ABSTRACT

Background: Printing press workers deal with printing inks that contain potentially hazardous chemicals and solvents. Present study was designed to determine the biochemical health profile of printing press workers.

Methods: Cross sectional study was conducted in 50 printing press (male) workers and 20 non-printing press age matched (male) workers, who were not exposed to printing press environment but lived all around the printing press area. Non-fasting blood samples were collected, from both Workers and Non-Workers, for determining full blood counts, lipid profile, Uric acid and Creatinine.

Results: Mean levels of cholesterol was 213 mg/dl (SD = ±13.0) vs 146 (SD = ±5.50), P = 0.04, triglycerides were 303 mg/dl (SD = ±16.9) vs 196 mg/dl (SD = ±7.13), P = 0.03 and LDL was 103 mmol/l (±3.32) vs 42.9 mmol/l (±2.57), P = 0.01 which were significantly higher in printing press workers than in Nonworkers. Additionally, mean total leukocyte count and total lymphocytes, were significantly higher 8590 (SD = ±830) vs. 7100 (SD = ±542) per cmm, P = 0.04 and 44.8 (SD = ±2.17)% vs. 33.1 (SD = ±1.85)% respectively, P = 0.02 while mean neutrophil count was significantly lower 46.3% (SD ± 1.97) vs. 59.7 % (SD ± 1.88) respectively, P = 0.03 in printing press workers than in Nonworkers.

Conclusion: Exposure to various chemicals in inks may put printing press workers at risk of poor biochemical profile which is an important risk factor for developing non-communicable diseases.
INTRODUCTION

Printing inks are mainly used in newspaper, making advertisement boards and writing and drawing images for other information. Four basic components are used to make printing inks which are pigments, resins, solvents and additives and are of two types according to their viscosity; liquid inks and paste inks. Printing press workers are exposed to potentially hazardous levels of these pigments and solvents (Beaulieu, 1978) (Volk et al., 2019) (Vineis and Magnani, 1985) (Michaels, Zoloth and Stern, 1991). A study from Denmark reported that workers from printing press were exposed to ~300 different substances, out of which ~26 were known or suspected carcinogens (Hadkhale et al., 2017).

Some important compounds in inks are iron oxides, titanium oxide, chromate, carbon black, molybdenum red pigments, cadmium pigments, zinc pigments, chromium oxide pigments, ultramarine blue, lead and iron blue (Kunjappu, 2001). Paraffin (petroleum-based wax) is an ingredient found in both toner and ink. Paraffin wax, considered fairly safe in solid form, releases many toxic chemicals (like toluene, formaldehyde, benzene and methyl ethyl ketone) when melted or burned (Winey and Vaia, 2007) which in turn have been associated with nose and throat irritation, acute bronchitis and even cancers (Fullilove, 1998).

A recent study reported high levels of lead, zinc and copper levels in workers occupationally exposed to printing compared to those who were not exposed to the printing environment (Agbenorku et al., 2012). The printing occupations, are therefore, related to an increased mortality and morbidity from various diseases, including dermatitis, asthma, hypertension, cancers and neurological disorders (Agbenorku et al., 2012) (Moss, Scott and Atherley, 1972) (Greenberg, 1972) (Leon, 1994). Therefore, it is plausible that occupational exposure to printing inks may cause disturbances in biochemical parameters. No such study yet done in Pakistani population, so we don’t know about the burden of occupation related diseases in printing workers, the aim of this study is to assess the biochemical health of printing press workers, who are exposed to potentially hazardous chemicals in the printing inks. Results of this study may help in formulating a guideline to public health department for ensuring health and safety of the workers in industrial workplace.

METHODS

DATA SOURCE

Data was collected from 50 printing press workers (Workers), employed in a printing press located in Chakdara, at Dir (Lower) and Batkhela, at Malakand, Khyber Pakhtunkhwa, and 20 non-printing press workers (Nonworkers), selected from the same neighborhood. All participants were male and aged 15 years or above. Participants who had physical limitations or systematic diseases like diabetes, cardiovascular diseases, renal diseases and liver diseases were excluded from the study.

Ethical approval was obtained from the Khyber Medical University Ethical Review Board (REF: DIRKMU/JUEC19). All participants gave an informed written consent for participation in the study, research was conducted in accordance with the Helsinki declaration.

