International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: 6.614
Available Online at www.journalijcar.org
Volume 7; Issue 7(C); July 2018; Page No. 14031-14035
DOI: http://dx.doi.org/10.24327/ijcar.2018.14035.2530

Research Article

DETERMINATION OF BENZENE METABOLITE PHENOL IN THE URINE AND ANALYSIS OF BLOOD PARAMETERS OF WORKERS EXPOSED TO BENZENE

Sanja Brekalo-Lazarevic[1,2]., Aida Begic[3]., Zahida Ademovic[4]*., Emir Horozic[4]

[1]Faculty of Medicine, University of Tuzla, Univerzitetska 1, 75 000 Tuzla, Bosnia and Herzegovina
[2]Public Health Institution, Health Centre "Dr Mustafa Sehovic", 75 000 Tuzla, Bosnia and Herzegovina
[3]Faculty of Pharmacy, University of Tuzla, Univerzitetska 8, 75 000 Tuzla, Bosnia and Herzegovina
[4]Faculty of Technology, University of Tuzla, Univerzitetska 8, 75 000 Tuzla, Bosnia and Herzegovina

A R T I C L E I N F O

Article History:

Received 5th April, 2018
Received in revised form 24th May, 2018 Accepted 20th June, 2018
Published online 28th July, 2018

Key words:

Benzene, phenol, biomarker, hematotoxicity, occupational exposure

A B S T R A C T

Determination of the benzene metabolite phenol in urine is a suitable biomarker for monitoring benzene exposure of industrial workers. The aim of the study was to determine the concentration of benzene metabolite phenol in the urine samples of exposed workers and changes in the blood count as the first sign of target organ toxicity. Urine and blood samples were collected from sixteen industry workers exposed to benzene in the area of Tuzla Canton, Bosnia and Herzegovina. The urine samples were analyzed by isocratic HPLC method after acid hydrolysis of urinary phenol conjugates and liquid-liquid extraction. High reproducibility was achieved and the recoveries from spiked urine samples were between 71 – 110 %. The phenol concentrations in samples were significantly higher in urine of industrial workers compared to controls (13.62 ± 17.18 mg/L for experimental group and 2.72 ± 2.83 mg/L for controls, $p<0.05$). Red blood cells and hematocrit were significantly lower in industrial workers ($p < 0.001$) and that the levels of creatinine and ALT activity were significantly different between the groups. Examination of biological fluids provides vital information on health hazards associated with exposure to occupational pollution. Phenol determination in urine was proven to be suitable biomarker of benzene exposure.

INTRODUCTION

Benzene is widely used organic chemical as a component of gasoline and as raw material in chemical industry. The use of benzene started with the development of the chemical industry in the late 19th century. It is volatile liquid, colorless, with mild aromatic odor. Benzene and its homologues, toluene and xylene are produced by distillation of tar and coal. Because of its wide use benzene ranks in the top 20 in production volume for chemicals produced in the United States.[1] Benzene is used in the manufacturing of rubbers, lubricants, dyes, detergents, drugs and pesticides. Thus, industrial processes are the main sources of benzene in the environment. Benzene levels in the air can be elevated by emissions from burning coal and oil, benzene waste, motor vehicle exhaust and evaporation from gasoline service stations.[1-2] The general population is exposed to small amounts of benzene mainly through breathing contaminated air. Individuals working in industries that make or use benzene are exposed to the highest levels of benzene (petrochemicals, petroleum refining, coke and coal industry, rubber tire manufacturing, printers and gas station employees). In the working environment, benzene and its homologues penetrate the body through the respiratory system or skin and rarely through the digestive system. It is partly removed from the body through exhalation. The remaining part enters the bloodstream and is further oxidized in the liver and bone marrow and eliminated by the urine in the form of metabolites - glucuronide and sulphate conjugates of phenol, catechol, hydroquinone, L-phenylmercapturic acid and trans,trans-muconic acid.[3] The conjugates of phenol and hydroquinone are major urinary metabolites of benzene.[4] The part of benzene that remains in the body is deposited in tissue and organs rich in lipoids (bone marrow, fat, liver, etc.) and is slowly secreted through oxidation by urine. The determination of concentration of certain benzene metabolites in urine is used as a biomarker of occupational or environmental exposure to benzene.[5-6] Complete blood counts and examination of bone marrow are used to determine harmful health effects of benzene exposure.

