INVESTIGATION OF HEMATOLOGICAL AND BIOCHEMICAL PROFILES OF TANNERY WORKERS EXPOSED TO CHROMIUM IN HAZARIBAGH, BANGLADESH

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Abstract

Occupational exposure to chromium used in mineral tanning processes cause adverse health effects on workers of leather tanning industries. This study aimed to evaluate the hematological and biochemical profiles in tannery workers of Hazaribagh, Bangladesh compared with a control group. A total of 225 participants, 121 tannery workers and 104 controls, were enrolled. All subjects completed interviewer-administered questionnaires; their physical health was examined, blood samples were collected and the hematological and biochemical parameters were analyzed. The tannery workers had mean duration of work exposure at tanneries for 13.1±9.7 years, and working hours per day of 10.7±2.3 which were significantly higher than 7.6±2.2 of the controls. Previous results showed long-term exposed tannery workers had significantly higher serum chromium concentrations than controls. The tanners had dermatological problems, infections on body surfaces, and respiratory ailments, among other complaints. The red blood cell count, hematocrit (48.91 %) and mean corpuscular hemoglobin concentration (29.39 g/dL) were lower in tannery workers but hemoglobin (14.58±1.30 g/dL) was significantly lower than in the controls (15.96±0.88 g/dL). The tanners had significantly lower neutrophil (51.31 %), higher lymphocyte (41.82 %), monocyte (2.44 %) and eosinophil (4.17 %) counts. Their mean creatinine and alkaline phosphatase values were normal but markers of liver damage, alanine transaminase (42.7±39.3 U/L) and aspartate transaminase (44.3±20.5 U/L), and liver dysfunction marker - bilirubin (1.04±0.85 mg/dL) levels were significantly higher. These findings suggest that exposure to chromium poses serious threats to the health of tannery workers who are at risk of toxicity related liver damage and hematological disorders.

Keywords: Tannery workers, Chromium, Liver damage, Hematological disorders, Bangladesh

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Abbreviations

alkaline phosphatase (ALP); alanine transaminase (ALT); aspartate transaminase (AST); chromium (Cr); mean corpuscular hemoglobin concentration (MCHC); mean corpuscular volume (MCV); ethylene diamine tetra acetic acid (EDTA).

Introduction

Hazaribagh, the largest tanning industrial area in Bangladesh which is situated on the south-western part of the capital city of Dhaka on the bank of the Buriganga river, has around 300 tanning units (Mohanta et al., 2010). Tannery industries use chemicals containing known or suspected carcinogens including hexavalent chromium (Cr(VI)) salts, arsenic, organic solvents such as benzene, formaldehyde, butyl acetate, ethanol, acetoacetate, toluene and acetone (Stem et al., 1987). Occupational workers in the tanneries are often challenged to chemical exposure due to poor maintenance, safety, hygiene, and ignorance about the hazards (Mohanta et al., 2012; Biswas and Rahman, 2013).

Despite its usefulness as an essential trace element required for normal energy metabolism, chromium poses a threat to mankind due to its toxic effects. There are two major forms of chromium of which Cr(VI) is about 1000 times more toxic than trivalent chromium (Cr(III)) (Zhang and Jin, 2006). Cr(III) is not very soluble and is immobilized by precipitation as hydroxides while Cr(VI) is toxic, soluble and easily transported to water resources (Kamaludeen et al., 2003). Occupational exposures often include mixed exposure to both Cr(III) and Cr(VI). Cr(VI) is structurally similar to sulfate and phosphate and thus highly absorbed through active transportation compared to Cr(III) (Costa, 1997).

The general population is exposed to Cr by inhaling ambient air, ingesting food, and drinking water containing chromium. Tannery workers are primarily exposed to chromium through the airways and skin contacts (De Flora et al., 1995). Cr(VI) salts used for leather preservation often bind with skin protein of the workers and cause hypersensitivity (Shahzad et al., 2006). Industrial exposure to Cr and its relation with increased mortality from respiratory cancer has been reported for decades (ATSDR, 2000). Cr exposure in tanning industry is attributed as possible reasons for impaired hepatic and renal functions among workers because of oxidative stress on body system (Khan et al., 2013). Oral Cr ingestion and poisoning may lead to hepatomegaly (Michie et al., 1991) and hepatic failure (Loubières et al., 1999).

