

## ARTICLE

## Metal Dust Exposure Caused Changes in Blood Indices and Serum Proteins

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## Competing interests

The authors have declared that no competing interests exist.

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## Abstract

**Background:** Amongst the industrial hubs in Pakistan, Wazirabad is renowned for its cutlery industry. Cutlery industry generates heaps of multi-metallic dust in whetting units during the processing of stain fewer steel tools. This dust comprises certain potentially toxic and even carcinogenic constituents, thus pose a serious health threat to the workers involved in its processing. Laborers health and safety is something quite non-seriously considered in most of the developing countries, no different is Pakistan. Present exploration was aimed at searching for the differences, in blood profile and quantitative serum protein profile of a group of laborers in cutlery industry that are directly and regularly exposed to multi-metallic dust.

**Materials and Methods:** After taking written consent from the participants, blood samples were drawn for hematological analysis and serum analysis. Hematological analysis was performed with hematological analyzer and serum was subjected to SDS gel electrophoresis for protein profiling.

**Results:** Statistically significant changes were observed in the number of RBCs, MCV, HCT and RDW, whereas platelet count was decreased in experimental groups when compared to control group. Serum protein profiling using SDS-PAGE revealed the protein fractions ranging from 73 to 287 kDa. Densitometric analysis has shown changes in the serum proteins of the subjects exposed to metal dust.

**Conclusion:** Chronic exposure to the metal dust induce changes in the hematological parameters as well as serum proteins. The industrial workers should ensure the use of industry specific personal safety equipment.

**Key words:** Health hazard, Hematology, Metals, Metal dust, Proteins, SDS-PAGE.

## Introduction

SMEs (small and medium-sized enterprises) have significant contribution towards progressive economy and regarded as backbone of developing countries economy. In Pakistan these SMEs are facing massive challenges to cope the economic growth (Chaudhry et al., 2018; Khowaja & Khaskheli, 2018). Sustained economic growth obligates industrial growth to generate employment opportunities as potential source of income. As per economic concerns, Punjab is the principal province of Islamic republic of Pakistan. Wazirabad is one of the seven industrial clusters in Punjab that is specialized for cutlery works manufacturing and export (Hussain et al., 2012; Khan, 2018) (Figure 1).

Extensive metal grinding chiefly of stainless steel is being practiced in cutlery manufacturing units. Among three principal units i.e., whetting unit (WHU), cleansing unit (CLU) and packing unit (PU), metal dust is chiefly generated in WHU. Improper exhaust and lack of safety measures are in practice over there. In course of exposure the path of introduction of metal into the body is related to its toxicity. Due to carcinogenic potential of some metals found in stainless steel, the workers have a high risk of developing cancer (Hussain et al., 2013; Mehmood & Sheikh, 2015; Santore et al., 2001). Metal constituent of welding fumes are responsible for probable pneumotoxic reactions. Significant levels of certain constituent metals of stainless steel like Ni (nickel) and Cr (chromium) are responsible for the induction of inflammation and lung damage age in animal models (Antonini et al., 2004).

Iron (Fe), copper (Cu), chromium (Cr) and cobalt (Co) in stainless steel are redox active metals that can cause oxidative stress in biological system leading to lipid peroxidation, protein modification and DNA damage (Jomova & Valko, 2011). Iron overload in circulation cause an increase in the serum transferrin iron-binding capacity (TIBC) and endogenous or environmental toxins may also increase due to iron build up (Breuer et al., 2000; Zecca et al., 2004). Lung damage has been reported due to exposure of iron particulate matter (Qazi et al., 2009).

In Wazirabad, certain health issues are of prime importance in relation with the exposure to metallic dust of cutlery industry due to which normal functioning of liver and kidney is also highly impaired (Hussain et al., 2013; Mehmood and Sheikh, 2015). The purpose of this study was to reveal the effects of direct exposure of metal dust to the worker's health.

## Materials and Methods

### Sampling and experimental design

Questionnaire based data was collected and blood samples were drawn from non-cutlery industry subjects as Control, subjects with no direct exposure and the subjects exposed to the metal dust. The workers were grouped into CIW2, CIW3 and CIW4 based on the exposure duration i.e., 1-13, 14-26 and 27-40 years of exposure, respectively.

### Hematological Analysis

Complete blood count analysis was carried out on an automated hematology analyzer (Model MEK-6318; Power input 190V A; 220-240 Volts; Nihon Kohden Corp.).

### SDS-PAGE for protein profiling

Serum proteins were size fractionized using SDS-PAGE according to the Laemmli method with slight modification (Abbasi et al., 2015; Laemmli, 1970). The densitometric analysis for electrophoretically resolved protein fractions was carried out by gel analyzer version 2010a.

