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CHANGES IN SOME BIOPHYSICAL AND BIOCHEMICAL PARAMETERS IN BLOOD AND URINE OF WORKERS CHRONICALLY EXPOSED TO BENZENE

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Abstract

Objective: Benzene may occur naturally as a component of petroleum, or may be manufactured synthetically. It is found in the environment as a contaminant from both human activities and natural processes, posing serious bio-hazards from chronic exposure.

Methods: A total of 330 individual were enrolled to study possible health hazards of benzene contamination; 265 males occupationally chronically exposed to low levels of benzene in their daily activity were compared to 65 healthy individuals of the same socio-economic standard. Benzene workers were divided between 45 workers in printing shops, 70 subjects dealing with benzene containing paints (painters), 75 subjects working in professions related to automotive work (autoworkers) and 75 car drivers.

Results: benzene itself was not detected in blood or urine of all participants, but the levels of its metabolites; phenol and t,t-muconic acid, were higher in the blood and urine samples in the group of benzene-exposed workers. The results also indicate that individuals in this group are under oxidative stress. However, neither the determined liver function nor the kidney function tests showed significant deviation from controls. However, the results of the biophysical hematological parameters, including the degree of hemolysis,

blood viscosity, RBCs aggregation and form factor were significantly deviated from normal.

Conclusion: The deviation of the determined biochemical and biophysical parameters from normal may predispose such workers to a variety of health problems. Early correction of the oxidative stress and the hematological parameters and improvement of working conditions are necessary to prevent their progress to more serious health conditions, especially in children and young adolescents working under similar conditions.

Running Title : Chronic exposure to benzene in work place

Keywords: Benzene exposure, hematological changes, oxidative stress

Introduction

Benzene is an important starting material for the manufacture of dyes, plastics, elastomers and nylon resins (CAPM/NCI), 1997; Spadaro & Renganathan, 1994; OHM/TADS, 1997). It occurs naturally as a component of gasoline (Patel et al., 2004), and is found in the environment as a contaminant from both human activities and natural processes (Reynolds et al., 2002). Exposure during refueling and driving, as well as the contribution of active and passive tobacco smoking, has been considered as part of the characterization of risk of the general population (Durate-Davidson et al., 2001). Benzene enters the body mainly through inhalation of contaminated air or through contact with the skin (Egeghy et al., 2003). It is both exhaled unchanged in the lungs and excreted as metabolites in the urine. Metabolism of benzene proceeds mainly through oxidation to epoxide which is either hydroxylated to phenol or converted to hydroquinone or benzoquinone. A second metabolic pathway involves conversion of benzene to t,t-muconoc acid (Rothman et al., 1998). Biomarkers of benzene used to estimate exposure and risk include: benzene in breath, blood and urine, its urinary metabolites: phenol, t,t-muconoc acid and S-phenylmercapturic acid; and blood protein adducts (Weasel ,2010).

The reported serious health hazards of moderate and long-term exposures of humans to benzene appear to be of hematologic, immunologic, genetic and malignant nature. Developmental and reproductive effects have also been noted (Zhang et al., 2010; Chen et al., 2000; Schnatter et al., 2010). The prevalence of leukemia and other hematologic diseases has been shown to be higher in benzene-exposed populations. However, it has been suggested that any risk of leukemia in the general population at low exposure concentrations is likely to be exceedingly small(Boogaard et al., 2011; Glass et al., 2004).

The present study was undertaken to evaluate the possible changes in some biochemical, biophysical and hematologic parameters in workers

exposed to low levels of benzene during their daily activity. Identification and early correction or prevention of possible health problems related to such exposure would help in maintaining good health of the workers, prevent reduction in working capacity and loss of working hours beside reducing the cost of medical care for affected workers

Materials And Methods

An all-male cohort of 330 individuals from the city of Alexandria was recruited to participate in the present study. This cohort included 65 healthy individuals (Group I) that served as a control group (age range 25-58 years) and 265 individuals (Group II) occupationally chronically exposed to low-levels of benzene in their daily activity during routine work (age range 23-60 years). The average benzene concentration measured in environmental air of the work place of all studied industrial settings in Alexandria, EGYPT; was 97.56 ± 88.12 ng/L (range: 4.69 – 260.86 ng/L) (Mohamed EI et al., 2013). The conditions of their work environment and working times were recorded. All participants were of the same socio-economic standard with similar living conditions and dietary habits. Exclusion criteria from the cohort included individuals suffering from endocrine diseases like diabetes or thyroid dysfunction. The Ethics Committee of the Medical Research Institute, Alexandria University; approved the study protocol and all experimental procedures were in accordance with the Helsinki Declaration of 1975, as revised in 1983.

