



## Differentially expressed plasma protein fractions in workers of dyeing unit at a textile industry and comparable controls

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### Abstract

Workers of textile industry are exposed to different hazardous chemicals that cause adverse effects on their health. As variations in plasma proteins are the best indicators of health status of the subjects, hence, we compared plasma protein fractions, resolved by SDS-PAGE of textile dyeing industry workers (n=30) with control subjects (n=30). Proteins were quantified by Total Lab Quant software and analyzed, statistically, by Student t-test. Thirteen protein fractions were detected ranging 250-17kDa. A significant increase in 132 and 53kDa fractions, whereas, a significant decrement in 87 and 66kDa fractions with a highly significant reduction in 112kDa fraction was observed. However, 250, 224, 43, 23, 19 and 17kDa protein fractions did not vary considerably. As some proteins circulate only during particular physiological or pathological circumstances and may serve as biomarkers of anomalies, the altered expressions of these biomolecules observed in textile dyeing industry workers may be strong predictors of different ailments.

**Keywords:** Dyeing unit; Electrophoresis; Proteins; SDS-PAGE; Textile industry workers

### Introduction

Textile environments are usually huge sources of countless organic and inorganic compounds like caustic soda, acetic acid, sulphuric acid, hydrochloric acid, fixing agents, softeners, filling agents, polyvinyl acetate (PNA), etc (Dabra *et al.* 2012).

Dyeing is one of the crucial parts in the textile sector wherein the workers usually have serious health problems like skin and respiratory disorders (Vlandreen *et al.* 2010). Workers in this environment are always been exposed to various harmful substances from different point and non point sources. Data have shown that majority of the laborers present in industrial environments are exposed to different volatile organic compounds (VOC) evolved from polythene, construction, dyeing, and many other building materials (Barro *et al.* 2009).

Common determinants of workplace hazards include lack of the use of self-protective equipments, active and passive exposure to cigarette smoke and socio-demographic as well as economic history of workers (Kamal *et al.* 2012).

Dyeing unit poses various threats to the ecosystem. It generates various toxic substances including colorants in the form of pollutants during various operational stages of textile

industry (Verma *et al.* 2012). These pollutants include formaldehyde, various dyes like azodyes and reactive dyes. These chemicals are hazardous to health and manifest different ailments in the subjects that are being exposed to these substances. Professional exposure to these chemicals is usually in the form of passive and active exposure as different working operational sites work in coordination with each respective part in sequential order. Proteins are involved in all cell functions, normal bodily growth and metabolism, hence, variations in these biomolecules cause abnormal functioning of cells and manifest different ailments. Therefore, the objective of the present preliminary study is to utilize SDS-PAGE (Sodium dodecylsulphate-Polyacrylamide Gel Electrophoresis) to explore potential protein biomarkers in workers occupationally exposed to textile dyeing unit.

### Materials and methods

Blood samples of textile dyeing male workers (n=30, age 20-45 years) were collected from a local textile unit. Age and sex matched controls of same socioeconomic status were sampled from the local population with the help of registered technician.

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The protocol was approved by Ethical Review Committee, University of the Punjab. A proforma was designed to collect the complete information about the medical condition of the exposed workers. All of the subjects, willingly and voluntarily, participated in the study. Complete information related to the study was collected from each participant by a comprehensive questionnaire. Inclusion criteria were 15 years exposure to textile environments and the absence of any co-morbidity like diabetes, hepatitis C, blood pressure, etc. Blood samples of both workers and controls were collected in pre-coated EDTA vacutainers and processed for plasma separation. Protein profile was analyzed by SDS-PAGE which resolves protein fractions on the basis of their molecular weights (Laemmli, 1970). Polyacrylamide gels (12 % and 6%) were prepared, for analyzing low and high molecular weight protein fractions, respectively. Selected samples were thawed in a water bath adjusted at 37 °C. Two µl of sample and 998µl of deionized water were dispensed in a falcon tube. Five ml of Bradford reagent was also added in the solution. Gently mix the whole ingredients and avoid foaming. The solutions were again incubated at 37°C for 15 minutes. Absorbances of individual samples were noted at 595nm and the protein concentration was determined by standard curve.

Loading dye containing, 0.2g of SDS (sodium dodecyl sulphate) 0.154g of DTT (dithiothreitol) 20 mg of bromophenol blue dye, 1ml of glycerol mixed in 0.5 ml of Tris (pH 6.8) was prepared. Final volume was made upto 10 ml by adding deionized water.