### Statistical analysis

The data was analyzed using Prism Graph pad 5 software (San Diego, CA). Statistical significance was calculated using one-way analysis of variance (ANOVA) and *Tukey posttest*. Significance was accepted at  $P < 0.05$  while results were shown as Mean  $\pm$  S.E.M. with  $n=10$ .

## Results

### Hematological Analysis

The number of RBCs was elevated in experimental groups when compared to the control group (Figure 2a). CIW3 experimental group exhibited the significant increase ( $P=0.0007$ ) as compared to other groups. Mean corpuscular volume (MCV) were significantly different among control and experimental groups (Figure 2b). Statistically substantial increase of 21.84%, 13.75 and 13.96% was observed in CIW1, CIW3 and CIW4 groups, respectively in MCV values with respect to the control group. Changes in white blood cells count, mean corpuscular hemoglobin (MCH), hemoglobin and mean platelet volume (MPV) were not statistically significant (Figure 2c-f).

Hematocrit (HCT) values were significantly changed in experimental groups as compared to controls (Figure 2g) with 11.22% higher in CIW4 experimental group ( $P=0.0068$ ) as compared to control. The changes in the red blood cell distribution width (RDW) were not statistically significant (Figure 2h).

The platelet count was significantly decreased to 50.5% in CIW3 experimental group ( $P=0.0004$ ) when compared to other experimental groups and control group (Figure 2i).

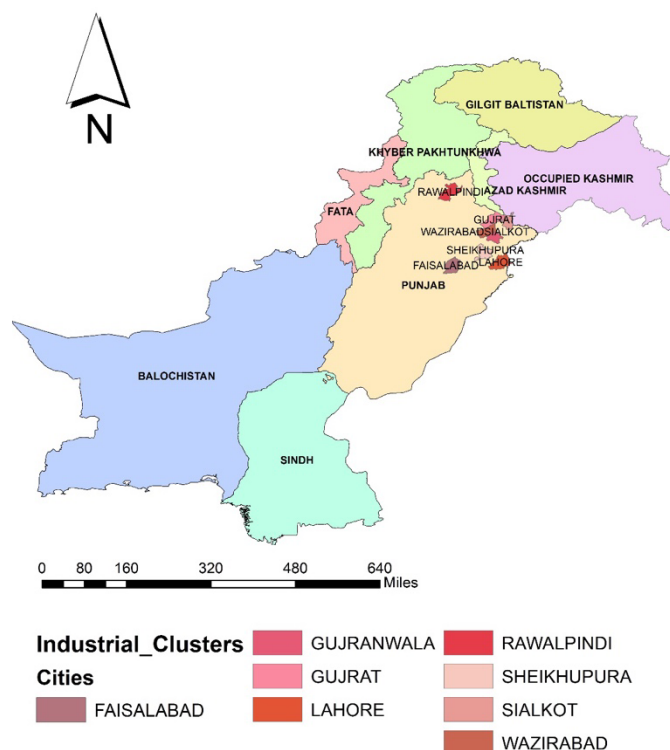


Figure 1: Seven industrial clusters in Punjab-Pakistan, amongst which Wazirabad is the hub of cutlery industry. (ESRI, 2010).

### SDS-PAGE analysis

Serum proteins profiled using a marker ladder ranging from 10 to 220 kDa, exhibited comparative protein fractions ranging from 73-287 kDa. The protein fractions of 188, 163, 115, 76 and 73 kDa were found in CIW2 as compared to control (113 and 78 kDa) and CIW1 (268, 213, 169, 114 and 79 kDa) groups. However, protein fractions of 207, 188, 165, 112, 78 and 73 kDa were present in CIW3 experimental group which was daily exposed to metal dust from 14-26 years. Bands of 287, 266, 211, 193, 164 and 73 kDa were resolved for CIW4 experimental group in comparison with marker (Figure 2).

## Discussion

This study was aimed to investigate the well-being of workers from cutlery factory. Several prevalent and supposed infections were cataloged in relation to the exposure duration and age of workers. Discrepancies in blood cell indices along with serum protein profiling upon exposure to multi-metallic dust in metallurgical workshop were explored in the current study.

