Urinary S-Phenylmercapturic Acid (S-PMA) Level as Biomarkers of Exposure to Benzene in Informal Shoes Industrial Workers, Cibaduyut Bandung

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Abstract

Urinary S-PMA is a biomarker that is most specific and sensitive to benzene exposure at any concentrations because it is not influenced by the exposure of other substances. The objective of this study was to analyze the association between urinary SPMA and individual characteristics in workers exposed to benzene. This study used cross-sectional design in five informal shoes industry in Cibaduyut. Urinary S-PMA level in 60 workers was determined using LC-MS/MS and individual characteristics data were collected by interview using questionnaires. The association between urinary S-PMA level and individual characteristics were analyzed using correlation (age and working time) and independent T-test (type of job, working hours per day, smoking status, and alcohol intake). The urinary S-PMA level from 33% workers was above BEI ACGIH (>25 µg/g creatinine). Types of job and working hours per day showed significant associations with urinary S-PMA level (p-value 0.036 and 0.033, respectively). Therefore, workers’ urinary S-PMA level indicates the presence of benzene exposure in the workplace.

Keywords: benzene; urinary S-phenylmercapturid acid; shoes industrial

1. INTRODUCTION

The development of informal shoes industry is growing very rapidly and chemicals such as organic solvents are used widely and freely. Organic solvents contained in shoe glue consist of benzene, xylene, ethyl benzene, toluene and n-hexane [13]. Benzene is one of the aromatic hydrocarbon compounds that have many uses for human life, especially for industries such as the rubber industry, oil refining, the footwear industry,
chemical industry, pharmaceutical industry, solvent paints, lubricants, detergents and pesticides, as well as components of crude oil [2, 3].

The use of benzene as a solvent in the industry both in developed countries and developing countries have started to be limited. This is because the benzene has effect of similar to human carcinogens [16–18]. However, occupational health problems have not been controlled, especially in developing countries [7, 12]. In addition, exposure to benzene is still found in air pollutants, such as burning wood/ forest, motor vehicle emissions, coal burning, smoke cigarettes, and gasoline fumes while charging at the fuel station [2, 11, 27]. Approximately 10 kg/ton benzene of manufacturing activity are released to the environment during production, distribution, and storage [5].

Workers exposed to benzene may be at risk of developing hematologic disorders [5, 21, 25, 30], cancers (such as acute and chronic lymphocytic leukemia, non-Hodgkin lymphoma, multiple myeloma, myelodysplastic syndrome and aplastic anemia) [4, 23, 29, 31, 33], chromosomal changes [37], as well as neurotoxicity disturbances [2]. The mechanism of the toxicity of benzene to produce immunotoxic effects may be associated with chromosomal damage, oxidative stress and DNA alteration [32]. Benzene can also damage the immune system by changing levels of antibody [28, 35].

Exposure to benzene is rapidly absorbed by the human body through the inhalation route, which makes exposure to benzene in the form of vapor the primary source of exposure [8]. In the process of metabolism in the liver, benzene produces some metabolites, i.e., phenol, catechol, hydroquinone, trans, trans-muconid acid (t.t-MA), and S-phenylmercapturic acid (SPMA) [19, 20]. Urinary SPMA is considered the most specific and sensitive biomarker for benzene exposure both at high and low concentrations because SPMA in urine is not influenced by other than exposure to benzene exposure than other benzene metabolites [14, 22, 26].

The level of individual susceptibility to exposure to benzene depends on the individual characteristics such as gender, age, smoking habits, lifestyle, working time, physical activity (exercise), nutrition status, health conditions, differences in the level of metabolism in the body, and working environment [24, 30]. Studies that measure the exposure through biomarker monitoring are important because biomarkers could describe the intensity of exposure during certain time intervals. Objectives of this study to analyze the correlation between urinary S-PMA level to the individual characteristics of workers.
2. METHOD

This study was conducted at five industries located in two areas, namely Bandung City (Cibaduyut Wetan and Cibaduyut Kidul Urban Villages) and Bandung Regency (Cangkuang Kulon and Sukamenak Villages). This study used cross-sectional design and aimed to measure the level of benzene exposure, using urinary S-PMA level with individual characteristics. The selection of industry was based on inclusion criteria such as: industry had been established for minimum one year, had the number of workers of minimum 10 people, shoes making process was conducted in workplace or in industry owner house, not in workers’ house. Study samples were then selected based on inclusion and exclusion criteria. Workers inclusion criteria were male, minimum age 18 years, and not performing or receiving blood donors in the last one year. The exclusion criteria were refusal of urinary and/or blood sample collection, refusal of being interviewed, being ill (fever/influenza), being unavailable in workplace when the study was conducted, and urinary creatinine level was less than 30 mg/dL or more than 300 mg/dL.

Workers’ urinary samples were collected once, which was in the end of working shift. Urinary SPMA level in 60 workers was determined by using LC-MS/MS [1]. Individual characteristic data (age, working time, working hours per day, type of job, smoking status and alcohol intake) were collected by interview. Bivariate data is analyzed by T-test and correlation. Differences were regarded as significant at \( P < 0.05 \). This study had passed ethical study procedure and was declared eligible to be conducted by Committee of Experts for Research and Ethics Research, Faculty of Public Health Universitas Indonesia on 1st June 2016, under the No. 170/UN2.F10/PPM.00.02/2016.

3. RESULTS

Urinary SPMA level showed the amount of exposure to benzene for each worker. Data of urinary S-PMA level of obtained workers were not normally distributed. Table 1 showed that mean of the urinary S-PMA level of the workers was \( 26.07 \pm 40.57 \mu g/g \) creatinine standard deviation (Table 1).

