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## **Serum Protein Electrophoresis Patterns in Sero-Positive Human Immunodeficiency Virus- Individuals with Chronic Kidney Disease**

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### **Abstract**

Various studies have reported a much higher incidence of monoclonal gammopathy among HIV–infected patients and also that serum protein electrophoresis patterns would be abnormal in HIV subjects. Hence this study was undertaken to study the pattern of serum protein electrophoresis in HIV- seropositive individuals with chronic kidney disease. Serum protein electrophoresis was performed in 100 HIV- seropositive individuals with chronic kidney disease and in 100 HIV-seronegative chronic kidney disease patients as controls. The mean age % HIV- seropositive individuals with chronic kidney disease was 57.2 years, with male-to-female ratio of 6:1. Of the HIV-seropositive patients, 42% showed polyclonal hypergammaglobulinaemia followed by nephrotic syndrome in 34% of patients. The HIV group also had hypo-gammaglobulinaemia in 8% of cases with only one case had monoclonal ‘M’ band. We found a remarkably high level (42%) of polyclonal hypergammaglobulinaemia in our patients compared with uninfected controls (28%).

**Key words:** HIV, electrophoresis, kidney, gammopathy

### **Introduction**

HIV/AIDS has now become a chronic treatable disease with far reaching economic and social consequences. The UNAIDS, the United Nations programme on HIV/AIDS, reports that India has the third-highest number of people living with HIV in the world with 2.1 million Indians accounting for about four out of 10 people infected with the deadly virus<sup>1,2</sup>. As per India HIV estimation 2015 report, National adult (15-49 years) HIV prevalence in India is estimated at 0.26% in 2015. The adult HIV prevalence is estimated between 0.22 to 0.30% among males and females. Prevalence is also high in the 15-49 age groups, indicating that AIDS still threatens the cream of society, those in the prime of their working life<sup>3</sup>.

As human immunodeficiency virus infected patients live longer while receiving antiretroviral therapy, kidney diseases have emerged as significant cause of morbidity and mortality among these patients. The incidence and spectrum of kidney diseases in HIV-infected patients have been altered by the widespread use of antiretroviral therapy<sup>4</sup>. Though the risk of End stage renal disease has been reduced and survival rate while undergoing dialysis has increased, risk factors for kidney disease are highly prevalent among HIV-infected patients and kidney disease remains a significant cause of morbidity and mortality.

Apart from the impaired renal function, HIV-infected patients are frequently associated with elevated serum protein, particularly the gammaglobulin fraction. Antibodies produced during HIV infection are polyclonal in nature and hypergammaglobulinemia is common which is characteristic of chronic inflammatory condition. Many conditions can cause an increase in the gamma region, but those which cause a homogenous spike like a peak in the gamma globulin zone, are of special interest<sup>5</sup>. This condition is called monoclonal gammopathies- a

debilitating malignancy, resulting from the proliferation of a single, usually malignant clone of plasma cells which produce either a single class of intact immunoglobulins, heavy chains or light chains or both. These proteins are called para proteins or M (monoclonal) proteins<sup>6</sup>.

The spectrum of disease ranges from monoclonal gammopathy of unknown significance (MGUS) to plasma cell leukaemia. It is very important to distinguish between Multiple Myeloma from MGUS due to the general nature of manifestation and moreover, the need for the therapy is also very much different in these two conditions.

Various reports indicate a much higher incidence of monoclonal gammopathy among human immunodeficiency virus (HIV)-infected patients and a significantly younger age at diagnosis. Recent guidelines from the International Myeloma Working Group have recommended the use of serum protein electrophoresis, immunofixation electrophoresis (IFE) and Free light chain assay (FLC) as the screening panel unless primary amyloidosis is suspected.

Evaluation of patients with chronic kidney disease typically includes serum and urine protein electrophoresis. As renal failure is a common mode of presentation in multiple myeloma and serum protein electrophoresis is valuable screening tool for evaluation of these patients with renal failure<sup>7,8</sup>. Serum electrophoresis (SPEP) and urine electrophoresis (UPEP) are done for baseline assessment of the amount and type of the myeloma, serial monitoring to document the level of response and for assessment of possible disease progression or relapse.