Brief exposure (5-10 minutes) to very high levels of benzene in air (10 000-20 000 ppm) can result in death. Lower levels (70-3000 ppm) can cause drowsiness, dizziness, rapid hearth

Corresponding author:* **Zahida Ademovic
Faculty of Technology, University of Tuzla, Univerzitetska 8, 75 000 Tuzla, Bosnia and Herzegovina

rate, headaches, tremors, confusion and unconsciousness. Long-term exposure to benzene can cause cancer of the blood-forming organs. The conducted animal studies on benzene carcinogenesis have shown that benzene is a strong carcinogen associated with high incidence of a variety of tumors.[7] International Agency for Cancer Research (IARC) has classified benzene as carcinogenic to humans (Group 1).[8]

Benzene and its homologues can cause acute and chronic toxic effects. Acute poisoning appears accidentally, when benzene vapor is presented in high concentrations. That could occur during the cleaning of benzene storage tanks or in the event of industrial disasters. In the case of acute poisoning the effects on central nervous system (CNS) predominate: drowsiness, headache, dizziness, nausea, vomiting, followed by "benzene narcosis" with respiratory and circulatory disorders, which is a comatose condition that could end with death.

Chronic exposure to lower concentrations of benzene causes hematological disorders. Early biomarkers of exposure to relatively low levels of benzene include depressed numbers of the circulating blood cell types (anemia, leukopenia and thrombocytopenia). Benzene-associated cytopenias vary and may involve a reduction in all three blood lines. With continued exposure it may progress to aplastic anemia, excessive bleeding, leukemia and damage to the immune system.[1] Aplastic anemia is characterized by reduction of all cellular elements in the peripheral blood and in the bone marrow, leading to fibrosis of bone marrow tissue.

The mechanism leading to benzene toxicity is not yet fully understood. It is known that both cancer and noncancer effects are caused by one or more reactive metabolites of benzene.[9] The predominant pathway of benzene metabolism involves nonenzymatic rearrangement to form phenol, which is the major initial product.[10] Phenol is oxidized in the presence of CYP2E1 to catechol and hydroquinone, which are oxidized via myeloperoxidase (MPO) to the reactive metabolites 1,2- and 1,4-benzoquinone.[11] Both catechol and hydroquinone may be converted to the reactive metabolite 1,2,4-benzenetriol via CYP2E1 catalysis.

In this cross-sectional study the effects of chronic exposure to benzene in working environment on blood parameters and liver and kidney functional status were examined. The primary aim was to determine the concentration of benzene metabolite phenol in the urine samples as well as changes in the blood count as the first sign of target organ toxicity. To our best knowledge, this is the first study of work-related exposure to benzene in Bosnia and Herzegovina. The results of this work will contribute to health and safety at the workplace and prevention of occupational diseases.

MATERIAL AND METHODS

Study population

The experimental group consisted of 16 male subjects working in the chemical industry in the Tuzla Canton. The control group consisted of 16 male subjects that are not occupationally exposed to benzene. The study was approved by the Ethic committee of the Institution Health Centre. All subjects gave written informed consent. The study was designed as a cross-sectional study.

Chemicals

Phenol reference standard, diethyl ether and acetic acid were purchased from Sigma-Aldrich, Germany, methanol HPLC grade was obtained from Bisolve Chimie, France. Hydrochloric acid and sodium hydroxide were purchased from Roth, Germany.

Blood test

Collected blood samples were analysed in biochemical laboratory of Occupational medicine in the Health Centre and were tested by an Automatic Biochemical Analyzer using standard laboratory procedures. Full blood count consisted of white blood cells (WBC), red blood cells (RBC) and platelet (PLT) count, hemoglobin (HGB), hematocrit (HCT) and mean corpuscular volume (MCV) determination. The liver functional test consisted of alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT) activities, total cholesterol (TC) and tryglicerides (TG) levels measurements. Also, level of direct and total bilirubin, urea, creatinine and glucose level were measured.

