# Serum Protein Electrophoresis: Any role in monitoring for Antiretroviral Therapy?

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## Abstract

**Background**: Developing world are always looking for monitoring tools during reagent shortage and equipments troubles which are very frequent. The aim of this study was to evaluate Serum Protein Electrophoresis (SPE) as a marker for assessing HIV treatment response.

**Methods**: A cross-sectional study was conducted with 220 participants in four distinct groups: Symptomatic HIV positive patients [specifically those on antiretroviral treatment (ART) versus those not on ART] asymptomatic HIV positive patients, and healthy blood donors. Five serum protein fractions (Albumin, Alpha-1, Alpha-2, Beta, and Gamma) were compared between these groups after measuring the density of the fractions.

**Results**: Concentration of gamma globulin was lowest among healthy blood donors, intermediate and comparable among asymptomatic HIV positive and symptomatic HIV positive on ART and highest among untreated symptomatic HIV positive. Concentration of gamma globulin was inversely correlated with the disease stage ($p < 0.001$).

**Conclusion**: In this study, conducted in a setting where the burden of infectious diseases is high, the density of gamma globulin and albumin fractions were significantly associated with HIV status, and among HIV positive patients, with stage of HIV disease and ART. These results suggest that the feasibility of using SPE for monitoring the response of ART in low resource settings should be further explored.

**Key words:** Serum Protein Electrophoresis (SPE); HIV; Anti-Retroviral Therapy (ARV); Mali

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## Introduction

Mortality and morbidity attributable to HIV/AIDS in Mali is considerable. Recent figures indicate approximately 11,000 deaths and 94,000 orphans in the country are due to HIV/AIDS[1]. In 2004, the Malian government launched a free antiretroviral therapy (ARV) program after surveys conducted in 2001 and 2006 indicated that prevalence of HIV infection in the general population was 1.7% and 1.3%, respectively[2]. Data from the Centers for Disease Control and Prevention (CDC) sentinel surveys indicateda spike in HIV incidence among female adolescents 15-19 years of age from 3.3% in 2002 to 4.1% in 2003[3].

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A fundamental lack of laboratory testing infrastructure and human resources make HIV diagnosis, treatment, and patient monitoring difficult in low-resource settings. Current standard clinical protocol for monitoring patients on antiretroviral (ARV) therapy dictates analysis of CD4 T cell counts, and plasma viral load. Since the availability of these complex and expensive laboratory tests remains limited in resource-poor countries, alternative approaches for monitoring of antiretroviral treatment have been explored in Mali as a surrogate where CD4 T cell count and plasma viral load tests are not available[4-8].

Serum protein electrophoresis (SPE) is designed to separate serum protein into five fractions (albumin, á-$_1$, á-$_2$, â and ã-globulin) to evaluate their individual concentration. HIV infection is frequently associated with elevated serum protein, particularly in the ã-globulin fraction[9-13]. Some studies have shown both hypo-albuminemia and hyper-proteinemia to be associated with a polyclonal ã-globulinemia in HIV seropositive patients as

compared to HIV seronegative patients [11, 13]. Polyclonal hyper ã-globulinemia has been reported as a characteristic of chronic inflammatory condition [14]. During an infection, the immune system reacts with the pathogen by generating pathogen-specific cells or antibodies. Immunoglobulin (Ig) is a component of ã-globulin, and HIV infection demonstrates serum hyper ã-globulinemia. The evaluation of this ã-globulin can be a surrogate for HIV treatment monitoring, particularly in resource-poor settings where CD4 and CD8 T cell monitoring is not available. Antibodies produced during HIV infection have been reported as polyclonal [10], and some of them may be directed to platelets leading to HIV-related thrombopenia, with no profound effect on virus replication [5] [6] [15] [16]. Current literature suggests that there is a difference in a density of the ã-globulin and albumin fractions between HIV positive and HIV negative patients [8,10]. The aim of this study was to compare the five serum protein fractions (albumin, á-$_1$, á-$_2$, â, and ã-globulin) between HIV seropositive and HIV seronegative blood donors. In a separate analysis, we compared protein fractions between symptomatic and asymptomatic HIV seropositive individuals to assess the impact of ARV treatment on the density of these five serum protein fractions. We further hypothesize that some potential serum protein fractions may be associated with the stage of disease.