Subjects in group II were subdivided according to their professions into the following categories: Forty five (45) subjects operating printing machines or working in printing shops, seventy (70) subjects dealing with benzene containing paints of, seventy five (75) car drivers, and seventy five (75) subjects working in professions related to automotive work (autoworkers) like gas stations, car body-shop repairs, car wash stations, or as mechanics. After explaining the objectives of the project and obtaining written consents a 5 milliliter blood sample was withdrawn, over EDTA, from each individual by qualified personnel under aseptic conditions and a spot urine sample was also obtained and kept in sealed containers. Benzene and its metabolites; phenol and t,t-muconic acid, were assayed in blood and urine by liquid chromatography (Olmos et al., 2006; Ducos et al., 1990). Analyses were carried out by injecting 25 µL in as high performance liquid chromatograph fitted with a C18 ODS reversed phase column, filled with Li Chrosorb C 18, 5 m (Merck), and a Shimadzo SPD 6 AV UV. Visible spectrophotometric detector set at 259 nm. The eluent was a solution of 1 % aqueous acetic acid / methanol (90/10), at a flow rate of one ml/minute. All concentrations were determined by external standardization with aqueous standards and were not corrected for recovery (mean recovery rate 90 %). The retention times for t,t-muconic and phenol were 15 min and13.6

respectively and the duration of an analytic run was 30 min. Both t,t-MA and phenol urinary concentrations were corrected according to the creatinine "normalization", as adopted by the NIOSH in the third edition of its Manual of analytical methods.

Urinary concentrations of both t,t-muconic acid and phenol were corrected by creatinine normalization. Creatinine in serum was assessed as a measure of kidney function and in urine for use in calculating the urinary excretion of phenol and t,t-muconic acid (Bartles et al., 1972). Liver function was evaluated by serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (Reitman & Frankel, 1957).

The degree of oxidative stress was assessed by determining total antioxidant activity (Biodiagnostic, Egypt). The principle of the method depends on the reaction of antioxidants in the sample with a defined amount of exogenously provided H_2O_2. The antioxidants in the tissue sample eliminate a certain amount of the added hydrogen peroxide. The residual H_2O_2 is determined calorimetrically by an enzymatic reaction involving the conversion of the exogenously added 3,5, dichloro–2–hydroxyl benzensulphonate to a pink colored product. The absorbance of each tissue sample was read at 505 nm against a blank in which the tissue was replaced with distilled water. In each tissue sample, the total antioxidant concentration was calculated according to the equation:

Total antioxidant concentration (mM/l)=(Abs of blank–Abs of sample)*3.33 (1)

The level of malondialdehyde (MDA) as a measure of lipid peroxidation was also determined by a Ready-for- use colorimetric kit (Biodiagnostic, Egypt) (Satoh , 1978; Ohkawa et al., 1979). A battery of biophysical tests was also run on the blood including relative viscosity (Abou-Elenein et al,1991), degree of hemolysis (Roper et al., 2001), and adhesive properties of RBCs by evaluating the aggregation shape parameter (ASP) by inclined slide microscopic technique, and form factor (FF), using the equation:

$$FF = \frac{PPA^2}{4\pi(PAA)} \quad (2)$$

1. Where FF is the form factor, PPA is the projected perimeter of all aggregates and PAA is the projected area of all aggregates (Elbelbese , 2005; Chen, 1995; Sanjay & Megha, 2004). All values are presented as the mean ± standard deviation and were analyzed by Statistical Package for Social Science (SPSS) version 10. Paired t-test was used to compare two mean parameter values for the same element and the level of significance was set at P value of 0.05 or less.

Results

The average benzene concentration measured in environmental air of the work place of all studied industrial settings in Alexandria, EGYPT; was 97.56 ± 88.12 ng/L (range: 4.69 – 260.86 ng/L) as measured by the electronic nose (Mohamed et al., 2013). The relatively higher levels were found in printing shops, where subjects work in confined areas.

