

Electrophoretic Analysis of Serum Proteins in Workers Exposed to Organic Acid Anhydrides (OAAs)

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Abstract– The Organic Acid Anhydrides (OAAs) are highly allergenic compounds used in many local industries of SITE Area Kotri, Sindh, Pakistan including: Textiles, Dyes, Plastics, Resins, Pharmaceuticals, Paper, Paints, Pesticides and Adhesives etc. The hazardous OAAs also called Haptens conjugate with proteins forming protein adducts (Complex) in exposed workers leading to harmful diseases like Conjunctivitis, Rhinitis, Asthma and even Cancer after long term exposure.

Method: This whole study is made to investigate the role of Serum proteins separated by Sodium Dodecyl Sulphate (SDS-PAGE) Electrophoresis on 10-15% gel and then stained with Coomassie Brilliant Blue (CBB-R 250) to detect proteins, which could serve as marker for the early detection of disease in exposed workers from local Dye factory SITE Area Kotri.

Results: The serum proteins resolved into clear sharp bands from 10kDa-200kDa in each group studied. The dominant band of 66kDa was prominent among many workers also showing weak bands at 14,21,45,98 & 135 KDa. However there were very weak and diffused bands seen at 66kDa in many normal cases along with 27,40,53,61,74,92 and 130kDa bands observed.

Conclusion: A dark band of 66kDa among exposed workers to OAAs indicate the folding of protein that shows abnormality and transformation of functions by forming an adduct leads to allergenic problems & symptoms.

Keywords– Analysis, Organic Acid Anhydrides, Serum Proteins and Complex

I. INTRODUCTION

Safe working environment is of crucial importance to the human health, especially for workers. In work-related diseases allergy is the most common increasing cause with Type-I and Type-IV allergy among industrial workers (Stefan R. Ahlfors et al). Organic Acid Anhydrides (OAAs) are hazardous industrial chemicals that can cause occupational asthma and hypersensitivity problems among exposed workers [1]-[2]. Kotri is commercial industrial center since 1968, which is spread over an area of about 1875 acres at the location right side of Indus River near Kotri Town. It was observed by survey that there are about 70 industrial units operational, 10 units non-functional and remaining under construction with open plots (Sohag M.A et al, 2005).

There are different types of Industrial units installed such as Textile (Spinning and Dyeing), Soap and Detergent, Paints, Electrical cables, Paper and Pulp, Pesticides, Ghee Plants,

Flour mills, Adhesives, Tobacco and Cigarette, Chemicals, Pharmaceuticals, Resins, Cotton wastes, Electrical Conductors and Plastic etc. These industries used most of common Organic acid anhydrides (OAAs) chemicals are Acetic anhydride (A.A), Maleic anhydride (M.A), Succinic anhydride (S.A), Trimellitic anhydride (T.M.A), and Phthalic anhydride (P.A). Some OAAs are very reactive at less exposure level only a few $\mu\text{g}/\text{m}^3$, which makes these compounds highly sensitizing chemicals for understanding allergy responses [3]-[5]. Organic Acid Anhydrides (OAAs), amines and isocyanates are also called haptens used mostly in the industries may conjugates with proteins forming adducts (complex) causing adverse health effects on the exposed workers due to specific IgE directed at epitopes on acid anhydride protein conjugates [6]-[10]. Approximately two-thirds of exposed workers show symptoms of diseases such as rhinitis, conjunctivitis, asthma and even cancer after long term exposure. It was also reported that Type-I allergy reaction occurs by the formation of specific IgE antibodies contributing to the asthma, urticaria, conjunctivitis, skin allergies and occupational rhinitis [11]-[16]. Serum albumin (SA) among all of the plasma proteins makes up about 45-55% of the proteins in plasma but after forming adducts (complex) with OAAs increases over up to 74% indicate potential role of Serum albumin (SA) compared with the other plasma proteins. The hapten have capability to modify proteins in type I as well as type IV allergies [17]-[25].

The aim of this study was to investigate the plasma proteins from a local dye industry SITE Area, Kotri workers exposed to OAAs to see variations compared with control (normal) samples of same age group using SDS-PAGE Electrophoresis. This SDS PAGE method is analysed according to Laemmli (Laemmli et al., 1970).

II. MATERIALS AND METHODS

The study includes total of 30 blood samples in which 15 blood samples were taken from workers employed at local dye manufacturing industry using OAAs. These workers were exposed to Phtalic anhydride (P.A), Succinic anhydride (S.A) and Acetic anhydride (A.A) mainly by vapors inhalation and skin contact during work. All of the workers had been exposed for about 10-12 years as mentioned in Questionnaire taken by workers. The main objective of this study is to

fractionate and identify blood plasma proteins responsible for work related sensitization and respiratory problems after forming adducts as reported earlier (Barker et al., 2000; Seema Rosqvist et al., 2000) for comparison with healthy group.

