

Clinical Utility of Electrophoretically Separated Serum Protein Fractions for Prediction of Myocardial Infarction

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Abstract.- In an investigation on molecular basis of pathogenesis in cardiovascular diseases the blood samples of forty patients diagnosed for myocardial infarction (MI) obtained from Punjab Institute of Cardiology, Lahore, Pakistan, and twenty healthy subjects of comparable age group without any history of cardiac ailment as control were used for study of protein profiles with sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) in first dimension. The amount and molecular weights of each of the protein fractions were determined by Gene Genius Bio-imaging Gel Documentation System. The protein fractions that showed significant variation were separated by using the technique of electroblotting and electroelution and run on isoelectric focusing (IEF) in second dimension to determine their isoelectric points. The most pertinent results in the comparison were the significant increase in apolipoprotein B, marked decrease in apolipoprotein A-I and transthyretin and high apolipoprotein B/apolipoprotein A-I ratio in the sera of patients of MI compared to healthy subjects. These results show that level of apolipoprotein A-I, apolipoprotein B, transthyretin and the apolipoprotein B/ apolipoprotein A-I ratio were the strong predictor of MI. It is also suggested that future MI events could be improved by measuring these proteins.

Key words: Myocardial infarction, apolipoprotein A-I, apolipoprotein B, transthyretin.

INTRODUCTION

Myocardial infarction (MI) is the major cause of death in the world. There are many factors which increase the risk of MI. In clinical practice traditional/conventional lipid measures are being used for the prediction of MI for long time. During the last several years interest has focused on serum proteins, particularly lipid transporting apolipoprotein B and apolipoprotein A-I for the prediction of MI risk (Walldius and Jungner, 2006). Practical advantage in favour of using apolipoprotein B and apolipoprotein A-I is that fasting is not required to analyze and interpret the value of apolipoprotein B and apolipoprotein A-I (Walldius and Jungner, 2007). Many reports have revealed that high plasma levels of apolipoprotein B, low plasma levels of apolipoprotein A-I and high apolipoprotein B/apolipoprotein A-I ratio were associated with higher risk of MI (Lanes *et al.*, 2007; Jungner *et al.*, 2006; St-Pierre *et al.*, 2006; Meisinger *et al.*, 2005; Walldius *et al.*, 2004).

Proteomics is a new technology that allow the detection and identification of several proteins in the

serum sample of MI patients. Presently two-dimensional gel electrophoresis (2-DE), with one dimension on isoelectric focusing (IEF) and other on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and vice versa, is mostly employed in such analysis. SDS-PAGE is likely to yield protein distribution profile consistent with the patient's clinical status (Andrews, 1986). In 2-DE constant differences were found in at least four different areas within the plasma protein map of MI patients (Mateos-Caceres *et al.*, 2004).

The aim of the present study was to investigate the protein patterns in the sera of MI patients and their comparison with those of healthy subjects through SDS-PAGE and 2-DE to find association between proteins and MI patient in our local population for the prediction and management of MI.

MATERIALS AND METHODS

Blood samples of forty patients with a diagnosis of MI (based upon clinical history, ECG and biochemical tests) were collected immediately after their admission to the Punjab Institute of Cardiology, Lahore, Pakistan. These patients were not on any cholesterol lowering drugs. Five ml blood was collected from each patient with the help of syringe. Blood samples of twenty healthy

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subjects with negative family history of CVD were also collected for use as controls. Serum was separated by centrifugation and stored at -70°C until used for analysis. For SDS-PAGE, the serum samples were diluted in phosphate buffer (pH 7.2) and proteins were denatured by heating with loading dye (1.54 g dithiothreitol, 2 g sodiumdodecyl sulfate, 8 mL of 1.0 M Tris HCl; pH 6.8, 10 mL of glycerol and 20 mg of bromophenol blue dye) in boiling water bath for two minutes before loading on the gel. Lyophilized mixture of proteins SDS-6H for high (205-45 kDa) and SDS VII-L for low (66-14.2 kDa) molecular weight proteins (Sigma Chemicals) were used as molecular weight markers. It was reconstituted, separately, in 1.5 mL of sample buffer (0.0625M Tris HCl pH 6.75, 2% SDS, 5% mercaptoethanol, 10% glycerol and 0.001% bromophenol blue). Heated in a boiling water bath for 2 minutes and stored, in aliquots, at -70°C .