Following entry into the blood stream, Cr(VI) ions are rapidly taken up by red blood cells (RBCs) and trapped through reduction inside the cell (ATSDR, 2000; Dayan and Paine, 2001). This may harm cellular integrity as Cr(VI) can induce RBC membrane scrambling through increasing Cr^{2+} ion entry and depletion of ATP concentration leading to the process eryptosis (Lupescu et al., 2011). There are clinical histories related to ingestion of Cr compounds which adversely affect the hematological status because of intravascular
hemolysis (Sharma et al., 1978). Evidence of anemia and thrombocytopenia is also found (Loubières et al., 1999). Acute dermal exposure of Cr in an electroplating worker showed alerting situation of leukocytosis and reduced hemoglobin (Lin et al., 2009). Cr accumulation in tannery workers also adversely affects iron metabolism (Kornhauser et al., 2002).

The occupational workers at tanneries are the worse suffers of toxic fumes and chemicals including Cr, and many kinds of microbes that enter their bodies from the putrefied animal hides. Cr(III) has been found to accumulate in the hair of the tannery workers (Randall and Gibson, 1989). Studies with the tannery workers of Kanpur in India and Hazaribagh in Bangladesh showed various health effects like respiratory and gastrointestinal tract problems and skin complaints (Rastogi et al., 2008; Islam et al., 2019). The tannery workers of Sialkot, Pakistan have been found highly exposed to Cr and showed hematological, hepatic and renal function impairment (Khan et al., 2013). A previous work reported prevalence of allergic diseases and elevated levels of serum immunoglobulin E in the tannery workers of Hazaribagh (Hossain and Islam, 2016). Cr exposure can affect certain biochemical parameters including glucose intolerance and lipid metabolism (Štupar et al., 1999). In view of inadequate data on health parameters of these workers, this study was undertaken to investigate the hematological and biochemical profiles of tannery workers exposed to chromium in Hazaribagh, Bangladesh.

Materials and Methods

Study Subjects

All the subjects included in this cross-sectional study were males; the female workers were excluded as they had very short duration of work exposure (<6 months). The tannery workers, working in 27 leather tanning industries of Hazaribagh, Dhaka, were enrolled in this study. A map of the study area had been shown elsewhere (Islam et al., 2015). A total of 121 tannery workers with at least 6 months of work exposure, and 104 unexposed subjects (non-tannery workers, not age-matched) volunteered to participate in this study as control subjects. They were academic and non-academic staffs of the university departments, gardeners, guards, cleaners and canteen staffs of student’s dormitory. Exclusion criteria included those suffering from impaired renal and liver functions and any other chronic conditions.

Data Collection from Studied Subjects

This study was conducted from July 2013 to August 2015. The general information of the studied subjects including age, level of education, number of family members, height, weight, waist to hip ratio,
duration of work exposure, working hour per day, types of work, blood pressure etc. were recorded on a preformed questionnaire form. Their physical health conditions were examined by an expert physician.

**Ethical Approval**

This study was approved by the ethical review committee (ERC) of the Faculty of Biological Sciences, University of Dhaka, Bangladesh. Before enrollment, each individual was informed about the objectives and significance of the study. Only the full consenting volunteers were included in the study. Simple random and availability sampling was applied to collect samples. The guidelines of the ERC were followed during physical health examination, interviewing of the participants and peripheral blood sample collection.

**Collection of Blood Samples**

About 6 mL of peripheral blood was collected from each participant, 3 mL was transferred in a purple capped vacuum tube containing di-potassium EDTA and the remainder was allowed to clot in a glass tube. Aliquots of fresh blood samples were used for the complete blood count. Following centrifugation, serum and plasma were collected in Eppendorf tubes and stored at -20°C until analyzed.

