A Study of Serum Protein Electrophoresis in Patients with Multiple Myeloma

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ABSTRACT:

BACKGROUND:

Multiple myeloma (MM) is caused by the malignant proliferation of plasma cells through the bone marrow. The clinical features are due to disordered immunoglobulin synthesis and/or secretion from the cell. The amount of protein in the vascular compartment depends on the balance between the rate of synthesis and catabolism or loss. Electrophoresis is a technique that separates compounds such as proteins according to their different electrical charge. In this way, five main groups of proteins namely albumin and the α_1, α_2, β, γ-globulins, may be distinguished after protein staining and may be visually compared with those in a normal control serum.

OBJECTIVE:

To evaluate total serum protein, serum albumin, serum globulin, and serum protein electrophoresis in Iraqi MM patients.

PATIENTS AND METHODS:

This study was conducted during the period from August 2012 until the end of November 2012. The patients were admitted to Medical City Hospital and all the measurements were performed in the Medical Teaching Laboratories in Baghdad. Total serum protein was measured by colorimetric Biuret method, while serum albumin was measured by bromocresol green method, serum globulin, and serum protein electrophoresis were measured in 25 patients with MM; their age range was (50-70) years and compared with 25 healthy controls.

RESULTS:

Total serum protein and serum globulin were significantly increased (86.76±17.05 g/l and 55.36±20.54 g/l respectively) in patients with MM, as compared with their controls, (P=0.0001). While a significant decreased was found in serum albumin (29.6±8.77 g/l) and albumin to globulin ratio (0.64±0.36 g/l) for patients with MM as compared with their controls, (P=0.0001).

CONCLUSION:

Abnormal concentrations of total serum protein, serum albumin, serum globulin, and M-band are associated with MM.

KEY WORDS: multiple myeloma,protein electrophoresis,total serum protein,albumin.

INTRODUCTION:

Multiple myeloma (MM) is a malignant disorder characterized by proliferation of single clone of plasma cells derived from B-cells in the bone marrow [(1)].

MM is a plasma cell malignancy that accounts for <1% of all cancers and for ~10% of the hematological malignancies. MM primarily affects older individuals with the median age of onset of ~65–70 years [(2)].

The most common symptoms are fatigue, bone pain, and recurrent infections [(3)]. MM is a neoplasia of plasma cells, hallmarked by tumor cell tropism for the bone marrow and production of monoclonal immunoglobulin (Ig) detectable in serum and/or urine [(4)]. The pathophysiology of MM begins with cytogenetic changes that occur in the plasma cell lineage within the bone marrow. Monoclonal expansion of myeloma plasma cells within the bone marrow interferes with the production of normal blood cells. Myeloma cells produce abnormal immunoglobulin (M protein), light chain proteins (κ and λ), and other factors, such as cytokines. Excessive M protein causes hyperviscosity of the blood; whereas excessive light chains cause end organ damage for example renal failure. Lesions of bone are largely caused by the release of cytokines that promote bone resorption via upregulation of osteoclast activity, differentiation, and maturation [(5)]. Unrestrained

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osteoclast activation leads to the release of mediators that stimulate further clonal proliferation of myeloma cells and subsequent tumor growth [6]. The result is a vicious cycle of bone destruction and tumor growth, leading to further bone destruction.

Amyloidosis is a rare complication of MM. It usually occurs in individuals whose plasma cells produce only Ig light chains. These Bence-Jones proteins combine with other serum proteins to form amyloidal protein [7].

Electrophoresis may be defined as the separation of charged particles in a uniform electric field. For a particular system of electrophoresis, the voltage is held constant as are the pH and ionic strength of the suspending medium [8].

The aim of the present study was to evaluate total serum protein, serum albumin, serum globulin, and serum protein electrophoresis in Iraqi MM patients.

PATIENTS AND METHODS:

This study was conducted during the period from August 2012 until the end of November 2012. The patients in this study were admitted to Medical City Hospital. They were diagnosed as MM patients after full investigations including bone marrow aspiration and biopsy. All the tests were performed in the Medical Teaching Laboratories in Baghdad/ proteins unit. Five milliliters of venous blood were obtained from 25 patients with MM and 25 healthy individuals as control group. Their age range was (50-70) years. Blood samples were transferred into plain tube, allowed to stand for 15 minutes at room temperature, centrifuged at 3500 rpm for 10 minutes. The resulting serum was separated and used for measurements of total serum protein, serum albumin, serum globulin, and serum protein electrophoresis. All patients were not subjected to any kind of treatments.

