Hepatic Dysfunction and Immune Suppression among Egyptian Workers Occupationally Exposed to Benzene


Abstract—Purpose: The aim of this study was to investigate the hepatic dysfunction and immunotoxicity of benzene exposure. Methods: Our study included 81 workers (61 male and 20 female) occupationally exposed to benzene and 83 workers (55 male and 28 female) were recruited as a control group. Blood samples were taken for the determination of alanine aminotransferase (ALT), aspartate aminotransferase (AST), Gamma glutamyl transferase (γGT), and alkaline phosphatase (ALP), in addition to estimation of immunoglobulins (IgG, IgA and IgM). Results: There was significant increase in the ALT, ALP, and γGT while AST showed insignificant increase in the exposed group compared with the controls. The values of both IgG and IgA were significantly lower, while the value of the IgM was significantly higher in the exposed group compared to the controls. Conclusion: Liver function tests and IgM are simple tests that can be used for the diagnosis of early liver injury and immune suppression.

Keywords: benzene; exposure; immunoglobulin; liver enzymes.

I. INTRODUCTION

Benzene is a toxic chemical that occurs naturally in the environment. Exposure to this chemical can result in a wide range of adverse effects, which can be acute or chronic and can be fatal. Benzene as an organic solvent has the potential to cause central nervous system (CNS) dysfunction, hematotoxicity, hepatotoxicity and nephrotoxicity. Common acute effects include epidermal, dermal and conjunctival irritation, headache, giddiness, drowsiness, vertigo, impaired balance, convulsions, collapse, transient irritation and CNS dysfunction. Occupational exposure to aromatic hydrocarbons may cause liver damage and elevated liver enzymes. The liver is more vulnerable to the hydrocarbons than bone marrow. The metabolism of benzene plays an important role in its toxicity. Benzene is metabolized, primarily in the liver, to a variety of hydroxylated and ring-opened products that are transported to the bone marrow where subsequent secondary metabolism occurs. Two potential mechanisms by which benzene metabolites may damage cellular macromolecules to induce toxicity include the covalent binding of reactive metabolites of benzene and the capacity of benzene metabolites to induce oxidative damage. Sex differences in the activity of glutathione-s-transferase in the liver have also been found to determine the toxicity of organic solvents including benzene. Activity to drug metabolizing enzymes via CYP450 in liver microsomes differs in male than female rats. The immune system is a collection of tissues, cells and molecules whose primary physiological function is to maintain the internal environment of the body by destroying invading infectious organisms. Repeated occupational benzene exposure over long periods of time may affect several parameters related to the immune system, including both innate and adaptive components. These effects include a decrease in serum immunoglobulins, the occurrence of an anti-benzene antibody response, a decrease in complement levels and alterations in subpopulations of lymphocytes. These studies investigated the possible associations between benzene exposure and acute effects on the immune system, measured by alterations in the level of circulating immunoglobulins, complement factors and subpopulations of lymphocytes. Benzene workers who were exposed to mixture of solvents including benzene showed increased serum immunoglobulin levels for IgM and decreased values for IgG and IgA. The decreased levels of immunoglobulin may represent suppression of immunoglobulin producing cells by benzene. Studies showed that intermediate to chronic occupational exposure to benzene induces a reduction in the levels of circulating leukocytes in the body. Leukopenia was found in a series of studies of workers exposed to benzene. Benzene decreases the formation of the β-lymphocytes that produce the serum immunoglobulins or antibodies. Exposure to benzene also decreases the ability of bone marrow cells to produce mature
β-lymphocytes in mice. Peripheral lymphocyte counts were depressed at all levels of exposure, whereas erythrocyte counts were depressed only at high levels.

II. METHODS

Our study included 81 workers (61 male and 20 female) occupationally exposed to benzene. They were working in Chini company in Cairo with a mean age of (37.5 ± 7.59) years and mean duration of exposure to benzene (15.51 ± 6.29) years.

The study was conducted in the drawing and decoration department of Chini factory. The main materials used are Metal oxides in the form of (powders), Liquid gold & Varnish, Turpentine & benzene as solvents and Hydrofluoric acid. The industrial process involves the following steps: Drawing & decoration are performed manually using metal oxides dissolved in solvents. Hydrofluoric acid is sometimes used to obtain impressed decorations. The final product is then transferred to furnaces for stabilization of drawings & decorations. The work was done manually in open system for 8 hours/day for 6 days weekly. They worked in big room with few exhaust ventilation systems. Also, eighty three healthy subjects (55 male and 28 female) of nearly the same age and socioeconomic status were recruited as a control group. Their mean age was (39.3 ± 5.41) years. Control subjects were chosen randomly from the office workers of the National Research Center. None of them had been occupationally exposed to benzene.

