Journal of Research in Biology

ISSN No: Print: 2231 –6280; Online: 2231- 6299

An International Scientific Research Journal

Original Research

Biochemical investigation - diagnostic tools of multiple myeloma

Authors:
Muthukumar N[1], John De Britto A[2], Harry Thomas Rodriquez A[3], Rekha Sunder[4].

Institution:
1. Associate Professor, Department of Pharmaceutical Biotechnology, Chilkur Balaji College of Pharmacy, Hyderabad.

2. Associate Professor, Department of Botany, St. Xaviers College, Tirunelveli.

3. Research Scholar, Department of Botany, St. Xaviers College, Tirunelveli.

4. Assistant Professor, Department of Pharmacology, Chilkur Balaji College of Pharmacy, Hyderabad.

ABSTRACT:

In this study biochemical technique was used as supportive tool for early diagnosis of multiple myeloma suspected cases. Samples of suspected cases were collected from histopathological diagnostic center. Serum calcium, serum albumin and Total protein were estimated. Bence jones protein was estimated in urine sample. Gel Electrophoresis was employed to resolve serum protein according to their molecular weight. The serum calcium level was very high (14.9 – 18mg/dl) than normal (9 -11mg/dl). When compared to normal serum albumin value, the test serum albumin level was decreased (2.7 – 3.2mg/dl). The total protein was increased because of the accumulation of para protein in blood stream. Light chain of immunoglobulins found as bence jones protein in the urine sample. The gel electrophoresis image revealed that the intensity of monoclonal protein ('M' Protein) was higher than other separated bands. The results suggested that this low cost biochemical technique may be used for the diagnostic purpose of Multiple myeloma at the earlier stage.

Keywords:
Multiple myeloma, biochemical investigation and Bence jones proteins.

Corresponding author:
Muthukumar Nallathambi.

Email:
muthukumar.nallathambi@gmail.com

Article Citation:
Muthukumar N, John De Britto A, Harry Thomas Rodriquez A and Rekha Sunder.
Biochemical investigation - diagnostic tools of multiple myeloma
Journal of Research in Biology (2014) 4(5): 1381-1386

Dates:
Received: 05 Dec 2012 **Accepted:** 02 Jan 2013 **Published:** 12 Jul 2014

Web Address:
http://jresearchbiology.com/documents/RA0459.pdf.

Journal of Research in Biology

INTRODUCTION

Multiple myeloma is a type of the blood cancer in which plasma cells are white blood cells that develop from B-lymphocytes in the bone marrow. Their normal function is to produce and secrete an antibody that maintains immunological function. Plasma cells that spread in the body become malignant, gets escaped from the normal growth controls and produce more cancerous cells that accumulate in the bone marrow. In some areas, the cancerous cells may grow rapidly and form a mass of cell called Plasmacytoma. If this tumors cell occurs in only one area it will be mentioned in the name of solitary Plasmacytomas. In almost all the cases, multiple plasmacytomas are found hence it is called by "multiple myeloma". The increase of myeloma cells in the bone marrow can have many serious effects in the body, including bone destruction, anemia, kidney dysfunction and high level of blood calcium. Bone fractures are dangerous as they occur in the spinal column. These fractures can compress or damage nerves and in some cases it causes paralysis. (Sirohi and Powles, 2004).

The development of multiple myeloma is associated with various chromosomal (cytogenetic) abnormalities, including extra or missing copies of particular chromosomes, and deletions or rearrangements of chromosome segments. The most common rearrangements involve genes that encode antibody proteins and genes that regulate cell growth oncogenes (Diane *et al*., 1997). Some common deletions involve genes that inhibit abnormal cell growth (tumour suppressors). The pathogenesis of multiple myeloma is thought to begin with genetic abnormalities that allow plasma cells to multiply uncontrollably and deposit in the bone marrow. In bone marrow, the malignant cells strongly attach with them and mix with stromal cells, collectively referred as the "Bone marrow microenvironment". The stromal cells and proteins contribute to the spread of cancer cells rapidly (Samson, 2001).

