Comparative analysis of serum protein electrophoresis' profiles of people infected with HIV and those not infected with HIV in Kinshasa

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ABSTRACT

Introduction: It is necessary to consider the analysis of electrophoresis' profiles of serum proteins as an alternative and less expensive for the biological monitoring of patients infected by HIV in countries with limited resources.

Objective: The aim of the study was to establish a comparison between the electrophoresis' profile of sera proteins of people infected by HIV naïve of treatment and people not infected by HIV.

Method: A transversal study was conducted at AMO-CONGO Kasa-Vubu in Kinshasa with people who came for a voluntary HIV screening test. Eighty one people, naïve of HAART, participated voluntarily in the study. HIV screening was systematically done according to WHO recommendations. Blood sample was obtained in a dry tube for the electrophoresis' profile of sera proteins, and in a tube with EDTA for numeration of CD4 for HIV positive patients. The statistical test of Chi-squared was used for qualitative data, and the test of Student for quantitative data.

Result: Out of 81 volunteers, 30 were confirmed HIV positive and 51 negative. The results obtained demonstrate that HIV infection is associated with a hyperprotidémie (60%), a hypoalbuminémie (100%) also a hypergammaglobulinemia (100%) according to CD4 level. No significant modification was observed for the alpha and beta-globulins.

Conclusion: The HIV infection induces some significant modifications of different fractions of sera proteins according to immune status.

Keywords: hyperprotidemia, hypergammaglobulinemia, hypoalbuminémie, total proteins, HIV

Introduction

Among the biological parameters most frequently asked by clinicians, the electrophoresis' profile of serum proteins is a marker of inflammation and various multiple infections[1]. Commonly used clinically, this analysis, low cost and affordable, reflects all the serum proteins. The latter provide a wealth of information in particular pathologies associated with inflammatory conditions of patients, protein malnutrition, the intestinal mal-absorption, reduced in various syntheses, the excessive catabolism or different losses[2]. Without many details on the clinical stage of patients, it has been shown that HIV infection induces changes in serum proteins[3-8]. With an estimated HIV prevalence of less than 5%, the city of Kinshasa has a problem for the management of infected patients[9-14]. The biological monitoring tests are not included in the management, are costly and supported by patients[12-14]. It is therefore necessary to consider the analysis of electrophoresis' profiles of serum proteins as an alternative and less expensive for the biological monitoring of patients within the reach of the target population.

To confirm the choice of the proposed work, it seemed important to compare the electrophoresis' profile in Kinshasa in people infected with HIV, treatment naive, with the electrophoresis' profile in non-infected with HIV.

Material and Method

Framework

This study is a cross-sectional study in outpatient treatment center for NGOs ACS AMO-Congo in the municipality of Kasa Vubu in the city of Kinshasa in the Democratic Republic of Congo from May 19 to June 15, 2010.

Patients

The volunteers who participated in this study were recruited among people who had come to the voluntary testing for HIV. The volunteers who were selected were in the age group 15 to 55, had no clinical AIDS-defining illness, not had unprotected sex in the three months preceding the test, had read and understood the interest of the study and signed informed consent. A total of 81personnes have participated in this study.

HIV status

Confirmation of HIV status of participants was conducted by three immunochrommatographiques tests as recommended by WHO and the protocol of the center. The three tests used for HIV status are: Determine ½, Unigold and Double Check.

Sample

The blood samples were collected from the vein in the elbow of the patient. Five milliliters of blood was collected in a dry tube to be centrifuged at 3000 g for 10 minutes to get the serum, 5 mL were collected in a tube with EDTA anticoagulant for lymphocyte count (CD4) on the

FACSCount™ only HIV + patients. The sera were stored at -20°C before being used the day after the sampling for electrophoresis.

