Original Article

Toxicity of xylene in occupationally exposed workers: A high-performance liquid chromatography analysis

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Abstract

Background: Xylene is one of the most commonly used solvents in industrial and medical technologies. Several health hazards of xylene have been documented in literature. Workers in certain forces appear to have the greatest potential for exposure to high concentrations of xylene – histopathology technicians and painters are two such groups. This study was undertaken with the aim to determine the level of xylene exposure and the various systemic health effects among these groups.

Methodology: The study was performed by analyzing the urine samples of the participants for methylhippuric acid, the established biomarker of xylene with the aid of high-performance liquid chromatography.

Results and Conclusion: The work hours per week of the occupationally exposed participants were statistically analyzed with that of the excretory values of the metabolites of xylene, and the *P* value was found to be highly significant. Various side effects of xylene including respiratory, dermatological, neurological and gastrointestinal symptoms were observed among the study groups.

Keywords: Biomarker, methylhippuric acid, occupational hazard, toxicity study

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INTRODUCTION

Xylene, chemically referred to as dimethylbenzene, occurs in the form of three isomers – meta, para and ortho. A mixture of these three forms is used for industrial purpose. Most of the chemicals including xylene are hazardous in nature. Many agencies including the Agency for Toxic Substances and Drug Registry (ATSDR) and Environmental Protection Agency have established the minimum risk level (MRL) for xylene as 0.1 ppm on the basis of animal studies and human occupational exposure reports. The health and safety authorities in most of the countries recommend a threshold limit of 100 ppm of xylene in the working atmosphere. Xylene metabolizes to methylhippuric acid (MHA) which can be measured in the urine of the exposed personnel.[1] The American Conference of Governmental Industrial Hygienists has put forth the biological exposure index (BEI) of several hazardous chemicals including xylene. The BEI of xylene is 1.5 g of MHA per gram of creatinine.[2]

Most of the xylene that enters the body leaves within 18 h following the end of the exposure. Significant amounts of xylene can get accumulated in the body on prolonged exposure as seen in occupationally exposed personnel. Several health hazards have been identified with acute and chronic xylene exposure. These can range from milder

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symptoms such as dizziness to severe lung congestion with focal areas of interalveolar hemorrhage followed by death. Chronic exposure often results in dizziness, eyes, nose and throat irritation.[1,3] This study was undertaken to quantify xylene exposure in two occupationally exposed groups and correlated with the various subjective symptoms due to its exposure.

METHODOLOGY

The study comprised a total of 45 samples of urine divided into four groups. Group I comprised five histopathology technicians with a 50-h work/week; Group II comprised ten histopathology technicians with a 30-h work/week. Group III comprised fifteen painters. Fifteen age-matched healthy volunteers were also included in the study (Group IV). Histopathology technicians with a minimum 10 years' experience and painters with a minimum 5 years' experience were only included in the study. Smokers and alcoholics were excluded from the study. Each individual was asked not to consume aspirin-like medications 48 h prior to the collection of their urine sample as it was found to be interfering with the excretion of the metabolites of xylene in the urine. A patient consent form in accordance with the ethics of the university was given to the participants along with a detailed questionnaire prior to the sample collection. Following the completion of the forms, the urine samples were collected in a sterile 100 ml polyethylene bottle with a few pellets of thymol crystals and transported immediately to the department of biomedical research and stored in a deep freezer at −40°C. The urine samples were processed as per the standard procedure (NIOSH method) prior to its analysis.[4] Three to five samples were analyzed per day using high-performance liquid chromatography (HPLC, PerkinElmer Series 200) with a ultraviolet detector. A personal computer (PC) was used as a recorder of data.

Standards of MHA (o- and m-) were purchased from Sigma-Aldrich chemicals, Germany. Freshly prepared standards of 2 and 3 MHAs were dissolved in the mobile phases of the HPLC and analyzed prior to every preinjection of the urine samples. The creatinine value of the samples was calculated using the kit method (Accurex). The urine samples were also subjected to routine urine analysis.