Myeloma cells stimulate stromal cells to form cytokines, which stimulates the growth of the myeloma cells and prevent a normal cell-death process known as apoptosis. Stromal cells, stimulate myeloma cells to produce growth factors that uplift the development of new blood vessels, or angiogenesis. Interactions between myeloma cells and the bone marrow microenvironment also organise high level formation of cytokines and other factors that activate bone-destroying cells called osteoclasts. Normal plasma cell makes antibodies or immuonglobulins (lg) that help to fight disease. Two long "heavy" chains and two shorter "light" chains are combined to form immunoglobulins. Myeloma cells also produce immunoglobulins, but these cells are monoclonal in nature. They also produce the same immunoglobulin protein in large accounts. The monoclonal (M) protein does not help to protect the body from infection. In addition, M protein can build up in organs such as the kidneys, causing serious damage overtime. Myeloma cells produce detectable levels of M protein (Gahrton *et al*., 2005). These patients are considered to have "non secretary" disease. Multiple myeloma can cause calcium elevation, renal dysfunction, anemia and bone disease. This sign and symptoms is commonly referred to as "CRAB" (Drewinko *et al*., 1981; Ashraf *et al*., 2003).

Research suggests possible associations with a decline in the immune system, genetic factors, and certain occupations exposure to certain chemicals and radiations. However there is no strong evidence to identify the prognostic factors. Hence the objective of study was carried out to concentrate and identify the prognostic and diagnostic factors, under biochemical investigations of human samples of multiple myeloma.

MATERIALS AND METHODS

Collection of samples

Samples were collected from SSS cancer Research Centre, Tirunelveli. Totally Six number of

samples were used for experimental studies.

Estimation of Serum Calcium

Preparation of dye solution (reagent 1)

Dye solution is prepared by adding 0.18 gm of methyl thymol Blue sodium salt, 6 gm of polyvinyl pyrrolidine, 7.2 gm of 8-hydroxy quinoline and 10 ml of concentrated Hydrochloric acid. Finally make up 1 litre with water.

Preparation of diluents (reagent 2)

Add 24 gm of sodium sulphite to 220 ml of ethanolamine. Finally make up 1 litre with water.

Preparation of working reagent

Mix equal volume of reagent (Ashraf *et al*., 2003; Sirohi and Powles, 2004)

Calcium standard (Stock solution) (10mmol/litre)

Place 1gm of dry calcium carbonate in a 1 litre of volumetric flask. Add 7.3 ml of concentrated Hydrochloric acid and makeup to 1 litre with distilled water.

Calcium Standard (working solution) (2.5 mmol/ litre)

To 25 ml of 10 mmol / litre standard solution, add 75 ml of distilled water.

Procedure

To 50 μl of test sample and add 3ml of working reagent in 6 test tubes. 5 μl of 2.5 mmol/litre standard and add 3ml of working reagent in another test tube. Read the absorbance against the working reagent as blank at 612 nm.

The concentration of calcium is given as =

$$\frac{Ab.Test}{Ab.Std} \times 2.5(mmol/litre)$$

Estimation of Serum albumin

Preparation of Bromocresol green reagent

Take 4.42 gm of succinic acid, 54 mg of Bromocresol green sodium salt and 50 mg of sodium azide in about 450 ml of water. Adjust PH to 4.2 using 5% succinic acid solution in water, finally make up to 500 ml.

Standard Protein

A Solution of Bovine Serum albumin containing 100 mg protein per ml.

Procedure

Take a test tube add 25μl of water, it acts as a blank and in the second test tube and add 25μl of standard protein solution. Add 25μl of serum to the other 6 tubes. Finally in all the tubes add 0.5 ml of Bromocresol green reagent. Mix and read the optical density at 628 nm.

Albumin in g/100 ml =

$$\frac{O.D\,of\,albu\min - O.D\,of\,blank}{O.D\,of\,s\tan dard - O.D\,of\,blank} \times \frac{0.05 \times 100}{1000 \times 0.025}$$

Estimation of Total Protein

Preparation of Alkaline sodium carbonate solution (reagent 'B')

Dissolve 2 gm of a Sodium Carbonate in 100 ml of 0.1N sodium hydroxide solution, prepare freshly

Preparation of coppersulphate – sodium potassium tartrate solution (reagent 'C')

Dissolve 0.5 gm / litre of copper sulphate in 1% of sodium potassium tartrate solution, prepare freshly.

Preparation of Alkaline copper reagent (reagent 'D')

Mix 50 ml of reagent 'B' and 1ml of reagent 'C' only on the day of use.

Procedure

Take 6 test tubes transfer 1 ml of 1N NAOH solution and heat up to 150°C

Suspend 1 ml of protein sample into the above 6 test tubes and wait for 4 to 5 minutes. Add 5 ml of reagent 'D' (alkaline copper sulphate) into 6 test tubes mix properly and leave this mixture at room temperature for 10 minutes. Add 0.5 ml of Folin-Ciocalteu reagent rapidly with immediate mixing. Leave it for 30 minutes, therefore measure the absorbance of solution at 660 nm.

