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Depletion of high abundance proteins in plasma of buffalo calves by SDS-PAGE of magnetic isolated anionic fraction

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Abstract
A variety of proteins and peptides are present in blood plasma which can be harnessed as biomarkers in disease diagnosis. Enzyme-linked assays are widely used for determination of some specific low abundance proteins in serum or plasma in diagnostic medicine which have various limitations. These assays need to be substituted by proteomic approach, as an aid in diagnostics. Proteome analysis is that essential tool which can be used for identification of plasma protein biomarkers. In plasma, albumin is the most abundant protein which hampers other low-abundant proteins which may prove to be important biomarker for various diseases. So it is very important to remove the excess of albumin and other proteins of high abundance for easy identification of proteins of low abundance. For this purpose, magnetic separation using cationic iron beads were used to deplete abundant proteins. These fractions were analysed by one-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (1-D SDS-PAGE) to monitor changes in major protein bands and optimize experimental conditions.

Keywords: Plasma proteins, magnetic separation, albumin depletion, buffalo calves

Introduction
Proteins and peptides as a result of several biochemical reactions are secreted in blood indicating alteration in biochemical and physiological status of the animal. So, these can act as biomarkers of disease and physiological status of an animal. For identification of disease-associated biomarkers, proteome analytical techniques are being used. The use of current proteomic approaches in the search for potential diagnostic/prognostic indicators in the plasma of animals is limited by highly abundant proteins (Georgiou *et al*., 2001) [4]. Albumin and immunoglobulins constitute more than 60% of the total plasma proteins, making the hundreds or even thousands of other proteins difficult to detect by two-dimensional electrophoresis (2-DE). Depletion of abundant plasma proteins will improve the resolution of less abundant proteins that may prove to be informative disease markers. Over several decades, many approaches have been used to remove abundant plasma proteins, by methods based on the high affinity of some abundant proteins for certain textile dyes, such as Cibacron-Blue and several derivatives (Gianazza and Arnaud, 1982; Raymackers *et al*., 2000) [5, 9].

Materials and Method
Blood collection and plasma separation
Ten apparently healthy buffalo calves of age up to six months were selected from buffalo farm, LUVAS, Hisar. Approximately 5 ml blood was collected from jugular vein using 10 ml disposable syringe aseptically for separation of plasma, the blood samples collected in centrifuge tube containing heparin were centrifuged at 3000 rpm for 10 minutes. The plasma samples were pooled and stored in aliquots till further analysis.

Sample preparation
100µl plasma was taken along with 100µl diluted iron oxide magnetic particles in a microcentrifuge tube and vortexed at full speed of table top vortex at room temperature for 5 minutes to magnetically separate rejected proteins (cationic) in solution (not bound to iron oxide particles). Washing was done twice with ultrapure water on vortex @ full speed RT for 2 minutes and followed by magnetic separation. Anionic proteins bound to iron oxide particles were used for SDS-PAGE analysis.

Analytical procedure

12% resolving and 4% stacking gels were polymerized between two glass plates in a gel caster, with a comb inserted at the top of the stacking gel to create the sample wells. After the gel was polymerized, the inner chamber was filled with running buffer and the comb was removed. The samples to be analyzed and the Unstained Protein Molecular Weight Marker (Clontech Takara #3452, 200kDa-6.5kDa) were mixed with sample buffer in 1:1 ratio. The mixtures were heated at 95 °C for 5 minutes and then cooled before loading. A constant electric field (90 Volt) was applied across the gel, causing the negatively-charged proteins to migrate across the gel towards the positive (+) electrode (anode). Depending on their size, each protein was moved differently through the gel matrix.

Results

The gel was stained with Coomassive blue dye. The molecular weights of the peptides were estimated by EZ Gel documentation system using Image lab software.

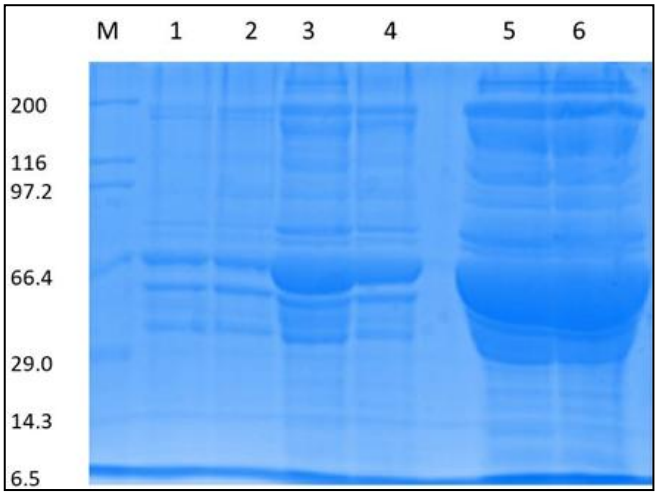

Fig 1: SDS-PAGE electrophoretic profile of anionic plasma proteins bound to iron oxide magnetic particles after albumin depletion from pooled plasma samples of buffalo calves.

Lane M: Molecular weight marker
Lane 1 to 4: Anionic proteins bound to iron oxide at different concentrations
Lane 5&6: Neat plasma at different concentrations

Lanes 1 to 4 are showing the depletion of albumin and clear separation of proteins of molecular weight between 66.4 to 29.0 kD. While in neat plasma samples albumin is hampering the separation of other low molecular weight proteins. Albumin has the molecular weight of approximately 70 kD which is more than 50 per cent of total protein suppress the clear separation of low molecular weight proteins on SDS PAGE separation. On magnetic separation of plasma proteins, anionic portion of proteins bound to iron oxide magnetic particles show clear excess albumin depletion thereby improving the separation of low abundant proteins in plasma samples.

Discussion

Albumin constitutes 30 to 50% of the total plasma protein having molecular weight of 66.4 kD with three homologous domains in structure (Nakajou *et al*., 2003) [7] containing 67% of the protein as α -helix with no β –sheet (Curry *et al*., 1998) [3]. Its concentration increases in dehydration while decreases in liver, kidney, gastrointestinal disease, acute phase response, malnutrition, Blood and plasma loss. The acute phase response is an integral component of the innate immune response (Beutler, 2004) [1] forming the first reaction of the host to pathogens and tissue damage. Monitoring the levels of the acute phase proteins (APP) can provide a way to assess the innate immune system's response to disease. But many acute phase proteins of diagnostic importance like α-1-fetoprotein, α-1-antithrombin III, α-2 –macroglobulin have molecular weights approximately similar to albumin which is present in excess and may hamper their clear separation. Therefore to assess the acute phase response proteins in various disease conditions excess albumin depletion can prove to be a useful tool. α-1-Fetoprotein, 65 kD molecular weight protein, increases in hepatoma, pregnancy, decreases in liver disease and chronic pulmonary disease. α -1 - Antithrombin III (Thrombin inhibitor) is also 65kD molecular weight protein which increases in acute phase response in cats and decreases in disseminated intravascular coagulation, protein-losing nephropathies, and enteropathies sepsis. Similarly, there are proteins of molecular weight 62 kD such as α 2 -Macroglobulin (Insulin binding protease inhibitor) and Protein C (Protease and anticoagulant) which show modulation in their concentrations in acute phase responses. There are several studies on individual serum proteins of domestic animals which show marked variation in their concentration during acute phase response in several clinical conditions (Ceron *et al*., 2005; Murata *et al*., 2004; Petersen *et al*., 2004) [2, 6, 8]. So monitoring the level and presence or absence of proteins of low abundance proteins can depict a picture in many disease conditions.

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