Expression of Low Molecular Weight Proteins in Patients with Leukaemia

N Sheikh[1], R Abid[1], AW Qureshi[1], T Basheer[2]

ABSTRACT

The current study is conducted to observe the differences in the level of low molecular weight proteins in the sera of patients with leukaemia in comparison to healthy subjects (control group). The sera of patients with leukaemia showed 15 peaks in the densitometric curve in comparison to the seven peaks of the controls. The peaks in the experimental samples that coincide with those in the control were of 134.14, 113.15, 76.06, 63.25, 48.07, 22.85 and 16.47 kDa molecular weights, respectively. Most of the new peaks appeared between the proteins of molecular weight 36–29 kDa in the experimental groups. Mean density of the 134.14 kDa protein band showed an increase in the protein in experimental groups I and II only whereas 113.15 and 22.85 kDa protein were increased in all experimental groups of patients with leukaemia. The expression of 76.06 and 63.25 kDa protein fraction was downregulated in the patients with leukaemia. A decline in the level of the protein of 48.07 kDa was observed in patients with leukaemia except in Group I. Unlike the other protein fractions, the level of the protein of 16.47 kDa was significantly ($p < 0.05$) increased with a maximum density in Group II. Intergroup (experimental) comparison revealed an increasing pattern of 95.44 and 89.21 kDa with maximum level in Group III sera. However, the protein fractions of 38.07 and 34.94 kDa varied in the serum with maximum density in Group IV. Protein fractions of 32.92 and 31.24 kDa were expressed in all age groups of patients with leukaemia with a maximum density in Group III whereas the percentage densities of 14.42 and 13.56 kDa protein were quite different. This preliminary study will provide a basis to study the role of different proteins in patients with leukaemia.

Keywords: Albumin, cancer, leukaemia, serum proteins

Expresión de las Proteínas de Bajo peso Molecular en Pacientes con Leucemia

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RESUMEN

El presente estudio se realiza con el fin de observar las diferencias en el nivel de proteínas de bajo peso molecular en los sueros de pacientes con leucemia, en comparación con pacientes sanos (grupo control). Los sueros de los pacientes con leucemia mostraron 15 picos en la curva densitométrica en comparación con los siete picos de los controles. Los picos en las muestras experimentales que coincidieron con aquéllos en los controles fueron de 134.14, 113.15, 76.06, 63.25, 48.07, 22.85 y 16.47 kDa de peso molecular, respectivamente. La mayoría de estos nuevos picos aparecían entre las proteínas de peso molecular 36–29 kDa en los grupos experimentales. La densidad promedio de la banda proteica de 134.14 kDa sólo mostró un aumento en los grupos experimentales I y II, mientras que las proteínas de 113.15 y 22.85 kDa experimentaron un aumento en todos los grupos experimentales de pacientes con leucemia. La expresión de las fracciones de proteína de 76.06 y 63.25 kDa experimentó una reducción negativa (downregulation) en los pacientes con leucemia. Se observó una disminución en el nivel de la proteína de 48.07 kDa en los pacientes con leucemia, excepto en el Grupo I. A diferencia de las otras fracciones proteicas, el nivel de la proteína de 16.47 kDa aumentó

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*significativamente (*p < *0.05) con una densidad máxima en el Grupo II. La comparación intergrupal (experimental) puso de manifiesto un patrón de aumento de 95.44 y 89.21 kDa con un nivel máximo en los sueros del Grupo III. Sin embargo, las fracciones proteicas de 38.07 y 34.94 kDa variaron en el suero con densidad máxima en el Grupo IV. Las fracciones proteicas de 32.92 y 31.24 kDa se expresaron en todos los grupos etarios de los pacientes con leucemia con una densidad máxima en el Grupo III, mientras que las densidades porcentuales del porcentaje de las proteínas de 14.42 y 13.56 kDa fueron bastante diferentes. Este estudio preliminar proporcionará la base para estudiar el papel de las diferentes proteínas en los pacientes con leucemia.*

