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Research Article

Electrophoretic Profile of Serum Proteins Using Capillary Technique in Patients Attending the Douala General Hospital, Cameroon

Judith Gwladys Ekwe Priso[1*], Jean Pierre Nda Mefo'o[1,2], Cécile Okalla Ebongue[1,2], Eveline Ngouadjeu Dongho Tsakeu[1,2], Catherine Akono Ndi [1,2], Gérard Beyiha [3], Marie Solange Doualla [3], Dieudonné Adiogo[2]

[1]Clinical Biology Laboratory, Douala General Hospital, Cameroon
[2]Department of Biological Sciences, Faculty of Medicine and Pharmaceutical Sciences, Douala, Cameroon
[3]Department of Clinical Sciences, Faculty of Medicine and Pharmaceutical Sciences, Douala, Cameroon

*Corresponding author:
Judith Gwladys Ekwe Priso,
Phone: +237 694 59 18 44
Email: epgwladys@yahoo.fr

Abstract

Background: Electrophoresis of serum proteins is an orientation examination routinely used in clinical practice. For a few years, agarose gel electrophoresis has tended to be replaced with capillary electrophoresis owing to an increase in the accuracy of results. However, this technique is uncommon and is not widely used in Cameroon.

Objectives: The research aimed at studying the electrophoretic profile of serum proteins using capillary technique among patients attending the Douala General Hospital, Cameroon.

Methods: Capillary electrophoresis was used to carry out tests on blood samples from any inpatients and outpatients and fasting for 8-12 hours. Capillary electrophoresis of serum samples was used for the separation of proteins into six fractions and the total protidemia of each serum samples was determined using the Biuret method. Results were interpreted by observing the shape of curves and quantitative variations in each fraction of the different serum proteins.

Results: A total of 311 patients participated in the study. The sampled population aged 50±18 years on average and consisted of 55.3% men and 44.7% women. All capillary electrophoresis profiles presented six protein fractions, namely, albumin, alpha (1 and 2), beta (1 and 2) and gamma globulins. Pathological disorders were diagnosed in 290 patients and 21 patients had normal results. Inflammatory syndromes accounted for 63.34% and monoclonal gammopathies for 10.29% the main pathological disorder identified.

Conclusion: Capillary electrophoresis provides a more precise identification of biological syndromes and clear distinction of the six fractions of each protein. Monoclonal profiles and inflammatory syndromes were well detected. A prevalence of 10.29% was determined for gammopathies.

Keywords: Electrophoresis profile, capillary technique, clinical utility, Douala

Background

Serum protein analysis through electrophoresis is useful in many pathological conditions to guide a diagnosis, define the severity of a disease or evaluate the effectiveness of a therapy (1). This technique is also helpful for better diagnostic and monitoring of some chronic inflammatory diseases and lymphomas, and even studying prognostic markers of severe hepatocellular insufficiency (2). Electrophoresis can be performed in several ways referred to as variants and consisting of cellulose acetate, paper, gel and capillary electrophoresis (3). For many years, it has been observed that capillary electrophoresis tends to replace its counterpart performed on agarose gel in many medical laboratories in Europe (4). Capillary electrophoresis allows very high-resolution separations of large molecules, i.e., proteins (3,5). In addition, the technique is better adapted to expectations of laboratory technicians due to rapidity reproducibility and quantitative considerations as well as not being labor-intensive (2,6). Capillary electrophoresis is poorly known and infrequently implemented in health facilities in African countries owing to its relatively high cost for these countries whose resources are limited (7). In Douala, the technic is routinely used nowhere except the General Hospital. This study was aimed at studying the electrophoresis profiles of serum proteins using capillary technic in patients admitted for consultation or hospitalized at the General Hospital of Douala, Cameroon.

Methods

Study Site and Design

A cross-sectional study was conducted from January 2013 to February 2014 at the Biochemistry Unit of the Clinical

Biology Laboratory at the Douala General Hospital, Cameroon.

Study Population and Sample Size
The study population consisted of male and female inpatients and outpatients of any age referred to the Douala General Hospital The recruitment of patients was random and inclusion criteria were the prescription of serum protein electrophoresis (SPE) by a medical doctor. These patients had to be fasting for 8-12 hours before protein electrophoresis examination.