RESULTS

The concentration of the metabolites in each urine sample was calculated by correlating the peak height of the standard solutions with that of the urine samples. The area under each curve was calculated and recorded as the concentration of the analytes (2 and 3 MHAs). It was found that all four groups of participants excreted the metabolites of xylene although there was nearly a 1000-fold increase in the occupationally exposed participant groups (Groups I, II and III) [Figure 1]. The mean excretion value of the xylene metabolites by the groups was analyzed statistically using ANOVA which was found to be highly significant with a $P = 0.000$ [Table 1]. *Post hoc* tests were performed for multiple comparisons among the groups using the Turkey's honestly significant difference method which showed that all the four groups were significantly different from each other in their values of excretion of xylene metabolites although histopathology technicians with a 30-h work/week (Group II) were found to be having nearly similar metabolite excretion value to that of the painters (Group III) [Table 2]. No significant gender-related differences were noted. Statistically significant difference in the excretion of xylene metabolites when compared with their work hours per week was observed among the occupationally exposed groups [Table 3]. The participants in these groups also reported the presence of systemic symptoms such as eye, nose and throat irritation, dizziness, drowsiness, abdominal pain and dermatitis revealed by the questionnaire filled by the participants prior to urine sample collection. Dizziness, drowsiness and anorexia were reported maximum among the Group I participants. Staggering gait was seen only in one participant in Group II. Abdominal pain was seen marginally more among the Group III participants. Percentage distribution of the subjective symptoms among the three study groups revealed that eye, nose and throat irritation was reported by most of the participants (90%), followed by dizziness (36.6%), abdominal pain (33%), anorexia and drowsiness (26.7%), nausea (6.6%) and staggering gait (3.3%) [Figure 2]. The excretion value of the xylene metabolites was compared with the usage of gloves among

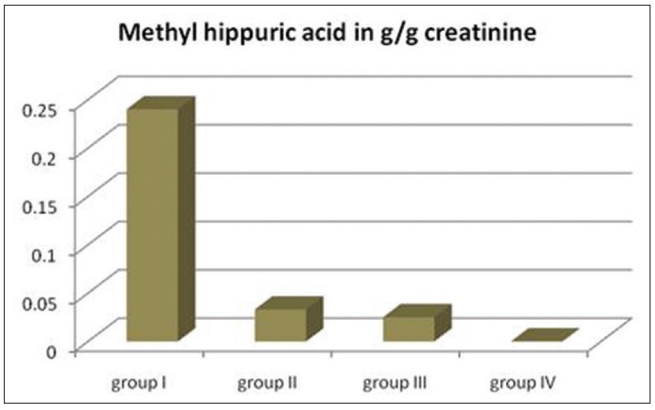

Figure 1: Mean excretion value of methylhippuric acid among the study groups

Table 1: The excretion values of the total methylhippuric acid among the four groups were statistically analyzed using ANOVA and found to be highly significant

Total g/g	*n*	Mean	SD	*P*
Group I	5	0.2400	0.05477	0.000 (significant)
Group II	10	0.0330	0.00823	
Group III	15	0.0253	0.01302	
Group IV	15	0.0002	0.00012	

#*P* value is significantly below the value of 0.05. SD: Standard deviation

Table 2: *Post hoc* test using Turkey's honestly significant difference method was performed for multiple comparisons among the groups

Group	Group	Mean difference	*P*
Group I	Group II	0.20700	0.000 (significant)
	Group III	0.21467	0.000 (significant)
	Group IV	0.23979	0.000 (significant)
Group II	Group I	−0.020700	0.000 (significant)
	Group III	0.00767	0.760 (not significant)
	Group IV	0.03279	0.001 (significant)
Group III	Group I	−0.23979	0.000 (significant)
	Group II	−0.00767	0.760
	Group IV	0.02512	0.005 (significant)
Group IV	Group I	−0.023979	0.000 (significant)
	Group II	−0.03279	0.001 (significant)
	Group III	−0.02512	0.005 (significant)

#*P* value is significantly below the value of 0.05

Table 3: The mean working hours of the groups were statistically compared with the excretion of methylhippuric acid and found to be highly significant

Mean work hours	*n*	Mean excretion	SD	*P*
50	5	0.24	0.05477	0.000
30	10	0.033	0.00823	(significant)
24	15	0.0253	0.01302	

#*P* value is significantly below the value of 0.05. SD: Standard deviation

the two groups of histopathological technicians, but no statistical significance could be observed.