Phenol determination

High Performance Liquid Chromatography (HPLC)

Phenol levels in urine were determined by High-Performance Liquid Chromatography (Shimadzu Corporation, Japan) gradient controlled system consisting of solvent delivery system, an autosampler, pump and UV-VIS SPD-20A detector. The performed separation method for analysis was compared to the previously described procedure.[12] Phenol was detected at 270 nm. The separation was carried out with a Selectra C18 column (4.6x250 mm, 5 μm) at the room temperature and a flow rate of 1 mL/min. Samples for HPLC analysis were filtered through membrane filter prior to injection. Isocratic separation was performed with a mobile phase consisting of distilled H_2O/methanol/acetic acid = 65/34/1 v/v %. Injection volume was 20 μl. For instrument control, data acquisition and data analysis, CLASS-VP 7.4 Software by Shimadzu was used.

Sample collection and preparation

Phenol levels were measured in urine collected after the workshift, transported and immediately analysed. The protocol for sample preparation for HPLC analysis was described previously.[13] Briefly, to 5 ml of urine in a glass tube 2 ml of concentrated HCl was added. The mixture was boiled for 60 min. After cooling, phenols were extracted with 4 ml of diethyl ether by shaking the tube for 1 min. Following centrifugation for 10 min at 1800 rpm, the organic phase was aspirated into 3 ml of 0.05 M NaOH in methanol. The resultant solution was evaporated to dryness and residues were dissolved in 0.5 ml of distilled water.

Statistical analysis

All statistical analyses were performed using SPSS Statistic software. One-way ANOVA and Tukey's test for post-hoc multiple comparisons were performed for statistical data analysis of phenol. Mann-Whitney U test was used for statistical data analysis of clinical variables. Clinical variables are reported as medians and IQR. Categorical variables are reported as numbers and percentages. Differences were considered statistically significant for $p<0.05$.

RESULTS AND DISCUSSION

The experimental group consisted of 16 male subjects ages 33-58 year, whereas the age of 16 male subjects in control group was between 24-55 years.

The applied isocratic HPLC-UV method for the determination of phenol was suitable as it did not require the use of special equipment or additional techniques such as steam distillation or derivatization. All recovery calculations were based on addition of phenol standard to urine.

However, in humans phenols are conjugated to sulphates and glucuronides by the normal functioning liver and also by colonic epithelial cells (predominantly as sulphates).[14] It was previously demonstrated that 84% of phenyl sulphate was recovered from urine after being hydrolysed with hydrochloric acid in a boiling bath for 60 min and extracted with dibutyl ether.[15] The procedure applied in this work was similar and sufficiently high recovery was achieved to enable making statistically valid comparisons between subjects and controls. Quantitative analysis was performed based on calibration curve of urine samples spiked with phenol prepared in distilled water, with concentrations in the range between 1 - 40 mg/L ($y = 9841x + 14794$, R^2=0.9973). The low levels of phenol detected approached the limits of detection. Identification of the phenol peaks obtained from urine samples was ensured by achieved high reproducibility for the selected chromatographic conditions (R.S.D for retention time 0.14 %).

Spiked samples were prepared to determine the efficiency of the extraction method in recovering phenols. The recoveries from spiked urine samples were between 71 – 110 %. It was shown that higher concentration yields lower recovery. The highest percent recovery of phenols was achieved for the spike of 5 mg/L (101%).

Levels of phenol, major initial benzene metabolite, were measured in urine samples of 16 industry workers as experimental group and 16 subjects working in an occupational setting without benzene exposure as control group. Previous studies reported a good correlation between benzene concentration at the work place and phenol concentration in urine.[16-17] The amount of phenol in urine analyzed by HPLC ranged from 2.8 – 63.4 mg/L in experimental group and 0 – 7.9 mg/L in control group. Phenol levels were found to be significantly higher in urine of workers compared to controls (13.62 ± 17.18 mg/L for experimental group and 2.72 ± 2.83 mg/L for controls, p=0.018) (Figure 1).