Questionnaire and Sample Collection
Prior to data collection, the objectives of the study were clearly explained to each patient and then the patient was asked to sign an informed consent form to participate in the study. Sociodemographic, clinical and paraclinical information was collected using a structured questionnaire within a 20-minute individual interview. Afterwards, a 5-mL whole blood sample was collected by venipuncture using vacutainer system and carefully transferred into a labelled dry tube.

Preparation of Serum Samples
Blood samples were centrifuged at 3000 rpm for 15 minutes until a clear and hemolysis- and fibrin-free liquid was obtained (8). Samples were then decanted in hemolysis tubes and stored at 2-8°C for up to 7 days for further analyses.

Sample Analysis
SPE was performed weekly on the Minicap™ automate (Sebia, France). To separate the proteins into fractions, charged molecules were moved with respect to their electrophoretic mobility in a buffer of constant pH, a voltage of 10- 30 kV and under an electroosmotic flux (9). It should be noted that the same samples were also utilized to determine total protein content. This was performed on CobasC311™ (Roche, Switzerland) using the Biuret method as described by Gornall et al (10) and the results were expressed in g/L.

Interpretation of the Results
The results of capillary electrophoresis were analyzed using the Phoresis™ version 7.0 software (Sebia, France). This software presents these results as curves. The curves were interpreted by describing their shape and commenting on the absolute and relative values obtained from different fractions (Figure 1). In addition, the subsequent analytical findings from the Sebia device were also taken into account in the interpretation.

Statistical Analysis
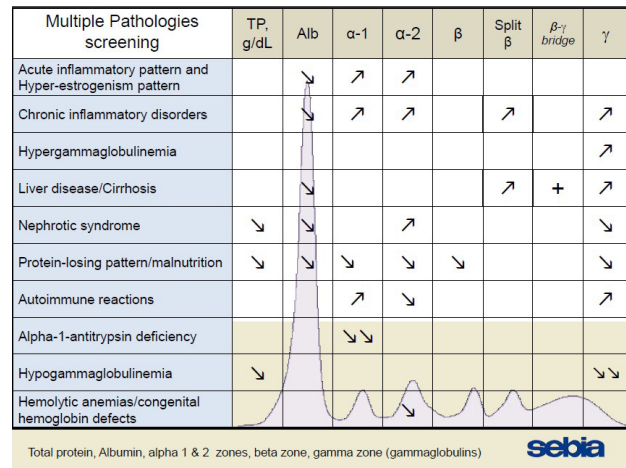Data were keyed, verified for consistency and coded in

Figure 1. Interpretation Table of Protein Electrophoresis Curves.

an Excel spreadsheet (Microsoft Office, 2010, USA) and then analyzed using the StatView™ software version 5.0 for Windows (SAS Institute, Inc., USA). Qualitative and quantitative variables were presented as percentage and mean ± standard deviation (SD), respectively. One-way analysis of variance (ANOVA) was used to compare the mean values of protidemia and different protein fractions. The significance level (P value) was considered to be less than 0.05.

Ethical Considerations
This study protocol was approved by the Institutional Review Board (IRB) of the National Committee on Research Ethics for Human Health in Cameroon. In addition, administrative and ethical clearances were issued by the Douala General Hospital.

Results
A total of 311 patients aged 50 ± 18 years on average and mainly consisting of men (55.30%) were included in the study.

SPE was prescribed by hematologist (32.00%), nephrologist (20.00%), hepato-gastroenterologist (16.00%), and general practitioner (13.00%). All patients had a history of some diseases including mostly diabetes, viral hepatitis and renal failure.

In total, the majority (93.20%) of patients presented protein electrophoresis-related pathological profiles, of which 74.50% and 18.70% were increase and decrease in globulin levels, respectively.

Total protidemia values ranged from 32 to 159.80 g/L (average: 75.08±15.21 g/L) (Table 1). The influence of gender on different protein fractions is summarized in Table 2. The values of beta 1 globulin fraction were significantly (P=0.001) higher in women (4.43±1.49 g/L) than in men (3.92±1.29 g/L). Hypoprotidemia and hyperprotidemia accounted for 11.50% and 28.61%, respectively (Table 3).