Sample Collection

This study was conducted from November 2010 to February 2011. The industry workers gave consent for giving their samples by their own free will and recorded in confidential files. The intravenous blood samples (10ml) were collected and made to clot by General Physician. After this serum was separated and centrifuged at about 4000r.m.p for about 25 minutes. Finally serum was collected and stored at 40°C prior to SDS-PAGE analysis.

Reagents for SDS-PAGE

All reagents are chemicals of high purity and purchased from Merck (Germany) and Sigma Aldrich.

Tris hydroxyl methyl aminimethane, $C_4H_{11}NO_3$.

Glycine (Amino Acetic Acid), $C_2H_5NO_2$

Acrylamide, C_3H_5NO

Bisacrylamide (N,N'-Methylenebisacrylamide), $C_7H_{10}N_2O_2$

SDS (Sodium Dodecyl Sulphate), $C_{12}H_{25}NaO_4S$.

APS (Ammonium per sulphate), $N_2H_8S_2O_8$

TEMED (N,N,N',N'-tetramethylethylenediamine), $C_6H_{16}N_2$

Chemicals for visualization of Gel

Bromophenol Blue (BPB), $C_{19}H_{10}Br_4O_5S$.

Glycerol, $C_3H_8O_3$

Coomassie Brilliant Blue (CBB), $C_{45}H_{44}N_3NaO_7S_2$

Butanol, $C_4H_{10}O$

2-Mercaptoethanol, $HS-CH_2CH_2OH$

Sample Preparation for serum samples

20µl of serum protein sample was added into 20µl of sample buffer (0.5 M tris HCl, pH 8), 30 % Glycerol, 5% SDS, 0.06% BPB and 2-Mercaptoethanol was boiled in tube for about 5min. After boiling then cooled this sample for about 5 minutes, finally centrifuged at 14000rpm at 4°C (5 minutes). 10µl of sample was applied on sample per well along with molecular weight marker sample.

Resolving gel (15%): It was prepared by mixing 2.5ml (1.5 M Tris HCl, pH 8.8), 1.4ml distilled water, 5.0ml of 30% acrylamide Bis, 1.0ml of 10% APS and 40µl TEMED.

Staking gel (4.5%): It was prepared by mixing 0.5 M Tris HCl pH 6.8, 1.8ml distilled water, 5.0ml of 30% Acrylamide, 10 % APS, 1% SDS and 40µl TEMED.

Preparation of Gel

Silicon tube, glass plate and comb were cleaned with ethanol then silicon tube and clip was set. Resolving gel was firstly poured up to about 6cm and layered with water for intercepting the air bubbles. When water was removed after clear intercepting between two layers then staking gel was poured and comb was inserted in staking gel.

SDS-PAGE Electrophoresis

Electrophoresis apparatus was fixed with glass plate and filled the upper tank with SDS running buffer. Then serum sample was loaded with molecular weight marker (10_180KDa) Fermentus pre staining protein ladder. After loading samples the power supply at 30mA (300V Constant current) samples were running for about 6 hrs. SDS-PAGE Electrophoresis will be stopped running when PBS line reached up to lower part of the gel.

Gel Staining and Decolorization

Gel was placed into staining gel solution (0.25%, CBB-R 250) for approx 35_40min with constantly shaking the gel. Finally the gel was washed with water and poured into decolorizing solution (7% acetic acid, 5% Methanol) for decolouring gel.

Data analysis of Gel

Gel reading and calculations was done by Gel documentation system of Bio-Rad for studying variations between workers and normal protein samples.

III. RESULTS

In our present study blood plasma samples were run on acrylamide gel of different concentrations (4-15%) in tris/glycine buffer (pH 8.3) for SDS-PAGE Electrophoresis. However there were good separation results came out on 12% Gel for serum samples as shown in electrograms given in figure 2 and figure 3 respectively. Coomassie brilliant blue (CBB-250) was used as standard stain (total protein) for detection of proteins separated by SDS-PAGE Electrophoresis. Further Gels were read by Gel documentation system Bio-Rad. The serum proteins resolved into clear sharp bands at 10-180KDa in each group studied. The electrogram in figure one showing bands at 27,40,53,61,74,92 and 130KDa in most of normal cases. There was a prominent band at 66 along with 14,21,45,98 and 135 KDa in many workers cases (as shown in figure two electrogram). The dark and dominant band at 66kDa found among many exposed industrial workers indicate the folding of protein showing abnormality and transformation of protein normal functions by forming an adduct (complex) with organic acid anhydrides (OAAs). This complex is elevated as dark band as compared to the control sample because this may possibly have merged in this serum albumin fraction as a single dark band. The complex formed by substitution reaction by exchange of reactants not by addition in formation of complex.