Polyacrylamide gels, 5 % for high and 12 % for low molecular weight proteins, were prepared (Laemmli, 1970). Protein marker and each of the samples were loaded in separate wells and gels were electrophoresed at a current supply of 20 mA and voltage of 200 volts in a cooling chamber maintained at 4°C . Electrophoresis was stopped, immediately, after dye seemed to diffuse in the buffer in the lower chamber. Following electrophoresis, the 5% gel was stained with coomassie brilliant blue for 30 minutes and 12 % gel for two hours. After staining the gels were destained until the clearance of blue background. Protein fractions of different molecular weights were visible in the form of blue bands on a transparent background. Gels were photographed and their images were saved for protein quantification by Gene Genius Bio-imaging Gel Documentation System that provides the data of molecular weights and the total area covered by each of the protein fractions. The data was employed in finding the enhancement or reduction and the appearance and disappearance of particular protein fractions for comparison of the healthy individuals and MI patients.

Samples containing significant quantities of desired protein fractions were run on SDS-PAGE and the unstained gel was electroblotted on polyvinylidene difluoride (PVDF) membrane

(Dunn, 1996 a). The required protein band from PVDF membrane was excised and electroeluted (Dunn, 1996 b). 10 μL solution D (10 % w/v sodiumdodecyl sulfate in 2.3 % w/v dithioerythritol) was added to 60 μL eluted protein solution, mixed and heated at 95°C for 5 minutes. Brought to room temperature and added 5 μL solution E (8 M urea, 4% CHAPS (3-[(3cholamidopropyl)dimethyl ammonio]-1-propanesulfonate), 40 mM Tris HCl and 65 mM dithioerythritol, traces of bromophenol blue). The eluted protein was subjected, afterwards, to isoelectric focusing (O'Farrell, 1975) in order to determine its isoelectric point/s.

RESULTS

Variations regarding enhancement of some of the protein fractions but reduction of few others in MI patients compared to healthy subjects were notable. No new fractions were, however, detected in MI group when compared to healthy group.

The comparison of protein fractions of myocardial infarction group with that of control group indicate that most of the protein fractions did not show any appreciable difference in their concentration. However, the protein fraction of 270 kDa showed highly significant increase in myocardial infarction group. The average percent raw volume of $2.45\pm 0.21\%$ and $4.20\pm 0.28\%$ were determined in control and myocardial infarction groups respectively showing 71 % elevation ($P<0.01$) in myocardial infarction group compared with the control group. A highly significant reduction of 41 % ($P<0.01$) was observed in 23 kDa fraction in myocardial infarction group when compared with normal group. The average raw volume exhibited by this fraction was $3.38\pm 0.25\%$ and $1.97\pm 0.27\%$ in control and myocardial infarction group respectively. Protein fractions of 17 and 14 kDa also exhibited significant decline of 22 % and 29 % respectively in myocardial infarction group ($P<0.05$). The percent raw volume values of these fractions in the control group were $1.34\pm 0.05\%$ and $0.96\pm 0.11\%$ respectively. The corresponding percentages in myocardial infarction group were estimated as $1.06\pm 0.32\%$ and $0.68\pm 0.09\%$ respectively (Table I; Fig. 1-2). The ratios of 270

kDa/23 kDa proteins, which showed highly significant differences in normal subjects and MI patients, were also determined in normal subjects and MI patients. The ratio of 270 kDa/23 kDa proteins was found to be 0.73 in normal subjects compared to 2.13 in MI patients (Table II).

weight and the isoelectric points, each of the proteins was then identified using human plasma protein map (Swiss 2DPAGE, 2002). Protein fractions of 270, 23 and 14 kDa were found to be apolipoprotein B, apolipoprotein A-I and transthyretin respectively.

Table I.- Average raw volumes (%) exhibited by electrophoretically separated serum protein fractions of control and myocardial infarction (MI) groups and their percentage differences.

Molecular weight of proteins (kDa)	Average raw volume (%) of protein fractions in control group	Average raw volume (%) of protein fractions in MI group	Percentage difference in protein fractions in MI group
270	2.45±0.21	4.20±0.28	71** ↑
190	7.37±0.30	6.78±0.36	08 ↓
186	3.50±0.26	3.20±0.20	09 ↓
135	4.25±0.23	4.31±0.24	02 ↑
115	5.23±0.37	5.32±0.25	02 ↑
100	3.25±0.24	3.38±0.22	04 ↑
77	10.21±0.23	10.53± 0.53	03 ↑
66	26.52±0.18	27.09±0.20	03 ↑
54	14.55±0.31	14.74±0.33	02 ↑
45	10.64±0.41	10.36±0.23	03 ↓
36	2.22±0.12	2.15±0.18	03 ↓
28	9.48±0.48	8.13±0.68	15 ↓
24	12.42±0.29	11.72±0.42	06 ↓
23	3.38±0.25	1.97±0.27	41** ↓
17	1.34±0.05	1.06±0.32	22* ↓
14	0.96±0.11	0.68±0.09	29* ↓