**Determination of Hematological Parameters**

Hemoglobin was determined by the cyanmethemoglobin method after oxidizing the hemoglobin content of blood by adding potassium ferricyanide reagent (Randox Laboratories Ltd., United Kingdom) and taking optical density readings in a Genesys 20 spectrophotometer (Thermo Scientific); the red blood cells (RBC) and white blood cells (WBC) were counted using hemocytometer; differential WBC counts were done by staining a smear of blood with Giemsa’s stain and counting at least 200 cells under the high power optics of an Olympus (CH-30RF200, Japan) microscope; the hematocrit (v/v), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular volume (MCV) were determined by calculations using hemoglobin, hematocrit and RBC data.

**Reagents**

All reagents used for the determination of glucose, and bilirubin were purchased from Randox Laboratories Ltd., UK; the creatinine assay Kit was obtained from Chemex, S.A., Spain; and ALP, ALT and AST assay Kits were from EMAPOL, Poland. All reagents used in this study were of analytical grade.

**Determination of Plasma Glucose, Creatinine and Bilirubin**

Glucose was determined after enzymatic oxidation of a plasma sample by glucose oxidase enzyme, and the concentration was determined from a standard graph constructed with known amounts of glucose. For the assay of creatinine, 100 µL of sample was mixed with 1000 µL of the working reagent consisting of equal volumes of picric acid (17.5 mmol/L) and sodium hydroxide (0.29 mol/L) and the value was determined by kinetic method, as described in the protocol. The albumin-bound bilirubin present in 100 µL of plasma
sample was released by reaction with 1000 µL of DCA reagent [2,4-dichloroaniline (3.0 mmol/L), hydrochloric acid (80 mmol/L), and a detergent (60 g/L) mixed with equal volume sodium nitrite, 3.0 mmol/L] to form a colored azobilirubin that was measured at 546 nm against the sample blank. The bilirubin concentration in the sample was determined according to the manufacturer’s protocol.

**Determination of ALP, ALT and AST Activities**

For the determination of alkaline phosphatase (ALP) activity in serum, 20 µL of the sample was added to 1000 µL of the working buffered substrate consisting of p-nitrophenyl-phosphate (53 mmol/L) in diethanolamine buffer (1 mol/L, pH 9.8), mixed well, and then absorbance was taken at 405 nm by using a Shimadzu UV-VIS spectrophotometer at 0, 1, 2, and 3 minutes. ALP activity was calculated as the mean absorbance change per min multiplied by correction factor 2760 and expressed in U/L. For the determination of alanine transaminase (ALT) activity, 100 µL of serum was added with 1000 µL of the working buffered substrate consisting of L-alanine in Tris buffer mixed with enzyme/coenzyme/α-oxoglutarate and the initial absorbance was taken against air after 1 min at 365 nm by using a Shimadzu UV-VIS spectrophotometer and then after 2 and 3 min interval. The mean absorbance change per min was used to calculate ALT activity in the sample and was expressed in U/L. Aspartate transaminase (AST) activity was determined by adding 100 µL of serum sample with 1000 µL of the working buffered substrate consisting of L-aspartate in Tris buffer mixed with enzyme/coenzyme/α-oxoglutarate and then following the same procedure as described for ALT.

**Statistical Analyses**

Data analyses were carried out using the Statistical Package for Social Sciences (version 17.0 for Windows, SPSS Inc., Chicago, USA). All results were expressed as mean±standard deviation. The Student’s t-test was used to compare demographic characteristics, hematological and biochemical profiles among the tannery workers and controls, and categorical data was compared by Chi square test. The results were considered significant when the value of p≤0.05.

**Results**

**Demographic Data of the Studied Participants**

The demographic data of the studied subjects including BMI, waist to hip ratio (WHR), number of family members, working hour per day, literacy, blood pressure etc. are compared in Table 1. The age of the tannery workers ranged from 15 to 65 years and that of the control subjects ranged from 19 to 60 years. The tanners had mean work duration at tanneries for 13.1±9.7 years ranging from 0.5 to 47 years. The body mass index (BMI, kg/m²) of the tanners varied from 15.7 to 28.9 while that of the control subjects ranged from 14.6 to 29.4. About 15% of the tannery workers were underweight (malnourished, BMI: <18.5) compared to 5% of the controls (p<0.05, χ² test), 18% were overweight (BMI: ≥25.0) compared to 21% of the controls, and
the remaining 67% of tanners were within the healthy range of BMI (18.5 to 24.99) compared to 74% of the controls. The level of education of the tanners was as follows: primary (54%), secondary (30%), higher secondary (3%), and the remaining 13% had no education. On the other hand, all control subjects received secondary education, some with higher degrees.