## Methods

### Study design

In this cross-sectional study, the National Blood Transfusion Center (CNTS) of Bamako compared the serum proteins levels of two main groups of subjects: HIV seropositive patients and HIV seronegative patients. HIV seropositive group was stratified in symptomatic and asymptomatic group. Finally, within symptomatic HIV seropositive patients, comparison was made between those currently undergoing ARV treatment versus those not on ARV (Fig.1). To distinguish betweensymptomatic and asymptomatic individuals, we used the WHO HIV disease stage classification. Potential participants were screened by clinical staff at the CNTS. Those who met WHO stage 1 criteria were classified as asymptomatic while those who met WHO stages 2, 3, and 4 were classified as symptomatic [17]. Inclusion criteria included: (1) willingness to participate in the study; (2) age between18-60 years; and (3) ability to give blood (no biological restrictions due to ongoing menstruation, pregnancy, or breastfeeding, and weight > 55 kg). We excluded persons with Hepatitis B, Hepatitis C or syphilis. The study population was randomly sampled using Epi-Info software (CDC, Atlanta, GA) by generating CNTS identification numbers amongst the pool of potential blood donors and from amongst HIV patients coming to the CNTS for routine biomedical testing. All willing and eligible study participants were asked to sign an informed consent before undergoing any study procedures. Approval for this study was obtained both from the Ethical Committee of the School of Medicine, Pharmacy and dentistry (FMPOS) at the University of Bamako, Mali and the UAB Institutional Review Board.The main outcome of interest of this study was the evaluation of the density serum protein fractions measured by serum protein electrophoresis.

### Data collection

For eligible participants, the study was explained by the CNTS physician and written informed consent was administered. Study participants completed a brief questionnaire on demographic data (age, gender, occupation and marital status). Participants were then admitted in the phlebotomy room where blood was obtained by venipuncture in serum separation tubes then centrifuged at 2600 rpm for 10 min. The obtained serum was transferred in a cryotube for further conservation in a -80$^0$C freezer. Any hemolysed or high lipidemic sera were rescheduled, so thatblood was drawn in good conditions. If the test could not be run the same day the sera were frozen at -80$^0$C and thawed at room temperature before the tests were run.

### Laboratory analysis

For HIV testing, the participants were screened with Genscreen version 2 ELISA test (Biorad). These results were confirmed with Immunocomb II HIV-1 and 2 Biospot test and the Western blot test (New Lav Blot HIV-1 and HIV-2). Serum protein electrophoresis (SPE) was performed on agarose gel (Kit hydragel protein EIK 20 from sebia®). Five peaks of serum protein fractions were displayed on agarose gel: albumin, á-$_1$ and á-$_2$ globulin, â-globulin (â$_1$, â$_2$) and ã-globulin and fixed with heat (80° C; 177° F). The electrophoretic gel was stained and the serum protein fractions were evaluated by the densitometry of the electrophoretic profile using 570nm as the wavelength. The total protein was measured by a colorimetric method called Biuret

method. The Protein Total liquicolor reagent from Human was used.