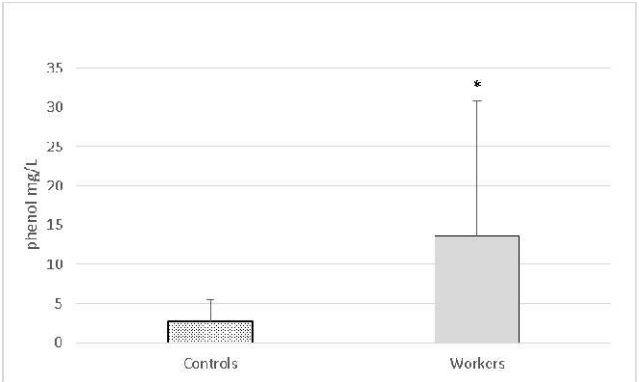

Figure 1 Phenol concentration in the urine samples.

The results are expressed as mean ± SD. Phenol concentration was expressed as mg/L. *Statistical significance was considered for $p \leq 0.05$.

The phenol concentrations measured in experimental group were higher than in the study conducted on gas station workers (phenol range 0.6 – 20.8 mg/L urine).[12] The urinary phenol concentration in unexposed individuals is less than 10 mg/L which is in agreement with reference value.[18-19]

The analyzed blood count of industrial workers and controls is presented in Table 1.

Table 1 Blood count and biochemical blood parameters of industrial workers as experimental group and controls.

Parameter		Median	25-75 Percentile	Minimum	Maximum	STD
WBC (x10^9/L)	Control	7.53	6.33-9.08	5.44	15.36	2.62
	Workers	6.55	5.53-7.48	5	10	1.31
RBC (x10^{12}/L)	Control	5.34	5.17-5.62	5.02	5.99	0.29
	Workers	4.94	4.78-5.23	4.41	5.62	0.31
HGB (g/L)	Control	154	150.75-166.5	148	178	9.18
	Workers	152.5	145-159.75	138	169	9.32
HCT	Control	0.467	0.459-0.505	0.45	0.53	0.03
	Workers	0.446	0.425-0.459	0.41	0.49	0.02
PLT (x10^9/L)	Control	242	219-269	184	306	36.34
	Workers	243	219.75-277.25	166	332	45.31
Glucose (mmol/l)	Control	5.25	4.9-5.75	4.9	8	0.81
	Workers	5.58	5.23-5.91	4.6	6.66	0.52
Urea (mmol/L)	Control	5	3.78-5.48	3	6.6	1.08
	Workers	5.43	4.85-5.78	3.49	7.57	0.91
Creatinine (S) (µmol/L)	Control	78.5	72.75-94.5	68	108	12
	Workers	99.07	96.74-114.22	92.08	125.88	11.08
Total bilirubin (µmol/L)	Control	11.65	7.68-15.2	6	23.9	5.48
	Workers	12.27	8.52-14.53	5.12	34.22	7.12
AST (U/L)	Control	22	18.25-27.75	16	49	8.48
	Workers	19.7	17.8-20.8	15.1	30.8	3.7
ALT (U/L)	Control	35	28.75-41.25	19	54	9.63
	Workers	22.5	12.1-29.9	10.8	65.3	14.04
GGT (U/I)	Control	26.5	18.75-36.25	14	159	36.02
	Workers	30.6	20.9-57.4	12.4	77.4	20.87

Benzene-associated cytopenias may involve a reduction in one to all three cellular elements of the blood. There was no significant difference in the count of white blood cells (WBCs) and platelets (PLT) in the experimental group compared to controls, whilst the difference in the count of red blood cells (RBCs) was significant ($p < 0.001$) (Figure 2).