Laboratories

The total serum proteins were assayed in triplicate by the colorimetric method called Biuret and measured by their absorbance at 540 nm using the spectrophotometer Spectrum SP-2100. The working solution consisted of sodium-potassium tartrate (15mmol/l), sodium hydroxide (100mmol/l), potassium iodide (5mmol/l) and copper sulphate (19mmol/l) and our standard is a bovine serum. The serum protein electrophoresis was carried out by series of seven samples of 90 Volt HydraGel (Kit HYDRAGEL PROTEIN (E) K20) for 22 minutes with direct current amperage from 12 ± 3 milliamperes. The different migrations of the electrophoresis were read by densitometry with a wavelength of 570 nm to define and quantify the percentage of relative concentrations of each fraction.

Statistical analysis

Data were entered using Excel and SPSS software. Statistical tests that were used in the work are the Student test for quantitative variables and Chi-square (X^2) for categorical variables. The significance (p) was chosen for the probability of $p < 0.05$. Results are expressed as mean ± standard deviation. The tables have been reformatted in Excel.

Ethical consideration

This study was approved by the ethics committee of the School of Public Health, University of Kinshasa as well as the center's director. Respect for the individual and the confidentiality of records were observed.

Results

Serology

Of the 81 sera collected from persons who had come to be screened in the center of AMO-Congo Kasa-Vubu, 30 were positive and 51 were negative for HIV. Table 1 shows the distribution of the HIV population by sex.

Values of serum proteins

Table 2 presents the different values compared to the protein distribution in population and serological results of means tests as associated t-statistics and chi-square. Table 3 shows the distribution of results compared to Congolese reference values[1]. Figure 1 shows a comparison of different protein fractions mean serum HIV + subjects compared to HIV-infected.

Values of CD4

The numbering of CD4 was performed for 30 HIV + patients. The average value of 283.5cells/µl

(range 10.0 to 937.0cells/µl). Figure 2 shows a comparison of mean serum protein HIV + subjects with CD4> 200 cells/µl and HIV + with CD4 <200 cells/µl.

Discussion

The present study analyzed the serum protein electrophoresis' profiles of people infected with HIV naive of treatment with those of non-infected with HIV in Kinshasa.

High values of total serum protein, high values of gamma globulin (γ-globulins), high values of total globulin and low albumin values are repeated in HIV +. In the group of subjects with HIV infection, we observed a hyperprotidémie in 60% of subjects, a hyper-γ globulin in 100% of subjects and hypoalbuminemia in 100% of subjects. Indeed, the hyper-γ globulin has been described as consistently associated with HIV infection but at varying intensities depending on the clinical situation. Some authors explain this phenomenon by the imbalance in the proportion of lymphocytes in favor of B lymphocytes and their polyclonal stimulation[7]. The hyperprotidémie and hyperglobulinemia resulting from the hyper-γ globulin. These large increases are markers of the presence of certain antibodies.

Hypoalbuminemia is the primary defect resulting from liver failure, inflammation, malnutrition, loss through leakage digestive, skin or urine, or excessive catabolism. It can serve as a marker for the nutritional status of patients.

The same observations were reported by S. A. AKPONA and al[2] in Benin, by JC Brelivet and al[3] in Burundi, by P. DJESSOU and al[4] in Ivory Coast, by F. Berkelman and al[5] and by E. Tangara[6] in Mali.

However, we observed a hypoprotinémie in an infected 3.4% of the population: this may be due to the progress of the disease. This observation was also made by P. DJESSOU and al[4].

Ninety percent of those infected have an albumin / globulin ratio relatively low. This decrease is due to report an increase in serum γ-globulin and a decrease in serum albumin. Remember that this report is physiologically for the albumin because it is the most abundant protein in serum. These results are consistent with the results of studies made by JC et al Brelivet in Burundi as well as those made by E. Tangara in Mali[3, 6].

The elevation of total serum protein and γ-globulin, and lower albumin are observed most consistently in most studies in HIV +. These parameters can be used as a method of monitoring the evolution of HIV infection due to the reproducibility of results.

The immunological level of patients, rates of CD4, plays a role in the variation of the different rates of protein fractions. Total protein and α -2 globulins are significantly different in the 2 groups ($p = 0.000$). The overall level of inflammation was higher in patients with a CD4 count below 200cells/µl. As against the albumin is slightly higher in patients with a CD4 count above 200cells/µl. This confirms that variations in these parameters are induced by the status of the subjects.