Table 1. Demographic characteristics of tannery workers and control subjects

<table>
<thead>
<tr>
<th>Parameters tested</th>
<th>Tannery workers, N=121</th>
<th>Control subjects, N=104</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>32.1±10.7</td>
<td>30.4±8.9</td>
</tr>
<tr>
<td>Body mass index (BMI, kg/m^2)</td>
<td>22.2±2.9</td>
<td>23.0±2.9</td>
</tr>
<tr>
<td>Waist to hip ratio (WHR)</td>
<td>0.91±0.06</td>
<td>0.89±0.05</td>
</tr>
<tr>
<td>No. of family members</td>
<td>4.32±1.48</td>
<td>4.25±1.27</td>
</tr>
<tr>
<td>Working hrs per day</td>
<td>10.7±2.3*</td>
<td>7.6±2.2</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>121.6±9.0</td>
<td>118.7±12.4</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>78.1±5.5</td>
<td>79.5±5.6</td>
</tr>
<tr>
<td>Pulse rate (beats per min)</td>
<td>76.6±4.8</td>
<td>81.3±13.5</td>
</tr>
<tr>
<td>Literacy (%)°</td>
<td>87**</td>
<td>100</td>
</tr>
</tbody>
</table>

*: p<0.05; **: p<0.01; °: Chi-square test.

Types of Work of the Tanners

Out of the total 121 tannery workers, 98 were directly working in the main tanning and finishing sections where they were being directly exposed to various corrosive chemicals including Cr salts used for tanning; of the remaining tanners, 13 were working inside the tanning units as machine operators and 10 were supervisors and other staffs who had indirect exposure to chromium salts (Table 2).

Table 2. Type of job category of the tannery workers

<table>
<thead>
<tr>
<th>Job category of tannery workers</th>
<th>No. of workers (N=121)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wet blue section</td>
<td>26</td>
</tr>
<tr>
<td>Drum section</td>
<td>20</td>
</tr>
<tr>
<td>Lime and other chemical treatment of raw hide</td>
<td>18</td>
</tr>
<tr>
<td>Leather shaving, splitting and spot removing</td>
<td>15</td>
</tr>
<tr>
<td>Machine operator</td>
<td>13</td>
</tr>
<tr>
<td>Color spray and washing</td>
<td>10</td>
</tr>
<tr>
<td>Supervisor and other staff</td>
<td>10</td>
</tr>
<tr>
<td>Vacuum drying, finishing, and buyer section</td>
<td>09</td>
</tr>
</tbody>
</table>

Health Examination of the Tannery Workers

About half of the tannery workers (49%) had rough skin along with itch and rash (dermatitis) on both limbs, 24% had decolorized skin which was clearly evident in workers exposed for as little as 0.5 yrs. A total of 9% of the tanners had reported food allergy, 15% had pain in different parts of the body, 14% had fungal
and bacterial infections on body surfaces, 13% reported respiratory problems while 6% had general weaknesses and 9% were anemic by their eye examination.

**Evaluation of Hematological Parameters of the Studied Subjects**

The blood samples of 80 tanners and 60 control subjects were analyzed for these parameters. The results are shown in Table 3. It was found that hemoglobin concentrations in the tannery workers varied from 11.33 to 16.73 g/dL and the mean value was significantly lower (p<0.01) than in the control subjects with values ranging from 14.16 to 16.91 g/dL. The RBC count in the tannery workers and control subjects varied from 3.28-5.68×10⁹ cells/mL and 3.92-5.60×10⁹ cells/mL, respectively. Their hematocrit ranged from about 43 to 58% compared to 45.8 to 58.3% in the control subjects; the MCHC was also lower but MCV was higher.

