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RESEARCH ARTICLE

Hemoglobin adducts as an important marker of chronic exposure to low concentration of 1, 3-butadiene

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Abstract

Background 1, 3-Butadiene is a famous industrial compound which occurs in gasoline and diesel exhaust and in cigarette smoke. Based on little evidence of carcinogenicity in laboratory animals and humans, it was classified as a probable carcinogen. The potential of exposure to gasoline and diesel exhaust containing these chemical compounds is very considerable in urban areas. According to studies, in estimation of workers' chronic exposure with biological samples, analysis of the concentration of related hemoglobin adducts seems to give the most valid estimation of exposure.

Methods This study designed to determine the level of chronic exposure in gas station workers and traffic policemen during routine work shift, by an appropriate biological marker. In this regards, 25 gas station workers, 25 policemen engaged in traffic control, and 25 occupationally non-exposed persons were studied. Blood samples were obtained after the work shift from each person. The level of selected hemoglobin adduct, 1- and 2-hydroxy-3-butenyl valine (MHBVal) was determined by using GC–MS after a modified Edman degradation and a further acetylation.

Results There were significant differences among the mean concentrations of MHBVal in blood samples of gas station workers, policemen and occupationally non-exposed persons. The mean airborne levels of 1, 3-Butadiene over 60 days, differed significantly among different studied groups (ANOVA: $p < 0.05$ and Kruskal-Wallis test: $p < 0.05$).

Conclusion There was a significant difference in MHBVal concentrations between job categories ($p < 0.05$ by ANOVA and Kruskal-Wallis test), and gas station workers and policemen were found to be probably the most exposed groups in this research.

Keywords 1, 3-butadiene · Hemoglobin adducts · Biomarker · Exposure assessment

Background

1, 3-Butadiene (BD) is an important hazardous chemical, which its potential health impacts are a complex pattern of carcinogenicity in different animals. It is a multi-site cancer-causing agent in different species with various potencies [1, 2]. Based on obvious evidence of carcinogenicity in different animals and partial evidence of carcinogenicity in human epidemiological studies, BD was classified as a probable carcinogen to humans (group 2A) (IARC, 1999) [3]. In 2002, the US Environmental Protection Agency announced that BD can cause cancer in human by inhalation [4]. Emissions of dangerous compounds such as BD and PAH by mobile sources, into the air and environment is considered as a great public health concern because of their serious side effects like carcinogenicity and increasing level of exposure capacity into society at all levels (rural, suburban and urban) every day. Many epidemiologic researches have showed greater cancer rates among urban population compared with suburban's [5, 6]. Air pollutants, such as poly aromatic hydrocarbons and 1,3-butadiene, are believed to be an important risk factors [7]. Exposure to incomplete combustion of diesel and gasoline has been a serious concern in many occupational fields, including petroleum industry, policemen and gas stations workers [8, 9]. According to researches, in estimation of workers' exposure to BD with biological samples, analysis of the concentration of major hemoglobin adduct of 1, 3-

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butadiene, 1- and 2-hydroxy-3-butenyl valine (MHBVal), in blood seems to give the most valid estimation of chronic exposure [10, 11]. In blood, in long term, BD can react with the *N*-terminal valine of hemoglobin which leads to the formation of MHBVal, a stable Hb adduct which can reflect chronic exposure of BD. Biological markers are useful tools for the assessment of the human health risks from chemical compounds exposure, especially for cancer, so in present study this hemoglobin adduct was selected as suitable biomarker of 1, 3-Butadiene chronic exposure. The main subject of this study was to determine the chronic exposure levels for traffic policemen and gas station workers during their routine work, by biological monitoring.

Materials and methods

Study population

Seventy-five healthy and nonsmoker men from Tehran, Iran, were enrolled in the study. The research population consisted of 25 traffic policemen enrolled in traffic control in six districts with medium to high traffic level, selected based on traffic stream information recorded by the Regional Agency for Environmental Protection and 25 gas station workers. All of the selected persons were men between 25 and 55 (mean 41) years of age and nonsmoker. For the estimation of any background levels which may originating from other activities, blood samples were collected from 25 occupationally non-exposed persons in the similar regions acted as controls.