Hence this study was to undertaken to review the serum protein electrophoresis patterns in HIV seropositive patients with chronic kidney disease (CKD).

## **Subjects and Methods**

A retrospective review of all the patients who had been referred for electrophoresis (SPEP) to Institute of NephroUrology, Bangalore from January 2016 to December 2016 was included in the study.

Baseline demographics, clinical history of the patients along with routine biochemical parameters and HIV enzyme linked immunosorbent assay (ELISA) results at the time of presentation/biopsy were included in the study. Patients without HIV results were excluded. Creatinine clearance (Crcl) was estimated in all the patients by Modification of Diet in Renal Disease (MDRD) formula.

Normal renal function was defined as  $GFR \geq 90 \text{ml/min/1.73m}^2$ , estimated using the MDRD formula. The definition of hematuria was  $\geq 5$  RBCs per high power field in the urinary sediment. Nephrotic syndrome was defined as proteinuria more than 3.5g/day with hypoalbuminemia, oedema and hyperlipidemia. Nephritic syndrome was defined as hematuria (usually with dysmorphic RBCs/ RBCs casts) with proteinuria less than 3.0g/day, hypertension and elevated serum creatinine. Chronic renal failure was defined as severe irreversible kidney damage and serum creatinine levels persistently above 1.5mg/dl.

For serum electrophoresis 5ml blood was drawn in plain tubes and sera were separated after the sample had clotted and stored till further analysis. Electrophoresis was run in batches on Helena Titan gel electrophoresis chamber. Sera were applied with the help of applicator on an agarose gel membrane as a support medium. Tris-Barbital buffer was set at pH 8.6. Line of application was directed towards cathode, and a constant volt of 80 Volts was applied for 30 minutes. Different protein fractions moved depending upon charge, molecular weight and size. Strips were dried in hot air oven. Then staining was done with Acid Blue stain for 5 minutes, followed by destaining to remove excess stain for 5 minutes. Densitometric scanning for quantification of different protein fractions and clinical correlation of the result interpretation was done using Platinum software supplied from Helena Biosciences (UK).

Serum total protein and albumin estimation were carried out spectrophotometrically, based on dye binding principle. The HIV test (antigen detection) was also done in batches by enzyme linked immunosorbent assay (ELISA) method. The HIV testing was done following standard NACO-approved guidelines.

## Results

A total of 100 patients who were HIV seropositive with chronic kidney disease were included in the study. Out of 100 patients 86 were male and 14 females with mean age of  $57.2 \pm 13.86$  years. An aged and sex matched 100 patients with HIV seronegative with chronic kidney disease were considered as controls. Major diseases diagnosed were subacute/ chronic infections, nephrotic syndrome, autoimmune disorders, malnutrition, liver disorders and paraproteinaemias. Results of serum total protein and albumin estimation by spectrophotometric analysis revealed broad results of total protein, albumin and total globulin. They revealed hypoalbuminaemia or hypergammaglobinaemia. These results were less diagnostic as compared to serum protein electrophoresis. The demographics and lab results are shown in table-1.

There was a decrease in all parameters suggesting hemodilution or malnutrition in five cases. An increased alpha-1 and alpha-2 globulins, decreased albumin and transferrin, consistent with acute inflammation was seen in five cases. Decreased albumin and haptoglobin with a polyclonal increase in gammaglobulins and beta-gamma bridging consistent with liver disease was present in seven cases. Very low albumin, transferrin and with increased alpha-2 globulin suggestive of protein loss pattern due to renal disease (nephrotic syndrome) in twenty four cases. Hypogammaglobulinemia due to immune deficiency, chemotherapy or B-cell neoplasm or due to corticosteroids and immuno suppressive treatments was seen in six cases. Polyclonal increase in immunoglobulins was seen in the 44 cases (44%), compared with uninfected controls (28%). While one patient clinically suspected for monoclonal gammopathies revealed a pattern consistent with multiple myeloma. The remaining eight cases were unremarkable. The patients serum electrophoretic patterns distribution is shown in table-2 and Fig. 1.