As human plasma contains varied amounts of proteins, hence, a stock solution was prepared for each of the samples by adding 2µl of plasma in 18µl of deionized water. Working dilutions were prepared by diluting calculated amount of each stock solution upto 20µl with deionized water. Added 20µl of loading dye to each of the working dilutions and vortexed. Each of the working dilutions contained equal amount of protein which was 100µg/40µl. Samples were heated at 95°C for 15 minutes to denature proteins. In each well of the gel 25µg/10µl of protein was loaded. Protein marker and each of the samples were loaded in separate wells. Gels were electrophoresed at a current supply of 30 mA and 200 V, in a cooling chamber maintained at 4°C until the dye reached the lower end of the gel. The gel was then stained with coomassie blue for 1 hour with constant agitation and destained afterwards until the clearance of blue background.

The gels were scanned for quantification of separated protein fractions by Total Lab Quant Software that provided the data of molecular weights and band percentage covered by each of the fractions. The data were analyzed using Student t-test and employed in finding the enhancement/reduction and

appearance/disappearance of particular protein fractions for comparison between the controls and the workers.

## Results

Variations in the plasma protein fractions of textile industry workers were remarkable when compared to control subjects. An overall comparison of plasma protein profiles of both control and workers groups indicated the expression of thirteen protein fractions ranging between 250-17kDa (Table 1). Protein fractions of 132 and 53kDa showed significant increase, whereas, 112, 87 and 66kDa protein fractions indicated a significant decrease in workers compared to control subjects. However, no significant variations were observed in 250, 224, 43, 23, 19 and 17kDa protein fractions in both of the comparable groups (Table I, Fig. 1).

## Discussion

Occupational health is the most concerned area in the world because these environments do have a lot of potentially hazardous pollutants which manifest various detrimental health related problems. Textile sector is the most frequently discussed working environment in the world. Workers in these environments possess different ailments that are attributed to the exposure of these chemical substances.

In present investigation, the protein profile of healthy subjects and dyeing unit workers was studied by SDS-PAGE. The protein fractions ranged between 250-17kDa in controls and the workers group.

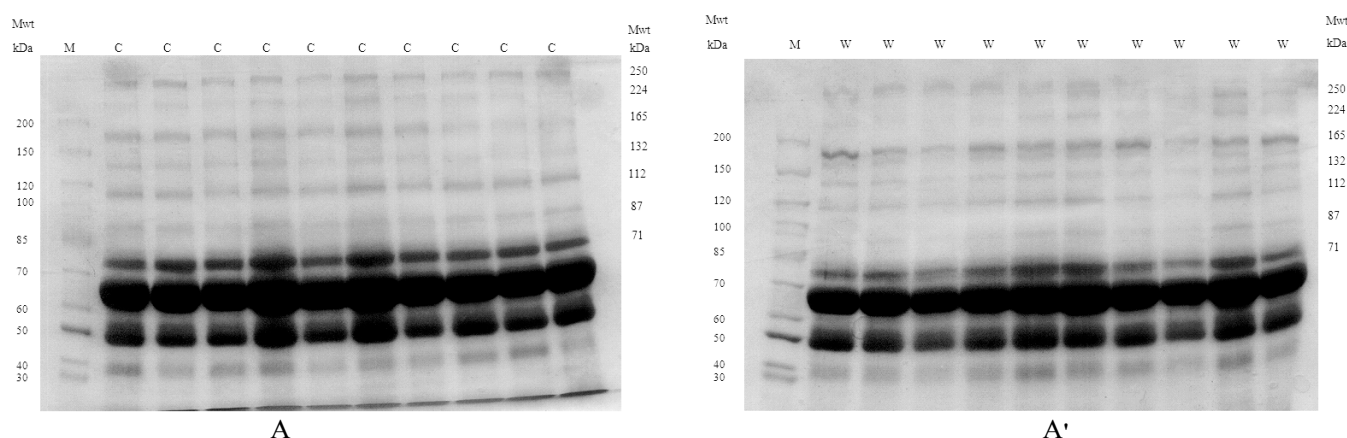
Protein fraction of 132kDa reported as ceruloplasmin, a glycoprotein in human serum, has shown significant elevation in workers compared to control group, in the present study (Mazumder *et al.* 1997). Ceruloplasmin is an antioxidant due to its acute phase reactant property, whose elevated concentration is observed in infection, inflammation, trauma, etc (Vassiliev *et al.* 2005). In workers of textile industry, different infections have been observed related to lungs and reproductive system such as respiratory disorders and trauma or cancer of reproductive organs. Increase of ceruloplasmin may indicate the risks of these disorders in workers of dyeing unit.