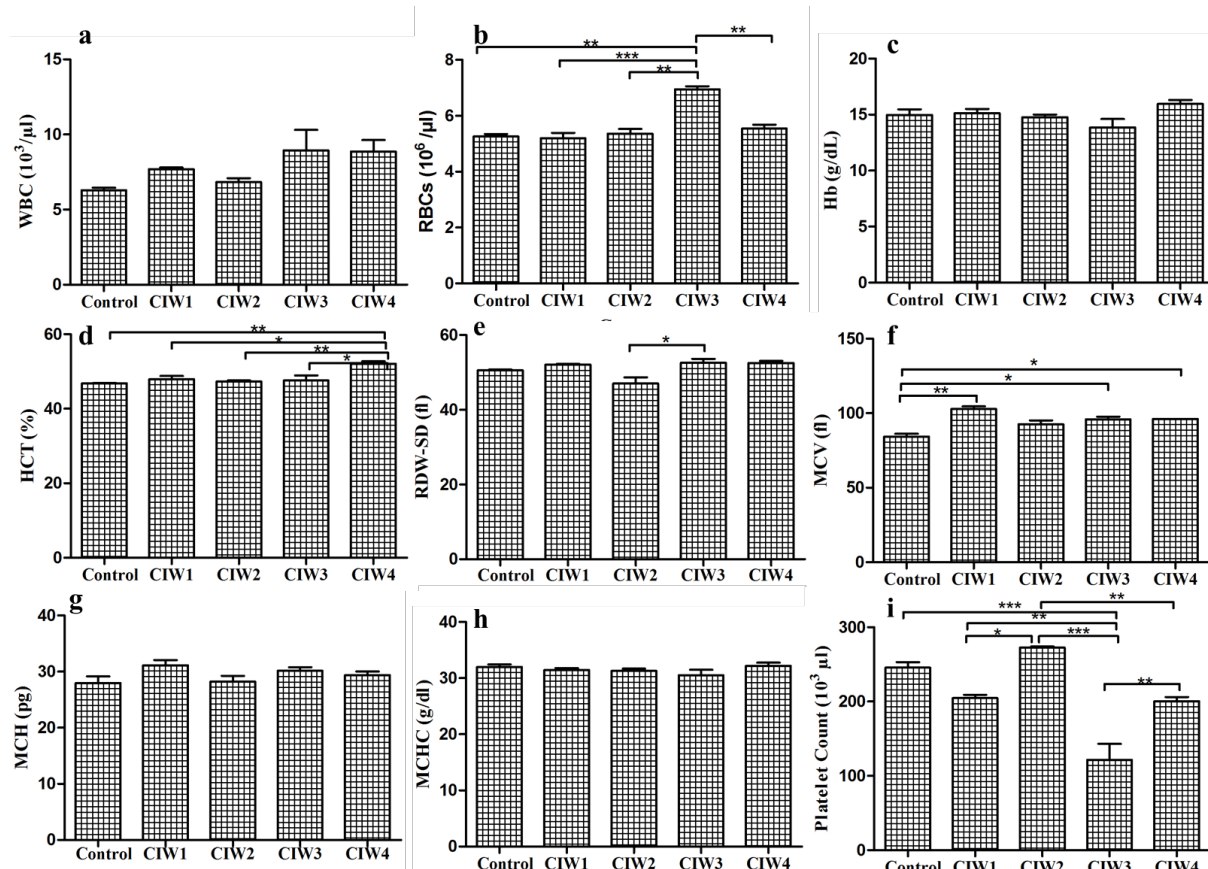


Figure 2: Variation of a (RBC Count), b (MCV), c (WBC count), d (MCH), e (hemoglobin), f (MPV), g (HCT), h (RDW) and i (platelet count) in the experimental groups as compared to control group. Results are Mean  $\pm$  SEM, analyzed by one-way ANOVA (post-hoc Tukey's test) with  $n = 10$ . Significance levels are \* $P \leq 0.05$ , \*\* $P \leq 0.01$ , \*\*\* $P \leq 0.001$