Both the exposed and the control groups were interviewed according to a previously prepared questionnaire which included detailed occupational past and present histories and their hobbies which might be a source of exposure to benzene (e.g. painting, printing…….). Subjects with history of liver disease, bilharzias, hypertension, diabetes mellitus, those with chronic kidney disease and regular drug users which might have deleterious effect on the liver or kidney (as analgesics, anti-inflammatory drugs, antibiotics, etc.) were excluded from both groups. Both groups were thoroughly clinically examined for signs and symptoms that could be related to benzene exposure. After taken written consents, five ml blood samples were collected from all participants in sterile dry tube, left to clot for 30 minute and then centrifuged at 3000 rpm for 10 minute. Separated sera were kept at -20 C for the following estimation:

- Serum aminotransferase (ALT and AST) were determined colorimetrically according to the method of Reitman and Frankel.
- Alkaline phosphatase (ALP) activity was determined by the kinetic method (optimized standard method) of Witt and Trendelenburg.
- Kinetic determination of γ-glutamyl transferase activity according to the method of Persijn. Determination of serum Immunoglobulins (IgG, IgM and IgA) using immunodiffusion plate.

III. RESULTS

Table I represents the percentage of dermatological manifestations in the form of itching and dermal affection lesions as eruption, erythema, blisters and dry scaly dermatitis among the exposed group compared with the controls. The differences were statistically significant only for itching and dry scaly dermatitis.

* Likelihood Ratio was used as > 20% of the cells was less than 5.

The percentage of musculoskeletal affection in the form of bone and joint pain of the exposed group showed highly significant difference in comparison with controls (table II). Also, the percentage of workers with bleeding tendency in the form of epistaxis and gingival bleeding showed statistically difference between both groups.

* Likelihood Ratio was used as > 20% of the cells was less than 5.
Table III showed significant increase in the values of ALT and ALP and γGT of the exposed group compared to the control group (P= 0.001), while AST shows insignificant increase in the exposed group compared with the controls (P > 0.05).

**TABLE III**

COMPARISON OF LIVER FUNCTION TESTS BETWEEN THE EXPOSED AND THE CONTROL GROUPS

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Exposed &lt;br&gt;(n= 81) &lt;br&gt;(Mean ± SD)</th>
<th>Controls &lt;br&gt;(n= 83) &lt;br&gt;(Mean ± SD)</th>
<th>t-test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (U/L)</td>
<td>28.94 ± 8.9</td>
<td>24.8 ± 4.5</td>
<td>2.513</td>
<td>0.001</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>27.2 ± 7.2</td>
<td>25.1 ± 4.9</td>
<td>1.972</td>
<td>NS</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>146.8 ± 39.5</td>
<td>110.9 ± 12.8</td>
<td>5.571</td>
<td>0.001</td>
</tr>
<tr>
<td>GGT (U/L)</td>
<td>25.9 ± 15.1</td>
<td>19.29 ± 5.02</td>
<td>2.439</td>
<td>0.001</td>
</tr>
</tbody>
</table>

ALT: Alanine aminotransferase  
AST: Aspartate aminotransferase  
ALP: Alkaline phosphatase  
GGT: Gamma glutamyltransferase

Table IV showed that ALT was significantly higher in exposed females than in control males. No significant difference in AST between four groups. γGT is significantly higher in exposed males than control males. ALP is significantly higher in exposed males and females than in controls; wither males or females.

