Original Research Article

Electrophoretic patterns of serum proteins in a chronic hemodialysis population

Abstract:

Objective*:* This study aimed to explore the benefit of monitoring patients on maintenance hemodialysis with serum protein electrophoresis (SPE) as a low-cost biologic test.

Patients and methods: It is a mono-center retrospective study, conducted between January 2017 and December 2020 among 100 patients at maintenance hemodialysis.

The patients' blood was collected after the hemodialysis session, and was analyzed for various biological tests. Tthe electrophoresis of serum proteins was analyzed by the ~~capillarys~~capillaries system from Sebia.

Results: Serum protein electrophoresis was normal in 36% of cases, showed an inflammatory syndrome in 24% of cases, hypergammaglobulinemia in 19% of patients, severe malnutrition profile in 13% of patients, anemic profile (8%) and nephrotic profile in 5% of cases.

The combined analysis of anthropometric ~~parametrs~~parameters, CRP, total cholesterol, albumin and serum protein electrophoresis results diagnose the complex inflammation malnutrition in 18% of patients.

The presence of an inflammatory syndrome at the SPE was associated with a positive CRP in 65% of cases (p = 0.002). The mean value of Albumin (g/l) with biochemical assay (bromocresol green method) is 39,8±3,83 versus 38,54±4,59 obtained with electrophoretic measurement. The discrepancy was observed more in the low concentrations of albumin (20 patients presented hypoalbuminemia in the SPE method versus 9% in the BCG method).

Conclusion: Serum protein electrophoresis is an inexpensive biological test that allows monitoring of protein metabolism in chronic hemodialysis patients. The diagnosis of inflammation and malnutrition sometimes lacks sensitivity or specificity, but the SPE is the test of choice for the diagnosis of monoclonal gammapathy.

Key words: Eelectrophoresis-, Hhemodialysis,- Iinflammation,- Mmalnutrition

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Introduction:

The prevalence of chronic kidney disease CKD is estimated to be 8–16% worldwide [1], with about 2 millions currently treated with hemodialysis (HD) [2]. Unfortunately, despite the technical advances in hemodialysis, the mortality of patients with end-stage renal disease (ESRD) is 10 to 30 times higher than that of the general population [3].

Protein-energy wasting (PEW) is a common complication of CKD and is thought to contribute to the high rates of morbidity and mortality observed in this population[4]. In fact, HD induces inadequate nutritional intake because of the decreased appetite and the increased dietary restrictions, thereby the persistent inflammation reduces the synthesis and half-life of serum albumin [5,6]. It is accepted now that malnutrition and inflammation occurs concomitantly, and researchers often use the term malnutrition-inflammation complex (MIC) to designate the combination of these two conditions in this population [7].

The biological monitoring of dialyzed patients aims to prevent the various complications arising from blood dialysis. In addition to other complications such as nutritional, hematologic, cardiovascular, and infectious problems [8].

Serum protein electrophoresis (SPE) is commonly used in CKD, it allows the qualitative evaluation of serum proteins and provides valuable information about their metabolism.
SPE can lead to the etiologic diagnosis by detecting monoclonal gammopathy which are frequently complicated by renal failure. Such as cast nephropathy, Randall's disease or amyloidosis or to explore an associated inflammatory syndrome [9].

This study aimed to explore the benefit of monitoring patients on maintenance hemodialysis with serum protein electrophoresis as a low-cost biologic test.

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This will provide the gap of the study.

Patients and methods:

It is a mono-center retrospective study, conducted between January 2014 and December 2017 among 100 patients undergoing hemodialysis at Atlas HD center in collaboration with the laboratory of biochemistry of military hospital Avicenna in Marrakech.
Blood specimen was obtained from each participant after dialysis. Laboratory testing was performed to determine complete blood count (CBC), total protein,

albumin, C-reactive protein (CRP), ferritin, creatinine, urea, total cholesterol, Triglyceride, HDL, LDL, alanine transaminase (ALT), aspartate transaminase (AST), gamma glutamyl transferase (GGT), alkaline phosphatase (ALP) as well as serological detection of hepatitis C virus (HCV) and hepatitis B virus (HBV).