Hypoalbuminaemia was due to nephrotic syndrome, cirrhosis liver, severe chronic malnutrition, and acute chronic infections or malignancy. Decrease in the alpha-1 globulin was because of hepatocellular insufficiency, malnutrition or protein loss, generally with concomitant decrease of albumin, alpha-2 and betaglobulins. Increase alpha-2 globulin mainly due to the inflammatory syndrome, by an increase of haptoglobin (the alpha-2 fraction is then greater than 15%), nephrotic syndrome by an often substantial increase of alpha-2 macroglobulin associated with hypoalbuminemia (due to urinary loss). Decrease in beta globulin was induced by hepatocellular insufficiency, malnutrition or protein loss related to a decrease in transferrin, complement-C3 consumption and also due to ageing of the serum sample. Hyper-betaglobulinemia was due to hypertransferrinemia, anemia or by increased beta lipoprotein and was also noted in cases with increased complement- C3 and inflammation.

Hypogamma-globulinemia usually secondary was associated with myeloma or due to corticosteroids and immune-suppressive treatments, chemotherapy or radiotherapy. While hypergamma-globulinemia was due to viral or bacterial infections, AIDS or autoimmune diseases. Monoclonal hypergammaglobulinemia showed sharp, narrow and homogeneous electrophoretic band, or bands if present under different polymerization forms, as a result of the presence of a monoclonal component. Oligoclonal hypergammaglobulinemia (several narrow and homogeneous bands) because of increase in some subclasses resulting in a particular oligoclonal pattern. These immunoglobulins correspond either to: auto-antibodies seen in some autoimmune diseases: rheumatoid arthritis, Sjogren's syndrome, lupus erythematosus. And also in individuals with HIV, viral hepatitis, meningitis, cytomegalovirus infections autoimmune responses in transplanted patients on immunosuppressive therapy

antibodies directed against viral proteins. The chart displays the percentage-wise distribution of the serum electrophoretic patterns.

## Discussion

With dramatic improvements in survival and disease progression in the era of combination antiretroviral therapy (ART), the risk for kidney injury has increased in HIV seropositive patients. Complications such as kidney, liver, and cardiac disease have largely replaced opportunistic infections as the leading causes of mortality in these individuals. A profound impairment of immune functions both the cellular and the humoral immune system is observed in human immunodeficiency virus seropositive patients, leading to severe depletion of lymphocyte functions and causing increased susceptibility to secondary and opportunistic infections<sup>9,10</sup>.

It has been reported that HIV seropositive patients have a significantly higher total protein level as compared to healthy individuals. Kapsenberg et al found a significant higher total protein levels amongst HIV positive pregnant women as compared to controls. These high levels of protein are due to hypergammaglobulinemia which is found among HIV seropositive patients followed by a significant decrease of the albumin fraction<sup>11</sup>.

The mechanisms inducing hypergammaglobulinemia in HIV infection are only partially known. The broad, diffuse increase in the gammaglobulin region is the result of the polyclonal plasma cell response to chronic antigenic stimulation. During an infection, the body responds by generating pathogen-specific cells or antibodies resulting in polyclonal hypergammaglobulinemia. Recently, it was proposed that the hypergammaglobulinemia in HIV infection can be caused by activated naive B cells, due to high and persistent antigen concentrations which present viral antigens in a BCR-independent manner. Activation driven by CD4<sup>+</sup> T cells, monocytes, and natural killer cells through CD40-CD40 ligand interaction and an inappropriate cytokine supply may play a significant role in inducing abnormal differentiation of B cells. Naive B cells from HIV-1-infected patients exhibited abnormal expression of the activation/differentiation markers CD70 and leukocyte-associated Ig-like receptor. In addition, HIV-1 itself may directly affect B-cell activation and dysfunction, inducing the appearance of a subset of CD21<sup>-</sup> B cells which have been proposed to contribute to increased antibody production. Naive B cells from patients which have been activated showed a significant increase in the intracellular immunoglobulin G content *ex vivo* and this activated phenotype correlated to hypergammaglobulinemia and to the ability of naive B cells from patients to secrete IgG *in vitro*. The reduction of memory B lymphocytes in HIV-1 infection correlates with defective humoral immunity and that hyperactivated naive B cells may represent the source of abnormal IgG production in HIV-1 infection<sup>12,13,14,15</sup>. A recent work by Hunziker et al has suggested that naive B cells represent an important source of hypergammaglobulinemia and autoantibody production in chronic viral infections. Naive B cells have been recently suggested as the major source of hypergammaglobulinemia in chronic viral infections. Hypergammaglobulinemia and defective humoral immunity are hallmarks of HIV-1 infection<sup>16</sup>.