DATA COLLECTION

Blood samples (4 ml) were collected aseptically from the radial vein of all press worker and Non press worker participants. For blood profiling, 1 ml of blood was transferred to heparin test tube. 3 ml blood was transferred to other test tube having no anticoagulant for isolation of serum for biochemistry test. The samples were immediately analyzed and those which were kept pending, transferred to −20°C refrigerator for storage. Test tubes were kept in slant position for centrifugation in centrifuge. After centrifugation the serum oozed out, and transferred to the other sterilized test tube. This Serum was then used for determining the different biochemical parameters amongst our participants. Blood samples were analyzed using the SYSMIX KX-21 (Japan) and Shimadzu Double Beam Spectrophotometer 1700 Pharma (Japan).

Following biochemical parameters were determined in all participants:

1. Full blood counts (Total red blood cells count [TRBC], Hemoglobin level [Hb], Hematocrit, mean cell hemoglobin [MCH], Mean corpuscular volume [MCV], Mean corpuscular hemoglobin concentration [MCHC], Total leukocyte count [TLC], Neutrophil’s count, Lymphocytes count and Platelet’s count).
Full blood count (FBC) is a set of medical laboratory tests that provide information about the cells in a person's blood. The CBC is often carried out as part of a medical assessment and can be used to monitor health or diagnose diseases. The results are interpreted by comparing them to reference ranges, which vary with sex and age. Conditions like anemia and thrombocytopenia are defined by abnormal complete blood count results.

2. Lipid profile (Cholesterol, Triglyceride, high-density lipoprotein [HDL] and low-density lipoprotein [LDL]).

A lipid profile or lipid panel is a panel of blood tests used to find abnormalities in lipids, such as cholesterol and triglycerides. The results of this test can identify certain genetic diseases and can determine approximate risks for cardiovascular disease, certain forms of pancreatitis, and other diseases.

3. Uric acid is a product of the metabolic breakdown of purine nucleotides and it is a normal component of urine. High blood concentrations of uric acid can lead to gout and are associated with other medical conditions, including diabetes and the formation of ammonium acid urate kidney stones.

4. Creatinine level which is a breakdown product of creatine phosphate from muscle and protein metabolism. It is released at a constant rate by the body (depending on muscle mass), it is the most commonly used indicator of renal function.

Data for all Worker (n = 50) and Nonworker (n = 20) participants was analyzed. Mean age of Workers and Nonworkers was ~27 years (p > 0.8). All participants were male and the minimum exposure time over a 24-hour period, for the printing press workers, was 5 hours while the average exposure time was 8 hours per 24 hours period. The mean years participants had been dedicated to printing press working was ~4 years. Most of the printing press workers did not wear protective gear in the worksite against the exposure to ink, aromatic organic solvent, and other chemicals.

STATISTICAL ANALYSIS

Data were statistically analyzed using GraphPad Software, CA, USA v. DEMO 05 (www.graphpad.com) and SPSS Inc., Chicago, IL, USA v. 22. Continuous (discrete) variables are presented as Mean (SD) and categorical variables as percentages. Comparisons between the Workers and Nonworkers were made using independent T test for the continuous variables and Chi square test for the categorical variables. P value of <0.05 was considered significant.

RESULTS

Different characteristics of printing press workers (age stratified), like exposure to chemicals/24 hours, experience (years), use of personal protective equipment (PPE), health problems before and after the job are described in Table 1.

LABORATORY INVESTIGATIONS

Lipid profile

Our analyses show a significant increase in blood cholesterol level among the printing press workers when compared with the Nonworkers (Figure 1). Mean cholesterol level for printing press workers was 213 (13.0) mg/dl compared to 146 (5.50) mg/dl in Nonworkers (p = 0.04). Similarly, a significant increase was observed in both triglyceride (Mean (SD): 303 (16.9) vs. 196 (7.13) mg/dl respectively, P = 0.03) and LDL (Mean (SD): 103 (3.32) vs. 42.9 (2.57) mmol/l respectively, P = 0.01) levels in printing press workers when compared to the Nonworkers (Table 2). Alternatively, HDL levels were lower, in printing press workers when compared to the Nonworkers, though the difference was not significant (Mean (SD): 50.7 (3.24) mg/dl vs. 59.7 (2.60) mg/dl P = 0.1) (Table 2 and Figure 1).