Serum protein electrophoresis was performed on capillary electrophoresis system from Sebia at pH 8.6.

~~we analyzed the data~~The data was analyzed using SPSS software version 16.0. Descriptive analysis of the sample as a whole was first done followed by expressing~~First, we performed a descriptive analysis of the sample as a whole; t~~ the quantitative variables ~~were expressed~~ as mean ± standard deviation, and finally ~~the~~ qualitative variables as frequencies and percentages.
The association between categories is measured by the Chi square test and the significance threshold was set at 0,05.
The imperative of informed consent was waived in light of the anonymous, retrospective, and observational character of this study.

Results:

This study comprised 100 patients, median age 53,13 years (±13,22). The total of the 100 patients (46 men and 54 women) completed the measurement of all the parameters at the baseline. The patients suffered from the following comorbidities: hypertension (33%), diabetes mellitus (18%), polycystic kidney disease (12%). The etiology of renal failure is vascular nephropathy in 21% and diabetes in 18%. The demographic variables, anthropometric measurements and laboratory parameters of the study subjects are depicted in the Table 1~~Table 1~~, and the serum protein electrophoresis results are resumed in the Table 2~~Table 2~~.

Table 1 : T~~t~~he demographic, clinic and biological characteristics of the patients.

	Mean	Max	Min	interquartile range	Normal range
Age (years)	53.13	6	83	13.22	
Age at start of HD (years)	50.44	6	78	14.05	
age of HD (months)	99.24	12	264	61.44	
BMI (Kg/M2)	24.46	15	37.77	5.52	20-25
urea (mmol/l)	22.16	11.33	53.16	6.83	2.5-7.5
creatinine (mmol/l)	918.31	1587.97	254.95	272.56	60-120
albumin (g/l)	39.8	49.1	29	3.83	35-50
cholesterol (mmol/l)	3.82	6.74	2.19	0.93	3.6-5.2
triglyceride (mmol/l)	1.53	6.17	0.49	0.82	<1.7

HDL (mmol/l)	0.98	2.17	0.49	0.31	>1.10
LDL (mmol/l)	2.19	4.31	0.9	0.74	1.6-4.4
ASAT (UI/l)	16.34	210	0.3	24.33	<50
ALAT (UI/l)	14.312	128	0.1	17.91	<65
PAL (UI/l)	133.7	1015	10.6	134.2	35-104
Hemoglobin (g/dl)	11.11	16.3	8.2	1.76	12-16
CRP (mg/l)	9.03	85	0.25	14.32	<5
ferritin (ng/l)	303.06	868	9	187.67	14-450

Table 2 : ~~r~~Results of serum protein electrophoresis:

parameter SPE	mean	max	Min	interquartile range	References
Total proteins (g/l)	70.79	88	57	5.61	66-83
Albumin (g/l)	38.54	48.59	26.56	4.59	35-47.6
Alpha 1 globulin (g/l)	2.62	6.3	1.05	0.96	2.10-4
Alpha 2 globulin (g/l)	7.62	12.37	5.02	1.31	5.10-9
Beta 1 globulin (g/l)	4.76	9.3	2.7	1.2	3.4-5.2
Beta 2 globulin (g/l)	4.1	10.64	2.06	1.35	2.3-4.7
Gammaglobulin (g/l)	13.4	23.66	7.69	3.27	7.00-14.00
Ratio Albumin/Globulin	1.21	1.87	0.59	0.25	1.2-1.8

Serum protein electrophoresis was normal in 36% of cases, showed an inflammatory syndrome in 24% of cases, hypergammaglobulinemia in 19% of patients and a severe malnutrition profile in 13% of cases, anemic profile (8%) and nephrotic profile in 5% of our patients. Figure 1~~Figure 1~~

Figure 1 : Syndromic profile of serum protein electrophoresis results:

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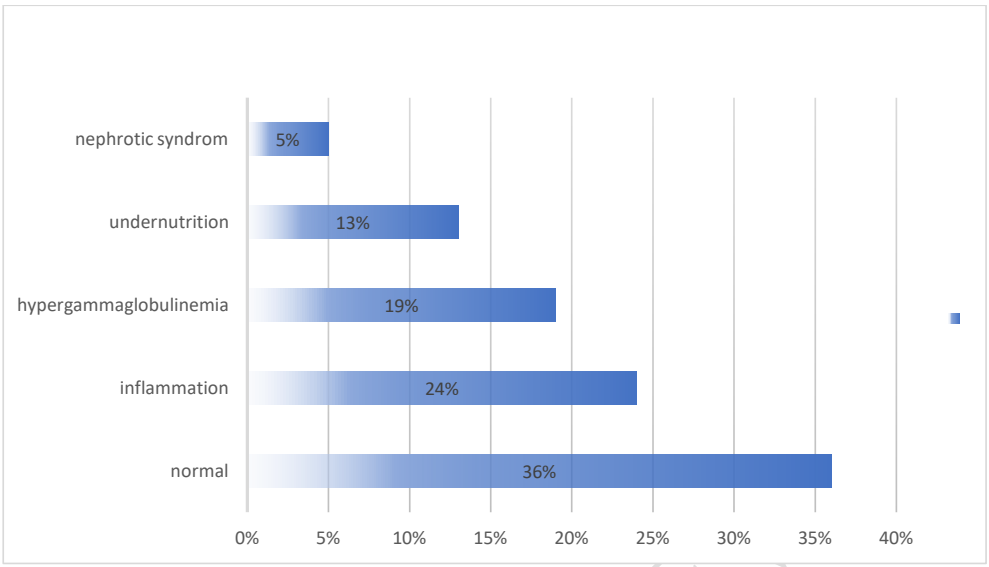

The presence of an inflammatory syndrome at the SPE was associated with a positive CRP in 65% of cases (p = 0.002).
The mean value of albumin (g/l) is 38,54±4,59 in electrophoretic measurement versus 39,8±3,83 obtained with biochemical quantification (bromocresol green method).
the combined analysis of BMI, CRP, total cholesterol, albumin and serum protein electrophoresis results diagnose the complex inflammation malnutrition in 18% of patients.

The prevalence of positive hepatitis C serology in our series was 6% and that of hepatitis B was 2%. the 2 diagnoses were often associated with an inflammatory syndrome and hypergammaglobulinemia in 2 patients with positive HCV.
There was no evidence of a monoclonal peak of gammaglobulins or dysgammaglobulinemia in any of the patients.

Discussion:

Protein metabolism is affected by chronic renal failure, regardless of its stage or the renal replacement therapy used (hemodialysis, peritoneal dialysis or renal transplantation). More than renal failure by itself, it is the conditions associated with it, primarily chronic inflammation, oxidative stress, uremia, metabolic acidosis and peripheral insulin resistance that led to catabolism and disturbances of protein metabolism [10].

Serum protein electrophoresis is a qualitative or semi-quantitative method, employed mostly in order to diagnose monoclonal gammopathy. Especially in the presence of renal failure that could be a direct consequence of the presence of an M component.

To the best of our knowledge, this is the first study assessing the protein electrophoretic patterns in hemodialysis patients, and we were focused on the contribution of serum protein electrophoresis in the exploration of protein metabolism in chronic hemodialysis patients and the distribution of electrophoretic profiles in this patient population.

Syndromic analysis of electrophoresis results showed that the inflammatory profile is the most prevalent in our hemodialysis population.

CKD is characterized as a low-grade chronically inflamed state that has five progressive stages. In the first two stages, patients are asymptomatic, thus may go undiagnosed[11]. As the disease progresses, end-stage renal disease (ESRD) ensues in which one needs long-term dialysis or a kidney transplant [11,12]. This chronic low-grade inflammation is attributed to several factors which include increased production of proinflammatory cytokines, oxidative stress, chronic and recurrent infections, fluid overload, sodium overload, and gut dysbiosis[12,13].

Acute inflammation is characterized by the hepatic synthesis of proteins of the acute phase of inflammation, also called acute phase reactants. These are α-1 antitrypsin, α-1 anti-chymotrypsin, and orosomucoid which are α-1 globulins, ceruloplasmin, and haptoglobin that migrate into α-2 globulin. when CRP, and complement constituents are β-globulin[14].