Measurements:

A- Total serum protein was measured by colorimetric Biuret method. Copper salts in alkaline medium react with the peptide bonds of the protein producing a violet color which is proportional to the amount of protein present [9].

(Normal value of total serum protein = 38-44 g/l).

B- Serum albumin was measured by bromocresol green (BCG) [10]. The measurement of serum albumin is based on its quantitative binding to the indicator (3, 3', 5, 5'-tetrabromo-m-cresol sulphonphthalein). The albumin BCG-complex absorbs maximally of 578 nm.

(Normal value of serum albumin = 66-82 g/l).

C- Serum globulin was measured using the following relation:

Total serum protein = Albumin + Globulin.

D- Serum protein electrophoresis was measured using the method of Bence-Jones protein (BJP). Cellulose acetate membranes are indicated for use in the electrophoretic separations of serum proteins. Electrophoretic separation exploits the speed of migration as determined by the protein charge. A typical protein bands indicate clinical significance [11].

Statistical Analysis:

All the statistical analysis was done by using SPSS program (version-10) and Excel application [12]. The suitable statistical methods were used in order to analyze and assess the results, they include the followings:

1- Descriptive statistics:

A) Statistical tables including observed frequencies and their percentage.

B) Summary statistic of the readings distribution (means ± SD).

C) Graphical presentation by (Bar–chart).

2- Inferential statistics: These were used to accept or reject the statistical hypotheses, they include the followings:

A) Chi-square (χ^2).

B) Student test (t-test). Data were expressed as (means ±SD); statistical significance was set at $P < 0.05$.

RESULTS:

Table (1) shows a significant increase in total serum protein and serum globulin in MM patients group, while a significant decrease was found in serum albumin and albumin to globulin (A/G) ratio as compared with their controls. The other protein fractions (α_1, α_2, β, and γ) were measured and compared with their healthy controls, table (2). When used with M-band, two groups can be identified: positive and negative M-band as compared with their healthy controls, table (3).

Table 1: Total serum protein, serum albumin, globulin, and (A/G) ratio in MM patients group and their controls.

Clinical data	Patients (n=25)	Healthy controls (n=25)	P value
Mean Total serum protein (g/l)	86.76±17.05	68.0±7.5	0.0001
Mean Serum albumin (g/l)	29.6±8.77	40.4±3.69	0.0001
Mean Serum globulin (g/l)	55.36±20.54	27.6±7.78	0.0001
Mean (A/G) ratio	0.64±0.36	1.59±0.52	0.0001

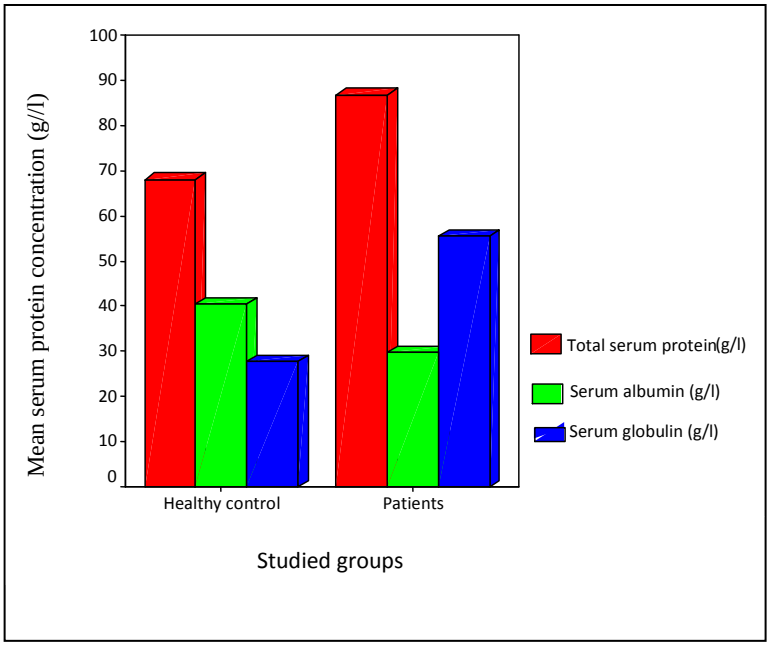

Figure 1: Total serum protein, serum albumin, and globulin in MM patients group and their controls.