Pathological disorder electrophoretic profiles are

Table 1. Range, Mean and Reference Values of Different Protein Fractions in Participants

Variables (g/L)	Maximum (g/L)	Minimum (g/L)	Mean ± SD (g/L)	Reference values (g/L)
Protidemia	159.80	32.00	75.08 ± 15.21	[60- 80]
Albuminemia	59.52	10.46	34.27 ± 8.95	[35- 45]
Alpha 1	10.79	1.50	4.07 ± 1.57	[2.10- 3.50]
Alpha 2	20.20	0.59	8.06 ± 2.60	[5.10- 8.50]
Beta 1	15.68	0.00	4.15 ± 1.41	[3.40- 5.20]
Beta 2	71.88	0.00	4.68 ± 5.72	[2.30- 4.70]
Gamma	95.56	2.20	19.88 ± 12.04	[8.00- 13.50]
A/G	9.79	0.14	1.64 ± 1.05	[0.70- 1.30]

SD, Standard deviation; A, albumin; G, globulin.

Table 2. Comparison of Mean Values of Protidemia and Protein Fractions Between Men and Women

Variables (g/L)	Female	Male	df	F	P
Protidemia	76.66 ± 14.55	73.77 ± 15.68	1	2.76	0.097
Albuminemia	34.83 ± 7.75	33.77 ± 9.80	1	1.07	0.301
Alpha 1	3.94 ± 1.34	4.18 ± 1.74	1	1.73	0.189
Alpha 2	7.89 ± 2.25	8.20 ± 2.86	1	1.10	0.294
Beta 1	4.43 ± 1.49	3.92 ± 1.29	1	10.61	0.001*
Beta 2	4.56 ± 6.05	4.78 ± 5.45	1	0.10	0.742
Gamma	21.01 ± 13.27	18.97 ± 10.95	1	2.20	0.138

Data are presented as mean ± standard deviation; analysis of variance was used to compare mean values.
*Statistically significant at P <0.05.

Table 3. Frequencies of Variation of Serum Proteins

Variables (g/L)	Increase		Decrease	
	No.	%	No.	%
Total proteins	89	28.66	36	11.57
Albumin	24	7.72	141	45.34
Alpha 1 globulins	169	54.34	62	19.93
Alpha 2 globulins	46	14.79	113	36.33
Beta 1 globulins	47	15.11	73	23.47
Beta 2 globulins	85	27.42	25	8.07
Gamma globulins	232	74.60	14	4.50
A/G	177	56.91	43	13.83

A, albumin; G, globulin

presented in Figure 2 and normal profiles in Figure 3. The main disorders identified were inflammatory syndromes (63.34%), monoclonal gammopathies (10.30%), and biclonal gammopathies (0.64%) (Figure 4). Hyper-gammaglobulinemia with polyclonal, monoclonal and biclonal profiles accounted for 86.20%, 12.00% and 0.90%, respectively. Thus, the overall prevalence of gamma disease was 10.30%.

Hypoprotidemia cases were mainly associated with hypoalbuminemia and hyper alpha-2 globulinemia nephrotic syndrome. It should be noted that a few cases of bisalbuminemia were found in the present study; cases of hypergammaglobulinemia and hypoammaglobulinemia were reported in electrophoretic profiles of HIV-infected patients.

Discussion

The profile of serum proteins from patients consulting at the Douala General Hospital were investigated using capillary electrophoresis in the present study.

The relatively small sample size in this study may be due to the fact that the capillary electrophoresis is not universally prescribed to all patients attending the Douala General Hospital. It is usually prescribed, as with our study, by specialists such as haematologists. The Canadian Society for Clinical Chemistry's Monoclonal Gammopathy Working Group met to standardize serum and urinary protein electrophoresis results and clinician haematologists were invited to this meeting (11).