Uric acid and Creatinine

Serum Uric acid and Creatinine were raised in printing press workers compared to the Nonworkers, however these results were not statistically different. Mean uric acid levels were 5.32 (0.70) µmol/l and 5.26 (0.732) µmol/l while mean creatinine levels were 0.97 (0.22) mg/dl and 0.87 (0.18) mg/dl in Workers and Nonworkers respectively (P > 0.05) (Table 2).
Results for Full blood count analysis are presented in Table 3. The TRBC, Hemoglobin levels, MCH, MCV and MCHC, although lower in Workers than in Nonworkers, were not statistically different between the two groups (all $p > 0.05$). Hematocrit (Figure 2) was lower in printing press workers compared to the Nonworkers ($p = 0.02$) (Table 3). In contrast, mean TLC and total lymphocytes were significantly higher in printing press workers than in Nonworkers (Mean (SD): 8590 (830) vs. 7100 (542) per cmm, $p = 0.04$ and 44.8 (2.17) % vs. 33.1 (1.85) % respectively, $p = 0.02$) (Figure 2). Furthermore, mean neutrophil count was significantly lower in printing press workers compared to the Unexposed (Mean (SD): 46.3 (1.97) % vs. 59.7 (1.88) % respectively, $P = 0.03$). Platelets were similar amongst Worker and Nonworker participants ($p > 0.05$).
DISCUSSION

In present study, effect of ink environments on the health of workers dealing with different ink hazards in printing press was evaluated. Findings of the current study showed that lipid disturbances were present in the printing press workers and the mean cholesterol, triglycerides and LDL levels were significantly higher in the workers than in Nonworkers. These lipid disturbances may be associated with the exposure to different toxin present in inks used by the workers.

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>PRINTING PRESS WORKERS</th>
<th>NON-PRINTING PRESS WORKERS</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>50</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Total RBC (Million/cmm)</td>
<td>4.39 (± 0.31)</td>
<td>4.57 (±0.21)</td>
<td>0.1</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>13.10 (±0.43)</td>
<td>13.91 (±0.23)</td>
<td>0.08</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>34.60 (±1.64)</td>
<td>44.90 (±2.51)</td>
<td>0.02</td>
</tr>
<tr>
<td>MCH (Pg/cell)</td>
<td>32.10 (±1.62)</td>
<td>42.60 (±1.91)</td>
<td>0.5</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>83.71 (±3.05)</td>
<td>95.34 (±2.54)</td>
<td>0.3</td>
</tr>
<tr>
<td>TLC (Per cmm)</td>
<td>8590 (±830)</td>
<td>7100 (±542)</td>
<td>0.04</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>46.31 (±1.97)</td>
<td>59.7 (±1.88)</td>
<td>0.03</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>44.80 (±2.17)</td>
<td>33.1 (±1.85)</td>
<td>0.02</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>33.22 (±1.71)</td>
<td>45.31 (±1.42)</td>
<td>0.4</td>
</tr>
<tr>
<td>Platelets (Per cmm)</td>
<td>306440 ± (29340)</td>
<td>292370 (±1603)</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Table 3 Comparison of Full blood counts amongst the Printing press workers and Non-printing press workers. Results are presented as Means (SD).

Figure 2 Comparison of (A) Hematocrit, (B) TLC, (C) Neutrophils, (D) Lymphocytes and (D) Platelets between Printing press workers and the non-printing press workers. Results for each participant is indicated along x-axis and concentrations of biochemical parameters along y-axis.
Lead and cadmium are present in printing inks and have been shown to alter the cholesterol level (Navas-Acien et al., 2004). In addition, synthetic resins, present in inks, have also been shown to increase cholesterol levels. Carbon black, another main ingredient of printing ink, effects the plasma triglyceride level (Lam et al., 2006). The Diethyl ether, ethyl acetate and ethyl hexane also increase the triglyceride level (Chan et al., 2006). Chromium, present in the inks, in addition to increasing the triglyceride level, also decreases the level of high-density lipoprotein and increases the level of low density lipoprotein (Preuss et al., 2000). Zinc has been shown to have similar effects too (Barter et al., 2007). Results of our study are consistent with these reported findings and suggest that the potential exposure to the chemicals in printing press workers may be the reason for higher levels of lipid disturbances in this group. Moreover, lipid disturbances are established risk factors for systemic diseases like metabolic syndrome, type 2 diabetes, cardiovascular diseases and peripheral arterial disease (Nawrot et al., 2002) (Staessen et al., 2000) (Hirsch et al., 2001) (Nash et al., 2003). Hence, printing press workers, dealing with ink toxins, may be at a higher risk of developing these chronic conditions than the non-printing press workers.