Palabras claves: Albúmina, cáncer, leucemia, proteínas séricas

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INTRODUCTION

Cancer is a disease in which individual mutant clones of cells begin by prospering at the expense of their neighbours, destroying the whole cellular society. Blood and lymphatic system play an important role in cancer spread to other parts of the body (1, 2). Leukaemia, characterized by increased numbers of white blood cells (WBC), is a type of blood cancer that starts in blood-forming tissue, the bone marrow and causes the production of large numbers of abnormal blood cells called leukaemia cells that enter the blood stream (3). Leu-kaemia has been a target of immunological studies in human cancer due to the fact that viable cancerous and normal hae-matopoietic cells become available for study from the same individual (4).

Leukaemia has markedly increased in the last three decades due to increasing amounts of ionizing radiation in the environment and other man-made additions to the natural background (5). Leukaemia is broadly categorized into four types.

Chronic lymphocytic leukaemia (CLL) is characterized by the morphologically mature but immunologically immature lymphocytes and is manifested by progressive accumulation of the cells in the blood, bone marrow and lymphatic tissues (6).

Chronic myeloid leukaemia (CML) is a myeloproliferative disorder of pleuripotent haematopoietic progenitor cells characterized by excessive proliferation and accumulation of granulocytes and occasionally red blood cells and platelets (7). Chronic myeloid leukaemia is due to changes in the genetic code of some of the cells in the bone marrow (8).

Acute lymphocytic leukaemia (ALL) is a malignant disorder of lymphoid progenitor cells, is manifested by the formation of unformed blasts that normally develop into lymphocytes. However, the blasts are abnormal and unable to develop and fight infections. The number of abnormal cells grow rapidly (9).

Acute myeloid leukaemia (AML) is characterized by an increase in the number of myeloid cells in the marrow and an arrest in their maturation frequently result in haematopoietic insufficiency *ie* granulocytopenia, thrombocytopenia or anaemia with or without leukocytosis (10).

The serum protein profile is strongly associated with the prevalence of cancer that could be of high diagnostic values for discriminating cancer patients and can be useful as a marker for detection of malignancy (11). Clinically useful data are obtained from fractionating the total protein which shows characteristic patterns in different types of leukaemia (12).

The present study is designed to investigate the serum protein profile of different age groups of patients with leukaemia in comparison to healthy subjects. The information obtained with the densitometric analysis will be very useful in understanding the pathophysiology of leukaemia and a way to overcome the risk of disease. Serum protein profile will guide professionals in the better diagnosis of the different types of leukaemia at different ages and determine the new therapeutic targets for the treatment of leukaemia.

SUBJECTS AND METHODS

Blood samples of patients with leukaemia were collected from INMOL (Institute of Nuclear Medicine and Oncology, Lahore) while blood samples of healthy persons were collected from Punjab University New Campus, Lahore. The study was designed to investigate the serum protein profile of the patients with leukaemia using SDS-PAGE. A total of 50 confirmed subjects were selected for each group.

The subjects had 5cc of blood extracted and the separated sera were stored at 20°C until used for SDS-PAGE. Polyacrylamide gel was prepared by using the method of Laemmli (13). Low molecular weight proteins were resolved on 12% gel. The quantification for electrophoretically separated protein fractions was carried out by Image J Gel Documentation System that provided the data based on percentage density of each fraction. The density of bands in a specific well was used to generate the densitometric graph to infer increase or decrease and appearance or disappearance of particular protein fractions as well as new protein fraction in comparison to the control group. Gene Genius Bio-imaging Gel Documentation System determined the molecular weights of the protein fractions of the samples.

