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Serum protein electrophoresis as an added value for disease control among HIV patients in a hospital setting in Cameroon

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In the resource-limited countries, CD4 count and viral load (VL) determination are not always systematic, due to the low follow-up coverage and/or limited access to optimal hospital facilities. The present study aimed at evaluating the clinical role of Serum Protein Electrophoresis (SPE) in HIV health care and disease control. An analytic study was conducted in a Cameroon reference's hospital, in HIV patients on antiretroviral treatment. Blood sample was collected to perform CD4 count, viral load, total proteins, and SPE through clinical capillary electrophoresis. SPE data were correlated to other results. ANOVA, Mann-Whitney test and Pearson's correlation were performed, at the threshold *P<0.05*. A total of 72 patients: 51 (70.8%) women and 21 (29.1%) men were included, with a mean age of 41.22±8.48 years. The electrophoretic profile showed that the majority of patients have hyperproteinemia (70.9%) and hyper-γ-globulinemia (89%). All the enrolled patients had low albumin/globulin ratio. A negative and significant correlation was observed between CD4 count and total proteins (r=-0.5, P<0.0001), and γ-globulin (r=-0.6, P<0.0001). The correlation was positive and significant with the albumin/globulin ratio (r=0.6, P<0.0001). Patients with VL≥250 Cp/mL, had a high total proteins and different fractions of globulins, compared to those with VL<250 Cp/mL. The perturbation of CD4 count and VL were associated with SPE data variations, which better describes patient's clinical status. SPE could be combined to the existing analyses to improve AIDS health care.

Key words: PLHIV, HIV/AIDS, serum proteins electrophoresis, disease control.

INTRODUCTION

Acquired Immune Deficiency Syndrome (AIDS) due to human immunodeficiency virus (HIV) is a major threat to the development of resource-limited countries. It is a poverty related disease that has destroyed many lives and contributed to maintain poverty. Sub-Saharan Africa with only 13.4% of the world population is the hardest hit region, home to nearly 70% of people living with HIV/AIDS. In 2016, there were 36.7 million people living

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with HIV globally, with 1.8 million people who became newly infected with HIV, while 1 million people died from AIDS-related illnesses (World Development Indicators, 2016; UNAIDS, 2017). Western and Central Africa are home to 6.1 million (about18%) of these infections, right after the Eastern and Southern Africa,19.4 million (52%)and before Asia and the Pacific, 5.1 million (14%) (UNAIDS, 2017). In Cameroon, the number of people living with HIV is estimated at 667,000 giving a prevalence of 4.3% over the country (National Institute of Statistics, 2011). Numerous plans to fight against HIV/AIDS have been implemented throughout the significant increase in the total number of Authorized Care Units (ACUs) within the country, and the free provision of antiretroviral treatment (ART) since 2007 nationwide. To meet the WHO standards of care, this free treatment ought to be accompanied by a deep psychological, clinical and biological follow-up. However, biological monitoring of people living with HIV (PLHIV) in resource-limited settings is not always systematic (Mbopi-Kewou et al., 2014). Given these challenges, WHO recommends lymphocytes typing approach and systematic CD4 cells count has so far helped improve the follow-up of PLHIV (Dieye et al., 2005). Sometimes, disturbances in some blood elements such as monocytes and lymphocytes might make difficult the interpretation of CD4 results and consequently jeopardize the management of HIV-infected people (Yéri, 2010). The other key monitoring parameter, the viral load determination, is limiting in the majority of patients with limited resources due to its non-affordable cost. Therefore, it is imperative to develop other cheaper and technically simpler methods for the follow-up of patients with complex biological results. Such methods include new biochemical markers that could be combined to the immunological parameters to better appraise the disease stage and/or progression (Pascale et al., 1997). Thereby serum proteins might be an interesting alternative to address such issue. Indeed, previous investigations carried out in other sub-Saharan African countries with limited resources (e.g. Nigeria and Democratic Republic of Congo) as well in South Africa, have shown the contribution of serum protein electrophoresis in the biological monitoring of PLHIV (Okogun et al., 2015; Adedeji et al., 2014; Kamangu et al., 2012). This approach is not as known in Cameroon as in other African countries especially its usefulness in the monitoring of HIV-infected people. The present study aimed at evaluating the role of serum protein electrophoresis in the monitoring and control of the disease in HIV infected patients.

