ABSTRACT
Sub-clinical impairment of liver function has been reported in textile dye workers in Abeokuta, Nigeria. In this study, we measured biomarkers of liver function in 68 dyers, 27 dye sellers and 22 handlers of finished products and determined whether assignment of job description would differentially affect liver function. The participants were age and sex matched with controls. Mean plasma levels of total protein, albumin and ALP were significantly lower (p<0.05) while ALT and AST (p<0.05) were significantly higher in the dyers than the control. Similar trend was observed in the levels of albumin, ALP and AST in the handlers of the finished product when compared with the control (p<0.05). In all the categories, levels of ALP were significantly lower than the control (p<0.05). The levels of total protein and albumin were reduced in the dyers than in the dye sellers (p = 0.013 and 0.005 respectively). Lower mean plasma levels of albumin was observed in the handlers of the finished product than that of the dye sellers (p= 0.005). Mean plasma level of AST was higher in the handlers of the finished product than that of the dye sellers p= 0.002. It appears that the dyers were most at risk followed by the handlers of the finished product and the sellers of dyes were the least at risk when considering the altered liver function.

Keywords: Job Category, Dye workers, Route of exposure, Liver Enzymes, Albumin

INTRODUCTION
Textile dyeing is a leading indigenous occupation in Abeokuta, Nigeria. It involves the use of vat dyes, characterized by the presence of a carbonyl functional group (C=O) with chromophores such as anthraquinoids and indigoids. Human exposure to dye can occur during its manufacture, sale and use. Dye powders can produce fine dusts, and dye solution can produce fine sprays of minute droplets that may be inhaled. During transfer of dyes from one container to another or scooping of dye, dye dust may gain access into the air and thus may be inhaled. When dye is being mixed with dye allied chemicals (sodium hydroxide and sodium hydrosulphite) and hot water during preparation for dyeing, there is a gaseous release into the air, which may be inhaled. Routes of exposure include inhalation (Sun et al 1987), dermal and ingestion (IARC 2012). Dermal exposure may occur when appropriate protective clothing is not used when handling dyes, as with a worker who is wearing a short sleeve blouse or shirt and working without gloves. Inhalation of gaseous emission may occur during the process of mixing the dye with allied chemicals and hot water. Ingestion is a relatively unlikely route of exposure to textile dyes but may mistakenly happen when hands are not properly washed before food or snack is taken at work place (National Institute for Occupational Safety and Health (NIOSH), 1980). Systemic effect may occur beyond the site of contact when the dye is absorbed into the bloodstream and distributed throughout the body.

Several health hazards associated with exposure to some textile dyes or dye intermediates have been recognized. These include dermatitis (Malinauskiene et al 2013), bladder cancer (Doi et al 2005) and functional disorders of liver (Zvezdai et al 1996). Furthermore, Soyinka et al (2007) reported sub-clinical effects of vat dyes on this same population of dye workers in Nigeria. Benzanthrone (BA) and 3 Bromobenzanthrone (3-BBA) are important dye intermediates used in the manufacture of various vat and disperse dyes. BA
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has been implicated as a cause of hepatic malfunctions and dermal lesions in dye workers. Serum amino transferases (ALT and AST) were found to be significantly increased in 3-BBA as well as in BA – and adrenal glands (Singh et al, 2003).

The experimental study of hair dye toxicity on rats done by Salih El-Amin et al (2014) showed significant increases in the activities of the liver enzymes GOT (AST), GPT (ALT) and ALP, and a significant decrease in total proteins. Imafidon and Okunrobo (2013) observed significant increases in ALT, AST, protein and albumin when they evaluated the effect of Sudan IV Dye on Liver function of albino rats.

The liver has been described as a metabolically active organ in the body, in fact the largest organ in the body, weighing 1 to 1.5 kg and representing 1.5 to 2.5% of the lean body mass. It is responsible for many vital life functions; these include synthesis, excretion, storage, defence and biotransformation (Hyder 2013). However, the liver has been associated with a lot of insult because of its location and roles in the human body thus causing dysfunction. Biochemical markers of liver dysfunction include serum bilirubin, alanine amino transferase, aspartate amino transferase, ratio of aminotransferases, alkaline phosphatase among others (Gowda et al 2009).