One-way ANOVA with Dunnett's post-test was performed using Graph Pad Prism version 4.00 for Windows (Graph Pad Software, San Diego California USA; www.graphpad.com). The patients were divided into four groups

on the basis of their ages. Group I included patients from 1–15 years, Group II from 16–30 years, Group III from 31–45 years and Group IV from 46–60 years.

RESULTS

Changes in serum protein profile were demonstrated by densitometric analysis of the protein bands fractionized on SDS-PAGE. Seven and fifteen peaks in the densitometric curve were observed in the sera of the controls and experimental groups, respectively. The experimental group shared seven peaks with the controls whereas the eight protein fractions appeared only in the experimental groups and not in the control group (Fig. 1). The seven peaks which the control and the experimental groups had in common had molecular weights 134.14, 113.15, 76.06, 63.25, 48.07, 22.85 and 16.47 kDa, respectively. Most of these new peaks appeared between the proteins of molecular weight 36-29 kDa in the experimental groups (Fig. 1).

The changes in the expression of proteins in leukaemia patients of different age groups compared with the control samples indicate a mean density of the 134.14 kDa protein band which revealed an increase in the protein in experimental groups I and II but a decrease in groups III and IV. Mean density of 113.15 kDa band revealed an increase in the specific protein in all experimental groups of patients with leukaemia. The expression of 76.06 and 63.25 kDa protein fraction was downregulated in the patients with leukaemia which was statistically highly significant ($p < 0.01$) when analysed by one way ANOVA (Table 1, Fig. 2A).

A non-significant decline in the level of protein of 48.07 kDa was observed in patients with leukaemia except in Group I. The level of the protein fractions of 22.85 kDa was increased significantly ($p < 0.05$) in all the experimental groups of patients with leukaemia in comparison to control. Unlike the other protein fractions, the level of the protein of 16.47 kDa was significantly ($p < 0.05$) increased when com-