MATERIALS AND METHODS

Study design, period, setting and population

An analytical study was carried out from August to November 2015 at the Day Care's Hospital of Douala Laquintinie Hospital (DLH) located in Douala city (Littoral region, Cameroon). DLH is a reference hospital in Cameroon, and its Day Care's Hospital, an Authorized Care Unit. The study population consisted of volunteers and consenting HIV patients, on ART for at least 12 months and under biological follow-up in DLH.

Sample size

Considering the HIV prevalence of 4.3% reported in Cameroon upon EDS-MICS 2011, the sample size was determined using the following formula: $n=PQ/D^2$, where n is the sample size required, Z_α the confidence level at the desired statistical significance, P is the HIV prevalence, $Q=1-P$ is the proportion of HIV negative participants and D^2 is the acceptable error. The minimum sample size was estimated as $n=63$ and majored to 72 persons with an effect size of about 1.25.

Inclusion criteria

Clinically asymptomatic HIV infected patients, 21 years old or above, who signed an informed consent form were included. Pregnant women were not included in the present study.

Sample collection

Alongside the routine analyses for HIV patients in the Day Care Hospital ($CD4^+$T-cell count and viral load, respectively), an additional 5 mL of blood sample was collected from each patient by venipuncture, in a dry tube. Serum was obtained by centrifugation at 3000 rpm for 10 min. Sera samples obtained were transferred into Eppendorf tubes and immediately stored at -20°C until electrophoresis analysis.

Ethical approval and consent to participate

The present study satisfied the National and International Ethical Standards. Prior to the study implementation, ethical clearance was obtained from the Research Ethics Committee of the University of Douala (Authorization N°263/09/2015/M); administrative authorization was also obtained from the Head of the Laquintinie Hospital (Authorization N°2782/AR/HLD/MA). In addition, informed consent was obtained from each enrolled participant. The study was conducted according to the CIOMS guidelines and complied with the Declaration of Helsinki (2013).

$CD4^+$ T-cells count

In laboratory of the DHL, $CD4^+$ T-cells lymphocytes were counted with a flow cytometer CyFlow® (Sysmex-Partec Görlitz, Germany) according to the manufacturer's instructions. Briefly, 20 µL of phycoerythrine-conjugated monoclonal antibody to human CD4 (mAb PE MEM241, Partec GmbH, Görlizt, Germany) were slightly mixed with 20 µL of whole blood into a test tube and incubated for 15 min at room temperature, protected from light. Then, 800 µL of no-lyse buffer were added to the mixture. After homogenizing the content, the tube was introduced into the CyFlow Counter for automatic counting.

Viral load determination

The viral load was performed at the Retro-virology Laboratory of the Laquintinie Hospital, using a quantitative PCR (qPCR) (GENERIC HIV Viral Load, Biocentric®, France). The threshold of detectability was 250 copies/mL.

Table 1. Baseline characteristics of the participants.

Variable	Categories	Frequency	%
Gender	Females	51	70.8
	Males	21	29.2
Age (years)	< 35	16	22.2
	[35 - 45[	36	50.0
	[45 - 51[	11	15.3
	≥ 51	9	12.5
Level of education	Primary	22	30.6
	Secondary	45	62.5
	University	5	6.9
Marital status	Single	35	48.6
	Married	27	37.5
	Widow(er)	8	11.1
	Divorced	2	2.8
HIV Subtypes	M	66	91.7
	O	6	8.3

Data are presented as frequency (percentages), M: Majority, O: outgrou**p.**

Central Laboratory of the Laquintinie Hospital, using the Biuret's method (BIOLABO kit, France), according to the manufacturer's instructions.