Protein fraction of 112kDa has shown highly significant decrease in workers compared to control group. This protein has been recognized as plasminogen and its declined level was reported in a disorder which causes pseudomembranes progression on mucosal surfaces, with subsequent end-organ impairment of the affected tissue (Metha and Shapiro, 2008). Pseudomembranes affect ear, gingiva, respiratory tract, skin

**Table I. Average percent raw volumes exhibited by various protein fractions in controls and workers. Values are Mean  $\pm$  SEM.**

Molecular weight of proteins	Average band percentage of protein fractions		Percentage difference of protein fractions in workers group	p-value
	Control group	Workers Group		
250	03.50 $\pm$ 0.16	02.90 $\pm$ 0.29	17.14 $\downarrow$	0.0535
224	03.10 $\pm$ 0.15	02.70 $\pm$ 0.25	12.90 $\downarrow$	0.1338
165	04.60 $\pm$ 0.13	04.60 $\pm$ 0.35	No change	0.9958
132	03.20 $\pm$ 0.08	03.60 $\pm$ 0.13	12.50 <sup>**</sup> $\uparrow$	0.0096
112	04.60 $\pm$ 0.10	03.50 $\pm$ 0.28	23.91 <sup>***</sup> $\downarrow$	0.0003
87	04.10 $\pm$ 0.19	03.30 $\pm$ 0.22	19.51 <sup>*</sup> $\downarrow$	0.011
71	11.00 $\pm$ 0.35	10.00 $\pm$ 0.38	9.09 $\downarrow$	0.179
66	36.00 $\pm$ 0.54	34.61 $\pm$ 0.78	5.50 <sup>*</sup> $\downarrow$	0.043
53	17.00 $\pm$ 0.46	19.00 $\pm$ 0.45	11.76 <sup>**</sup> $\uparrow$	0.003
43	12.00 $\pm$ 0.26	11.00 $\pm$ 0.24	8.30 $\downarrow$	0.2244
23	16.00 $\pm$ 0.48	16.00 $\pm$ 0.40	No change	0.4037
19	04.30 $\pm$ 0.17	04.00 $\pm$ 0.22	6.96 $\downarrow$	0.3535
17	02.70 $\pm$ 0.11	02.70 $\pm$ 0.14	No change	0.9719

$\downarrow$  Decrease,  $\uparrow$  Increase, Significant at \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$



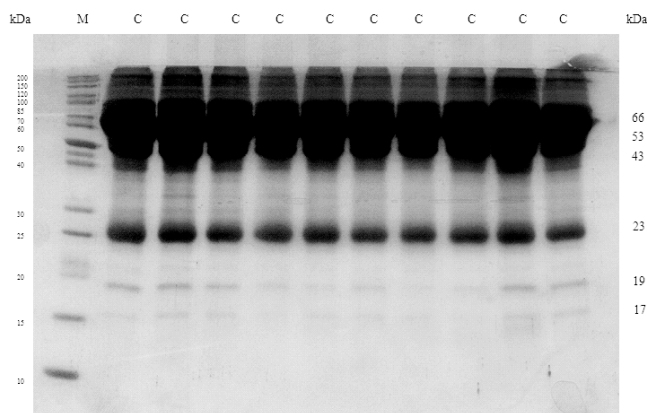
**Fig. 1. Photographs showing electrophoretically resolved high molecular weight (A, A') plasma protein fractions in distinct bands in control subjects (C) and workers group (W).**

**M= Protein markers with known molecular (Mwt) in kilodaltons (kDa).**

and renal collecting system. It has been reported by dermatologists that textile dyes may cause skin reactions (Hatch and Maibach, 1995). Studies have also shown that the highest frequency of chronic respiratory diseases like dyspnea, rhinitis, sinusitis, cough, hoarseness and chronic phlegm was observed in exposed workers than in healthy controls (Zuskin *et al.* 1997).