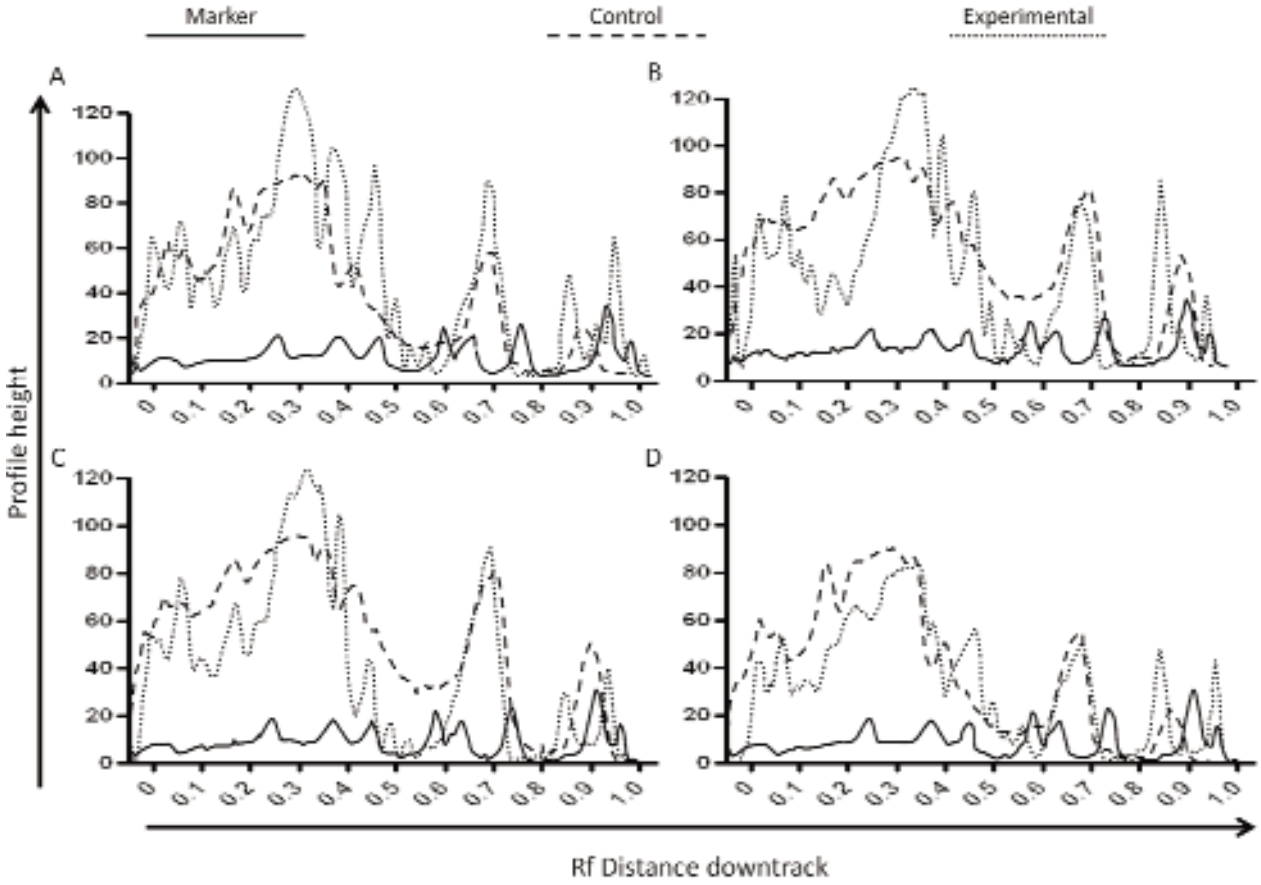

Fig. 1: The densitometric curves of the protein bands fractionized on SDS-PAGE from group I (A), group II (B), group III (C) and group IV (D). The solid line represents the marker, dashed line is the densitometric curve from control samples and the dotted line corresponds to the density of proteins in the experimental samples. The experimental curves show a disrupted pattern of protein.

Table 1: Comparison of the percentage densities of different protein fractions with the control. The values are represented as mean ± SEM

Molecular weight (kDa) → / Age Groups ↓	134.14	113.15	76.06	63.25	48.07	22.85	16.47
Control	7.11 ± 1.14	6.61 ± 0.59	17.26 ± 1.07	46.07 ± 1.08	8.37 ± 1.52	10.94 ± 0.26	1.27 ± 0.36
I	9.29 ± 1.30	10.08 ± 1.46	9.75 ± 0.81	31.96 ± 7.01	11.90 ± 0.00	11.12 ± 1.70	2.67 ± 0.00
II	7.39 ± 1.80	9.93 ± 1.15	10.26 ± 1.05	32.20 ± 1.06	5.32 ± 0.77	11.60 ± 1.53	4.39 ± 0.68
III	5.24 ± 0.94	9.46 ± 0.71	9.38 ± 0.63	36.08 ± 1.55	6.43 ± 0.90	15.31 ± 1.06	1.94 ± 0.31
IV	3.68 ± 0.91	7.02 ± 0.40	12.37 ± 0.00	38.92 ± 4.55	7.02 ± 2.15	12.50 ± 2.33	2.96 ± 0.67