Bence - jones protein identification

Bradshaw's test

Few ml of urine was mixed with few ml of concentrated HCL in a test tube Bence - Jones protein is precipitated by the acid giving a white ring at the junction of two fluids. The heating test should be used to confirm since slightly positive test may be obtained. If other proteins such as albumin are present in considerable amount with Bence – Jones protein the test will still be positive after diluting the urine.

Heating Test

Urine sample was taken in test tube and filled upto two thirds of its volume. Test tubes were placed in water bath and heated slowly. A thermometer was dipped in water bath and the appearance of urine sample was noted carefully between the ranges of temperature 40 – 60°C. A slight cloud was appeared when the urine sample was heated to the boiling point. A few drops of acetic acid was added and this will dissolve phosphates and that was separated out. If a precipitate forms at the boiling point, it is due to albumin. The urine sample was boiled with few drops of dilute acetic acid, and it was filtered rapidly and repeat the test on the cooled filtrate.

RESULTS AND DISCUSSION

Serum Calcium

In multiple myeloma, the bone destruction occurs and the calcium present in the bones was mobilized to the serum. So the level of serum calcium is high in multiple myeloma and it leads to the kidney failure (or) renal dysfunction. The serum calcium level found to be markedly increased in the six collected samples. The normal serum calcium level is 9-11 mg/dL but the collected samples showed a much increased value (14.9-18 mg/dL). Hypercalcemia has been associated with multiple myeloma and it is a main marker in the identification of multiple myeloma (Gindler and King, 1972).

Serum albumin

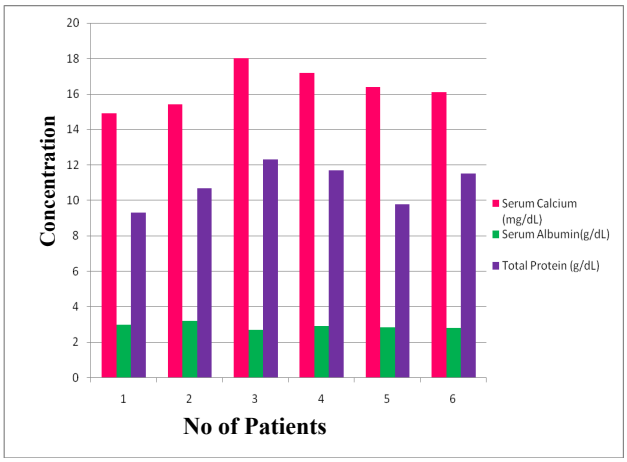
Six samples were used for the analysis of serum albumin. The individual results were found out and the results could be compared and tabulated. Generally the serum albumin level was decreased in multiple myeloma cases, because of the excretion of excess protein in urine. The data given in table no.1 revealed that the serum albumin level was found to be markedly decreased. The range of decreased level was 2.7 – 3.2 g/dL (Figure -1). The normal range of serum albumin was 3.5 – 5.5 g/dL (Samson, 2001).

Total Protein

Six experimental samples were used for the analysis of total protein and the individual results were found out and tabulated. The total protein level was increased in multiple myeloma, because of the accumulation of para protein in blood serum. The data given in table no.1 revealed that the total protein level were found to be markedly increased in the all six collected samples. The increased range of collected samples were 9.3 – 12.3 g/dL, and the normal level of total protein ranges being 6-8 g/dL (Lowry *et al*., 1951; Samson, 2001).

Table No.1.

Patients	Serum Calcium (mg/dL)	Serum Albumin (g/dL)	Total Protein (g/dL)
1	14.9	2.98	9.3
2	15.4	3.2	10.7
3	18	2.7	12.3
4	17.2	2.93	11.7
5	16.4	2.87	9.8
6	16.1	2.82	11.5



Bence-Jones protein

The protein content of the sample was precipitated when it has been subjected to slowly increased temperature from 40-60°C and subsequently the precipitate has redissolved at the boiling temperature of 100°C. The same precipitate reappeared on cooling. Bence Jones protein has a different property compared with other proteins, because it precipitates at the temperature of 40-60°C and the precipitate re- dissolves at 100°C. The same precipitate reappears when it is subjected to cooling. It is the only prominent confirmative test for the identification of Bence Jones protein. This biochemical analysis is used for finding of any such Bence Jones protein in the urine sample (Gindler *et al*., 1972 and Meyler, 1936). Multiple myeloma is a proliferation of plasma cell and most myelomas produce complete immunoglobulin molecules. Excessive amounts of immunoglobulin fragments were also produced in all cases. Light chains were found in urine known as Bence-Jones protein.