Serum protein electrophoresis (SPE)

Clinical capillary serum protein electrophoresis was performed using Helena Electrophoresis system according to the manufacturer's instructions (HELENA BIOSCIENCES® England). This tool allowed to obtain: albumin, α1-globulins, α2-globulins, β-globulins, γ-globulins fractions as well as the albumin/globulin ratio.

Statistical analysis

Statistical analyses were performed with SPSS version 16.0 for Windows. Mean ± standard deviation (SD) and frequencies were used to summarize descriptive statistics. Analysis of variance (ANOVA) was used to compare differences for the normally distributed variables; the Kruskal-Wallis test was also used. The Mann-Whitney was used to compare mean values between two groups for small sample size. The Pearson's correlation was used to depict the variation trend between two continue quantitative variables. Statistical significance was set at *P<0.05.*

RESULTS

A total of 72 patients, made up of 51 (70.8%) women and 21 (29.1%) men were included in the present study, giving a sex ratio of 2.4 in favor of women. Half of participants were aged between 35 and 45 years old (50.0%). The majority of the participants were with a secondary level of education (62.5%) and were single (48.6%). HIV type 1 was found to be responsible for all infection cases and subtype M represented 91.7% (Table 1).

Fifty-one (70.9%) of total hyperproteinemia 102.8±17.8 g/L and 5 (6.9%) of total hypoproteinemia 49.3±9.5 g/L were observed. 31 (43.1%) had hypoalbuminie 29±5g/L, followed by 7 (9.7%) of hyperalbuminia 54.1±2.6g/L. 24 (33.3%) had hyper α1-globulinemia 5.3±1.2 g/L. 21 (29.2%) had hyper α2-globulinemia 13.4±2.6 g/L and 7 (9.7%) had hypo α2-globulinemia 3.8±1.4 g/L. 30 (41.7%) had hyper β-globulinemia 17.9±4.0 g/L and 2 (2.8%) had hypo β-globulinemia 4.9±0.4 g/L. 64 (89%) had hyper γ-globulinemia 30.6 ± 11.2 g/L. All patients (100%) had a low albumin/globulin ratio 0.4±0.1 (Table 2).

In the present sample, 7 (9.7%) had CD4 count below 200 cells/µL, 12 (16.7%) had CD4 between 200 and 350 cells/µL, 15 (20.8%) had CD4 between 350 and 500 cells/µL and 38 (52.8%) had CD4 values greater than 500 cells/µL (Figure 1).

A significant decrease of CD4 T-cells was associated with a high concentration of γ-globulin ($p<0.0001$), whereas this decrease was associated with a low concentration of albumin ($p<0.0001$) and low albumin/globulin ratio ($p<0.0001$), respectively (Table 3). A negative and significant correlation was observed and linked to the decrease in total protein ($r=-0.5$, $P<0.0001$), α1-globulins ($r=-0.3$; $P=0.0157$), α2-globulins ($r=-0.3$; $P=0.0061$), β-globulins ($r=-0.3$; $P=0.0252$) and γ-globulins ($r=-0.6$; $P<0.0001$) fractions, respectively, whereas a positive and significant correlation related

Table 2. Serum protein electrophoresis profile.