The blood samples of 100 tannery workers and 80 control subjects were investigated for the total and differential WBC counts. The mean total WBC count of the study groups was within the normal range (4.0×10⁶ cells/mL-11.0×10⁶ cells/mL) but the differential counts showed 40% of the tanners had neutropenia (having <50% neutrophils) and 64% had lymphocytosis (having >40% lymphocytes). The mean neutrophil to lymphocyte ratio was 1.3 in the tanners compared to 1.9 in the controls. The percentages of lymphocytes, monocytes and eosinophils were significantly higher while neutrophils were significantly lower in the tannery workers than in the controls (Table 3).

**Table 3. Evaluation of hematological profiles of the tannery workers and control subjects**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control subjects, N=60</th>
<th>Tannery workers, N=80</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>15.96±0.88</td>
<td>14.58±1.30**</td>
</tr>
<tr>
<td>RBC count (10⁹ per mL)</td>
<td>4.59±0.53</td>
<td>4.53±0.69</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>52.30±2.89</td>
<td>48.91±8.09</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>113.58±12.55</td>
<td>119.80±23.97</td>
</tr>
<tr>
<td>MCHC (g/dL)</td>
<td>30.02±1.66</td>
<td>29.39±3.69</td>
</tr>
<tr>
<td>WBC count (10⁶ per mL)</td>
<td>7.36±1.06</td>
<td>7.41±1.79</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>62.03±6.40</td>
<td>51.31±7.04***</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>32.68±6.20</td>
<td>41.82±6.88***</td>
</tr>
<tr>
<td>Monocytes (%)</td>
<td>2.11±0.61</td>
<td>2.44±1.04**</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>2.96±0.85</td>
<td>4.17±2.84***</td>
</tr>
<tr>
<td>Basophils (%)</td>
<td>0.18±0.30</td>
<td>0.12±0.27</td>
</tr>
</tbody>
</table>

**; p<0.01; ***; p<0.001; N=100 for tanners, and 80 for controls for the total and differential WBC data.
Levels of Plasma Glucose, Creatinine and Bilirubin in the Studied Subjects

The random plasma glucose level of 75.6% of the tannery workers was normal (normal range: 70-140 mg/dL), 16.7% was lower (<70 mg/dL), and 7.7% was higher compared to only 7.6% of the controls with lower level of plasma glucose. In the tannery workers, the mean level of plasma creatinine, which is a measure of kidney function, was within the normal range (Table 4). On the other hand, the total plasma bilirubin level, which is a measure of liver function, varied widely from 0.11-4.89 mg/dL in the tannery workers compared to 0.03-0.92 mg/dL in the control subjects (reference range: 0.1-1.2 mg/dL). It was found that about 31% of the tannery workers had abnormally high level of total bilirubin, and the mean value of the population was significantly higher than the control subjects (Table 4).

Activity of serum ALP, ALT and AST in the Studied subjects

About 99% of the tannery workers had normal serum ALP activity (normal range: 20-140 U/L) and only 1% showed higher activity. On the other hand, all of the control subjects showed normal ALP activity. In the case of ALT, which is an important liver function test, about 36% of the tannery workers showed abnormally high serum ALT activity (normal value: ≤ 41 U/L). The mean ALT activity of the tanners was significantly higher than the control subjects. Similarly, 61% of the tannery workers had abnormally high serum AST activity (normal value: ≤ 37 U/L); the mean AST activity of the tannery workers was significantly higher than the controls. Activities of all these clinically important enzymes are compared in Table 4.