Serum protein electrophoresis is a well established, inexpensive diagnostic test routinely used in clinical laboratories for screening protein abnormalities in various biological fluids (serum, urine, CSF). The technique resolves major proteins of serum into patterns that may be highly specific for some diseases. Serum protein electrophoresis is advised in any patient with an elevated total protein, especially those with elevated globulin level relative to albumin, or any signs and symptoms suggestive of an underlying plasma cell disorder such as multiple myeloma, Waldenstrom's macroglobulinemia, or primary amyloidosis. Many sub specialties include serum protein electrophoresis as a screening tool in the evaluation for numerous clinical conditions<sup>17,18</sup>.

Generally large percentage of HIV-seropositive patients show striking polyclonal hypergammaglobulinemia and/or oligoclonal banding but studies have reported different patterns of electrophoretograms in this population

ranging from visually unremarkable to showing only subtle abnormalities. A. E. Zemlin et al have shown that both hypoalbuminemia and hyperproteinemia associated with a polyclonal gammaglobulinemia in HIV-seropositive patients is the result of generalized B cell stimulation towards infection. The polyclonal hypergammaglobulinaemia correlated significantly with lower CD4+ counts. The evaluation of this gammaglobulin has been reported as a surrogate for HIV treatment monitoring, particularly in resource poor settings where CD4 and CD8 T cell monitoring is not available. Current literature also suggests that there is a difference in a density of the gammaglobulin and albumin fractions between HIV positive and HIV negative patients<sup>17</sup>.

Prevalence of monoclonal gammopathy in healthy subjects is between 1% and 2% and varies by age, while a reported risk of myeloma in HIV-seropositive patients is higher according to different studies. Occasionally polyclonal gammopathies can be large, producing bands that give an impression of monoclonality. Also due to profoundly immunosuppressed by their disease, acquired immune-deficiency syndrome (AIDS) patients have a complex pattern with occasionally massive polyclonal increases in gammaglobulin, often accompanied by oligoclonal, or occasionally monoclonal gammopathies<sup>18,19</sup>.

The HIV-seropositive patients also show hypogammaglobulinemia (usually less than 250 mg/dl of IgG) commonly seen in adults with lymphoreticular disorders, after chemotherapy/immunosuppressive therapy, as well as in hypoproteinemic states. Our study showed a remarkably high level (44%) of polyclonal hypergammaglobulinaemia in our patients compared with uninfected controls (14%) while 6% of cases showed hypogammaglobulinaemia. Only one case (1% of total) had monoclonal band though the reported cases of myeloma HIV-seropositive patients is higher according to studies from Fernando et al. Studies have also reported oligoclonal gammopathy with two, three, or more small discrete bands reflecting greater expansion of particular B-cell clones. Some of these bands have been shown to have specificity for human immunodeficiency virus antigens. Sinclair et al have reported a positive correlation between the presence of oligoclonal bands and the degree of anti-human immunodeficiency virus (HIV) antibody positivity<sup>20</sup>. In their study, they found that 43 per cent of patients who were HIV antibody positive had oligoclonal bands by serum protein electrophoresis. These patterns are the result of immune suppression due to decreased CD4 lymphocytes and extraordinary antigenic stimulation by the many infections<sup>21,22</sup>.