↑ Increase; ↓ Decrease; * p<0.05; **p<0.01

The protein fractions showing considerable variations were subjected to isoelectric focusing. High molecular weight protein fraction of 270 kDa was resolved into two bands corresponding to isoelectric points 5.9 and 6.1 (Fig. 3A). Amongst low molecular weight proteins the fraction of 23 kDa was resolved into three fractions whose isoelectric points were determined as of 5.1, 5.3 and 5.5 (Fig. 3B). The fraction of 14 kDa resolved into two bands corresponding to isoelectric points 5.3 and 5.5 (Fig. 3C).

From the above data regarding the molecular

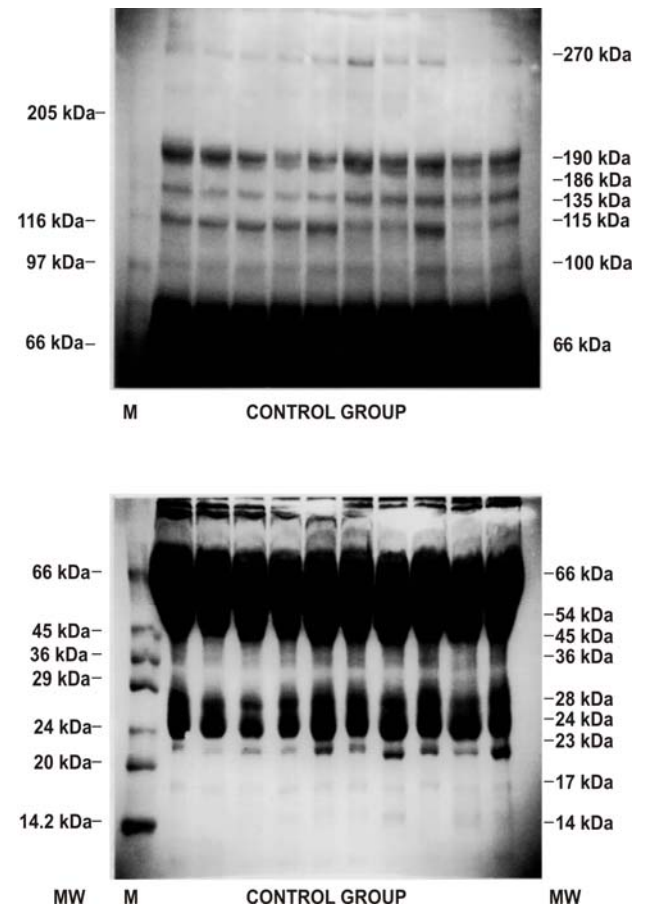


Fig. 1. SDS-PAGE of serum of control subjects. (M indicates protein markers; MW indicates molecular weight of protein markers on left side and serum protein fractions on right side; 5 % gel above and 12 % gel below).

Table II.- Apolipoprotein B/Apolipoprotein A-I ratio in control and myocardial infarction (MI) groups.

Category	Apolipo-protein B	Apolipo-protein A-I	Apo B/Apo A-I ratio
Control group	2.45	3.38	0.73
MI group	4.20	1.97	2.13