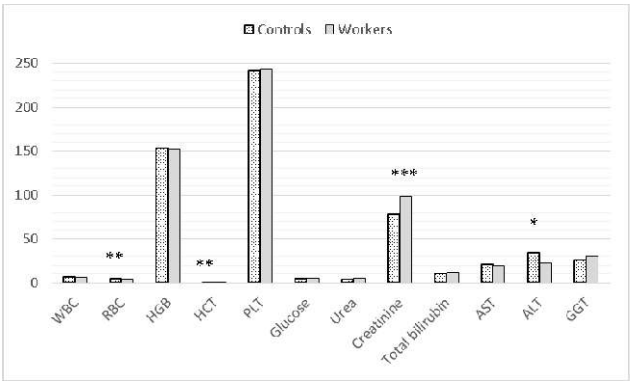

Figure 2 Blood count and biochemical blood parameters of industrial workers and controls.

The results are expressed as medians. *Statistical significance was considered for $p \leq 0.05$. Level of significance is labeled as follows: *$p < 0.01$, **$p < 0.001$ and ***$p < 0.0001$.

Even so, the number of RBCs remained within the reference values (median ± STD: 4.94 ± 0.31 x10^{12}/L and 5.34 ± 0.29 x10^{12}/L for industrial workers and controls, respectively) (Table 1). Hemoglobin level was found to be similar in the blood of industrial workers and controls, whilst hematocrit of industrial workers was significantly lower compared to controls ($p < 0.001$) (Figure 2). The median hematocrit also remained within the reference values (median ± STD: 0.446 ± 0.02 and 0.467 ± 0.03 for industrial workers and controls, respectively) (Figure 2, Table 1). Previous study reported a

statistically significant decrease in total RBC, WBC, PLT and hematocrit for a group of subjects exposed to benzene in the workplace (median 8-hour time-weighted average TWA of 31 ppm) for an average of 6.3 years in China.[20] This study however showed no decrease in the relative number of circulating blood cells of tested subjects. Measured benzene air concentration in selected work areas in this study was between TWA of 1 to 7.5 ppm. The results are in compliance with the previous study of 303 workers exposed to less than 1 ppm of benzene for up to 26 years, with no signs of changes in blood or any case of leukemia.[21] It is evident that chronic exposure to high levels of benzene (more than 50 ppm) is associated with the development of anemia and leukopenia, but such association is not found for lower benzene concentration (less than 10 ppm).[22]

All performed biochemical blood tests were found to be within the reference values. Statistical analysis showed that the levels of creatinine and ALT activity were significantly different compared to control (Figure 2).

The limitations of this study are that there was no personal monitoring for benzene exposure. Also, there are no records for baseline and periodic medical examination of workers to identify changes that could be attributed to benzene exposure. This study is a small-scale study, which limits the generalization of results to the total population of workers exposed to benzene.

CONCLUSION

Exposure to benzene in industrial work environment still poses a significant health hazard. The effects of long-term benzene exposure on blood are clearly dependent on the level of the exposure. Even though phenol determination in urine was proven to be suitable biomarker of benzene exposure, further studies on the relationship between the urinary phenol and benzene exposure as well as health effects on exposed workers are necessary.

Acknowledgment

This work was financially supported by grant from the Federal Ministry of Education and Science, Bosnia and Herzegovina in 2017.