Following the dye work in Abeokuta, it was observed that some of the dye workers change from one category of job to another such as dyeing of textile to selling of the finished product. Often times, those who perform the textile dyeing, do this intensively within a short period of time to gather money to engage in other aspects of the work, for example, selling of the finished products. It is generally believed that the dyers are more prone to health risks than other categories of dye workers (personal communication). No known study has been able to relate this claim. This study was therefore aimed at investigating the biochemical risks, involved in various categories of dye work in Abeokuta, with respect to liver function in dye exposed workers, and specific objectives of determining the effect of vat dyes on liver functions in various categories of dye workers and comparing the effects among the various job categories and control.

MATERIALS AND METHODS

Participants: The study involved one hundred and seventeen (117) test subjects (Occupationally exposed to textile dye) and control (unexposed) subject. There were three categories of dye workers, the dyers i.e (those who were involved in the dyeing process, n=68), the sellers of dyes (those who sell the dyestuff, n=27) and the handlers of the finished products (those who handle the dyed textile material (Adire) such as those who beat the Adire material into shape and those who sell the Adire material n=22). Minimum duration of exposure was one (1) year. The mean age of the exposed group was 42.53 ±1.3 (years) ranging from 18-70 years. The control consisted of 60 subjects with mean age of 38.78±1.93 (years) ranging from 18-70 years.

The dye exposed participants were located in Itoku, Abeokuta, South West, Nigeria and the control subjects were selected within Abeokuta environ away from the dye users. From both the study and control groups, children and pregnant mothers were excluded. Those who reside in the vicinity of “adire makers” were also excluded

Ethical approval: The approval for this study was received from the Scientific and Ethical Review Committee of Olabisi Onabanjo University Teaching Hospital, Sagamu, Ogun State.

Data Collection: An appointment was booked and a meeting was held with the Leader of the Association of dye workers, who was briefed about the aims and objectives of the study, after which she granted permission to enter into the community of dye workers. Other members of the study group were later informed about the study.

Interviewer-administered structured questionnaire was used to collect information on socio-demographic, environmental and occupational characteristics.

Blood sample collection and Biochemical analysis: Blood samples were collected from participants who gave their consent during the interview. About 10ml of venous blood was obtained from the ante-cubital vein with pyrogen free syringes and needles (Becton Dickinson S.A. Spain) into lithium heparin bottles. These were stoppered, mixed gently and the samples centrifuged at 700Xg for 5 minutes. The plasma were separated and stored at -20°C for the various analyses using the following methods: Determination of total protein (Biuret method; Reinhold, 1953), albumin (Bromocresol green method; Doumas, Watson and Biggs, 1971.), total Bilirubin (colorimetric method; Jendrassik and Grof. 1938), Alanine amino transferase and Aspartate amino transferase activities (Colorimetric method of Reitman & Frankel, 1957) and alkaline phosphatase. (Nitrophenylphosphate kit method; Bessey.Lowry and Brock 1946). The reagents were obtained from Randox laboratories Ltd, United Kingdom.

Statistical Analyses: Statistical Package for Social Sciences (SPSS), version 11.0 was used for data analyses. ANOVA was used to test the association between more than two groups of continuous variables. Post hoc test was used to show relationship between the categories. Results were expressed as means ± (SEM). P values ≤ 0.05 were considered significant.

RESULTS

Statistical comparison of mean plasma levels of parameters of liver function among the various job categories of the exposed group (dyers of textiles, sellers of dyes, handlers of finished products) and control showed significant changes in total protein, albumin, ALT, AST and ALP (P< 0.05). No significant difference was observed in plasma concentrations of total bilirubin of all the exposed categories and the control (P> 0.05) (Table 1). Also, no significant difference was seen in the plasma levels of total bilirubin when comparison was made between the various job categories to show relationship between one another (note this was omitted in table 2).