Benzene was not detected in the blood or in the urine of all subjects tested, (**Table 1**). Also, t,t-muconic acid was not found in the blood of controls but present in the urine at a level of 0.03+0.02 mg/g creatinine (mean + SD). The variability of the concentration of t,t-muconic acid in the blood and urine of exposed workers was high. The concentrations in the blood averaged 0.88±0.43 mg/L and in the urine 0.99±0.42 mg/g creatinine. Phenol, on the other hand, was detectable in all blood and urine samples, with mean concentrations in the blood and urine of exposed workers 64.8% and 79.5% above control values respectively.

Low level benzene pollution resulted in increased oxidative stress in exposed workers. The determined parameters were indicative of such condition, (**Table 2**). The mean malondialdehyde (MDA) level was 45.7% higher than the corresponding control values. The highest MDA concentrations were detected in the drivers and printers (87.0% and 35.5% above control respectively). In the mean time, the antioxidant capacity was low averaging 36.6% less than control. The lowest levels were again obtained in the subgroup of printers averaging 94.4% below control value. Despite the stressful conditions, both liver function represented by serum ALT and AST, and kidney function, represented by serum creatinine, were within normal values. The only exception was the mean serum creatinine in the subgroup of printers, which was slightly higher (1.45 mg/dl) than the clinically acceptable normal range (1.2 mg/dl).

The biophysical properties of blood were affected by chronic exposure to low levels of benzene. The tendency for hemolysis of RBCs was higher in the blood samples withdrawn from benzene-exposed workers, (**Table 3**). On the average, hemolysis in the exposed workers samples was 1.6 times that in samples from the control subjects. The highest hemolysis was observed in samples from those working in printing shops averaging more than 2.6 times the control. The mean relative viscosity of blood, (**Table 3**), was 94% higher in exposed workers. The greatest change was observed in those working as drivers, where the RBCs viscosity more than doubled. Other factors affecting blood viscosity were also abnormal in benzene-exposed workers. The aggregation shape parameter increased by 38.7% and the form factor by 24% above control values. As presented in Figure 1 and Figure 2 there were significant correlations between RBCs relative viscosity

and RBCs form factor (r = 0.52), as well as between the changes in RBCs aggregation and the RBCs form factor (r = 0.78).

Discussion

Benzene has been measured in the environment and is commonly emitted in several industrial and transportation settings. As a component of petroleum products, including gasoline and as a trace impurity in industrial products, it results in continued occupational exposure, especially in small, uncontrolled workshops in developing countries (Weasel ,2010). Determination of the extent of exposure to benzene represented a problem in the present study. The difficulties in logistics, the seasonal changes in working hours and the inability to take samples at set times necessitated the use of random sampling. Most of the subjects worked outdoors and accordingly were not exposed to constant or near constant levels of benzene all the time, except those who worked in printing shops.

Following inhalation, benzene is distributed throughout the body, and animal data indicate it may distribute preferentially to adipose tissue due to its lipophilicity (Sato & Fujiwara,1975; Rickert et al., 1979). It has been suggested that benzene elimination in humans appears to follow a two compartment model, with half-lives of around 1 hour and 24 hours (Sherwood & Carter, 1970). However, the half-life of benzene in blood has been reported to vary depending on the duration and magnitude of exposure, and the concentration may be different in venous and arterial blood (Sherwood, 1988; Kalf et al., 1989) This may result from redistribution of benzene back into the blood causing a relatively long elimination half-life. In this case, the inability to detect benzene itself in the blood or urine of workers exposed to low concentrations may be due to two reasons; rapid distribution into adipose tissues with slow redistribution into the blood, and rapid oxidation of the small amounts in the blood mainly by liver enzymes. Accordingly, it was decided to use the levels of benzene metabolites; phenol and t,t-muconic acid, as indicators for the extent of exposure. Spot blood and urine samples reasonably reflected the extent of such exposure. The urine data on benzene metabolites were of particular interest because collection of spot urine samples represented a non-invasive technique that encouraged participation and minimized dropouts.

The blood levels of the benzene metabolites; phenol and t,t-muconic acid, were obviously higher in the group of benzene-exposed workers. This probably indicates the higher rate of contact with benzene-contaminated environment and consequently increased possibility of deleterious consequences of such exposure. The high variability in the results of benzene metabolites, as judged by large standard deviations, may be due to variations in both the level of pollution and duration of exposure. The benzene metabolites in the blood and urine of control individuals probably

resulted from incidental exposure and accordingly reflected the level of air pollution by car exhaust and other contaminants in city streets. Liver function tests did not reveal any statistically significant differences between benzene-exposed workers and control subjects. It was also noted that none of the subjects in the cohort tested showed serum AST or ALT values indicative of viral hepatitis (B or C), in view of the reports (Waked et al., 1995; Geoger et al.,2000), of the high incidence of this clinical problem in the Egyptian population. This raises the question whether contracting such diseases would be related to the disease history, geographical location or the original home locality of the individual, whether urban or rural.

