Evaluation of Occupational Exposure to Toxic Metals using Fingernails as Biological Indicators

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ABSTRACT
The importance of fingernails as bioindicators for assessing occupational hazard from high risk female workers working in the match, cracker and beedi factories in and around Virudhunagar district of South Tamilnadu, India was studied. Fingernails of different age group female workers were collected, chemically treated, dried and analysed for the concentrations of different heavy metals Cd, Cr, Cu and Pb by atomic absorption spectrophotometer. Student t-test was used to find out the statistical significance of mean values between different age groups. The levels of lead in the finger nails of fire workers (81.5 μg g⁻¹), match workers (51.6 μg g⁻¹) and beedi workers (44.2 μg g⁻¹) were higher compared to other metals. In beedi workers Cd (3.86 μg g⁻¹) and Cr (2.72 μg g⁻¹) were particularly higher than the normal level of control subject not exposed in occupational working environment. The level of copper concentration (93.42 μg g⁻¹) was higher in match workers than the normal level of the control subject. From the study it is clear that there was significant positive correlation between their age and years of working experience with the concentration of the metals. In conclusion, this study proved that fingernails could be used successfully as a biological indicator for the assessment of heavy metal pollution.

Key words: Metal exposure, fingernails, workers, pollution, atomic absorption spectrophotometer

INTRODUCTION
Modern industrialization causes environmental pollution affecting air, land or soil and water eco-systems which in turn affect the health of the people who interact with them. The main threats to human health from heavy metals are associated with exposure to lead, cadmium, arsenic and mercury. Exposure to heavy metals continues and is even increasing in some parts of the world, in particular in less developed countries, though emissions have declined in most developed countries over the last 100 years (Jarup, 2003). Male and female workers especially adults working in industries like metal and mining are severely exposed to many toxic metals and mainly affected when they inhale metals in air. They may have the symptoms like damage of the respiratory tract leading to bronchitis and in severe cases pulmonary edema, where fluid fills the lungs and leads to death. Children are suffered when inhale airborne metallic particulates and ingest metals dissolved in food and in drinking water. Hence it is important to determine the metal concentrations in industrial workers to monitor and assess their impact on their health (Satake et al., 1997;
Nath, 2000). The occupational exposure to chromium leading to lung cancer has been reported
(Michaels et al., 2006). Not only occupationally exposed workers but the non occupational persons
may also suffer due to increased metal pollutants in the environment.

For biological monitoring of heavy metals various samples blood, hair, teeth, urine, erythrocytes
including fingernails and toenails have been collected and analysed from occupationally
exposed workers and non occupationally exposed persons (Stern et al., 1998; D'ilio et al., 2000;
Morton et al., 2004; Popovic et al., 2005; Afridi et al., 2006). These scientists have assessed the
levels of toxic heavy metals mercury, chromium, lead, cadmium and nickel in different age groups.
As most of them are being used by the human body as micronutrients for physiological activity,
high concentration may lead to diseases. The exposure to cadmium can lead to glomerular and bone
damage and also lead to increased cancer risks and mortality in environmentally exposed
populations (Jarup and Akesson, 2009). As sampling and subsequent analysis of fingernails for
toxic metals in tissues is very easy and they can provide a continuous record of trace element
concentrations of the body, they have been used as samples (Mehra and Juneja, 2005; Rashid and
Hossam, 2007). Eventhough many have already reported nails from different workers as a
bioindicator, we report the use of fingernails as bioindicators for assessing occupational hazard from
high risk female workers working in the match, cracker and beedi factories in and around
Virudhunagar district of South Tamilnadu, India.

MATERIALS AND METHODS

Sample collection: Fingernails were collected from female workers from the match, cracker and
beedi factories in and around Virudhunagar district of South Tamilnadu, India. The study was
carried out in the Department of Biotechnology, Centre for Biological Sciences, KSR College,
Tiruchengode, India in 2009.

At first before the sample collection, the hands of the workers were washed with distilled water
and metals free medicated soap followed by drying with sterile clean tissue paper to remove any
external contamination. Using sterile stainless steel scissors fingernail samples from 15-50 years
old female workers were collected. Nails collected from each person were sealed in sterile plastic
cover for analysis. The personal and medical history details including age, personal habitats, place
of residence, occupation and any possible prior metal exposure were obtained through a
questionnaire.

Sample preparation: The nails were scrapped and cleaned with Triton X-100, a nonionic
detergent to remove attached dust particles and washed according to a standardized washing
procedure described by Gammelgaard et al. (1991). Finally the nails were soaked in acetone to
remove external contamination, rinsed five times with deionized water, dried in an oven and stored
in a desiccator. For wet acid digestion, the dried nail samples were digested with 10 mL of 6:1
mixture of concentrated nitric and perchloric acid kept overnight at room temperature to prevent
excessive foaming and subsequently heated at 160-180°C until the mixture was water clear and
less than 1 mL of the solution remained. Each sample solution was then diluted with 0.1 N nitric
acid (Mehra and Juneja, 2005).