Table 4. Comparison of plasma glucose, creatinine and bilirubin levels, and activities of serum enzymes ALP, ALT and AST in tannery workers and control subjects

<table>
<thead>
<tr>
<th>Parameters tested</th>
<th>Tannery workers, N=80</th>
<th>Control subjects, N=60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dL)</td>
<td>99.6±24.3***</td>
<td>120.4±24.9</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>1.03±0.25</td>
<td>0.94±0.22</td>
</tr>
<tr>
<td>Bilirubin (mg/dL)</td>
<td>1.04±0.85***</td>
<td>0.41±0.24</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>52.8±22.9</td>
<td>53.5±25.9</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>42.7±39.3***</td>
<td>25.1±20.0</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>44.3±20.5***</td>
<td>24.5±7.7</td>
</tr>
</tbody>
</table>

***: p≤0.001.
Discussion

This study was conducted to evaluate certain hematological and biochemical functions of occupationally exposed tannery workers of Hazaribagh. The tanners, exposed to Cr salts used in mineral tanning processes, had duration of work exposure for 13.1±9.7 years with a mean working time per day of 10.7±2.3 hrs including overtime which had increased the risk of adverse health effects upon them. The nutritional status of the tannery workers, as assessed by BMI and waist to hip ratio, and family size was similar to that of the control population. Although the mean age of the tanners was slightly higher than the controls (p>0.05), the parameters investigated in this study did not vary much among the controls of different age.

A study conducted on the tannery workers of Slovenia showed that the total Cr content in tannery air (1-54 µg/m³) was higher in comparison to ambient air (4-6 ng/m³). As a result of accumulation, the tanners were showing elevated levels of Cr in different tissues and body fluids (Štupar et al., 1999). Our recent study revealed that the long-term exposed tannery workers of Hazaribagh had significantly higher serum Cr concentrations than the control subjects (Islam et al., 2019). However, in that study the levels of Cr in the tanners (∼27 µg/dL) and controls (∼7.4 µg/dL) were much higher than the maximum permissible level in blood, which was 3.0 µg/dL (ATSDR, 2008). Higher level of plasma Cr has been reported in a study conducted on the tannery workers of Kasur, Pakistan (Ahsan et al., 2006).

It has been reported that the tannery solid wastes containing high amount of Cr are converted to protein-concentrate, which is used as poultry feed, fish feed, and in the production of organic fertilizers in Bangladesh, and this extremely hazardous practice has become a common phenomenon in the Hazaribagh tannery area of Dhaka city (Hossain et al., 2007). There could be an accumulation of larger amount of Cr in the ecosystem which could give a higher value in serum since the control subjects enrolled in this study were also from the Dhaka city. It may be mentioned here that due to increased concern over the health effects of Cr exposure and environmental pollution caused by tannery wastes, most of the tanneries of Hazaribagh have recently been relocated to a newly built leather industrial estate in Savar.

One study reported that a large number of workers at the tanneries of Hazaribagh suffered from gastrointestinal (58%), dermatological (31%) and other diseases that could be related to pollution at work place and that 90% of them died before the age of 50 years (Maurice, 2001). Study conducted on the tannery workers of Sialkot, Pakistan showed that the workers suffer from skin rash, chronic bronchitis, gastritis and conjunctivitis (Khan et al., 2013). The tannery workers of Kanpur, India also have been reported to suffer from significantly higher low back pain, asthma, chronic bronchitis and dermatitis (Öry et al., 2011). In agreement with a recent study on the tannery workers of Hazaribagh (Islam et al., 2019), the present study
also found a large number of tanners had dermatological problems including dermatitis and decolorized skin on limbs, body pain, microbial infections on body surfaces, and respiratory problems. The prevalence of respiratory problems (asthma) was also found in the tannery workers of Karachi, Pakistan (Shahzad et al., 2006).

The present study found significantly lower hemoglobin content in the tannery workers while their RBC count, hematocrit and MCHC values were lower than the controls. However, their mean MCV 119.8±23.97 fL (normal value: 80-100 fL) although was not significantly higher than controls but suggested signs of macrocytic anemia. During physical health examination, 9% of the tanners showed signs of anemia while 6% reported weakness which was consistent with laboratory findings of lower hemoglobin contents. However, a previous study found significantly lower RBC count in the tanners (Ramzan et al., 2011), which could be due to toxic effect of Cr. It has been suggested that Cr competes with plasma iron for binding with apo-transferrin and hampers cellular uptake of iron which may affect related parameters such as hemoglobin and hematocrit levels (Moshtaghie et al., 1992). The present study found lower neutrophil to lymphocyte ratio in the tannery workers which could be due to loss of neutrophils while fighting with infections on body surface caused by toxic effect of Cr exposure.