Recent studies have demonstrated a nephrotoxic role of lead and Cadmium. Cadmium has been associated with kidney tubular damage and shown to negatively affect the urinary metabolites (Järup and Åkesson, 2009) (Lin et al., 2003). Cadmium also causes bone damage, either directly or indirectly as a result of renal dysfunction (Vaziri, Ding and Ni, 2001). Lead and Cadmium have also been reported to induce renal dysfunction by increasing oxidative stress, affecting endothelial function and promoting inflammation (Lin et al., 2003). Although the mean Uric acid and Creatinine levels in our study participants are higher amongst printing press workers compared to the Nonworkers, results did not reach significance level which may be due to the small sample size.

Aromatic organic solvents (such as toluene, ethyl benzene and xylene), common components of printing inks, effect the TRBC, hemoglobin and hematocrit levels (Irwin et al., 1997). Aromatic solvent effects blood production by affecting the bone marrow and may increase the risk of hematopoietic diseases (Kang et al., 2005). In addition, other blood disorders like leukemias and aplastic anemia have also been associated with exposure to aromatic solvents found in printing inks (Hecht, 2003) (Leon, Thomas and Hutchings, 1994). Our results are in line with the above stated evidence as printing press workers had a significantly lower hematocrit in contrast to non-printing press workers. Furthermore, comparing the full blood count results between Workers and Nonworkers showed that the former is more likely to be anemic.

Our results reported higher TLC and lymphocytes in printing press workers as compared to Nonworkers. Research studies have demonstrated higher TLC in workers occupationally exposed to lead and other industrial chemicals (Peng et al., 2002)(Kollias et al., 1999) and in printing press workers specifically (Clark, 2007). Raised TLC and lymphocytes are established markers of chronic inflammation (Kollias et al., 1999). An increase in TLC observed in workers may be associated upper respiratory tract infections (Vermeulen et al., 2002).

In addition to biochemical parameters, we also evaluated the presence of allergic conditions in printing press workers and the non-printing press workers. Numerous allergic conditions, including eye irritation, rhinitis, and allergic skin reactions, are shown to be consistently higher among workers in the printing process than other workers (Yu et al., 2004) (Zeliger, 2003) (Kukull et al., 1995). Persistent exposure to the toxic inks may lead to asthma (Burge et al., 1985). In line with this data, our results show that compared to the Nonworkers, the proportion of printing press workers with allergic conditions was higher.

**STRENGTHS AND LIMITATIONS OF THE STUDY**

This is the first study to study the biochemical health profile of printing press workers in KP, Pakistan. Our results have highlighted the possible exposure and related harmful effects amongst the printing press workers in Pakistan, which is consistent with the available literature. A comparison group was used in analysis and both, the printing press workers and the Nonworkers, were age and sex matched. Additionally, the Nonworkers were chosen from the population living around the printing press area but not working in the printing press. Hence the Nonworkers are likely from a similar socioeconomic background as the Workers.
One of the limitations of our study is the small sample size which may be insufficient to detect small significant differences between the two comparison groups. Secondly, the effect of other potential confounders has not been assessed. Dyslipidemia and the variation of full blood counts are affected by life-style behaviors (eating behaviors, exercise, drinking, obesity, etc.), infections and genetic factors. Future studies should study the effect of these potential risk factors on the health of printing press workers. Moreover, the study design is cross sectional, so caution must be taken before drawing any causal inferences.

CONCLUSIONS AND RECOMMENDATIONS

In summary, results obtained in the present study, indicate that workers of a printing press, who are potentially exposed to toxic inks in printing press, are at an increased risk of having biochemical disturbances. These findings are consistent with previously published studies. Furthermore, the biochemical changes observed in printing press workers in our study need to be elucidated further, using prospective as well as intervention studies with adequate sample size, so that the workers who are dealing with potentially toxic inks can be prevented from the harmful effects of these chemicals. This may not only help in the prevention of acute allergic conditions but also in the prevention of more serious chronic conditions like metabolic syndrome, cardiovascular diseases and associated complications in this population. In addition, a detailed assessment of the working conditions in a printing press is required. For instance, information on the type of inks, aromatic organic solvent, and other chemicals being used in the press.

Results of our study may contribute towards formulating guideline to public health department for ensuring health and safety of industrial workers. Measures should be taken to promote the health and safety of printing press workers. Printing press workers should be encouraged to wear mask and gloves while being exposed to the toxic inks and practice thorough hand washing. Awareness campaigns, about the hazards of being exposed to potentially toxic chemicals in printing press environment, are needed. Government should be involved actively, and the public health department should ensure the health and safety of workers in an industrial workplace.

ETHICS AND CONSENT

All participants gave an informed written consent for participation in the study.

COMPETING INTERESTS

The authors have no competing interests to declare.

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