**TABLE IV**

COMPARISON OF LIVER FUNCTION TESTS IN EXPOSED MALES AND FEMALES AND THEIR CONTROLS

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Exposed &lt;br&gt;(Male (61)) &lt;br&gt;(Mean ± SD)</th>
<th>Controls &lt;br&gt;(Female (20)) &lt;br&gt;(Mean ± SD)</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT</td>
<td>28.56 ± 8.4</td>
<td>29.95 ± 10.4</td>
<td>0.36</td>
</tr>
<tr>
<td>LSD</td>
<td>(–)</td>
<td>(CM)</td>
<td>NS</td>
</tr>
<tr>
<td>AST</td>
<td>27.10 ± 7.2</td>
<td>27.50 ± 7.8</td>
<td>0.04</td>
</tr>
<tr>
<td>LSD</td>
<td>(–)</td>
<td>(–)</td>
<td>NS</td>
</tr>
<tr>
<td>GG T</td>
<td>27.20 ± 15.6</td>
<td>22.06 ± 12.8</td>
<td>0.03</td>
</tr>
<tr>
<td>LSD</td>
<td>(CM)</td>
<td>(–)</td>
<td>NS</td>
</tr>
<tr>
<td>ALP</td>
<td>146.3 ± 39.7</td>
<td>148.0 ± 39.6</td>
<td>0.01</td>
</tr>
<tr>
<td>LSD</td>
<td>(CM,CF)</td>
<td>(CM,EF)</td>
<td>NS</td>
</tr>
</tbody>
</table>

M=mean, SD=standard deviation


Table V showed that the levels of both immunoglobulins (IgG and IgA) were significantly lower in the exposed than in the controls (P= 0.05). On the other hand, the level of the IgM for exposed group was significantly increase compared to the controls (P= 0.001).

**TABLE V**

COMPARISON OF IMMUNOLOGICAL PARAMETERS BETWEEN EXPOSED AND CONTROL GROUPS

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Exposed &lt;br&gt;(n= 81) &lt;br&gt;(Mean±SD)</th>
<th>Controls &lt;br&gt;(n= 83) &lt;br&gt;(Mean±SD)</th>
<th>t-test</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgA(U/L)</td>
<td>1.69 ± 0.63</td>
<td>1.90 ± 0.64</td>
<td>2.002</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>IgM (U/L)</td>
<td>2.16 ± 0.97</td>
<td>1.41 ± 0.47</td>
<td>3.409</td>
<td>0.001</td>
</tr>
<tr>
<td>IgG (U/L)</td>
<td>14.50 ± 2.41</td>
<td>15.27 ± 2.18</td>
<td>1.977</td>
<td>0.05</td>
</tr>
</tbody>
</table>

No significant difference in IgG and IgA between the four groups. IgM of exposed groups were significantly higher in both males and females compared to the controls; males or females (Table VI)

**TABLE VI**

COMPARISON OF IMMUNOLOGICULIN LEVELS BETWEEN EXPOSED AND CONTROLS GROUPS ACCORDING TO GENDER

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Exposed &lt;br&gt;(Male (61))</th>
<th>Controls &lt;br&gt;(Male (55))</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG (U/L)</td>
<td>14.5 ± 2.4</td>
<td>15.1 ± 2.1</td>
<td>0.015</td>
</tr>
<tr>
<td>IgM (U/L)</td>
<td>2.4 ± 0.9</td>
<td>1.8 ± 0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>IgA (U/L)</td>
<td>1.7 ± 0.6</td>
<td>1.9 ± 0.7</td>
<td>0.6</td>
</tr>
</tbody>
</table>


IV. DISCUSSION

In a previous study on the same workers (at the same time) the urinary level of t.t muconic acid (as a biomarker of benzene in urine) was elevated in benzene exposed workers (0.22 ± 0.48) especially in the smoking group than the controls (0.043 + 0.008)²³. A high percentage of our workers were suffering from skin lesions in the form of itching, eruption and dry scaly dermatitis. Many authors found a high prevalence of skin irritation among the workers occupationally exposed to benzene ²⁶. ²⁷. In this research some of our workers were suffering from gastrointestinal tract troubles in the form of anorexia, nausea and vomiting and constipation and/or diarrhea. This was explained by the gastritis produced by organic solvents ²⁶. ²⁷. This was also documented by Yu et al. ²⁷ who observed that mixtures of organic solvents containing benzene are powerful irritants to the mucous membranes, producing gastroenteritis with nausea and vomiting. Furthermore, our results were in agreement with
those of Sheng et al. 28 who reported that workers exposed to a mixture of organic solvents suffered from GIT troubles which might be due to relaxation of smooth muscles leading to diarrhea. Eye irritation and blurred vision were a common complaint among workers in this study. The same results were obtained by ATSDR 2 and Chatterjee et al. 32 who detected a high incidence of eye irritation among exposed painters. In our study 24.7% of exposed workers were suffering from musculoskeletal affection in the form of bone and joint pain which was in accordance with Yu et al. 27. In this work, we found that 12.3% and 17.3% of the exposed workers complained of bleeding tendency in the form of epistaxis and gingival bleeding, respectively. This finding was explained by ATSDR 2 which stated that the reduction in the components of the blood can cause excessive bleeding due to lack of platelets.