Conclusion

HIV infection induces significant changes in total protein, albumin and γ-globulin in treatment-naïve patients according to level immunity.

In our case, this model does not aim to replace the existing system, but should be seen as a complementary tool to better understand some of the phenomena involved in the body due to HIV infection. Monitoring the electrophoresis' profile of serum proteins can be used to monitor the disease, especially in the case of a therapeutic care and biological follow-up in countries with limited resources.

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Conflict of Interest: None declared.

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Table 1: Population per sex

	VIH+		VIH-		TOTAL	
	Number	**%**	**Number**	**%**	**Number**	**%**
MALES	12	14,8	24	29,6	36	44,4
FEMALES	18	22,2	27	33,4	45	55,6
TOTAL	30	37,0	51	63,0	81	100

Table 2: Proteins values in the population

	VIH+			VIH-			*p
	Mean	Maxima	Minima	Mean	Maxima	Minima	
Total Proteins	84,10	122,90	28,00	74,74	89,00	47,30	‡ 0,000
Albumin	45,62	57,11	29,98	58,42	69,42	46,97	† 0,000
Globulin	54,38	79,03	42,88	41,58	53,03	30,57	† 0,000
α-1-Glob.	1,940	7,780	0,000	1,770	10,430	0,640	† 9,348
α-2-Glob.	9,074	15,050	2,190	9,380	12,620	5,050	† 0,921
β-1-Glob.	5,768	11,590	1,150	7,917	11,190	4,600	† 0,481
β-2-Glob.	3,132	8,830	0,700	3,096	5,910	1,300	† 1,738
γ-Glob.	34,470	65,430	17,610	19,420	25,570	10,360	† 0,000
Alb/Glob	0,869	1,330	0,430	1,438	2,270	0,890	† 3,333

Alb. = Albumin

Glob. = Globulin

*Calculated values on SPSS 10.0 for Windows

‡Test of Student

†Test of Chi-Square

Table 3: Proteins according the Congolese (DRC) Values

Values	VIH+ (30)	VIH- (51)
Total sera Proteins: 62 – 82 g/l		
Low	1 (3,4 %)	2 (3,9 %)
Normal	11 (36,6 %)	**43 (84,3 %)**
High	**18 (60 %)**	6 (11,8 %)
Albumin : 59,7 – 70,6 %		
Low	**30 (100 %)**	**30 (58,8 %)**
Normal	0	21 (41,2 %)
High	0	0
Alpha-1 : 1,4 – 2,7 %		
Low	**11 (36,6 %)**	17 (33,3 %)
Normal	10 (33,4 %)	**31 (60,8 %)**
High	9 (30 %)	3 (5,9 %)
Alpha-2 : 7,2 – 11,1 %		
Low	**11 (36,6 %)**	5 (9,8 %)
Normal	**11 (36,6 %)**	**37 (72,6 %)**
High	8 (26,6 %)	9 (17,6 %)
Beta-1 : 6,0 – 9,3 %		
Low	**16 (54 %)**	4 (7,8 %)
Normal	12 (40 %)	**42 (82,4 %)**
High	2 (6 %)	5 (9,8 %)
Beta-2 : 2,0 – 5,5 %		
Low	9 (30 %)	4 (7,8 %)
Normal	**18 (60 %)**	**45 (88,3 %)**
High	3 (10 %)	2 (3,9 %)
Gamma : 8,4 – 16,3 %		
Low	0	0
Normal	0	11 (21,6 %)
High	**30 (100 %)**	**40 (78,4 %)**
Albumin/Globulin : 1,14 – 2,70		
Low	**27 (90 %)**	0
Normal	3 (10 %)	**44 (86,3 %)**
High	0	7 (13,7 %)