References

1. ATSDR. Toxicological profile for benzene (2007).
2. WHO (2010) Exposure to benzene: A major public health concern. Geneva, World Health Organization (http://www.who.int/ipcs/features/benzene.pdf).
3. Waidyanatha S, Rothman N, Li G, Smith MT, Yin S, Rappaport SM, Rapid determination of six urinary benzene metabolites in occupationally exposed and unexposed subjects. *Anal Biochem* 2004;327(2):184-99.
4. Wells MS, Nerland DE. Hematotoxicity and concentration-dependent conjugation of phenol in mice following inhalation exposure to benzene. *Toxicol Lett* 1991;56:159-166.
5. Kouniali A, Cicolella A, Gonzalez-Flesca N, Dujardin R, Gehanno JF, Bois FY. Environmental benzene exposure assessment for parent-child Pairs in Rouen, *France. Sci. Total Environm* 2003;308:73-82.
6. Melikian AA, Qu O, Shore R, Li G, Li H, Jin X, Wang B. Personal exposure to different levels of benzene and its relationships to the urinary metabolites S-phenylmecapturic acid and trans-muconic acid. *J Chromatgr B Analyt Technol Biomed Life Sci* 2002;778(1-2):211-221.
7. Maltoni C, Gilliberti A, Conti G, Belpoggi F. Benzene, an experimental multipotential carcinogen: Results of the long-term bioassays performed at the Bologna Institute of Oncology. *Health Perspect* 1989;82:109-124.
8. International Agency for Research on Cancer, IARC (1987) - Summaries & Evaluations: Benzene (Group 1). Lyon, p. 120. IARC Monographs on the Carcinogenicity of Chemicals to Humans, Supplement 7. http://www.inchem.org/documents/iarc/suppl7/benzene.html
9. Yardley-Jones A, Anderon D, Parke DV. The toxicity of benzene and its metabolism and molecular pathology in human risk assessment. Br J Ind Med 1991;48:437-444.
10. Parke DV, Williams RT. Studied in detoxication 49. The metabolism of benzene containing [^{14}C1] benzene. *Biochem J* 1953;54:231-238.
11. Nebert DW, Roe AL, Vandale SE. NAD(P)H: Quinone oxidoreductase (NQO1) polymorfism, exposure to benzene, and predisposition to disease: A huGE review. Genet Med 2002;4(2):62-70.
12. Alawi MA. Determination of benzene and its metabolite phenol in the urine samples of gas-station workers in the greater Amman municipality. *Dirasat* 2008;35(2):175-179.
13. Birkett AM, Jones GP, Muir JG. Simple high-performance liquid chromatography analysis of phenol and p-cresol in urine and feces. *J Chromatography* B 1995;674:187-191.
14. Ramakrishna BS, Roberts-Thomson IC, Pannall PR, Roediger WEW. Impaired sulphation of phenol by the colonic mucosa in quiescent and active ulcerative colitis. *Gut* 1991;32:46-49.
15. Eadsforth CV, Coveney PC. Measurement of phenol in urine using a high-performance liquid chromatographic method. *Analyst* 1984;109:175-176.
16. Syafar M, Wahab AW. Analysis of benzene concentration effects at workplace to the phenol concentration in urine of painting workshop labors in Makassar, Indonesia, *Int J Sci: Basic Appl Res* 2015; 21,439-445.
17. Waidyanatha S, Rothman N, Fustinoni S, Smith MT, Hayes RB, Bechtold W, Dosemeci M, Guilan L, Yin S, Rappaport SM. Urinary benzene as a biomarker of exposure among occupationally exposed and unexposed subjects, *Carcinogenesis* 2001;22:279–286.
18. Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological Profile for Phenol (Update). Public Health Service, U.S. Department of Health and Human Services, Atlanta, GA. 1998.
19. Braier L, Levy A, Dror K, Pardo A. Benzene in blood and phenol in urine in monitoring benzene exposure in industry. *Am J Ind Med.* 1981;2(2):119-23.
20. Rothman N, Li GL, Dosemeci M, Bechtold WE, Marti GE, Wang YZ, Linet M, Xi LQ, Lu W, Smith MT, et al. Hematotoxicity among Chinese workers heavily exposed to benzene. *Am J Ind Med*. 1996;29(3):236–246.

21. Tsai SP, Wen CP, Weiss NS, Wong O, McClellan WA, Gibson RL. Retrospective mortality and medical surveillance studies of workers in benzene areas of rafineries. *J Occup Med* 1983;25:685-692.

22. Kipen HM, Cody RP, Goldstein BD. Use of longitudinal analysis of peripheral blood counts to validate historical reconstrutions of benzene exposure. *Environ Health* 1989;82:199-2016.

How to cite this article:

Sanja Brekalo-Lazarevic *et al* (2018) 'Determination of Benzene Metabolite phenol in the Urine and Analysis of Blood Parameters of Workers Exposed to Benzene', *International Journal of Current Advanced Research,* 07(7), pp. 14031-14035. DOI: http://dx.doi.org/10.24327/ijcar.2018.14035.2530

****