Determination of toxic metals: The concentrations of the metals were determined by using
Atomic Absorption Spectrophotometer (AAS) with three slot high solid burner and acetylene air
flame (Perkin-Elmer model 403). A series of standards were prepared in deionised water for
instrumental calibration by diluting commercial standards containing 1000 ppm of the metals. All
Reagents used were of analytical grade, free from metal contamination and purchased from Merck (India). A number of blanks were prepared for minimization of contaminated errors. The main instrumental parameters such as bandwidth, lamp current and wavelength for estimation of metals by AAS were set up separately for each metal.

**Statistical analysis:** All the values of metal level in nails were statistically presented as arithmetic mean in μg g⁻¹ with standard deviation and tabulated. The statistical significance of mean values between different age groups was determined by applying student t-test. The levels of significance was set at p<0.05.

**RESULTS AND DISCUSSION**

In beedi workers, Cd (3.86 μg g⁻¹) and Cr (2.72 μg g⁻¹) levels were particularly higher than the normal level (0.25 μg g⁻¹), who were not exposed to occupational working environment. The concentration of these metals have been gradually increased based on their years of experience (Table 1). These metals have extreme persistence and higher half-life period in the human body. Chromium toxicity can lead to vomiting, diarrhea, blood loss into the digestive tract and cardiovascular shock followed by liver and kidney necrosis (WHO, 1988). The principal chronic toxic effect of cadmium is on the kidney leading to renal failure and also cause emphysema, prostate and lung cancer (Landrigan, 1982). Chattopadhyay et al. (1990) and Sukumar and Subramanian (1992) reported higher bioaccumulation of Cd in male workers compared to female workers. This is due to smoking inhalation. The higher Cr concentration obtained may be attributed to its incorporation in tobacco leaves from the soil is responsible for skin lesions (Mido and Satake, 1995).

The levels of lead concentration in fire workers (81.5 μg g⁻¹) were particularly higher than the normal subjects (15 μg g⁻¹) who were not exposed in occupational working environment (Table 2).

**Table 1:** Determination of toxic metals in twenty finger nail samples of female fire workers

<table>
<thead>
<tr>
<th>Years of experience</th>
<th>Chromium</th>
<th>Copper</th>
<th>Cadmium</th>
<th>Lead</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age 15-20</td>
<td>5</td>
<td>0.23±0.18</td>
<td>9.90±1.74</td>
<td>0.21±0.17</td>
</tr>
<tr>
<td>Age 20-25</td>
<td>10</td>
<td>0.28±0.19</td>
<td>10.20±4.96</td>
<td>0.23±0.18</td>
</tr>
<tr>
<td>Age 25-30</td>
<td>15</td>
<td>0.34±0.22</td>
<td>11.60±5.21</td>
<td>0.25±0.19</td>
</tr>
<tr>
<td>Age 30-35</td>
<td>20</td>
<td>0.38±0.24</td>
<td>12.70±5.45</td>
<td>0.28±0.21</td>
</tr>
<tr>
<td>Age 35-40</td>
<td>25</td>
<td>0.45±0.27</td>
<td>13.60±5.75</td>
<td>0.32±0.26</td>
</tr>
<tr>
<td>Age 40-45</td>
<td>30</td>
<td>0.47±0.28</td>
<td>14.21±6.87</td>
<td>0.37±2.29</td>
</tr>
<tr>
<td>Age 45-50</td>
<td>35</td>
<td>0.49±0.29</td>
<td>16.21±6.01</td>
<td>0.40±0.29</td>
</tr>
</tbody>
</table>

All the values in Mean (μg g⁻¹±SD). *Values are significant at p<0.05

**Table 2:** Determination of toxic metals in twenty finger nail samples of female match workers

<table>
<thead>
<tr>
<th>Years of experience</th>
<th>Chromium</th>
<th>Copper</th>
<th>Cadmium</th>
<th>Lead</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age 15-20</td>
<td>5</td>
<td>0.18±0.16</td>
<td>25.60±8.12</td>
<td>0.16±0.12</td>
</tr>
<tr>
<td>Age 20-25</td>
<td>10</td>
<td>0.18±0.16</td>
<td>36.70±8.82*</td>
<td>0.19±0.14</td>
</tr>
<tr>
<td>Age 25-30</td>
<td>15</td>
<td>0.19±0.17</td>
<td>48.60±11.52*</td>
<td>0.22±0.16</td>
</tr>
<tr>
<td>Age 30-35</td>
<td>20</td>
<td>0.21±0.19</td>
<td>65.70±16.72*</td>
<td>0.22±0.15</td>
</tr>
<tr>
<td>Age 35-40</td>
<td>25</td>
<td>0.22±0.19</td>
<td>72.60±19.21*</td>
<td>0.26±0.18</td>
</tr>
<tr>
<td>Age 40-45</td>
<td>30</td>
<td>0.24±0.19</td>
<td>85.21±25.25*</td>
<td>0.25±0.17</td>
</tr>
<tr>
<td>Age 45-50</td>
<td>35</td>
<td>0.26±0.21</td>
<td>93.42±30.21*</td>
<td>0.27±0.19</td>
</tr>
</tbody>
</table>