Table 2 shows the relationship between various job categories and controls as well as with one another, with regards to parameters of liver function.
Liver function in dye workers

Table 1:
Plasma concentrations of total protein, albumin, total bilirubin, ALP, AST and ALT in the exposed and control groups

<table>
<thead>
<tr>
<th>Group/Parameters</th>
<th>Dyers</th>
<th>Sellers of Dye</th>
<th>Handlers of finished products</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Protein (g/L)</td>
<td>74.0 ± 1.60</td>
<td>80.4 ± 0.40</td>
<td>76.8 ± 1.90</td>
<td>83.8 ± 1.10</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>37.8 ± 1.60</td>
<td>44.9 ± 2.00</td>
<td>35.9 ± 2.10</td>
<td>41.7 ± 1.10</td>
</tr>
<tr>
<td>Total Bilirubin (µmol/L)</td>
<td>6.50± 0.68</td>
<td>6.50 ± 0.68</td>
<td>9.23 ± 3.08</td>
<td>4.79 ± 0.51</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>72.6±3.29</td>
<td>65.26±4.73</td>
<td>70.18±6.22</td>
<td>92.17±4.38</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>8.69 ± 0.97</td>
<td>6.85 ± 0.67</td>
<td>6.41 ± 0.75</td>
<td>5.08 ± 0.32</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>10.37±0.57</td>
<td>8.67 ± 0.71</td>
<td>12.14±1.80</td>
<td>8.19±0.51</td>
</tr>
</tbody>
</table>

The results are presented as means ± standard error of means. Values are significant at p ≤ 0.05; * represents significant values; p = probability.

Table 2:
Comparison of plasma levels of total protein, albumin, alkaline phosphatase, alanine aminotransferase and aspartate amino transferase among various job categories and control.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group/Parameters</th>
<th>Paired groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein (g/L)</td>
<td>Dyers/Sellers</td>
<td>0.013*</td>
</tr>
<tr>
<td></td>
<td>Dyers/Control</td>
<td>0.000*</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>Dyers and Sellers</td>
<td>0.005*</td>
</tr>
<tr>
<td></td>
<td>Dyers and Control</td>
<td>0.045*</td>
</tr>
<tr>
<td></td>
<td>Sellers/Handlers</td>
<td>0.005*</td>
</tr>
<tr>
<td></td>
<td>Handlers/Control</td>
<td>0.034*</td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>Dyers/Control</td>
<td>0.000*</td>
</tr>
<tr>
<td></td>
<td>Sellers/Control</td>
<td>0.000*</td>
</tr>
<tr>
<td></td>
<td>Handlers/Control</td>
<td>0.003*</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>Dyers/Control</td>
<td>0.000*</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>Dyers/Control</td>
<td>0.014*</td>
</tr>
<tr>
<td></td>
<td>Sellers/Handlers</td>
<td>0.016*</td>
</tr>
<tr>
<td></td>
<td>Handlers/Control</td>
<td>0.002*</td>
</tr>
</tbody>
</table>

Values are significant at p ≤ 0.05; * represents significant values; p = probability;

Mean plasma level of total protein in dyers was observed to be significantly different from that of the sellers and control (p< 0.05), with lower values in the dyers than either of the seller or control. No significant differences were observed between sellers and control; dyers and handlers of the finished products and between sellers and handlers of finished product. (p>0.05)

Significant differences were also observed between mean plasma levels of albumin in dyers compared with sellers, dyers compared with control, sellers compared with handlers of the finished products and handlers of the finished product compared with control. Dyers were found to have lower mean values than either the sellers or control (p<0.05), while handlers appeared to have lower mean values than either the sellers or the control. No significant differences were observed between each of sellers and control and dyers and handlers of the finished products (p>0.05).

The mean of the plasma ALP in each of the job category among the exposed group was found to be significantly different from the control (p<0.05), with each of them having a lower mean value than the control. No significant differences were observed between dyers and sellers; dyers and handlers of the finished products and between sellers and handlers of the finished product (p>0.05).