According to Table 2, there was no significant association between urinary S-PMA level with the variables of age and working time. Variable of age and working time affected urinary S-PMA level by 1.8% and 5.0%, respectively. In Table 3, there was significant association between urinary S-PMA level with the type of job \( (p \ value 0.036) \) and working hours per day \( (p \ value 0.033) \).
<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>Median</th>
<th>SD</th>
<th>Min-Maks</th>
<th>95% CI</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S-PMA level (µg/g creatinine)</td>
<td>26.07</td>
<td>10.54</td>
<td>40.57</td>
<td>0.18 - 211.82</td>
<td>15.59 – 36.55</td>
<td></td>
</tr>
<tr>
<td>&gt;25 µg/g creatinine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>20 (33.3)</td>
</tr>
<tr>
<td>≤ 25 µg/g creatinine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>40 (66.7)</td>
</tr>
</tbody>
</table>

**TABLE 2: Correlation Analysis of Urinary S-PMA Level with Benzene Concentration, Age and Working Time.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>SD</th>
<th>r</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>36.92</td>
<td>12.55</td>
<td>0.133</td>
<td>0.311</td>
</tr>
<tr>
<td>Working time</td>
<td>13.80</td>
<td>12.89</td>
<td>0.223</td>
<td>0.087</td>
</tr>
</tbody>
</table>

**4. DISCUSSION**

Urinary S-PMA levels in the shoe industry workers were still below normal limits (<25 µg/g creatinine). Low levels of S-PMA urine might be due to the loss of compound due to the evaporation process or because of contamination. This not only occurred in the measurement of S-PMA but also occurred in the other benzene metabolites (t, TMA,

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>SE</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of job</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Gluing</td>
<td>35</td>
<td>35.32</td>
<td>49.56</td>
<td>8.37</td>
<td>0.036</td>
</tr>
<tr>
<td>- Non-gluing</td>
<td>25</td>
<td>13.12</td>
<td>16.37</td>
<td>3.27</td>
<td></td>
</tr>
<tr>
<td>Working hours per day</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- &gt;8 hours</td>
<td>50</td>
<td>21.11</td>
<td>25.47</td>
<td>3.60</td>
<td>0.033</td>
</tr>
<tr>
<td>- ≤8 hours</td>
<td>10</td>
<td>50.88</td>
<td>80.24</td>
<td>25.37</td>
<td></td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Yes</td>
<td>46</td>
<td>20.84</td>
<td>25.53</td>
<td>3.76</td>
<td>0.070</td>
</tr>
<tr>
<td>- No</td>
<td>14</td>
<td>43.25</td>
<td>69.27</td>
<td>18.51</td>
<td></td>
</tr>
<tr>
<td>Alcohol intake</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Yes</td>
<td>11</td>
<td>26.30</td>
<td>26.02</td>
<td>7.85</td>
<td>0.983</td>
</tr>
<tr>
<td>- No</td>
<td>49</td>
<td>26.02</td>
<td>43.38</td>
<td>6.19</td>
<td></td>
</tr>
</tbody>
</table>
phenol, hydroquinone, catechol) during the measurement using urine samples [1]. The results of other studies in the shoe industry in China showed that workers exposed to high concentrations of benzene will have S-PMA levels >25 µg/g creatinine more than the workers exposed to benzene at low concentrations [22].

In this study there was no significant association between age and working time with urinary SPMA level. This is because urinary S-PMA level is also very influenced by life style, such as smoking habits seen by differences in smoking intensity and the number of cigarettes consumed per day and/or alcohol intake [14, 34].

Other factors that affect urinary S-PMA level is the type of job and working hours per day. This type of job investigated in this study was workers gluing and workers non-gluing. Gluing has higher risk of exposure to benzene because the job is widely using shoe glue, while non-gluing type is dealing more with administrative work. Most workers in the informal shoes industry work hours more than 8 hours per day, which result in workers spending more time in the workplace. This is because the manufacturer of shoes are employing handmade technique which takes longer time in the manufacturing process.

Other studies showed that urinary S-PMA level was significantly higher on workers who smoked than those who did not [14, 34]. However, this study showed that there was no difference between the mean of urinary SPMA level and smoking status. This was because all workers were placed in one room, so that workers who did not smoke might have had inhaled smoke from workers who smoked. Smoking can affect both S-PMA metabolites, t, t-MA, hydroquinone, catechol, and phenol. The metabolite levels can reach five times higher in smokers than non-smokers. Smoking can be considered as the main source of benzene intake. Among the chemical compounds contained in cigarette smoke, benzene is the most powerful leukemogenic agent [10].

Alcohol intake can affect urinary S-PMA level [6], but this study showed that no significant mean difference urinary S-PMA level between workers who consumed alcohol and those who did not. It might be because alcohol-consuming workers only drank occasionally.

Unlike smoking who was practiced daily, alcohol consumption was not routinely done.

Alcoholic drinks contain ethanol. When consumed, ethanol intake and exposure to benzene in the body result in the formation of liver isoenzymes of cytochrome P-450 (CYP2E1). As a result, ethanol can increase the toxicity of benzene by increasing the rate of formation of benzene metabolites [36].
5. CONCLUSION

Individual characteristics such as age and working time can affect urinary S-PMA level. Studies that measure the exposure through biomarker monitoring are important to do, because biomarkers could describe the intensity of exposure during certain time intervals. Urinary SPMA can be used for biological monitoring in the workplace environment, so as to minimize health risks in the work environment and to ensure safety for workers’ health. Further researches can analyze the relationship between the levels of urinary S-PMA with occupational health due to benzene exposure.

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