Characterization of Protein In Serum By Slab-Gel Electrophoresis

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Abstract- *Data on protein fractions in the serum of 64 cases of anemia were studied and another normal 2 cases were used as control. About 5 ml of non-heparinized blood was collected in the age group of 20-30 and serum was isolated from the whole blood by coagulation. Protein profile of the serum in 10 µg/well concentration of serum was studied by SDS-PAGE. Totally 8 samples were collected from various age groups (between 20-24, and 27- 30 years) respectively. Relative and absolute values respectively of the protein fractions of anemia cases were compared with control samples. It is to be noted that there appears to be no consistent difference between anemia and normal ones.*

Keywords- peptides, protein, proteomics, serum

I. INTRODUCTION

Serum is potentially the most valuable specimen for biomarker elucidation (Ardekani*et al.,* 2002). Serum is attracting increasing interest in proteomics, which is currently striving to broadly characterize the protein constituents. At first glance, serum presents many beneficial attributes for proteomic investigation because it has a high protein content (60-80 mg/ml), with many of these proteins being secreted and shed from cells and tissues (Sasaki *et al*., 2002; Kennedy, 2002). The protein content of serum primarily includes certain proteins such as albumin, transferrin, haptoglobulin, immunoglobulin and lipoprotein (Turner and Hulme, 1970).

Serum protein electrophoresis is a laboratory examination that is widely used to identify patients with multiple myeloma and other disorders. Many subspecialists include serum protein electrophoresis screening in the initial evaluation for numerous clinical conditions. Electrophoresis is a method of separating proteins based on their physical properties. Serum is placed on a specific medium, and a charge is applied and the net charge (positive or negative) and the size and shape of the protein are used in differentiating various serum proteins (Jacob and Cole, 2000). Several subsets of serum protein electrophoresis are also available. Review of literature on characterization of protein pattern in serum reveal that such a study in serum of anemia is meager. The electrophoretic study of the serum is aims in verifying the number, band area and pattern of protein fractions in anemic serum samples.

II. MATERIALS AND METHODS

Serum samples were obtained from healthy as well as anemic individuals. Routinely collected 66 blood samples were studied. About 5 ml of non-heparinized blood was collected from Tambaram Government hospital, Chennai, in the age group of 20-30 and serum was isolated from the whole blood by coagulation. Totally 8 samples were collected from each age groups (20, 22, 23 24, 27, 28, 29 and 30 years) respectively. The collected serum samples were immediately used for SDS-PAGE analysis.

III. RESULTS

Data on protein fractions in the serum of 64 cases of anemia were studied and another normal 2 cases were used as control. Protein profiles of the serum in 10 µg/well concentration of serum were studied by SDS-PAGE. Analysis of molecular weight and relative mobility factor (R_f) of protein marker showed that 10% resolving gel of SDS-PAGE was able to resolve 5-7 bands at concentration of 10 µg/well which contain high and low bands with molecular weight of 205,000 - 3000 KDa.

Totally 8 samples were collected from the various age groups of 20, 22, 23, 24, 27, 28, 29 and 30 years old. Relative and absolute values respectively of the protein fractions of anemia cases were compared with control samples. It is to be noted that there appears to be no consistent difference between anemia and normal ones.

The pattern of distribution of protein bands was cumulatively presented in percentage in all age groups of data revealed that high molecular weight protein bands are minimal than the other. Particularly, 29-20 KDa bands are in higher percentage in all age groups. It is interestingly to note that 28 year old person's serum showed all type of protein bands. The percentage of protein bands were one with 100% in 29 KDa in 20 year old persons, only one with 205-97 KDa in 22 year old persons, only one with 100% percentage in 66-14 KDa in 23

year old person, only one with 100% in 29 KDa in 24 year old person, three with 100% in 205-97 KDa, 66-43 and 29-20 KDa on 27 year old person, one with 100% in 14-6 KDa in 28 year old persons, one in 100% in 29 KDa in 29 year old people and one with 100% in 30 year old persons. Comparatively, the protein band with 29 KDa noted to be in high percentage in all age groups (Table: 1).