DISCUSSION

The significant differences observed in the parameters of liver function among the textile dyers and the controls can probably be attributed to textile dye exposure in the exposed subjects. The dyers were constantly exposed to dye solution which can produce fine sprays of minute droplets that may be inhaled as well as to dye powders which can produce fine dusts which is inhalable Elms et al (2003). This is similar to the finding of Sun et al (1987) who studied rats that were exposed to dye aerosol, changes were reported in the lungs of the rats as a result of inhalation of the two commercial dyes. Furthermore, visiting the workplaces of these dyers during the dyeing process, it was observed that the dyers eat snacks on the job. They really do not have separate place of eating, this attitude may promote accidental ingestion of dyes among these dye workers. In addition, their outlook during work shows that their legs, hands, shoes and clothes are seemingly decorated with textile dyes, all these may allow for entry of dye through the skin. Some of these dye workers who probably have spent years on this occupation often boast that they are used to the occupation and that it did not kill their parents from whom they inherited the job. This attitude gives room to carelessness and may prevent them from practicing safety precautions at work. This is in agreement with Akintayo (2013) who reported low prevalence of good knowledge and appropriate attitude (3.7% and 4.2% respectively) among these dyers. He in fact found out that the prevalence of using nose cover was only 29%. Apart from the possibility of the dyers being exposed through inhalation route, there is likelihood of dermal exposure. This may further be encouraged by the poor knowledge of hazard, health and safety associated with dye work as reported by Akintayo 2013, Paramasivam (2010) reported that dyers in India were not aware that dye could affect the organs of their body such as heart, liver and kidneys.
apart from only the skin, since they have experience of dermatitis.

Similar to the dyers, the handlers of the finished products have significant changes in their mean plasma levels of albumin, ALP and AST when compared with the levels in control. Though the handlers of the finished products are not directly handling textile dye, they are however found located in the same vicinity with the dyers. This potentially exposes them to the gas emission as it goes into the environment. This is similar to the report of Jo et al (2004) who observed that residents in the neighbourhood of Daegu Dyeing Industrial Complex were exposed to elevated outdoor toluene levels compared with residents further away from such a source. Kamal et al (2012) also reported that residents surrounding industrial sites also face significant health risks due to indirect chemical exposure. Furthermore, dye dusts that persist in the environment can be inhaled or settle on their bodies or on their clothes and enter into the system through the skin. During the process of dyeing, there is usually a gaseous emission released into the atmosphere with the characteristic smell of rotten egg (from the allied chemical used i.e sodium hydrosulphite). It is possible for people within the vicinity of the dyers to perceive the odour generated by the ongoing dyeing process. This therefore indicates that the handlers of the finished products may passively be inhaling the gaseous emission similar to that of tobacco smoking by non smokers (Peto and Doll 1986, Glantz et al 1991). There is also possibility of the presence of textile dye remnants on the finished products being handled by the handlers of the finished products after the cloth material might have been dyed, rinsed, dried and beaten to shape so as to pack it ready for sale.

The similarity that exists between the sellers of dyes and controls with regards to the mean levels of major parameters of liver function however makes it possible to exonerate the dye dust as the culprit responsible for the observed effect. The sellers of dyes are located away from the places of dyeing unlike the handlers of the finished products and the dyers. They are not so exposed to the gaseous emission. The sellers of dyed fabric included among the handlers of finished products also regularly come around to the dyers to either drop fabrics to be dyed or pick them when ready for sale. The dyers however go to purchase dye away from the dying sheds.

Job category is seen to have effects on various parameters used as markers of liver function. Levels of albumin which indicate the synthetic power of the liver are seen to decrease in the textile dyer and in the handlers of the finished product but, not in the sellers of dyes when compared with levels in the control. This probably indicates the association of altered synthetic ability with these two job categories. Textile dyers are exposed to dye dust as well as to gaseous emission. The handlers by virtue of their job location are also exposed to the gaseous emission, being in the same vicinity or in the same environment with the dyers. The sellers of dyes were not observed to have this same effect; this may likely be due to the absence of gaseous emission. They work with the dye dust and are not involved in the dyeing process which generates the gaseous emission. Similar derangements were observed with the plasma mean level of total protein and this can be explained in the same way. Comparing this risk in the dyers and in the sellers of dyes, it was observed that the liver of the dyers was associated with reduced synthetic ability and this can be further linked to the probable presence of the gaseous emission. Similar event happened between the sellers and handlers of the finished product, when the risk was compared. The handlers have reduced plasma levels of albumin indicating reduced synthesis which also may be linked with the presence of the gaseous emission.