Electrophoretic abnormalities were observed in profiles of most patients. This finding may be explained by the fact
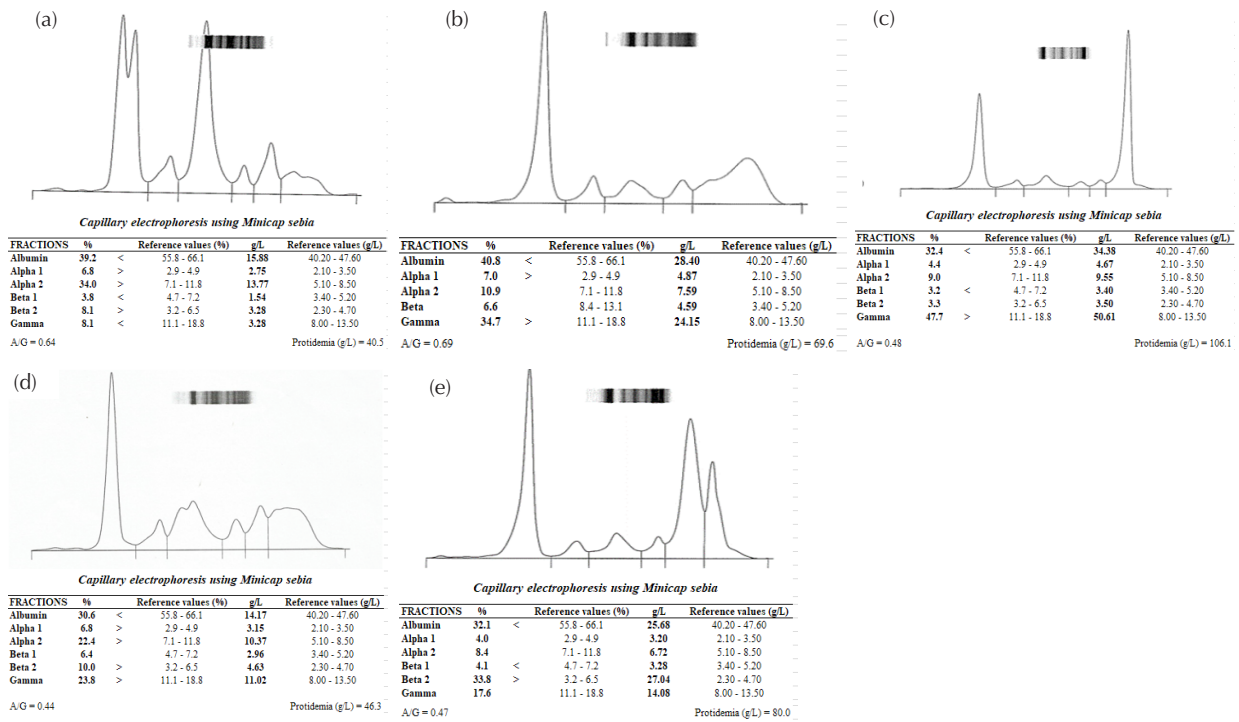
Figure 2. Serum Protein Electrophoresis Profiles Obtained on MINICAP. (a) Profile compatible with probable nephrotic syndrome + bisalbuminemia. (b) Profile compatible with cirrhosis . (c) Profile compatible with monoclonal gammopathy. (d) Profile compatible with chronic inflammatory syndrome. (e) Profile compatible with biclonal gammopathy.

that a large proportion of them had a history of diseases such as diabetes, hepatitis and myeloma carcinoma. These pathologies are known to significantly influence the electrophoretic profile of serum proteins (12).

The method allowed for quantification of different protein fractions from each patient based on the values of total protidemia. Albert et al pointed out the importance of such a quantification in order to improve the quality of management through the diagnosis of latent immunological disorders whose diagnosis is impossible or difficult with classic electrophoresis methods (13).

Beta 1 globulin fraction levels were found to increase in some patients. This fraction consists mainly of

hemopexin and transferrin as previously described elsewhere. Thus, any increase in the level of this fraction indicates a hypertransferrinemia (iron deficiency anemia) (2,3). Furthermore, the serum levels of this fraction were significantly higher in women than in men. This finding may confirm the commonly reported iron deficiency in African populations, mainly women (14). In addition, a study by Serraj et al (15) shows that iron deficiency affects more than half (52%) of all women in resource-limited countries.

The use of automated capillary technology has allowed a combination between rapid separation in free solution and the distinction between 6 fractions consisting of α1-, α2-, β1-, β2-, and γ-globulins. Moreover, the finest shape of curves obtained facilitated the definitive diagnosis of diseases such as monoclonal, biclonal gammapathies, nephrotic syndromes, hypo-gammaglobulinemia, and rare conditions such as bisalbuminemia. These results are consistent with those of Le Carrer and Bach-Ngohou (3). Besides, no interference was reported during experiments, and therefore factors such as high bilirubin levels, hemolysis and exogenous substances had no influence on the electrophoretic profile (16,17).