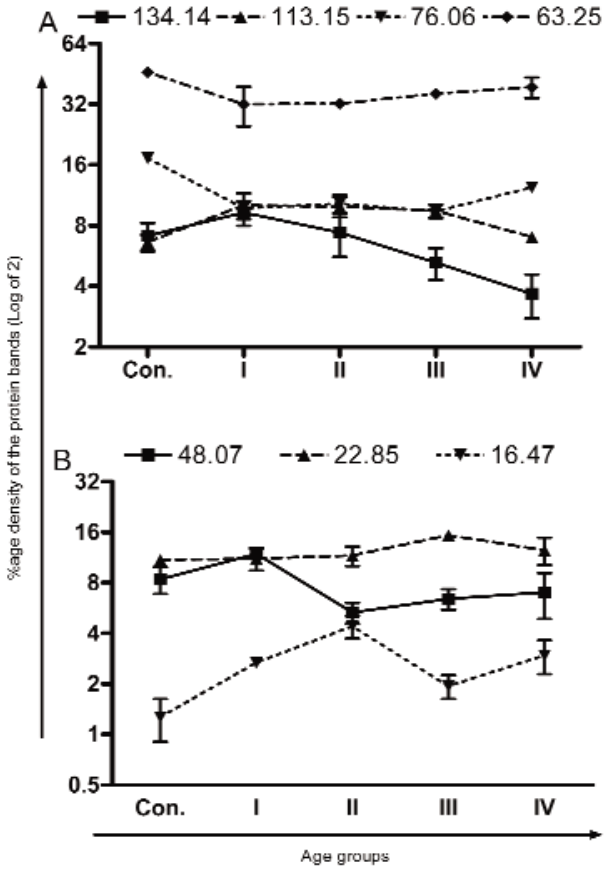

Fig. 2: Changes in the density of different molecular weight proteins (A: 134.14, 113.15, 76.06 and 63.25 kDa, B; 48.07, 22.85 and 16.47 kDa) in patients with leukaemia categorized in different age groups in comparison with the control group. The figure explains the relative density of different protein bands along with the changes in different age groups.

pared with the control group. The maximum density of the protein fraction was observed in Group II (Fig. 2B).

Besides these changes in the expression of the protein in patients with leukaemia, about eight different protein fractions were observed which were not present in the control group. Therefore, intergroup (experimental) comparison of these proteins was studied as no control value was available. The expression pattern of the proteins and their intergroup (experimental) comparison is shown in Table 2. Intergroup comparison of fractions of 95.44 and 89.21 kDa revealed an increasing pattern with maximum level in Group III sera.

The protein fractions of 38.07 and 34.94 kDa varied in terms of the density of specific bands reflecting their levels in the serum. However, intergroup comparison has shown that the maximum level of these two fractions was in Group IV with the density of 34.94 kDa protein fractions remained almost identical in all age groups of leukaemia.

A comparison of the two protein fractions, 32.92 and 31.24 kDa, in different age groups revealed their expression in all age groups of patients with leukaemia with a maximum density in Group III. A fraction of 14.42 and 13.56 kDa appeared in the experimental groups, however, the percentage densities of the two fractions was quite different (Fig. 3B).

DISCUSSION

Serum proteins are useful indicators for initial screening of any abnormal function, inflammation and diseased condition. The expression of different proteins can vary depending on the age of the person. The protein profile of the patients with leukaemia in the present study revealed a decrease in albumin concentration in comparison to the controls. The decreased albumin level results either from depressed synthesis or increased losses of albumin. A decrease in albumin syn-

Table 2: Intergroup comparison of the percentage densities of different protein fractions. The values are represented as mean ± SEM

Molecular weight (kDa) → / Age Groups ↓	95.44	89.21	38.07	34.94	32.92	31.24	14.42	13.56
I	4.36 ± 1.36	4.96 ± 0.00	5.75 ± 0.00	2.10 ± 0.34	1.58 ± 0.24	1.47 ± 0.30	0.60 ± 0.00	1.33 ± 0.31
II	4.67 ± 0.85	5.12 ± 0.47	5.26 ± 1.27	2.28 ± 0.41	1.48 ± 0.14	1.73 ± 0.26	0.31 ± 0.07	1.27 ± 0.32
III	5.35 ± 0.71	6.87 ± 0.89	4.82 ± 0.63	2.07 ± 0.16	1.80 ± 0.31	2.33 ± 0.38	0.44 ± 0.06	1.38 ± 0.13
IV	3.38 ± 0.55	5.56 ± 0.20	6.58 ± 2.00	2.27 ± 0.50	1.11 ± 0.09	1.76 ± 0.00	0.49 ± 0.00	1.70 ± 0.37