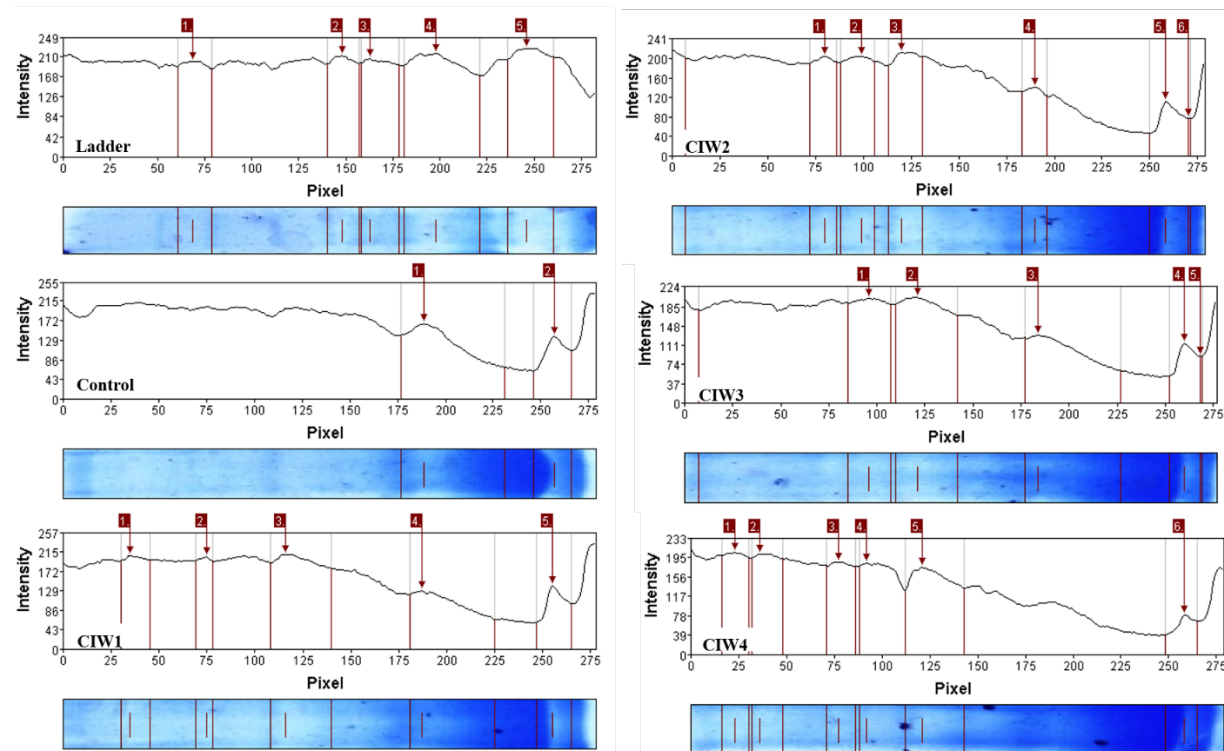


Figure 3: One dimensional SDS-PAGE and densitometric comparison of electrophoretically resolved serum proteins of residents of Wazirabad with no direct exposure to metallic dust (CIW1), 1-13 years of exposure (CIW2), 14-26 years of exposure (CIW3) and 27-40 years of exposure (CIW4) compared with healthy population of Lahore (Control) and protein ladder.



Red blood cells count showed significant variations in the experimental groups as compared to control group, and this increase may be attributed to the increase in erythropoietin (EPO), that is a positive hepatic acute phase protein synthesized during inflammation (Ramadori et al., 2010). The MCV has been considerably augmented in CIW3 and CIW4 experimental groups. Macrocytosis, a pathological status, can be marked through a rise in MCV. Persuasive source of macrocytosis is folate deficiency and liver diseases. (Munker et al., 2007). An increase in MCV in alcoholic liver disease finds its correlation with decreased erythrocyte deformability (Shiraishi et al., 1993). WBCs count was typical in all groups. WBCs count varies from person to person with up to 14% variation in a person at times (England & Bain, 1976).

The changes in MCH level were non-significant. No statistically significant deviations were elucidated during the measurement of hemoglobin concentration in experimental groups in contrast with control groups. MPV were normal in course of this study. MPV quantifies the consistency of size of the platelets and usually used to differentially diagnose thrombocytopenia. The increase in the HCT may be due to escalation of certain physiological conditions like in myeloproliferative disorders, Dengue Shock Syndrome, polycythemia, hypoxia, and chronic obstructive disease. Increased EPO may be a contributor to increase the HCT (Ramadori et al., 2010). Coefficient of inequality of the erythrocyte volume distribution is expressed in terms of RDW (red cell distribution width) (Turgeon, 2005).

Considerable changes were observed in level of RDW. Statistically significant drop was found in platelet count of members of experimental groups in comparison with control group. Platelet count is important for estimating the blood clotting abnormalities. A decline up to  $<20 \times 10^3/\text{mm}^3$  might be concomitant with persistent bleeding, petechiae and ecchymosis (Fischbach & Dunning, 2009). For the screening of purity, execution of molecular mass and identification of proportion of proteins in complex mixtures SDS-PAGE is largely in practice (Corley, 2005). Protein bands of 73 kDa to 287 kDa were reported and disclosed remarkable fluctuations in concentration of serum proteins. Increased level of plasma proteins may find its connection with high risk of many ailments like ischemic heart disease (Kamath & Lip, 2003).

## Conclusion

Present work revealed significant potential of metal dust to induce remarkable changes in blood profile of the workers of the concerned industry due to chronic exposure. Changes in hematology and profiling of serum proteins of workers indicates that anomalies may be due to the metal dust exposure leading to health concerns. Accordingly, proper education and use of suitable safety equipment is highly recommended.

## Author Contributions

The authors confirm contribution to the paper as follows: study conception and design: NS; data collection: RM; analysis and interpretation of results: RM, NS; draft manuscript preparation: RM; Overall supervision of the study: NS. All authors reviewed the results and approved the final version of the manuscript.

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