Variable	Category	Frequency	%	Mean ± SD	Overall mean ± SD
T. Protein (g/L)	Low (<60)	5	6.9	49.3±9.5	92.7±23
	Normal (60-80)	16	22.2	73.6±6	
	High (>80)	51	70.9	102.8±17.8.	
Alb (g/L)	Low (<35)	31	43.1	29±5	37.6±9.3
	Normal (35-50)	34	47.2	42.1±4.1	
	High (>50)	7	9.7	54.1±2.6	
α-1 Glob (g/L)	Low (<1.0)	0	0.00	NA	3.7±1.4
	Normal (1.0-4.0)	48	66.7	2.9±0.7	
	High (>4.0)	24	33.3	5.3±1.2	
α-2 Glob (g/L)	Low (<5.0)	7	9.7	3.8±1.4	9.4±3.5
	Normal (5.0-11.0)	44	61.1	8.7±1.7	
	High (>11.0)	21	29.2	13.4±2.6	
β-Glob (g/L)	Low (<6.0)	2	2.8	4.9±0.4	12.9±5.1
	Normal (6.0-13.0)	40	55.6	9.7±2.0	
	High (>13.0)	30	41.7	17.9±4.0	
γ-Glob (g/L)	Low (<7.0)	0	0.00	NA	28.8 ± 11.9
	Normal (7.00-16.0)	8	11.1	13.6 ± 2.1	
	High (>16.0)	64	89	30.6 ± 11.2	
Alb/glob	Low (<1.1)	72	100	0.4±0.1	0.4±0.1.
	Normal (1.1-2.7)	0	0	NA	
	High (>2.7)	0	0	NA	

Data are presented as frequency, percentage and mean±standard deviation; SD: Standard deviation; NA: not available; T. Protein: total protein; α-1 Glob: alpha-1globulin; α-2 Glob: alpha-2 Globulin; β-Glob: beta-globulin; γ-Glob: gamma globulin; Alb: albumin; Alb/Glob: albumin globulin ratio.

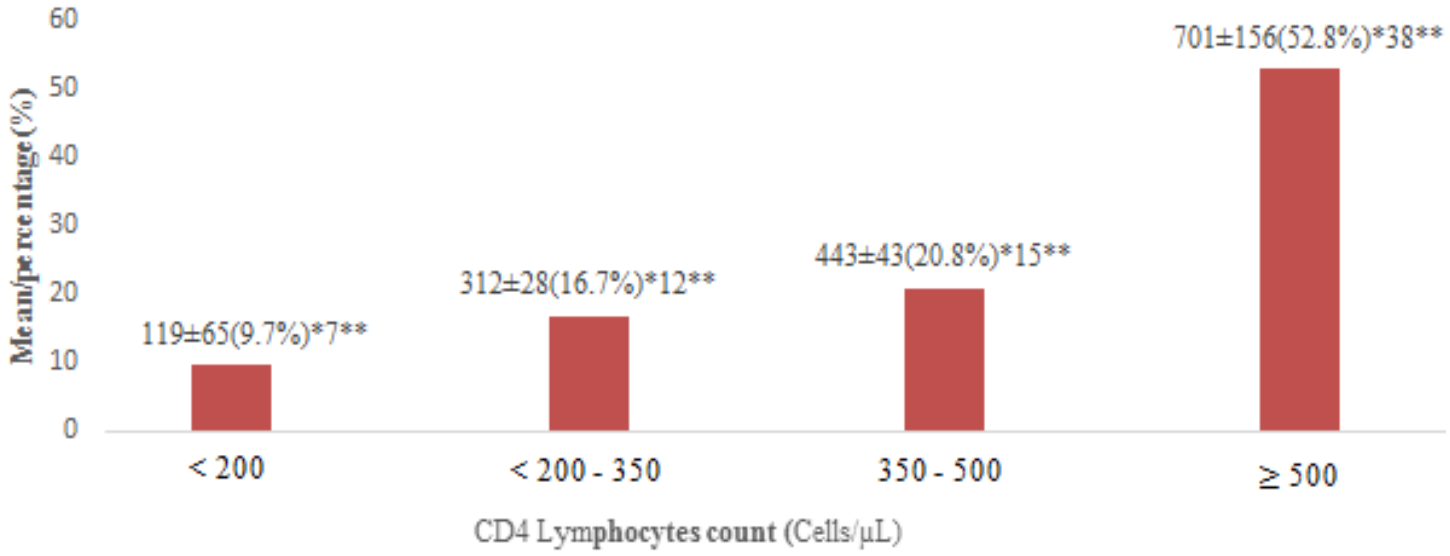

Figure 1. Profile of CD4 of the population with regaed to CD4 group.

Table 3. Variation of parameters of serum protein electrophoretic with regard to CD4+ T cells count.