Protein fraction of 87kDa showed significant decrease in workers compared to control group. It is referred to as serotransferrin found in some external secretions and blood. In physiological and pathological conditions, important alterations of plasma serotransferrin concentration are observed (Inoue *et al.* 1993). Serotransferrin, identified in the bladder tumor donors group was found to be decreased

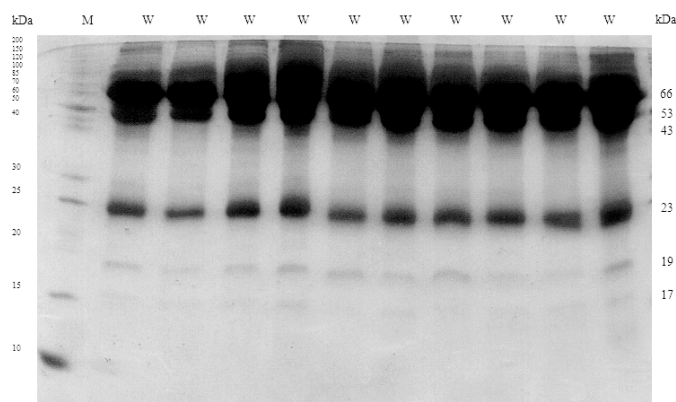
as compared to healthy group (Kreunin *et al.* 2007). Textile industry workers have been exposed to dyes encompassing aromatic amine derivatives for many years. Some of these compounds have the ability to induce tumors, particularly, urinary bladder tumors in humans (IARC, 1982; Hicks *et al.* 1982). The declined level of serotransferrin observed in workers group of the present investigation can be the risk factor for bladder cancer in textile workers.



B

## Conclusion

The up-regulation and down-regulation of different protein fractions is attributed to risk of different diseases. Such as the up-regulation of 132kDa as observed in our study is a risk factor of lungs and reproductive system impairments. Moreover, the protein fraction of 53kDa is up-regulated which is marker of allergic asthma. The



B'

**Fig. 2. Photographs showing electrophoretically resolved low molecular weight (B, B') plasma protein fractions in distinct bands in control subjects (C) and workers group (W).**

**M= Protein markers with known molecular (Mwt) in kilodaltons (kDa).**

In our study, protein fraction of 66kDa (albumin) was found to be declined in workers group in comparison to control subjects. It is reported that decline in albumin concentration of 2.5 g/l predicts coronary heart disease (CHD) and increased mortality in elderly men. Serum albumin quantity less than normal range may occur in various disorders (Weijenberg *et al.* 1997). Among these ailments are those attributed to malabsorption and malnutrition, in such cases protein is either not properly metabolized in food or is depleted through the gastrointestinal tract (Waldmann, 1972). Moreover, reduced albumin quantity is frequently evident in patients with liver ailments, cancer and sepsis (Ballmer *et al.* 1993; Pasanisi *et al.* 2001; Ruot *et al.* 2000).

Protein fraction of 53kDa exhibited a significant elevation in workers group as compared to control individuals. Estimate of the molecular mass of the soluble CD14 by SDS-PAGE is considered to be 50-53kDa (Bazil *et al.* 1986). *In vivo*, higher levels of soluble CD14 have been reported in bronchoalveolar lavage (BAL) fluid in pulmonary disorders such as tuberculosis, sarcoidosis, allergic alveolitis and idiopathic pulmonary fibrosis (Heitbrock and Ulevitch, 1993; Pforte *et al.* 1994; Striz *et al.* 1995; Hoheisel *et al.* 1995). Elevated level of soluble CD14 is a marker of allergic asthma, as observed in workers group of the present study.

down-regulation of 112kDa in present study is associated with skin disorders which are prominent in the workers of dyeing unit. The down-regulation of 87kDa is a risk factor for bladder cancer, while the down-regulation of 66kDa is a marker of cardiac and liver ailments.

Hence, workers in dyeing unit are at a risk of cardiac, liver, skin and respiratory disorders. Preventive measures such as the use of gloves, masks, a proper covered uniform, and large sized glasses must be strictly observed in workers of dyeing unit. Work hours should be reduced to minimize the duration of exposure. Further, workers should be screened regularly for manifestation of various disorders so that an early prediction and effective treatment strategies may be opted to improve the quality of their lives.

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## Declaration of conflicting interest

The authors declare no conflict of interest with respect to authorship and publication of this article.

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