Sampling

The environmental exposure assessments were done during June until August 2019, every 20 days. Radiello passive samplers were used for measuring personal exposure to BD [12, 13]. Four samples were gathered from each person. Every collecting day, after work shift, the chemical substances collected on sorbing cartridges were desorbed by thermal desorption method and analyzed by GC/MS. Air concentration (expressed in μg m^{-3}) was calculated using the following equation:

$$Conc.\left(\mu g/m^3\right) = \frac{m(\mu g) \times 10^3}{Q(L/\min) \times t(\min)}$$

Where Conc. = concentration of BD in breathing air, m = mass of analytes determined in desorbing solvent; t = exposure time (www.sigma-aldrich.com/radiello, 2010); Q = uptake rate of substances (30.5 mL min-1 for BD) [13]. Blood samples were collected on first and 60th day, at the end of the work shift. Blood samples were stored under 4 °C in a cool box until they were gotten by the laboratory, where the erythrocytes were isolated from the blood samples and washed with isotonic saline two times and, frozen (−70 °C) until analysis.

Analysis of blood samples

The blood samples of 75 volunteers were collected and analyzed for their MHBVal concentrations according to a modified Edman degradation and a further acetylation [14]. At first, to isolate globin, frozen washed erythrocytes were thawed and diluted with an equal volume of distilled, deionized water, and then globin was isolated according to the method of Törnqvist et al. The extracted globin was derivatized according to the modified Edman degradation. [15]. Samples were then acetylated by the method of Pérez et al. [16]. Acetylated samples were reconstituted in 50 μL toluene and were analyzed by GC–MS. The concentration of MHBVal was expressed as (pmol/g) globin.

Statistics

Mean concentration of MHBVal in blood samples among three groups (occupationally non-exposed persons, gas station workers and policemen) were analyzed and since the distribution of data was not normal, the statistical analysis was done by means of two methods: Kruskal-Wallis test and analysis of variance (one- way ANOVA) followed by Scheff's post hoc test. Results were indicated as mean ± S.D. and 95% confidence intervals. The level of significance was set to 0.05 and p values >0.05 were considered to be nonsignificant.

Results

Table 1 shows the mean levels of environmental measurements (μgm^{-3}) in the three groups of occupationally non exposed persons, traffic policemen and gas station workers during 60 working days. The mean value for exposure of 1, 3-Butadiene in breathing zone of the two groups of traffic policemen, gas station workers were 24.76 and 13.82 μgm^{-3}, respectively, which were significantly higher than the occupationally non exposed groups (1.66 μgm^{-3}) but below the 2000 ACGIH TLV(TWA) (2.21 mg m^{-3}) (OSHA, 2000) [17].

Table 2 shows the results of the mean blood concentrations of MHBVal ((pmol/g) globin) in the three groups of workers in two different samples collected: before starting the study on first day and at the end of the study after 60 days. The blood levels of MHBVal in samples of subjects with jobs associated with exposure to gasoline (gas station workers and policemen) were compared with the no or low exposure group. The mean blood concentration of MHBVal in policemen was significantly greater than that the other groups. In addition, the mean levels of MHBVal in the occupationally non-exposed group

Table 1 The mean value (SD) for exposure of 1, 3-Butadiene in breathing zone

Groups	Sample size (n)	1, 3-Butadiene (μgm^{-3})	Pvalue*
Traffic policemen,	25	24.76 (25.33)	0.011
Gas station workers	25	13.82 (17.54)	
occupationally nonexposed persons	25	1.66 (1.48)	

*Significance level: ≤0.05

on first and 60th day were 0.655 and 0.648 (pmol/g) globin, respectively which were lower than occupationally exposed groups and statistically significant differences could be observed ($p \leq 0.05$). The concentration of MHBVal did not increase significantly in none of the groups after 60 days. The slight increases are observed in policemen and gas station workers which are not statistically significant. Significant correlations between mean level of BD in breathing zone and blood concentration of MHBVal was found in this study ($r = 0.576$, $p \leq 0.05$).