It is important to look for potential monoclonal gammopathy in a patient with an infectious disease as infections are a frequent feature of patients with multiple myeloma. Monoclonal Gammopathy of Undetermined Significance is known to have an increased risk of subsequent development of haematologic malignancies, especially multiple myeloma and it has been recently demonstrated that myeloma is always preceded by a MGUS phase<sup>23</sup>.

In our study all the patients presented with renal failure, majority of the cases had acute renal failure followed by chronic renal failure and nephrotic syndrome. The kidney acts as a filter, eliminating only a few molecules and leaving most of the proteins in the bloodstream. When monoclonal protein is present in serum, often the excess of free light chains will be found in the urine as Bence Jones protein, which appears as M spike. It is necessary then to confirm its presence and to determine its type by identifying which types of heavy chains and light chains are involved in its structure. Knowing the type of M-protein is important in establishing a diagnosis and in monitoring the patient. Up-to 50% of newly diagnosed patients present with decrease in GFR and many require dialysis. Despite progress in therapy regimes the median survival rates with conventional treatment remain no more than 2-3 years. Monoclonal gammopathies in AIDS patients have been seen together with myeloma and lymphomas. As acute renal failure is the most common renal disease preceding the diagnosis of myeloma, it is

necessary to look for potential renal impairment in these patients as reversal of renal function can be achieved with appropriate therapy and hemodialysis treatment<sup>24,25</sup>.

Our study has a number of flaws that make it difficult to reach firm conclusions regarding HIV-associated monoclonal gammopathy. Weaknesses include study's retrospective nature with small sample size and, there was no distinction between symptomatic or asymptomatic HIV cases. Also worth noting is that we identified one patient with M-paraproteinemia out of a clinic population of 200 patients including the controls. This certainly represents an underestimated prevalence of this condition in our clinic population, because serum protein electrophoresis testing is only performed in the event of a clinical suspicion.

### Conclusion

Only one patient of the HIV-positive group had a monoclonal band. Future longitudinal studies will be important to determine the prognostic value of these findings.

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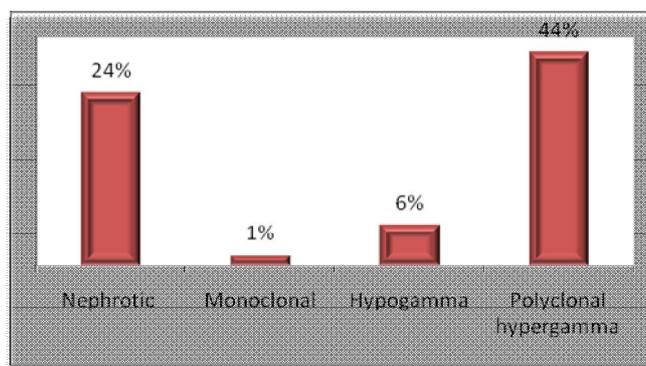
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**TABLE-1** showing the demographics and laboratory results

Demographics	CKD	CKD with HIV-seropositive
Age (years)	56.4 ± 12.5	57.2±13.86
Serum creatinine (mg/dL)	5.96 ± 3.45	6.5 ± 3.1
Hemoglobin (g/dL)	8.74 ± 2.9	10.02±1.6
Total protein (g/dL)	6.14 ± 2.2	6.87±2.07
Globulin (g/dL)	3.42 ± 1.9	3.9±1.3
Urine Protein (mg/dL)	233.5 ± 132.4	137.5±42.5

**TABLE-2** showing the patient distribution of different serum electrophoretic patterns.

Protein Electrophoresis Pattern	No. of Patients (%)
Nephrotic syndrome	24 ( 24%)
Monoclonal band	1 (1%)
Hypogammaglobulinemic	6 (6%)
Polyclonal hypergammaglobulinemic	44 (44%)



**Fig 1:** Graph showing the patient distribution of different serum electrophoretic patterns