The results of serum creatinine were not indicative of the presence of renal problems, except in the small subgroup of printers, exposed to relatively higher benzene levels, in whom the mean serum creatinine value was slightly higher (1.45 mg/dl) than the acceptable upper normal limit (1.2 mg/dl).The exposure of this subgroup was probably higher than the rest of the cohort because they worked regular hours in confined areas.

It is clear that the benzene-exposed workers are under oxidative stress. The blood level of malondialdehyde (MDA) is elevated and the total antioxidant activity is depressed. Oxidative stress causes cellular damage that is implicated in many chronic diseases such as diabetes and cancer among many others (Aydin et al., 2001; Durackova, 2010; Khalade et al.,2010; Huff ,2007), and may predispose exposed workers to occupational diseases. Either benzene itself, as an organic solvent or more probably the increased oxidative stress in the blood of exposed workers apparently affected the integrity of the RBCs lipoid membrane. This was reflected in increased tendency toward membrane breakage and hemolysis.

Blood is considered as a non-Newtonian fluid and its viscosity is therefore variable at any given temperature, depending on the shear rate. At low shear rate, RBCs can aggregate and form one-dimensional stack-of-coins-like rouleaux or three-dimensional aggregates. The aggregates are formed because the electrostatic repulsion of RBCs is overcome by the presence of macromolecules which aggregate the cells (Popel et al., 2005). The formation of aggregates is dependent on the concentration of fibrinogen and globulin in plasma (Fung, 1993). RBCs may exhibit reduced deformability and stronger aggregation in many pathological situations. One of the important factors affecting viscosity of blood is RBCs aggregation since aggregate (rouleaux) formation increases blood viscosity. This process is particularly important in the microcirculation, since such aggregates can dramatically increase effective blood viscosity and affect hemodynamics (Popel et al., 2005). All parameters tested; including RBCs form factor (FF) and aggregation shape parameter (ASP) that predispose to higher blood viscosity are increased in the present benzene-exposed cohort.

It is clear from the above discussion that disturbance in the mentioned biochemical and biophysical parameters can be an underlying cause of some serious hazards resulting from work in professions that necessitate chronic exposure to low levels of benzene. Special attention should be directed to child labor in this case, which is a common practice sometimes. Beside the health hazards, it is possible that the dexterity, cognitive function and manual skills of children and young adolescents working in such professions would be hindered affecting the quality of their lives and the development of their careers.

Conclusion

The biochemical and hematological changes found in the present study indicate that exposure, even to low variable levels of benzene, may be detrimental to the health and well-being of exposed workers. We believe that the oxidative stress and the hemodynamic changes may hinder the ability to acquire skills in children and young adolescents working in such professions. Measures to reduce oxidative stress that may be behind many of the observed changes should be considered. The effect on female workers is not known, especially those working in petroleum refineries or in benzene production facilities.

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Table (1): Benzene, Phenol, and trans,trans, muconic acid concentrations as markers of the extend of exposure to benzene in control and benzene exposed subjects.

		Benzene		Phenol		Trans, trans, muconic acid	
		Urine	Blood	Urine	Blood	Urine	Blood
			mg/l	mg/g creatinine	mg/l	mg/g creatinine	mg/l
Control	Mean	ND	ND	1.85	1.08	0.03	ND
	± SD			±1.30	±0.7	±0.02	±
Exposed	Mean	ND	ND	3.32*	1.78*	0.99*	0.88
	± SD			±1.20	±0.6	±0.42	±0.43

- Significant ($P < 0.001$)

Table (2) : Biochemical parameters of serum in control and benzene exposed subjects.

Biochemical parameters	Control	Total exposed workers	Printers	Painters	Drivers	Auto-workers
MDA mM / ml	1.38 ± 1.27	2.01 ±1.62*	1.87 ±1.54*	1.65 ±1.68*	2.58 ±1.59*	1.50 ±1.38*
Total anti-oxidant mM/l	1.42 ±0.54	0.90 ±0.64*	0.08 ±0.06*	0.85 ±0.49*	1.2 ±0.68*	0.77 ±0.54*
AST (U/l)	9.85 ±5.06	10.64 ±6.95	10.75 ±2.49	10.9 ±3.98	10.14 ±5.09	8.89 ±4.59
ALT (U/l)	6.40 ±2.41	6.55 ±2.91	6.13 ±1.89	6.62 ±2.13	6.48 ±3.51	6.15 ±1.91
Serum creatinine mg/dl	1.02 ±0.41	0.96 ±0.22	1.45 ±0.82	0.94 ±0.32	0.91 ±0.44	1.05 ±0.76

ALT : Alanine aminotransferase
AST : Aspartate aminotransferase
Data presented as Mean ± SD.