The inflammatory electrophoretic profile in SPE is characterized by hyper α-globulinemia. When it associates hypoalbuminemia, hyper β-globulinemia and hyper γ-globulinemia with it, it testifies to the chronicity of the inflammatory phenomenon[14].

Detection of inflammation among adults with CKD is commonly identified by Tumor Necrosis Factor-alpha (TNFα), Interleukin-6 (IL-6) and C-reactive protein (CRP)[11,15]. CRP, especially, has been associated with an increased risk of cardiovascular disease, cardiovascular morbidity, and mortality risk in this population[3,8,13,15,16].
Over half of adults with advanced stages (3–5) of CKD have elevated levels of CRP and the prevalence is even higher, at 35–65%, in adults undergoing chronic hemodialysis [6,17].

In our study, the prevalence of a positive CRP is 37%, while PSE shows an inflammatory electropherogram in 24% of cases. In fact, among patients with an inflammatory syndrome on SPE, 65% (n = 19) had a positive CRP. The concordance between the 2 biological tests is statistically significant. Nevertheless, the CRP is much more sensitive and easier to handle for the monitoring of inflammation in hemodialysis patients.
The increase of CRP variate usually within the marginal zone between norm and pathology (3-10 mg/L),testifying the chronic low grade inflammation[18].

We diagnosed severe malnutrition in 13% of our patients. Several studies have evidenced malnutrition in 23%-76% of patients on hemodialysis (HD) and in 18%-50% of patients on peritoneal dialysis [19].The wide variation in malnutrition prevalence in patients on HD may be attributed to the different assessment methods. Those methods could be subjective (clinical history and nutritional physical examination) or objective (anthropometry, biochemical exams, and bioelectrical impedance).

The major sign of malnutrition is hypoalbuminemia. In our study, the mean value of albumin (g/l) is 38,54±4,59 in electrophoretic measurement versus 39,8±3,83 obtained with biochemical quantification. This founds agrees with several authors who have found a difference of 0.5 to o.6 g/dl between the 2 methods, with a tendency for the bromocresol green dying method to find higher values than the electrophoresis method especially in low concentrations[20,21].

Malnutrition among HD patients is attributed to several factors including limited dietary intake, dialysis-related nutrient loss, oxidative stress, metabolic acidosis, hyper-metabolism, and chronic inflammation-induced protein breakdown[6,22]
The development of sarcopenia and cachexia in ESRD is both a consequence and a cause of systemic diseases. [18,23]. In fact, conventional cardiovascular risk factors such as hypercholesterolemia or hypertension do not strongly affect mortality in patients with CKD. However, low serum albumin, poor protein intake, and low BMI or weight loss are strong predictors of mortality in HD patients[24].

Hypergamamglobulonemia is the result of non-specific plasma cell activation. It is often encountered during viral or bacterial infections, autoimmune conditions, or sometimes without determined reason [25].

In conclusion, SPE is an inexpensive biological test that allows a general assessment of protein metabolism in hemodialysis patients. Although the quantification of the different protein fractions is global, and lacks of precision. the syndromic study of the electropherograms gives precious information and allows a correct monitoring of the nutritional and inflammatory state of the patients.

References:

[1] Jha V, Garcia-Garcia G, Iseki K, Li Z, Naicker S, Plattner B, et al. Chronic kidney disease: global dimension and perspectives. The Lancet 2013;382:260–72. https://doi.org/10.1016/S0140-6736(13)60687-X.

[2] Figliuzzi M, Remuzzi G, Remuzzi A. Renal bioengineering with scaffolds generated from rat and pig kidneys. Nephron Exp Nephrol 2014;126:113. https://doi.org/10.1159/000360683.

[3] Moreau-Gaudry X, Jean G, Genet L, Lataillade D, Legrand E, Kuentz F, et al. A simple protein-energy wasting score predicts survival in maintenance hemodialysis patients. J Ren Nutr Off J Counc Ren Nutr Natl Kidney Found 2014;24:395–400. https://doi.org/10.1053/j.jrn.2014.06.008.