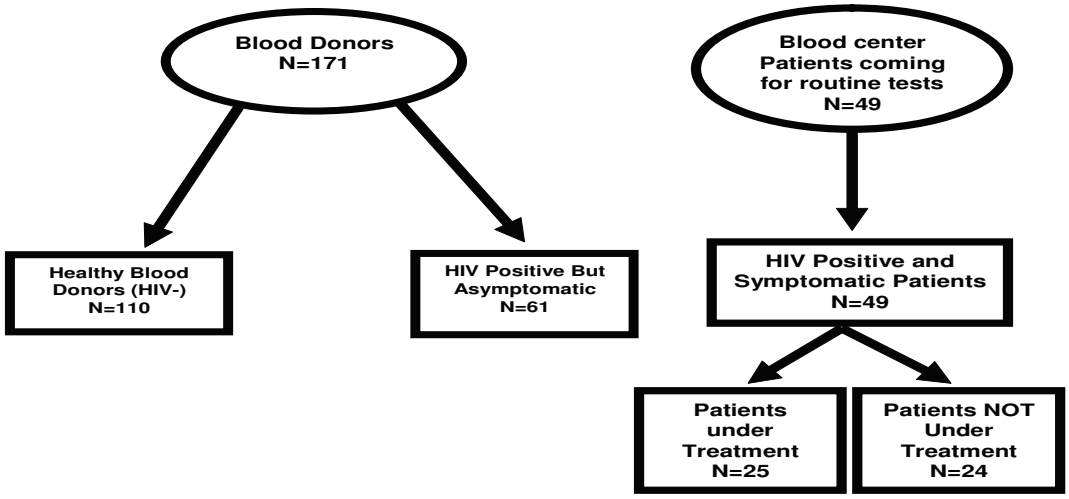
**Statistical analysis**

The data were entered and analyzed on Epi-Info 6.04 (CDC, Atlanta, GA). The proportions of patientswere compared using a chi-square test. The mean values of the serum proteins fraction were compared between symptomatic and asymptomatic HIV-infected patients and healthy blood donors then symptomatic patient under ARV and not under ARV. These comparisons were done using independent t-tests. P-values were used to detect significant statistical differences between mean values and proportions at a level of $p < 0.05$.

## Results

A total of 212 subjects were enrolled in this study, including 49 HIV seropositive symptomatic patients, 61 HIV seropositive, asymptomatic patients and 110 healthy controls (Figure 1). Within the 110 HIV seropositive patients, 49 (44.5%) were symptomatic; of those, 25 (51%) were on antiretroviral therapy. HIV-1 was the dominant subtype, found among 106 (96.4%) of the HIV seropositive patients (data not shown).

**Figure 1: Participants enrollment scheme and description and number of participant in each groups compared in the study**




Almost 70% were males (151), Almost 60% of the HIV seropositive patients belonged to 26-35 age group. The rest were equally distributed in the other age-groups. There were 60% males in the in theHIV seropositive subpopulation.

The mean proportion that each fraction represents in the total protein was calculated and compared between HIV positive and healthy blood donors (Table 1). All mean values of SPE fractions other than â-globulin fraction demonstrated significant differences between these two groups ($p<0.001$). We compared symptomatic patients and asymptomatic HIV seropositive patients (Table 2).

**Table 1: Serum protein electrophoresis results comparison between HIV positive and HIV Negative patients**

| SPE results | HIV Positive (N=110) Mean | HIV Negative (N=110) |
|---|---|---|
| Total protein (g/l)** | 102.83 | 81.31 |
| Albumin/Globulin Ratio | 1.04 | 1.71 |
| Protein Fraction | | |
| Albumin (%)** | 48.63 | 62.02 |
| Alpha-1 (%)** | 3.05 | 2.65 |
| Alpha-2 (%)** | 9.67 | 8.43 |
| Beta (%) | 10.79 | 10.45 |
| Gamma (%)** | 29.17 | 16.14 |

**%**, Percentage of the protein fraction in the total protides; ** $p < 0.0001$

**Table 2: Serum protein electrophoresis results comparison between HIV positive asymptomatic and symptomatic patients**

| SPE results | Asymptomatic patient (N=61) | Mean | Symptomatic patient (N=49) |
|---|---|---|---|
| **Total protein (g/l)** | 99.15 | | 107.43 |
| Albumin/Globulin Ratio** | 1.19 | | 0.86 |
| Protein Fraction Albumin (%)** | 53 | | 43.2 |
| Alpha-1 (%) | 2.79 | | 3.36 |
| Alpha-2 (%)* | 9.15 | | 10.33 |
| Beta (%) | 10.36 | | 10.57 |
| Gamma (%)** | 26.08 | | 33.02 |

**%**, Percentage of the protein fraction in the total protein; **p < 0.0001; *p = 0.05

In this comparison, the albumin/globulin ratio and the albumin fraction showed a significant difference between these two groups. The gamma-globulin fraction also showed a significant difference between the two groups. (p < 0.001).