- Sig. at $P < 0.05$.

Table (3) : Biophysical parameters of of RBCs of control and benzene exposed subjects.

Autoworkers	Drivers	Painters	Printers	Total exposed	Control	Biophysical parameters
1.99* ±0.64	2.47* ±0.63	2.39* ±0.60	1.90* ±0.26	2.29* ±0.63	1.21 ±0.40	Relative viscosity
4.67* ±0.92	4.67* ±0.88	5.20* ±1.72	8.47* ±1.97	5.16* ±1.66	3.20 ±0.32	Degree of hemolysis
198.01* ±17.55	193.17* ±25.6	195.94* ±21.41	174.47* ±20.27	193.97* ±22.85	156.43 ±16.51	FF
0.87* ±0.1	0.87* ±0.15	0.86* ±0.09	0.79* ±0.07	0.86* ±0.12	0.62 ±0.09	ASP

FF: Form Factor
ASP: Aggregation Shape Parameter

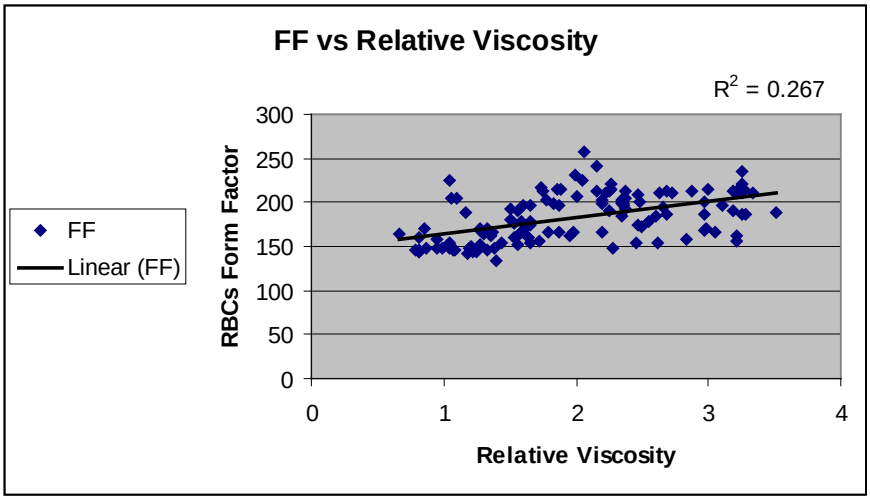

Fig. 1: Correlation between the RBCs Form Factor (FF), and RBCs Relative Viscosity

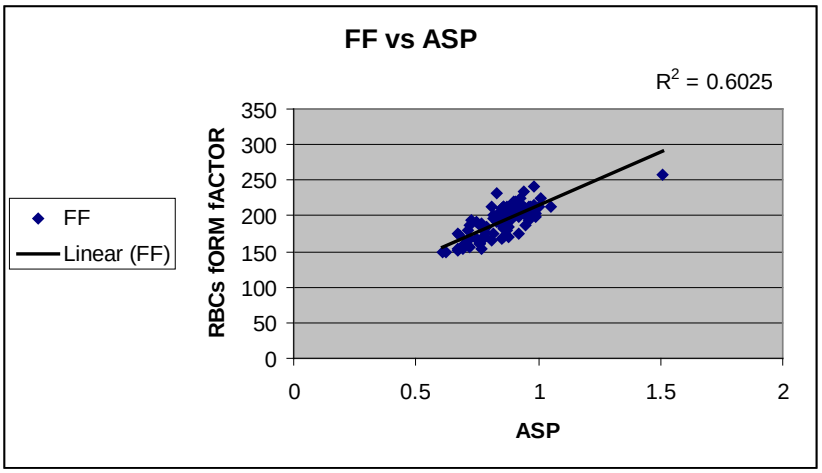

Fig. 2: Correlation between RBCs Form Factor (FF) and RBCs Aggregation Shape Parameter (ASP)