedical Company) and total protein immunoglobulins; IgM, IgG, IgA [10], IgE [11] and nitric oxide [12].

Heparin 0.5% tubes used for preparation of hemolysate by Dightonone after washing erythrocytes by physiological saline as described by Korrnburg and Korecker [13] This hemolysate was subjected for quantitative determination of erythrocyte catalase [14,15], total super oxide dismutase [16], reduced glutathione [17], glutathione peroxidase [18] and malondialdehyde [19].

**Statistical analysis:** Data analysis was expressed as mean ± S.E. and analyzed for statistical significance by one-way ANOVA followed by Tukey's post-hoc test for multiple comparisons, using SPSS program for Windows version 22.0 (SPSS Inc., Chicago, USA). Values were considered statistically significant at P < 0.05 correlations between the measured variables were assessed by linear regression analysis by the least squares method.

### 3. Results

Results obtained in **Table 1** showed a significant decrease in serum Copper, Zinc, Iron and Lead levels (P < 0.05) in fuel station workers exposed to benzene vapours for 2 and 4 years and highly significant reductions in Cu, Zn after 6 and more than 6 years of exposure when compared to control. This significant decrease was still noticeable in the 2nd sample after 6 months of the first one. Also, a highly significant decrease was found in serum immunoglobulins IgG and IgA levels at (P < 0.05) in spite of the increase in IgM and IgE and serum Lead and Cadmium levels in workers exposed to benzene vapours for 2 and 4 years and highly significant increase after 6 and more than 6 years of exposure when compared to control, these significant changes were persisted in the 2nd sample. Moreover, serum nitric oxide showed a significant decrease at (P < 0.05) in workers exposed to benzene vapours for 2 and 4 years and highly significant decrease after 6 and more than 6 years of exposure in comparison with control and also, NO levels were still decreased significantly in 2nd sample after 6 months. Serum MPO levels showed a significant increasing in workers exposed to benzene vapours for 2 and 4 years and highly significant increasing (P < 0.05) after 6 and more than 6 years of exposure when compared to control in the first and second samples.

The data recorded in **Table 2** showed; a significant decrease in RBCs SOD, CAT, GPx and GSH levels (P < 0.05) in fuel station workers exposed to benzene vapours for 2 and 4 years and highly significant reduction in RBCs SOD, CAT, GPx and GSH levels after 6 and more than 6 years of exposure when compared to control. This significant decreasing still registered in the 2nd sample after 6 months from first sample. Despite of increasing of Lipid peroxidation, MDA levels in workers exposed to benzene vapours for 2 and 4 years and highly significant decreases in serum MPO and total protein immunoglobulins IgM and IgE and serum Lead and Cadmium levels found in serum immunoglobulines IgG and IgA levels at (P < 0.05) in first one. Also, a highly significant decrease in serum SOD, CAT, GPx and GSH levels after 6 and more than 6 years of exposure when compared to control. This significant decreasing still registered in the 2nd sample after 6 months from first sample.
significant increase after 6 and more than 6 years of exposure when compared to control and also, MDA levels were still increased significantly in 2nd sample.

4. Discussion

Risk of benzene exposure is mostly related to four main components; benzene, toluene, ethylene and xylene that known as BTEx [20]. However, benzene was found to be the most hazardous component due to mutagenic and carcinogenic effects of its metabolites [22]. Spotting on fuel station workers who are chronically exposed to benzene vapours seemed necessary, as properly achieved in the present study.

Moderate and long term exposures to benzene which carry the risk of numerous health problems including inorganic elements in such hazards. Our results was in agreement with [23] who found that, all of the accumulated metals are proposed to contribute to oxidative stress by different mechanisms. The underlying mechanisms of their toxicity involve formation of the superoxide radicals, hydroxyl radicals and other reactive oxygen species (ROS). Increased formation of ROS overwhelms body antioxidant protection [24], which may be the underlying cause of the decreased antioxidant activity observed in the present study, leading to possible induction of numerous conditions detrimental to health.