IV. DISCUSSION

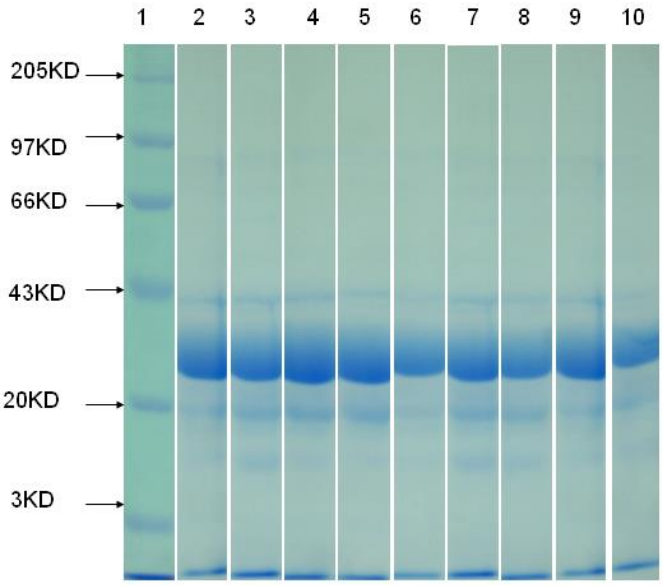
Serum protein electrophoresis is used to identify patients with multiple myeloma and other serum protein disorders (O' Connell *et al*., 2005). Acrylamide gel disc electrophoresis was used for the routine clinical laboratory examination of human serum lipoproteins (Mitsuo and Junichi, 1972). Serum protein electrophoresis in acrylamide gel patterns was done in normal human subjects (Peacock, 1965).Sodium dodocylsulphate polyacrylamide gel electrophoresis (SDS – PAGE) is a high-resolution one dimensional technique, suitable for analytical resolution of thepolypeptide constituents of complex protein mixtures (Laemmli, 1970).

Table 1: Frequency distribution (in %) of the protein fractions in samples

Age groups	MOLECULAR WEIGHT(KDa)							
	205,000 - 97,400	97,400 - 66,000	66,000 - 43,000	43,000 - 29,000	29,000 - 20,100	20,100 - 14,300	14,300 - 6,500	6,500 - 3,000
20	0	0	0	87.5	100	75	75	0
22	100	0	0	0	0	0	0	0
23	0	0	87.5	100	25	75	25	0
24	0	0	0	0	100	37.5	0	0
27	100	0	100	0	100	0	0	0
28	12.5	62.5	37.5	37.5	25	87.5	100	87.5
29	0	0	0	62.5	100	0	62.5	0
30	0	0	0	62.5	100	75	62.5	0

Such an analysis was carried out in the present study, which is in accordance with earlier work. Electrophoretic patterns of analyzed human serum or plasma usually do not show components that migrate faster than the albumin. Human serum proteins were evaluated by SDS-PAGE after protein denaturation in the presence or absence of 2-mercaptoethanol. In human and domestic animal medicine,electrophoresis of plasma proteins can provide information about chronic or acute inflammatory processes in the patient and may help the clinician to determine appropriate treatment. Electrophoresis also has been utilized for diagnosing other diseases such as hepatitis and nephritis (Cray and Tatum, 1998).Low molecular weight human serum proteins, peptides and other small componentshave been associated with pathological conditions such as cancer (Petricoin*et al*., 2002) diabetes (Basso

Plate: 6.1 Data to show the band area of serum protein at the age 20



Lane 1 = Molecular weight marker
Lane 2 = Control sample

et al., 2002) and cardiovascular and infectious diseases (Rubin and Merchant, 2000) and likely reflect the state of the underlying cell or tissue.One of the purposes of this study was to develop an efficient fractionation method to enable the identification of components of the serum proteins. Serum is one of the most difficult proteomic samples to characterize. The ability to do so promises rich information regarding the histological state of a patient and its analysis using proteomic techniques is being counted on for the discovery of reliable disease biomarkers.The present study further reveal that the fractions vary in relation to age in all anemic cases and particularly the number and percentage of protein bands were relatively high in the age of 28 than the others.

V. CONCLUSION

Further identification of such bands by Western blot technique and configuration of these proteins may be of high value to interpret the impact of anemic conditions in the physiological and pathological conditions of the youth in India.

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