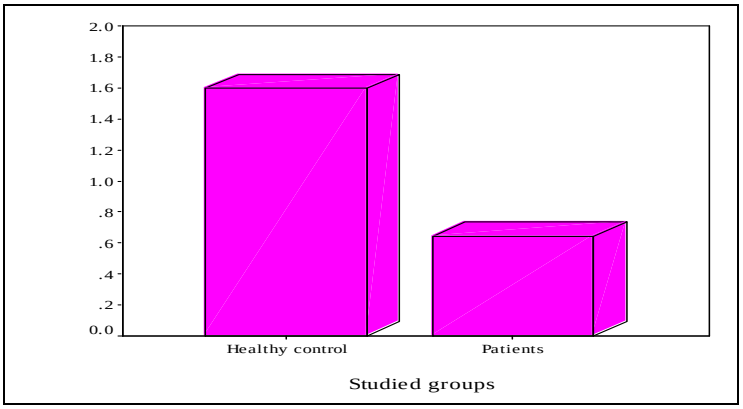

Figure 2: (A/G) ratio in MM patients group and their controls

Table 2: Serum protein electrophoresis for MM patients group and their controls.

Protein electrophoresis	Patients (n=25)			Controls (n=25)	Chi-square	P value
	Normal	Increased	Decreased	Normal		
Albumin	9 (36%)	-	16 (64%)	25 (100%)	19.862	0.001
α-$_1$	12 (48%)	1 (4%)	12 (48%)	25 (100%)	14.625	0.001
α-$_2$	9 (36%)	8 (32%)	8 (32%)	25 (100%)	19.862	0.001
β	25 (100%)	-	-	25 (100%)	0.556	0.456
γ	11 (44%)	14 (56%)	-	25 (100%)	11.647	0.001

Table 3: M-Band in study groups.

M-Band	Patients (n=25)	Controls (n=25)
Positive	16 (64%)	-
Negative	9 (36%)	25 (100%)

DISCUSSION:

Multiple myeloma is the most common primary cancer of the bones in adults [13]. Only marked changes of major constituents, such as albumin and Igs, are likely to alter total protein concentrations significantly. Low serum albumin levels may also be related to patient nutritional and performance status [14].

This study found that the serum albumin level reflected the severity of multiple myeloma, high concentration of serum globulin, and total serum protein all of which were significantly related to low albumin levels, are known to be associated with disease severity in multiple myeloma [15]. Also, most individual proteins a part from albumin contribute little to the total protein concentration; therefore, quite a large percentage change in the concentration of one may not cause a detectable change in the total protein concentration. Raised plasma total protein concentrations as shown in present results, may be due to loss of protein-free fluid, or excessive stasis during venepuncture, or a major increase in the concentration of one or more of the Igs, including paraproteins. A low plasma albumin concentration may be due to dilution or redistriction. Hypoalbuminemia may therefore be due to malnutrition, or impairment of synthesis. Normally there is a little more albumin than globulin and the ratio is greater than 1. A ratio less than 1 or much greater than 1 give clues about problems in the body. However, changes within the different globulin classes cannot be evaluated unless serum protein electrophoresis is performed [16].

The biochemical changes include the stimulation of synthesis of the so-called acute phase proteins, with a rise in the α_1-globulin and α_2 - globulin fractions. The plasma concentrations of these proteins reflect the activity of the inflammatory responses, and increased plasma viscosity characteristic of such response [17].

β_2-microglobulin is a low molecular weight protein that form a part of the human lymphocyte antigen on the surface of all nucleated cells. The protein is readily filtered by glomeruli, and plasma concentrations are normally low. In mylomytosis, the plasma β_2-microglobulin concentration is an index of the extent of the disease and of the prognosis [18].

CONCLUSION:

Low serum albumin levels in MM patients are associated with clinical factors indicative of increased disease severity.

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