Variable	CD4 lymphocytes (cells/µL)				H	P
	<200 (n=7)	200-350 (n=12)	350-500 (n=15)	>500 (n=38)		
T. Protein	97.7±21.8	114.2±22.7	98.4±18.1	81.8±19.1	17.58	0.0005
Alb	28±12.3	36.7±3.1	40.7±5.1	43.9±5.1	27.26	<0.0001
α-1 Glob	3.4±1	4.2±1.0	3.8±1	4.1±1	8.10	0.0440
α-2 Glob	10.8±3.4	13.3±3.2	14.8±3.2	14.1±3.4	12.09	0.0071
β-Glob	11.9±4.4	13.5±3.2	14.8±3.2	14.1±3.4	7.26	0.0638
γ-Glob	41.2±8.7	35.3±6.5	30.7±7.1	27.9±6.4	28.56	<0.0001
Alb/Glob	0.3±0.1	0.4±0.0	0.4±0.1	0.4±0.1	28.34	<0.0001

Data are presented as mean ± standard deviation, T. Protein: Total Protein; α-1 Glob: alpha-1globulin; α-2 Glob: alpha-2 Globulin; β-Glob: beta-globulin; γ-Glob: gamma globulin; Alb: albumin,; Glob: globulin; H: Kruskal-Wallis test. Significance was set at P<0.05.

Table 4. Correlation between CD4 T cells count and serum proteins fractions.

Variable	R	95% CI	P-value
T. Protein	-0.5	-0.64 to -0.28	<0.0001
Alb	-0.2	-0.38 to 0.15	0.1991
α-1 Glob	-0.3	-0.48 to -0.06	0.0157
α-2 Glob	-0.3	- 0.51 to -0.09	0.0061
β-Glob	-0.3	-0.47 to -0.03	0.0252
γ-Glob	-0.6	-0.75 to -0.47	<0.0001
Alb/Glo	0.6	0.37 to 0.70	<0.0001

T. Protein: Total Protein; α-1 Glob: alpha-1globulin; α-2 Glob: alpha-2 Globulin; β-Glob: beta-globulin; γ-Glob: gamma globin; Alb: albumin; Glob: globulin; R: Pearson's correlation coefficient.95%CI: Confidence interval at 95%, Significance was set at P < 0.05.

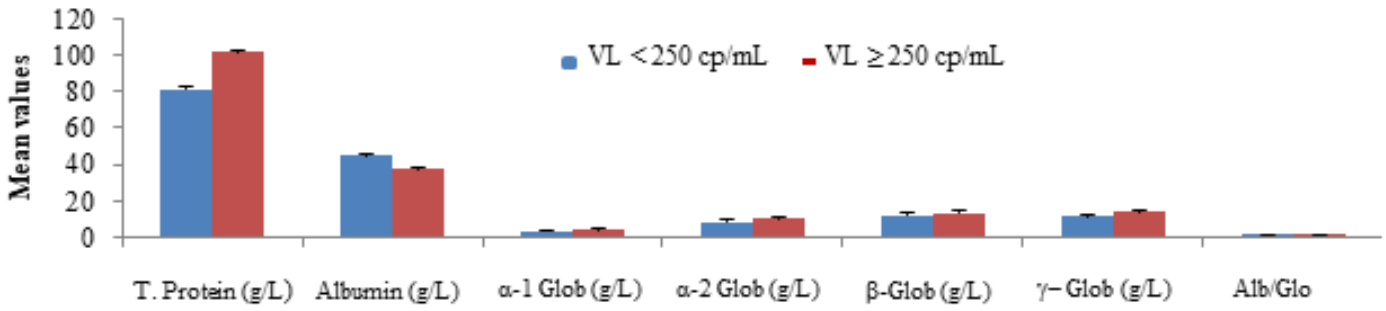

Figure 2. Relationship between the Serum Protein Electrophoretic (SPE) and viral load (VL) VL ≥ 250 copies/mL: Detectable, VL < 250 copies/mL: Undetectable.

to the increase in albumin/globulin ratio (r=0.6; P<0.0001) (Table 4) was noticed.

