A STUDY OF SOME OF THE ERYTHROCYTE MEMBRANE ASSOCIATED PROTEINS IN HEALTHY INDIVIDUALS AND LEUKEMIA PATIENTS USING ONE DIMENSIONAL SODIUM DODECYL SULFATE POLY ACRYLAMIDE GEL ELECTROPHORESIS (1 D SDS – PAGE) AND MATRIX ASSISTED LASER DESORPTION IONIZATION TIME OF FLIGHT (MALDI – TOF) MASS SPECTROMETRY

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Abstract:

Analysis of red blood cell (erythrocyte) membrane associated proteins of normal persons and leukemia patients in Sri Lanka is presented in this thesis. The main aim of this research is to analyze erythrocyte membrane associated proteins using One Dimensional Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (1 D SDS-PAGE) and Matrix-Assisted Laser Desorption Ionization Time OF Flight (MALDI-TOF) Mass Spectrometry (MS). Since the introduction of MS in protein chemistry, Peptide Mass Fingerprinting (PMF) became the method of choice to identify different proteins in biological samples.

Blood samples of normal persons and leukemia patients were chosen for this study. Following washing, osmotic lysis and centrifugation, erythrocyte membranes related to leukemia patients and normal persons were isolated and subjected to 1D SDS – PAGE. Silver staining was performed to visualize the separated proteins. The separated protein bands dissected out from the gel and were subjected to digestion by proteolytic enzyme, trypsin, and the resulting peptide mixture was analyzed by MALDI-TOF mass spectrometry. The experimental mass values were matched with theoretical mass values in the protein databases and identification was made using software.

α and β spectrins are the major membrane associated proteins and were successfully identified in healthy persons using this technology. The theoretical molecular weight of α and β spectrins are 279.91 KDa and 246.32 KDa respectively. Experimental molecular weights of those two proteins were 281.04 KDa and 247.16 KDa. Molecular weights obtained by mass spectrometric methods were more accurate than those calculated by gel electrophoretic methods. Glyceraldehyde -3-phosphate dehydrogenase (GAPDH) was identified in patients of two types of leukemia with molecular weights of 36.21 KDa and 36.22 KDa. It had been identified as an erythrocyte membrane associated proteins with similar molecular weights in previous studies. α and β spectrins were identified from different types of leukemia patients and their molecular weights were similar to molecular weights obtained from normal persons. Although mature erythrocyte does not contain a nucleus, two nuclear proteins namely Dbf 4 related factor 1 isoform 2 and Cyclin E isoform 2 were identified. Those two proteins may be derived as contaminants of white cells or nuclei containing erythroblasts peripheral blood such as reticulocytes. One of the most abundant erythrocyte membrane associated protein actin was identified and the molecular weight obtained was similar to that of previously reported. About 60 different gel pieces were analyzed by MALDI-TOF and PMF, only a few could be identified. Most of them were identified as human 'keratins. The most probable reason was the contamination. Even though great amount of care and precautions was taken when handling of samples during the whole process, contamination could not be completely avoided. In certain instances proteins were not identified even after obtaining high quality MALDI-TOF spectra. In 1D SDS-PAGE a single protein band could contain one or more proteins. Therefore PMF may fail to identify some proteins when working with mixtures.