All the values in Mean (μg g⁻¹±SD). *Values are significant at p<0.05
Table 3: Determination of toxic metals in twenty finger nail samples of beedi workers

<table>
<thead>
<tr>
<th>Age</th>
<th>Years of experience</th>
<th>Chromium</th>
<th>Copper</th>
<th>Cadmium</th>
<th>Lead</th>
</tr>
</thead>
<tbody>
<tr>
<td>15-20</td>
<td>5</td>
<td>0.72±0.21</td>
<td>12.5±5.98</td>
<td>0.78±0.29</td>
<td>18.5±6.32</td>
</tr>
<tr>
<td>20-25</td>
<td>10</td>
<td>0.84±0.28</td>
<td>16.2±6.21</td>
<td>0.88±0.31</td>
<td>22.6±7.65</td>
</tr>
<tr>
<td>25-30</td>
<td>15</td>
<td>0.98±0.35</td>
<td>20.2±7.01</td>
<td>1.21±0.34*</td>
<td>33.7±8.13</td>
</tr>
<tr>
<td>30-35</td>
<td>20</td>
<td>1.30±0.38*</td>
<td>21.2±7.25</td>
<td>1.65±0.38*</td>
<td>30.5±8.96*</td>
</tr>
<tr>
<td>35-40</td>
<td>25</td>
<td>2.25±0.39*</td>
<td>23.2±7.85</td>
<td>2.32±0.39*</td>
<td>35.8±9.65*</td>
</tr>
<tr>
<td>40-45</td>
<td>30</td>
<td>2.62±0.41*</td>
<td>24.1±7.90</td>
<td>2.92±0.41*</td>
<td>39.5±10.25*</td>
</tr>
<tr>
<td>45-50</td>
<td>35</td>
<td>2.72±0.46*</td>
<td>27.4±8.01</td>
<td>3.86±0.59</td>
<td>44.2±12.57</td>
</tr>
</tbody>
</table>

All the values in Mean (µg g⁻¹ ±SD). *Values are significant at p<0.05

At extreme conditions it may affect central nervous system causing encephalopathy in children at a concentration of 80 µg dL⁻¹ or greater characterized by symptoms, ataxia, coma and convulsions. The adverse effects of Pb exposure on pregnancy out come including increased risks of spontaneous abortion (Bojra-Aburto et al., 1999) and an increase in preterm births and skill births (McMichael et al., 1986) have been reported. The level of copper (93.42 µg g⁻¹) was higher in match workers than the normal subjects (9.6 µg g⁻¹) who were not exposed occupational working environment. The metal concentration has been gradually increased based on their years of working experience (Table 3). This metal is added in more concentrations during the production of crackers. Trace amounts of copper are required in the synthesis of hemoglobin and several human enzymes. It is an essential nutrient homeostatic mechanism for regulating the body levels of copper ions over a wide range of dietary intake. If very large doses are consumed and homeostatic mechanisms break down, signs of acute toxicity include vomiting, diarrhea, jaundice and urinary problems and green hair coloration will appear; inhaling copper dust appears to be more hazardous to health (Cox, 1991). Similar results have been reported by Chaudhary et al. (1995) who determined many metals in finger nails by Instrumental Neutron Activation Analysis (INAA). In contrast to our study, Rashed and Hossam (2007) from their study involving fingernails of different sexes and ages of school children, adults and workers from polluted and unpolluted areas located at the West Nile of Aswan, Egypt have reported significant higher concentrations of Cd and Pb (in children) and Cd, Cu and Pb (in adults and workers) than in those from unpolluted ones where metal concentration depends on human sex but little on the ages.

CONCLUSIONS

As toxic metals are more soluble in water, the aquatic life is affected which in turn will affect humans. Many metals such as Cd, Pb are associated with the acid rain problem thus create health hazard for people. These heavy metals exposed in the environment thus cause pollution. As workers are frequently exposed to such metals periodical analysis is must to know about the toxic level in order to prevent disorders. The present investigation thus reveals significant high levels of metals in the nail samples of the occupational workers studied and the concentration of the metals increases with their age and years of exposure.

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REFERENCES