In about all six experimental cases excess light chain is the only abnormality in protein which indicates the presence of myeloma. These can be considered as biochemical identification for multiple myeloma.

Serum protein electrophoresis

The plasma cells produce homogenous immunoglobulins that are identified in serum protein electrophoresis as a mono clonal protein. So the mono clonal protein could be considered as a most characteristic finding of multiple myeloma in serum protein electrophoresis. In normal individuals the band is much lower and broader but in multiple myeloma the 'M' protein shows narrow intense band compared with the normal. (O'Connell *et al*., 2005).

DISCUSSION

This study provided the preliminary diagnosis of Multiple myeloma by using fundamental biochemical techniques. Myeloma cells produce antibodies similar to that of normal plasma cells but they are monoclonal in nature. In multiple myeloma cases the serum calcium level is high (14.9 -18mg/dL) as compare to normal case (9-11mg/dL). Myeloma cells cause a massive destruction of bone marrow cells and elevate blood calcium level. The normal range of serum albumin is 3.5 -5.5g/dL .

Whereas in multiple myeloma cases it was found to be decreased (2.7-3.2g/dL). Protein in blood stream plays an important role in determining multiple myeloma severity. In this case the total protein level was high (9.3-12.3g/dL) than the normal case (6-8gm/dL) (Samson, 2001). Identification of Bence - Jones protein in urine sample is a preliminary confirmation test for multiple myeloma. In all cases a precipitate was appeared in urine sample at a temperature between 40-60°C and redissolved at 100°C. Monoclonal proteins secreted by plasma cells were detected by using gel electrophoresis. The narrow intense band was detected in multiple myeloma sample whereas the band was broader in normal case.

Experimental samples showed increased serum calcium, total protein level and decreased serum albumin level. Bence Jones protein precipitate was present in all experimental samples. Compared to all experimental samples, case no:3 is the mostly affected case, due to the increased level of serum calcium, total protein and decreased level of serum albumin than other cases. The median age at diagnosis of multiple myeloma was above 65. Hence most of the patients die prior to identify the clear symptoms. In order to identify the stage or severity of multiple myeloma, there is a need for essential and simplified biochemical technique.

CONCLUSION

There fore based on our investigations we concluded that the various types of biochemical techniques have been used for this project gave a good supportive tool for the identification of multiple myeloma patient severity and we believe that this low

cost method is useful for the diagnostic purpose of multiple myeloma at earlier stage.

REFERENCES

Ashraf M, Abdullah J, Ariff AR, Biswal BM, Mokhtar I and Othman NH. 2003. Unilateral Exophthalmos caused by an extramedullary plasmacytoma: A Case Report. Malaysian journal of medical sciences. 10(1): 95-99.

Sirohi B and Powles R. 2004. Multiple myeloma. The lancet. 363(9412): 875-887. www.the lancet.com.

Drewinko B, Alexanian R, Boyer H, Barlogie B and Rubinow SI. 1981. The growth fraction of human myeloma cells. Blood. 57(2): 333-338.

Diane F. Jelinek, Kjersti M. Aagaard-Tillery, Bonnie K. Arendt, Arora T, Tschumper RC and Westendorf JJ. 1997. Differential Human multiple myeloma cell line responsiveness to interferon- alpha. Analysis of transcription factor activation and interleukin 6 receptor expression. The Journal of clinical investigation. 99(3):447-456.

Gahrton G, Brian GM Durie and Diana M. Samson. 2005. Multiple myeloma and related disorders. New England Journal of Medicine. 352(23): 2460-2461.

Samson D. 2001. Diagnosis and management of multiple myeloma. British journal of Haematology. 115(3): 522-540.

Gindler M and King JD. 1972. Chemical method for determination of calcium in serum. Am. J. Clin.Path., 58: 376-382.

Lowry OH, Rosebrough NJ, Farr AL and Randall R J. 1951. Protein measurement with the folin phenol reagent. J.Biol.Chem., 193(1): 265-275.

Meyler L MD. 1936. Bence- jones' protein. Arch Intern Med. 57(4):708-713.

O'Connell TX, Horita TJ and Kasravi B. 2005. Understanding and interpreting serum protein electrophoresis. Am Fam Physician. 71(1): 105-112.