This may conclude that is organic acid anhydride (OAAs) or haptene, but not any other new protein formed after binding of OAAs which is very important for inhibition process.

For the confirmation of this complex, it is even suggested that these samples can be analyzed by 2D-PAGE followed by immunoblotting analysis by separating proteins on the basis of their isoelectric point (pI); that has an advantage over SDS-PAGE Electrophoresis.

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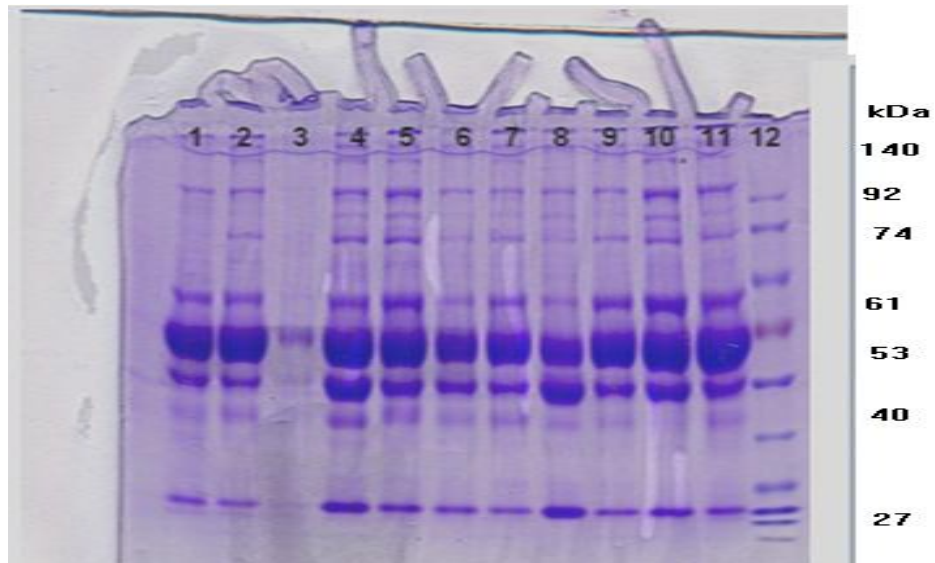


Figure 1: Electrogram showing different banding pattern of normal (serum) samples along with 12- Molecular weight Marker

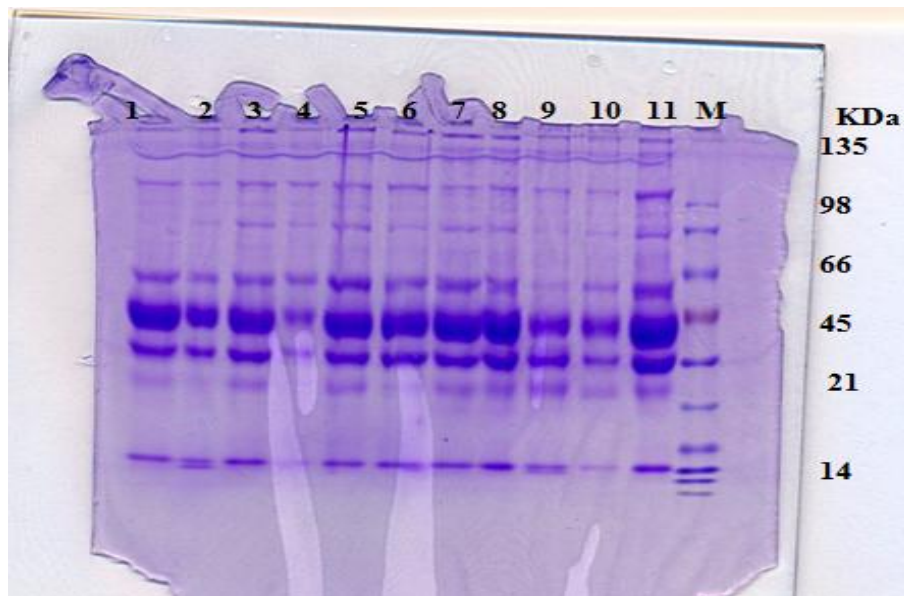


Figure 2: Electrogram showing banding pattern in dye industry workers with marker