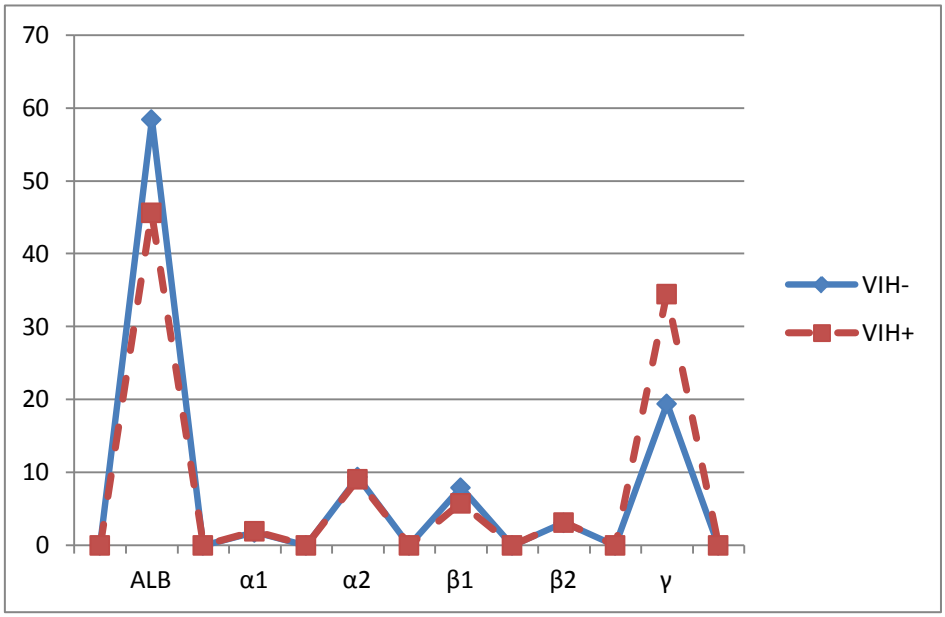

Figure 1: Comparison of proteins among HIV+ and HIV- patients

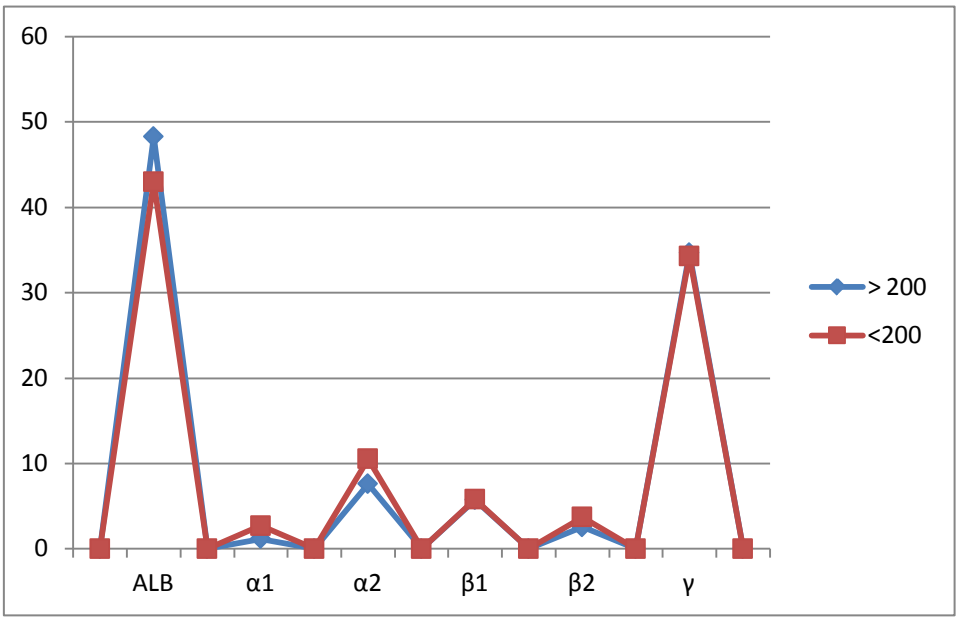

Figure 2: Comparison of proteins among HIV+ with CD4>200 cells/µl and HIV+ with CD4<200 cells/µl