Results obtained in this study showed significant decrease in copper and zinc metal levels in comparison to control group. This came in agreement with Mona et al. [25], who reported that; both trace elements copper and zinc were significantly lower compared to peoples that are not exposed to fuels. These results are consistence with [21] who reported that; long to moderate benzene exposed individuals under oxidative stress due to decreased levels of antioxidants, including copper and zinc, in the plasma and red blood cells. In this study, copper and zinc were reduced by 51% and 56%, respectively, than lower limit of the accepted reference intervals and amounted 63% and 64% decrease to control individuals. Zinc and Copper are essential antioxidants in the human body [26]. These trace elements are essential component of many enzymes and proteins involved in protection against oxidative stress damage. Zinc deficiency associated with reduced immunity [27], DNA damage and increases other metals induced oxidative toxicity [28]. Copper is essential micronutrients for normal growth and protection of many organs and electron transport [29]. Copper deficiency increases risk of cellular oxidative damage due to decreased activity of superoxide dismutase (SOD) [30]). The balance between antioxidant, such as copper and zinc, and rate of free radicals levels are essential factor to prevent organs and tissue from oxidative damages [31]. The deficiency of copper and zinc antioxidants observed in petroleum fuel stations workers indicates the increasing of oxidative stress in such populations showed in previous results of our study.

The most risky toxic metal those lead which can cause abnormal alterations in the functioning of many vital organs [32] and associated with increased risk of hematological malignancies. Unfortunately, the majority of the workers are neglecting or lacking the protective safety measures such as; facemasks, protective cloths, this carelessness makes them more susceptible to those toxic fumes. Lead exposure may increase the susceptibility of membranes by altering their integrity via causing deterioration of their components [33]. Cadmium metal was found to be elevated in the blood benzene exposed individuals. Increase in lipid peroxidation represented by increased MDA level, has been observed in experimental animals treated with cadmium [34]. While cadmium itself is unable to generate free radicals directly, indirect generation of various radicals has been reported. Such mechanism involves displacement of other redox-active metals from their binding sites thus increasing their free form and enhancing their capability of producing free radicals [35], found that; Fuel station workers who have exposed to benzene showed highly significant reductions in total antioxidant capacity (TAC) activities and high significant increased plasma MDA levels [36]. Malondialdehyde (MDA) is one of the end-products of the peroxidation of membrane lipids caused by ROS formation, especially by the superoxide ion. This is in agreement with other studies which illustrated that Benzene exposure has been associated with increases in the overall formation of MDA [37].

MPO is one of the most promising biomarkers of oxidative stress for clinical cardiologists [38]. Elevated circulating MPO levels have been found to be associated with the presence of CAD. In our present study, high MPO levels were able to predict increased risk of developing CAD in benzene vapours exposed workers, this was in agreement with Meuwese et al., [39] who stated that; high MPO levels were able to predict increased risk of developing coronary artery diseases (CAD) in healthy individuals. Most of attention was directed toward gasoline related immunotoxicity through decreasing number of immunoglobulins (IgA, IgG) which are often measured to give information about immune system homeostasis [40]. Current study recorded remarkable decreases in IgA and IgG levels with significantly elevated levels of serum IgM and IgE 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 [41]. Decreased levels of immunoglobulins in gasoline exposed workers were explained to either suppression of immunoglobulin producing cells or decreased cell mediated immunity [3].

5. Conclusion

By the end of this study we concluded that, benzene vapours inhalation lead to potential biochemical and immunological disorders appeared as elevation of serum Lead, Cadmium, (MPO) and (MDA) levels. Also, markedly decreases in IgA and IgG, along with increases in levels of IgM and IgE. Meanwhile, reduction in some other biochemical parameters including; Copper, Zinc and Iron, (SOD), (CAT) enzyme
levels and glutathione (GSH) content. This probably through po-
tentiating oxidative stress and inflammatory pathways.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rmcr.2019.100836.

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