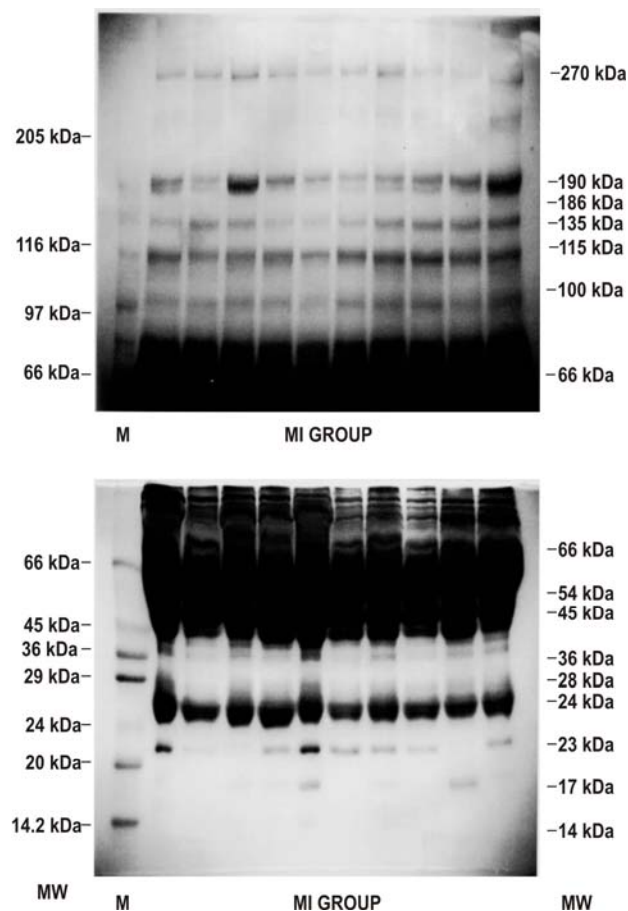


Fig. 2. SDS-PAGE of serum of MI patients. (M indicates protein markers; MW indicates molecular weight of protein markers on left side and serum protein fractions on right side; 5 % gel above and 12 % gel below).

DISCUSSION

The present study was undertaken to find the variations in the serum protein profiles in the patients of MI in local population. In recent years proteomics is a rapidly growing research area. It has increased the understanding of many diseases and protein composition represents the functional status of biological compartment. Due to resolution and sensitivity the technique of 2-DE is a powerful tool for the analysis and detection of proteins from complex biological sources (O'Farrell, 1975). Variations in the serum protein profile in MI were therefore detected by SDS-PAGE in first dimension. IEF was performed in second dimension of those

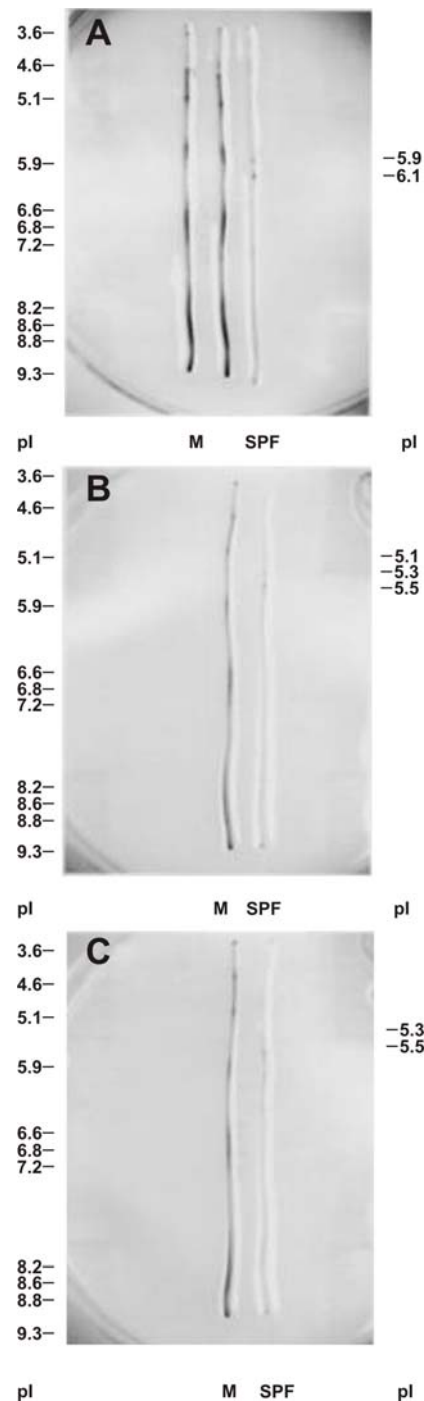


Fig. 3. IEF gels showing distinct bands of 270 kDa, 23 kDa and 14 kDa protein fractions in Fig. A, B and C respectively. (M indicates protein markers; SPF indicates serum protein fractions; pI indicates isoelectric points of protein markers on left side and serum protein fractions on right side).

proteins which exhibited significant variations and could be used for prediction and management of MI.