A study on the action of leukocytes following uptake of radiolabelled Cr showed both eosinophils and neutrophils caused rapid release of Cr from erythrocytes following phagocytosis (Sanderson and Thomas, 1978). Overwhelming infections of the tannery workers from putrefied fleshing and inhalation of fungal spores and hyphae from the raw hide may cause rapid usage of neutrophils for phagocytosis followed by destruction which leads to neutropenia. Lymphocytosis may be associated with conditions like increased viral, bacterial and fungal infections. Eosinophilia occurs in parasitic and fungal infections, food allergies, and skin disorders. In this study, 14% of the tannery workers were suffering from different types of bacterial and fungal infections on body surfaces, which could lead to adverse health conditions.

In agreement with a previous study showing plasma glucose lowering ability of Cr (Anderson, 1998), the present study found 16.7% of the tanners with less than 70 mg/dL of random glucose compared to 7.6% of the control subjects, and the whole population of tanners showed significantly lower plasma glucose. However, this finding differed with another study conducted on the tannery workers in Kasur (Ahsan et al., 2006). In the present study the plasma creatinine level of the tannery workers was not significantly different from the control population, suggesting no direct effect of Cr on kidney function. This study observed that although kidney disease is often cited as one of the adverse effects of Cr, chronic renal disease due to occupational or environmental exposure to Cr has not been found, as reported earlier by Wedeen and Qian
However, Cr-induced early changes in renal function were found among ferrochromium-producing workers (Wang et al., 1994).

The present study found no significant change in ALP activity between the tanners and controls although one study showed significant decrease in ALP activity in the men tannery workers while women workers showed an increase in ALP (Ahsan et al., 2006). A report on inhibition of acid phosphatase, adenosine triphosphatase and succinic dehydrogenase after administration of Cr(III) and Cr(IV) was known (Behari et al., 1978). On the other hand, significant increase in ALP activity after lead intoxication was observed (Nehru and Kaushal, 1993). However, these discrepancies in different studies could be due to variation in duration and level of Cr (or Pb) exposure.

There are conflicting reports on the effect of Cr on liver function, which is assessed by the release of liver specific enzymes ALT and AST in the circulation, and elevated bilirubin levels. An old study reported that ALT and AST enzymatic activities were higher in tannery workers compared to workers in the shoe factory (Bavazzano et al., 1981); another study found no significant differences in ALT and AST values of the tannery workers and controls (Uyanik, 2001). The study by Ahsan et al. (2006) showed variable patterns in ALT and AST activities in the tannery workers, with an overall significant increase in all age and gender groups when compared with the control population but the values remained well within the normal range.

In the current study, the liver enzymes ALT and AST showed significantly higher activities in serum which might be associated with possible liver damage as 61% and 36% of the tannery workers had higher AST and ALT activities, respectively. Increased ALT activity may be associated with possible liver cell damage while increased AST activity may indicate liver function impairment as well as cardiovascular problems. The most important finding of the present study was 31% of the tanners with higher levels of total bilirubin in plasma (>1.2 mg/dL), which indicated excess bilirubin not cleared by liver. Collectively, these findings confirmed liver function impairment in the tannery workers.

Conclusion

Chromium, an important health risk factor for the occupationally exposed tannery workers of Hazaribagh, caused gastrointestinal, dermatological, and respiratory problems, among others, and significantly lowered hemoglobin content in blood while affecting other hematological parameters. Chronic exposure to chromium significantly elevated bilirubin level, as well as activities of liver enzymes ALT and AST in the circulation, suggesting liver function impairment of the tannery workers.
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Authors’ contribution

LNI conceived the idea and designed the study, provided expertise and supervised the whole work, wrote and contributed to the critical revision of the manuscript and intellectual contents. AR and MFR are the investigators of the work who helped in writing and critical revision of the manuscript and were involved in data collection of the study. AHMNN contributed to the critical discussion of the results and participated in data collection and analysis. JA was involved in data collection, processing of blood samples and verified the laboratory results. All authors read and approved the final manuscript.

Disclosure statement:
The authors declare no potential conflict of interest.

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