In this study, a positive correlation was observed between protidemia and gamma globulin fraction on the one hand and albuminemia and total protidemia on the other hand. Albumin is the main protein (55%-60%), thus any variation in this protein in a certain direction will translate by a variation in the same direction (18).
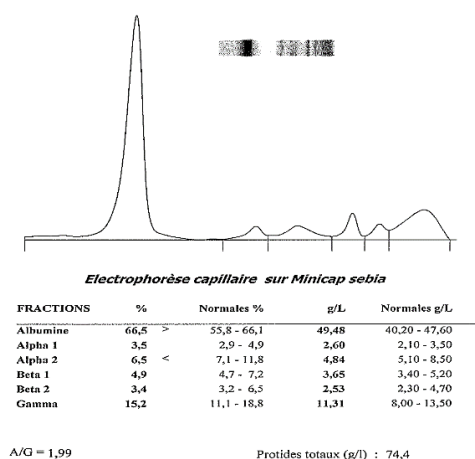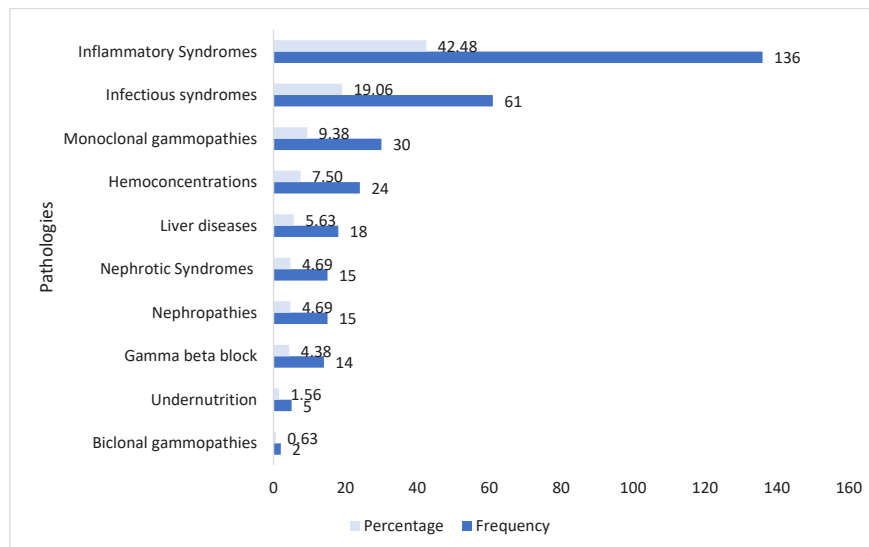

Figure 3. Normal Profile.

Figure 4. Pathologies Revealed by Capillary Electrophoresis.

One patient was reported to suffer from bisalbuminemia in this study, which is in line with the study of Hajoui et al (12), in which one 62-year inpatient was observed. Bisalbuminemia is an electrophoretic abnormality characterized by the duplication of albumin on the obtained profile (18). Capillary electrophoresis allowed the diagnosis of this particularly rare abnormality that is misdiagnosed by means of conventional agarose gel electrophoresis and whose pathophysiology is elusive (19). Inflammatory syndrome was the main observation in this study. The high proportion of patients with increased alpha and beta globulins may likely explain this finding. Guo et al (20) have defined the inflammation as a consequence of either the direct actions of infectious pathogens or indirect those of molecules exerted during their activation or of the molecules derived from host proteins.

Beta 1 and 2 fractions were well distinct on all electrophoretic profiles allowing for the detection of monoclonal gamma diseases in the beta 2 area, which has already been reported by Szymanowicz et al (7). To be noted, these authors stressed on the fact that the appearance of beta 2 globulins higher than beta 1 globulins should be interpreted with caution. We found 10.29% of patients to have monoclonal gammopathies that may be attributed to an improved detection of monoclonal component by capillary electrophoresis. Indeed, the observation of monoclonal peaks is done more accurately using capillary technique than its counterpart agarose-dependent as there is no varying binding affinity between proteins and dyes (21).

Conclusion

Capillary electrophoresis results are sufficiently precise for the identification of biological syndromes and clearly differentiate the six fractions of each protein. Monoclonal profiles and inflammatory syndromes were well-revealed. We found a prevalence of 10.29% for monoclonal gammopathies. The present study showed the utilization of capillary electrophoresis could detect underlying immunological pathologies that are likely to be misdiagnosed with conventional electrophoresis methods routinely used in most health facilities in Cameroon. The implementation of this technique, as first line strategy, in the health facilities could be greatly helpful in diagnosis even though certain interferences may exist during manipulation and should be taken into account.

Conflict of Interest Disclosures

The authors declare no conflicts of interest.

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