Discussion

In this work, exposure to BD, which is carcinogenic substance in urban air from incomplete combustion of gasoline and diesel have been investigated, by using personal air monitoring, as well as through the use of biological markers of exposure. The levels of individual exposure in traffic policemen and gas station workers (mean concentration were 24.76 and 13.82 $\mu gm\text{-}^3$, respectively) were far below the OSHA permissible exposure limits, 8-h' time weight average (TWA) of 2.2 mgm^{-3} for BD (OSHA, 2000) [17]. However, because BD is carcinogen and can cause DNA damage, NIOSH suggests that exposure should be reduced to protect people's health from chronic side effects [18]. Sapkota et al. conducted a study about exposure assessment of BD through ambient air in toll booth workers in an urban area in Baltimore, Maryland, US. Their reported concentration (mean concentration = 2.88 μgm^{-3}) was lower than our results in this study which indicated higher emissions of BD from vehicles in central districts of Tehran that are highly traffic congested [19]. The levels of individual exposure of BD in this study were similar to the results of Fustinoni et al. from Italy [20]. Difference in many of environment elements, such as traffic characteristics, quality of fuel, difference in physical activity in the workplace, building characteristics of the area, and meteorological conditions may cause the differences in the levels of individual exposure to BD. Different biomarkers of exposure to BD were studied to evaluate suitability for use as indicator of exposure to this compound through air. A few number of biomonitoring researches has been done in order to find a suitable biomarker for investigation about chronic exposure of BD through ambient air. Biomolecules, such as electrophilic metabolites bound to DNA or hemoglobin, can be used to estimate human exposure to BD. Many DNA adducts of BD epoxide metabolites have been reported in the different studies. Adducts that appear to be formed in the highest amounts are those which react with the N-terminal valine of hemoglobin (Hb). These adducts are formed by reaction of BD metabolites with the N-terminal valine of Hb. Formation of MHBVal adducts depends upon the concentration of free BD metabolites that are available to react with Hb. The concentrations of BD metabolites are determined by their relative rates of formation and removal or the activation/detoxication ratio. Formation of DNA adducts by carcinogen compounds or their DNA-reactive metabolites is considered as an initial step in carcinogenesis. If information is well known about the DNA adducts responsible for initiation of disease such as cancer, then one can estimate the molecular dose of a carcinogenic compound by measuring the concentration of DNA adducts induced by a given exposure [21].

In this study, blood concentration of MHBVal was found to be a sutiable biomarker of the levels of 1,3-butadiene exposure in urban air. Blood concentration of MHBVal could significantly distinguish between exposed and nonexposed persons and showed a good correlation with personal total 1,3-butadiene exposure [22, 23]. The mean blood concentration of MHBVal in policemen and gas station workers were significantly greater than that the nonexposed group. Levels of MHBVal reported by Boogaard et al. were lower than those in this work, despite the level of individual exposure being

Table 2 Mean blood concentrations (S.D.) of MHBVal in three groups ($n = 25$) on first and 60th day of study

Time of sampling	Groups			Pvalue*
	Policemen	Gas station workers	Occupationally non-exposed persons	
First Day	3.528 (0.822)	1.956 (0.423)	0.655 (0.112)	0.001
60th Day	3.684 (0.937)	2.036 (0.516)	0.648 (0.098)	0.001

*Significance level: ≤0.05

quite similar [23]. Reasons behind these differences may include the impacts of genetic polymorphisms or different analytical methods or differing lifestyles between people in these nations. The results of this study showed that blood levels of MHBVal in traffic policemen and gas station workers were slightly but not significantly elevated after 60 days, indicating a complex metabolism of 1,3-butadiene at low levels.

Conclusion

In conclusion, these results demonstrated that traffic policemen, who are exposed chronically to BD at the roadside in central of Tehran, are possibly at a greater risk for development of diseases like cancer than other groups. The quality of fuel, high rate of passenger cars, Traffic jam conditions, and increased rate of exposure would result a higher risk for groups such as policemen. Further researches need to focus on the lifestyle and genetic factors that may affect the background levels of blood MHBVal concentration.

Abbreviations *IARC,* International Agency for Research on Cancer; *BD,* 1, 3-Butadiene; *MHBVal,* 1- and 2-hydroxy-3-butenyl valine,; *TWA,* Time weight average

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Authors' contributions RA and NR participated in the design of the study. RA did the analyses and FI interpreted the analyzed results. NR was the main investigator, supervised the work, drafted and revised the paper critically for important intellectual content and compiled the work in accordance with journal format. All authors have read and approved the final manuscript.

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Availability of data and materials The data will not be shared with a reason.

Declarations

Ethics approval and consent to participate The research protocol was approved by Ethics Committee of Tehran University of Medical Sciences (IR.TUMS.SPH.REC.1396.2335).

Consent for publication Not applicable.

Competing interests The authors declare that they have no competing interests.

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