In patients with undetectable viral load (less than 250 copies/mL), a significant decrease in total protein concentration (p<0.0001), α1-globulin (p<0.0098), α2-globulin (p<0.0176) and γ-globulins (p<0.0297) were observed while a significant increase in albumin concentration (p<0.0001) and albumin/globulin ratio (p<0.0001) was observed in patients with viral load above 250 copies/mL (Figure 2).

DISCUSSION

In the present study, the majority of participants were female (70.8%). This result is in line with a previous study carried out in the Laquintinie Hospital in which 64.6% were women, with a sex ratio of 0.54 in favor of women

(Ramjee and Daniels, 2013; Essomba et al., 2015). This feminization of the pandemics might be due to the biological, epidemiological and sociocultural factors that characterize women's vulnerability as far as HIV infection is concerned in sub-Saharan Africa. In fact, women are more at risk for HIV infection than their male counterparts (Ramjee and Daniels, 2013). However, Kandi (2013) observed in a previous study that 56% of the population were male, more numerous than women (Kandi, 2013).

In the present study, half of participants were aged between 35 and 45 years. This is similar to some previous studies in which 49.2 and 27.5% aged between 30-44 and 35-44 years, respectively were found (Mbanya et al., 2002; Essomba et al., 2015). These results show that 35 to 45 years are the hardest hit ages as far as HIV infection is concerned in Cameroon.

The hyperproteinemia (102.8±17.8 g/L) obtained in this study is similar to some studies that found 84.10 and 98.3±14.8 g/L in HIV asymptomatic patients on treatment and naïve of treatment, respectively (Adedeji et al., 2014). This increase in total proteins might be due to the increase in some group of immunoglobulins, and therefore to the disease progression. These results are in contrast with a previous study that observed a decrease in total protein level in HIV patients (Okpa et al., 2015).

A low concentration in albumin (29±5 g/L) was observed in 31 (43.05%) patients. These results are similar to the findings from Kamangu et al. (2012) who observed a decrease in albumin in 45.62% of patients. The low albumin levels might be due to some chronic inflammation associated with HIV infection (Mehta et al., 2006). An increase in α1 and α2-globulins as well as the β-globulins fractions were observed. This might be due to the presence of an acute inflammation. A hyper γ-globulinemia was observed in the present study. These results corroborate previous findings with a high γ-globulinemia in all HIV participants (Onyango et al., 2011). Actually, the γ-globulin fraction of the electrophoregram constitutes the primary arm of the humoral immune responses. Therefore, a persistent high γ-globulin fractions on effective control of HIV disease progression suggests a compensatory phenomenon of the deficient cell mediated immunity associated with HIV infection.

In the present study, the albumin/globulin ratio was low in all patients. These findings are similar to a previous study in which a low albumin/globulin ratio was observed in 90% of participants (Adedeji et al., 2014). Other studies reported the same phenomenon of HIV infection associated with a low albumin/globulin ratio (Uchenna et al., 2015).

The high viral load obtained, which characterizes the disease progression could induce a significant increase in the levels of total protein, different fractions of globulins and decrease in albumin and albumin/globulin ratio compared to those with a low viral load. Hence, the SPE markers are closely related to the different variations of CD4 cells count and viral load.

Conclusions

This study aimed at evaluating the role of serum protein electrophoresis (SPE) in the monitoring and control of the disease progression in HIV infected patients. The high total serum protein and globulin fractions, and the low albumin observed in HIV patients show that HIV infection causes significant changes in total protein, albumin, α, β, γ-globulins and albumin/globulin ratio fractions in correlation with the disease progression. This model should be seen as a complementary tool to better understand some phenomena during the HIV infection. SPE profile could be recommended as additional tool in countries with limited resources.

RECOMMENDATIONS

Based on the present research findings, it is necessary to integrate the serum proteins electrophoresis (SPE) to the existing analyses to improve AIDS health care.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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