DISCUSSION

A strong association between urinary MHA concentration and xylene exposure has been well documented in a study by Inoue *et al*., in a group of 175 Chinese workers exposed predominantly to xylene.[5] In our study, the excretion value of all the participants was well within the limits of the BEI. It was found that the healthy volunteers too excreted xylene metabolites, but it was found to be nearly a 1000-fold lesser than the occupationally exposed participants which is attributed to vehicular pollution in the urban areas of the city as concluded in a study on traffic wardens in Milan by Buratti *et al*. in the study it was found that the excretion of xylene metabolites increased in proportion to an increase in work hours owing to longer hours of exposure to xylene.[6] No significant difference in urinary excretion of metabolites among the histopathology technicians could be seen with relation to the usage of gloves at workplace.

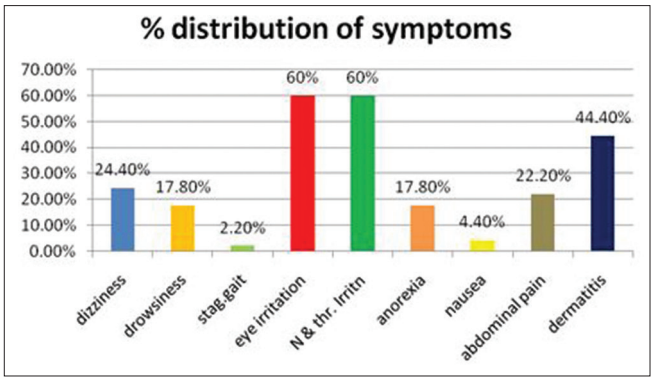

Figure 2: Percentage distribution of the symptoms of xylene exposure among the study participants

The usage of gloves could not be monitored continuously. Therefore, we could contemplate that the technicians have probably not utilized the gloves continuously at all times when working with xylene. Engström *et al*. have demonstrated that dermal absorption of xylene in humans can occur by direct contact with the compound and that higher levels of xylene were found in the venous blood drawn from the contaminated area when compared with mixed venous blood.[7] Palmer and Rycroft reported the occurrence of urticaria in a female cytology worker exposed to xylene.[8] Gunasekar *et al*. have concluded in their study that dermal exposure to xylene enhances the production of interleukin-1α and inducible nitric oxide synthase that serve as early indicators of skin irritation.[9]

One of the largest chronic occupational xylene exposure study in 175 Chinese workers performed by Uchida *et al*. revealed the presence of subjective symptoms such as eye, nose and throat irritation, dizziness and floating sensation which was also seen among our occupationally exposed group.[10] Hine and Zuidema reported the occurrence of slight-to-moderate eye irritation in rabbits when instilled with 0.1 ml of mixed xylene.[11] Goldie has reported a case of dryness of the throat in painters exposed to xylene vapors.[12] Carpenter *et al*. reported that dizziness was the most frequent neurological complaint of participants exposed to xylene.[13] Savolainen and Pfäffli attributed the liposolubility of xylene in the neuronal membrane to its neurotoxicity.[14] The Agency for Toxic Substances and Disease Registry (ATSDR) has come up with a minimum risk value (MRL) of 0.1 ppm for xylene[1,3] which corresponds to 0.0015 g/g creatinine above which systemic and local side effects of xylene become evident. This was observed in the occupationally exposed participants in our study.

Following an acute exposure to xylene, it is advisable to shift the person to a well-ventilated room away from the source of exposure. Gastric lavage, hemodialysis

and hemoperfusion are some of the techniques used for speedy removal of large doses of xylene. These techniques can help in reversal of acute systemic toxicity due to xylene exposure. Personnel protection using impervious clothing, face shields, gloves and safety glasses can help in controlling acute and chronic xylene exposure to a large extent. Installation and maintenance of eyewash stations and quick-drench facilities closer to work areas is needed for handling emergencies.[1,7,10]

Limitations

In the study, the exposure rate (time-related exposure in ppm) of xylene could not be determined as the ambient level of xylene in the atmosphere (e.g., laboratory rooms) using passive diffusion vapor monitors could not be evaluated.

CONCLUSION

With this study, we conclude that xylene exposure can be constantly monitored using the urinary excretion of its metabolites. Periodic biological monitoring of the workers' body fluids to detect if xylene exposure is within limits is recommended as their health and safety is of utmost importance. Personnel who work with such compounds need to be educated of the various health effects of xylene and advised of ways to reduce its occupational exposure.

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Conflicts of interest

There are no conflicts of interest.

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