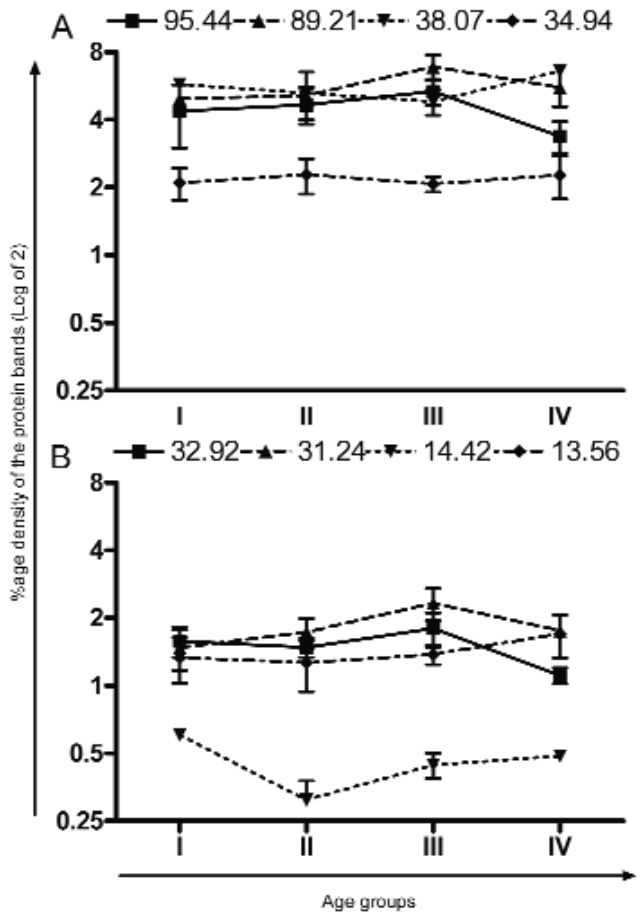

Fig. 3: Density of the protein bands present in patients with leukaemia with variable density and molecular weights (A: 95.44, 89.21, 38.07 and 34.94 kDa, B*:* 32.92, 31.24, 14.42 and 13.56 kDa). Intergroup comparison of these protein bands reflects the difference in the expression level. These bands could not be compared and analysed with control subjects as these proteins were not present in control subjects.

thesis mainly reflects end-stage liver disease, intestinal malabsorption syndromes and protein calorie malnutrition. Nephrotic syndrome and severe burns are the examples of albumin loss. The consequences of a decrease in serum albumin result in a shift of fluid from the intravascular to the interstitial space, resulting in intravascular volume depletion and oedema formation (12). Besides albumin, the protein profile varies considerably in patients, at different ages, suffering from anaemia. Expression of four protein fractions that fall between 36 kDa and 29 kDa in all the patients highlight the need to perform detailed study at protein levels using advanced techniques in proteomics. Previously, variations have been found in patients with leukaemia using paper electrophoresis. The serum proteins varied from normal control levels within the normal range but there were large variations with low albumin and gamma globulin (14).

Serum protein electrophoresis (SPEP) was used to test the CLL patients and was useful in reporting that a higher proportion of the patients under study with hypergammaglobulinaemia were black, and patients with hypergammaglobulinaemia and monoclonal gammopathy were more likely to die within the first year. However, no association was found between SPEP pattern and a clinical staging classification for CLL (15).

These results provide preliminary information about the serum protein profile of patients with leukaemia. However, using the latest and advanced techniques in proteomics, it is possible to identify and sequence the proteins that alter in patients with leukaemia. It is therefore suggested that in order to define a marker protein and changes in the regulatory proteins, the studies should be expanded at transcriptional and translational level. Further, a comparative analysis is mandatory to study the effectiveness of different drugs or treatment methods used for treatment of leukaemia.

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