Wiwanitkit et al. 20 reported that the toxicity of benzene could cause bone marrow depression and leukemogenesis resulting in damage to multiple classes of hematopoietic cells and hematopoietic functions. The elevated serum AST, ALT, ALP and γGT activities were in accordance with many previous studies on workers exposed to benzene. Perez et al. 8 who studied workers occupationally exposed to hydrocarbons including benzene reported similar findings. Wu et al. [30] and Chen et al. 31 found elevation in liver enzymes (AST, ALT, ALP and γGT) in cook oven workers exposed to aromatic hydrocarbons and explained that the elevation may be related to the combined effect. Nunes & Pereira 32 revealed a significant increase in AST and ALP in workers exposed to solvents in car repainting shops. Also Fernandez & Orono 33 found increase in some liver enzymes in mixed organic solvents in petrochemical industry. The significant rise in both transaminases (AST and ALT) could be considered as an indication of necrosis and might be used for early detection and monitoring of hepatocellular liver injury as reported by Kim 3 Solter34 and Kim 35. On contrary our data revealed that serum alkaline phosphatase showed a significant increase among workers exposed to benzene compared to the control group. These findings agreed with those reported by Fernandez & Orono 33, Nunes & Pereira 32 and Chen et al. 31. Elevation of serum ALP activities might be due to overproduction or release of the enzyme from the liver cells in response to diverse stimuli of hepatocellular injury as explained by Wu et al. 30. γGT is considered more sensitive indicator not only for hepatocellular injury but also for hepatic cholestasis due to chemically induced hepatocyte or biliary damage as reported by Solter 36 and Wu et al. 37 reported that the adverse hepatic effect may be caused by a mixture of hazards, rather than a unique identifiable chemical.

The principle biological function of the immunoglobulins is to provide the body with a defense against infections. 2–36. Our work revealed decreased level of immunoglobulins (IgG and IgA) and increased level of IgM. The high work-load might have influenced the concentrations of the immunoglobulins in exposed workers as expressed by Kirkeleit et al. 37 who found a significant decrease in serum IgM, IgA and IgG of tank workers exposed to benzene. Furthermore, Loi & Johanson 38, Csanady & Filszer 39, Nadeau et al.40 and Zimmer et al.41 reported that physical activity during exposure has been reported to increase the blood concentration of organic solvents due to increased pulmonary ventilation and cardiac output. The published literature provides no major support for a toxic effect of benzene on immunoglobulin production. Suppression of IgA and IgG accompanied by an increased level of IgM has been reported in painters occupationally exposed to benzene, toluene and xylene 40 whereas another cohort of painters exposed to organic solvents, including benzene, had significantly lower IgM than referents 11. Bogadi-Sare & Zavalic 41 found no difference in IgA, IgM or IgG among benzene exposed shoe workers and controls but reported a significant association between IgG and benzene exposure in the working environment. Wu et al. 42 found that serum IgA level was significantly lower in cook oven workers; they suggested that polycyclic aromatic hydrocarbon exposure may alter the immunoresponses. Moreover, we cannot exclude that the combined exposure or specific exposure to other compounds in the work atmosphere might have caused the reported immunosuppression, such as polycyclic aromatic hydrocarbons 43–45. The clinical significance of these findings is unknown, and further studies on a larger number of subjects are warranted. Cigarette smoking is a known source of exposure to benzene. A synergistic effect of smoking on the immunosuppressive effects of solvents has been suggested by Lan et al. 39 and Kirkeleit et al. 12, but in our results we did not find any relations between smoking index and the immunoglobulins IgA, IgM and IgG.

There was insignificant difference in IgG and IgA between the exposed males and females compared with their corresponding controls. On the other hand IgM level of exposed groups were significantly higher in both males and females compared to their controls and this was in Kirkeleit et al. 12. Concerning the duration of exposure to benzene, the statistical data showed no significant change with any parameter. This might be attributed to the fact that the duration of exposure to benzene was not sufficient enough to affect these parameters.

V. CONCLUSIONS

Being simple and non-expensive, liver function tests (ALT, AST and especially ALP) can be used in the screening of workers exposed to benzene or organic solvents to diagnose liver injury at early stage. Also IgM may be considered an early marker to detect immune suppression in these workers.

References