Those HIV positive patients undergoing ARV treatment versus those not under ARV treatment was not significantly different regarding total protein level and the albumin/globulin ratio. Albumin protein fraction, á1-globulin protein fraction (p<0.0001) and the mean value of the ã-globulin were also significantly different between these sub-categories of patients (Table 3). These results showed a significant increasing of the percentage of the albumin and a significant decreasing of the gamma-globulin fractions after ARV initiation (Table 3).

**Table 3: Serum protein electrophoresis results comparison between HIV positive under ARV treatment and no ARV treatment**

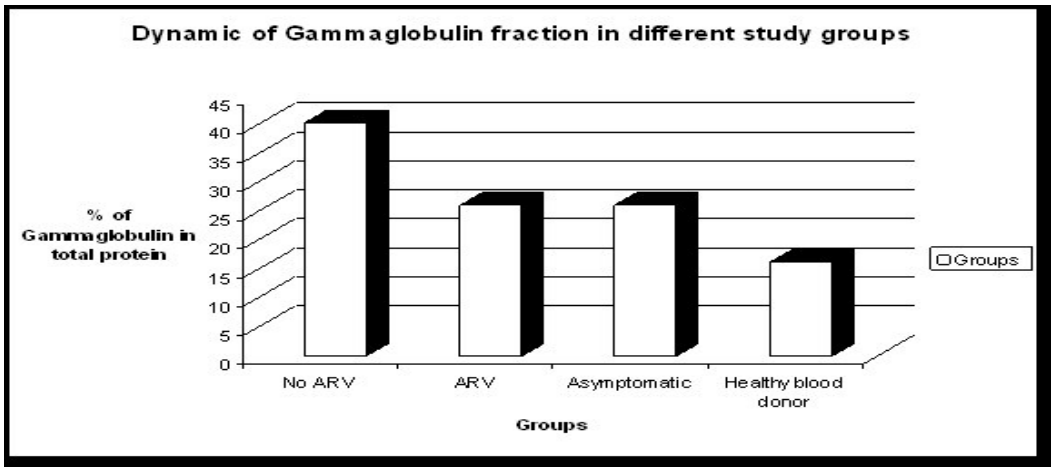
| SPEresults | ARV treatment (N=25) | Mean | No ARV treatment (N=24) |
|---|---|---|---|
| Total Protein (g/l) | 104.88 | | 110.08 |
| Albumin/Globulin Ratio | 1.08 | | 0.62 |
| Protein FractionAlbumin (%)** | 51.52 | | 34.54 |
| Alpha-1 (%)** | 2.56 | | 4.2 |
| Alpha-2 (%) | 9.64 | | 11.04 |
| Beta (%) | 10.06 | | 10.17 |
| Gamma (%)** | 26.04 | | 40.29 |

**%**, Percentage of the protein fraction in the total protein; **p < 0.0001

## Discussion

The goal of this study was to assess the serum protein electrophoretogram between HIV positive patients and healthy blood donors. We also did a comparison within HIV infected groups (asymptomatic versus symptomatic; those on ARV treatment versus those not on ARV treatment). The goal of theses comparison were to link changes in SPE patterns to the HIV disease stage and effects ARV treatment by analyzing total serum protein level and the fraction of several proteins after electrophoresis. HIV seropositive patients had a significantly higher total protein level as compared to healthy blood donors (102.3g/l and 81.3g/l) (p<0.001). Akpona et al examined 29 HIV positive patients in Benin and found lower protein levels, but these were still significantly different between HIV seropositive and HIV seronegative patients (77.93g/l and 75g/l) (p<0.05)[10]. Similar results was reported by Kapsenberg et al who found significant higher total protein levels amongst HIV positive pregnant women as compared to controls[13]. This hyper ã-globulinemia was described by Montagnier et al in 1989 [18]. This high level of protein level can be explained by a hyper ã-globulinemia which we found among HIV seropositive patients followed by a significant decrease of the albumin fraction. A similar study in Benin showed a significant increasing of ã-globulin fraction from 21.6g/l to 38.49g/l (p < 0.001)[10]. The ã-globulin fraction was compared between HIV seropositive symptomatic groups under ARV treatment versus those not on ARV treatments (Table 3). Our results showed that the group on ARV treatment had a significantly lower level of ã-globulin fraction (p < 0.001). Changes in concentration of ã-globulin fraction in HIV seropositive group and HIV seronegative group are shown (Fig. 2).