With regard to the hepatocellular integrity, mean plasma levels of AST increased in the dyers and in the handlers, showing a likelihood of sub-clinical hepatocellular damage in association with the gaseous emission being released during the dyeing process. Comparing the risk of hepatocellular damage in the sellers and the handlers, there appeared to be a level of sub-clinical impairment in the handlers of the finished product which is not present in the sellers of dye (no significant difference between levels in control and sellers), this also can probably be linked to the presence of gaseous emission that is associated with the location of handlers of finished products compared with just the presence of the dye dust that the sellers are exposed to. It is however observed that with regards to comparison of levels of ALT in different job categories and control, alteration in levels occurred only in the dyers and not even with the handlers; this can be explained by increased susceptibility in the dyers when both the handlers and the dyers are compared and this may likely be that the dyers are exposed to more concentration of both the dye dust and the gaseous emission.

All the job categories were observed to have reduced plasma levels of ALP associated with the dye work, both dye dust and gaseous emission are probably implicated. The possible explanation for lowered plasma level of ALP could be sub-clinical reduction in synthesis. It will therefore be worthwhile to estimate plasma zinc level, in occupational exposure to vat dyes. This is because ALP is said to be a zinc-containing enzyme (Prasad, 1996), a reduction in plasma zinc level may therefore strengthen the suggested mechanism of reduction. Reduced ALP has also been associated with reduction in the levels of vitamin C (Mahmoodian 1996). According to Das et al (1994), administration of benzanthrone, a vat dye resulted in Vitamin C depletion in the liver, adrenals and blood serum. The reduced ALP observed in this study can therefore be explained from the likely association of reduced Vitamin C with exposure to vat dyes, this may therefore be responsible for the reduced synthesis of ALP, thus bringing about reduction in the plasma ALP level. According to Das (1994) extra supplementation of Vitamin C could attenuate the toxic manifestations of Benzanthrone. It may also be good to study the levels of Vitamin C among these dye workers.

This study has shown that dye work which involves the release of gaseous emission is associated with sub-clinical reduction in synthetic ability of the liver and hepatocellular damage. It will be interesting to study the route of exposure in textile dye work as it applies to various job categories. Soyinka et al (2007) studied the entirety of the dye workers as the exposed subjects and reported sub-clinical impairment of liver function. This study however showed similar effects only with the dyers and the handlers of the finished products and not with the sellers of dyes. This shows that workers in different job categories within an occupation may not be similarly affected. This may be due to exposure to different
chemicals or nature of the chemical (Singhi et al 2005), differences in occupational setting, different route of exposure and differences in quantum of exposure (Elms et al 2003). Also it does not mean that a particular category is not exposed as it was in the study of Hon et al (2011) who observed that most numbers of the job categories in hospital is exposed to antineoplastic drugs. Similarly, in the semi conductor industries, studies have demonstrated effects of job categories (Gold et al 1995)

The study showed that there is sub-clinical hepatocellular damage and reduction in the synthetic abilities of the dyers and handlers of the finished product (who are located in the same vicinity with dyers) but not in the sellers of dyes. It appeared that the dyers were the most affected, followed by the handlers of the finished products while the least affected were the sellers of dyes.

Acknowledgement
We hereby acknowledge Professor O.A. Dada who was the Director of the Centre for Research in Reproductive Health (CRRH), Sagamu, where the laboratory analysis for this study was carried out. He supplied the entire laboratory requirement. Members of his staff are hereby appreciated for their support in carrying out the analysis.

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