In present study apolipoprotein B (270 kDa) have been found to be elevated in the patients of MI compared to the control subjects. This protein showed an increase of 71 % in patients of MI. These results are in agreement with observations of many researchers/investigators who have reported an elevation of apolipoprotein B in MI patients and suggested that higher level of apolipoprotein B were strong predictor of MI (Benn *et al.*, 2007; Dirisamer *et al.*, 2006; Pischon *et al.*, 2005; Corsetti *et al.*, 2004; Simes *et al.*, 2002; Walldius *et al.*, 2001; Moss *et al.*, 1999; Luc *et al.*, 1996; Jadhav *et al.*, 1994). Due to this property apolipoprotein B considered as atherogenic. The atherogenic nature of apolipoprotein B is due to the fact that it only occurs in atherogenic lipoprotein, mainly LDL, which is also called as "bad" LDL (Walldius and Jungner, 2004). Therefore it is suggested that the higher level of apolipoprotein B may be used for prediction of MI in our local population.

Apolipoprotein A-I (23 kDa) exhibited the trend of reduction in the patients of MI. Reduction of 41 % was found in patients of MI. Many researchers demonstrated lower levels of apolipoprotein A-I in patients of MI and suggested that low level of apolipoprotein A-I is better risk marker in predicting MI (Walldius and Jungner, 2007; Ingelsson *et al.*, 2007; Walldius and Jungner, 2006; Thogersen *et al.*, 2004; Averina *et al.*, 2004; Simes *et al.*, 2002; Luc *et al.*, 2002; Walldius *et al.*, 2001; Moss *et al.*, 1999; Marques-Vidal *et al.*, 1995; Jadhav *et al.*, 1994; Mbewu *et al.*, 1993; Linden *et al.*, 1989; Fager *et al.*, 1980). It is suggested that apolipoprotein A-I might be of greatest value in diagnosis and treatment in MI patients who have common lipid abnormality but have normal or low concentration of LDL-cholesterol (Walldius *et al.*, 2001). The low level of apolipoprotein A-I in MI patients support the studies which showed that apolipoprotein A-I is cardioprotective (Gu *et al.*, 2007). This cardioprotective nature of apolipoprotein A-I is bound to antiatherogenic lipoprotein HDL also called "good" HDL (Walldius and Jungner, 2004).

Our results demonstrated high apolipoprotein B/apolipoprotein A-I ratio in MI patients (2.13)

compared to normal subjects (0.73). These results are in line with many studies in which high ratio of apolipoprotein B/apolipoprotein A-I was observed and strongly predicted for MI (McQueen *et al.*, 2008; Sniderman and Faraj, 2007; Ingelsson *et al.*, 2007; Jungner *et al.*, 2006; Meisinger *et al.*, 2005; Walldius *et al.*, 2004). Studies also revealed that prevalence of MI was significantly higher among subjects with apolipoprotein B/apoipoprotein A-I ratio exceeding one compared to those with ratio less than one (Jadhav and Kadam, 2004). Our results also showed the ratio of apolipoprotein B/apolipoprotein A-I higher than one in MI patients and less than one in normal subjects. In prospective risk studies it has been shown that apolipoprotein B/apolipoprotein A-I ratio reflect cholesterol balance between atherogenic (LDL) and antiatherogenic lipoprotein (HDL) and is better marker in predicting MI (Walldius and Jungner, 2007).

Transthyretin (14kDa) decreased significantly in patients of MI. The decrease of transthyretin was 29 % in patients of MI. Other studies also exhibited significant decreases in the concentration of transthyretin in patients of MI (Goldwasser *et al.*, 1993; Bodmann *et al.*, 1993; Oehler *et al.*, 1988; Franklyn *et al.*, 1984). Studies also showed that S-sulfated transthyretin was found to be responsive to MI (Kiernan *et al.*, 2006)

This pattern of variations in apolipoprotein B, apolipoprotein A-I and transthyretin in MI patients is similar to most of the population of the world. However, some researchers reported different protein patterns in some populations of the world (Dirisamer, 2006; Zeman *et al.*, 1995; Mbewu *et al.*, 1993; Bausserman *et al.*, 1989). These differences may be due to environmental and genetic differences of the populations.

The results of present study indicate that in MI patient alteration in protein fractions occur as a result of displaced molecular homeostasis. Presently the technique of electrophoresis, particularly two-dimensional gel electrophoresis, due to its resolution and sensitivity is very suitable for the detection of protein variations. These variations (elevation or reduction) in proteins can be employed for the prediction of MI. However further studies are required, particularly in patients at high risk of MI,

to determine whether MI can be predicted by variations in these proteins.

The present study also suggest that the technique of electrophoresis, particularly two-dimensional gel electrophoresis, should be further developed/improved for further biochemical investigations in MI for its further and better understanding on better developed methodology in our Pakistani population where the work on the diagnosis of MI is merely conventional and highly underdeveloped.

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