**Figure 2: Dynamic of the percentage of ã-globulin fraction in the total protein of the four groups of patients**




ARV therapy may decrease the ã-globulin fraction but did not reach the normal donors ã-globulin fraction level. In 2004, Chiappini et al also described the same hyper ã-globulinemia phenomenon among HIV positive children in Italy, which was significantly reduced after ARV treatment initiation from 40.29% of the total protein to 26.04% ($p<0.001$) [14]. There the authors concluded that combined antiretroviral therapy leads to the reduction of hyper-immunoglobulinemia [14]. This restoration of the gamma-globulin level after ARV treatment initiation can be interpreted by a significant reduction of viral replication in these patients, resulting in an improvement of health. In ourstudy, the immunofixation result found that this hyper-globulinemia was polyclonal (data not shown) while Kapsenberg detected an oligoclonal bands against a polyclonal background in addition to the raised of the ã-globulin fraction for HIV seropositive patients [13]. The dynamic profile of the ã-globulin and albumin fraction with HIV+ patients may be associated with disease stage and is partially restored after ARV treatment initiation because it did not reach the level found in the HIV negative group. The polyclonal gamma-globulinemia is characteristic of chronic inflammatory condition generally created by viral infection.

The scale-up of ARV treatment in developing countries over the past decade has greatly reduced HIV mortality and morbidity. This scale-up must be followed by the implementation of laboratory monitoring for patients in these areas. Many developing countries are creating policies to facilitate access to viral load PCR testing and T-cell count for HIV patients, but infrastructure and training challenges remain. This study was designed to explore alternative solutions when the gold standard tests are not available for HIV treatment assessment. The ã-globulin and the albumin fractions can be used to assess treatment response. The SPE is widely used to diagnose many diseases in the clinics for years ago and its cost is much lower than the CD4 count and viral load. However, due to the unavailability of the viral load test and the CD4 T cell count during the study period, we could not correlate this finding with the gold standard monitoring test for HIV patients. Further follow-up studies should be done to evaluate the correlation of SPE, T-cell count and viral load in the resource poor settings. Further studies should investigate the specificity of the antibodies in the gamma-globulin fraction. These antibodies can be evaluated for their ability to neutralize the virus and set up a basis for vaccine research.

This study has many limitations, including the unavailability of CD4 T cell count, and viral load during the study period which meant that we could not look for a correlation between SPE fractions, CD4 T cell count and viral load. The cross-sectional study design allows only a snapshot of the measures. Another potential limitation is our definition of "healthy blood donors" which meant that donors were free of HIV, Hepatitis B, Hepatitis C, and syphilis, but did not account for other possible diseases. Because CNTS only has the ability to screen blood for those particular diseases, the healthy blood donors may have had other infections which could have impacted the ã-globulin fraction dynamic in HIV infected patients. Last but not least, protein fraction concentrations can change with age and this

may have adversely affected our results as there was not an even age distribution among our study groups.

## Conclusion

In SPE during HIV infection, these changes may be partial compared to healthy individuals. Therefore, serum protein electrophoresis may be considered as an alternative in monitoring HIV patients in low-resource settings. Extended evaluation is needed to set a core algorithm for this test which would be adapted for each resource poor setting.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