[4] Kovesdy CP, Kalantar-Zadeh K. Why is protein-energy wasting associated with mortality in chronic kidney disease? Semin Nephrol 2009;29:3–14. https://doi.org/10.1016/j.semnephrol.2008.10.002.

[5] Bramania PK, Ruggajo P, Bramania R, Mahmoud M, Furia FF. Prevalence of malnutrition inflammation complex syndrome among patients on maintenance haemodialysis at Muhimbili National Hospital in Tanzania: a cross-sectional study. BMC Nephrol 2020;21:521. https://doi.org/10.1186/s12882-020-02171-3.

[6] Jankowska M, Cobo G, Lindholm B, Stenvinkel P. Inflammation and Protein-Energy Wasting in the Uremic Milieu. Contrib Nephrol 2017;191:58–71. https://doi.org/10.1159/000479256.

[7] Kanda E, Lopes MB, Tsuruya K, Hirakata H, Iseki K, Karaboyas A, et al. The combination of malnutrition-inflammation and functional status limitations is associated with mortality in hemodialysis patients. Sci Rep 2021;11:1582. https://doi.org/10.1038/s41598-020-80716-0.

[8] Gidenne S, Ceppa F, Robino C, Sarret D, Burnat P. Biological monitoring of hemodialysis. Ann Biol Clin (Paris) 2000;58:663–74.

[9] Presle A, Bertocchio J-P, Schneider N, Maquart F-X, Ramont L, Oudart J-B. An acute monoclonal gammopathy? Ann Biol Clin (Paris) 2015;73:185–9. https://doi.org/10.1684/abc.2015.1034.

[10] Masson E. Métabolisme protéique et insuffisance rénale chronique. EM-Consulte n.d. https://www.em-consulte.com/article/24119/metabolisme-proteique-et-insuffisance-renale-chron (accessed January 17, 2022).

[11] Webster AC, Nagler EV, Morton RL, Masson P. Chronic Kidney Disease. Lancet Lond Engl 2017;389:1238–52. https://doi.org/10.1016/S0140-6736(16)32064-5.

[12] Aycart DF, Acevedo S, Eguiguren-Jimenez L, Andrade JM. Influence of Plant and Animal Proteins on Inflammation Markers among Adults with Chronic Kidney Disease: A Systematic Review and Meta-Analysis. Nutrients 2021;13:1660. https://doi.org/10.3390/nu13051660.
[13] Cobo G, Lindholm B, Stenvinkel P. Chronic inflammation in end-stage renal disease and dialysis. Nephrol Dial Transplant Off Publ Eur Dial Transpl Assoc - Eur Ren Assoc 2018;33:iii35–40. https://doi.org/10.1093/ndt/gfy175.
[14] Szymanowicz A, Cartier B, Couaillac J-P, Gibaud C, Poulin G, Rivière H, et al. Proposition de commentaires interprétatifs prêts à l'emploi pour l'électrophorèse des protéines sériques. Ann Biol Clin (Paris) 2006;64:367–80.
[15] Del Giudice M, Gangestad SW. Rethinking IL-6 and CRP: Why they are more than inflammatory biomarkers, and why it matters. Brain Behav Immun 2018;70:61–75. https://doi.org/10.1016/j.bbi.2018.02.013.
[16] Zimmermann J, Herrlinger S, Pruy A, Metzger T, Wanner C. Inflammation enhances cardiovascular risk and mortality in hemodialysis patients. Kidney Int 1999;55:648–58. https://doi.org/10.1046/j.1523-1755.1999.00273.x.
[17] Dashti N, Einollahi N, Nabatchian F, Moradi Sarabi M, Zarebavani M. Significance of albumin and C-reactive protein variations in 300 end stage renal disease patients in Tehran University of Medical Sciences Hospitals during year 2010. Acta Med Iran 2012;50:197–202.
[18] Gusev E, Solomatina L, Zhuravleva Y, Sarapultsev A. The Pathogenesis of End-Stage Renal Disease from the Standpoint of the Theory of General Pathological Processes of Inflammation. Int J Mol Sci 2021;22:11453. https://doi.org/10.3390/ijms222111453.
[19] Oliveira CMC de, Kubrusly M, Mota RS, Silva CAB da, Oliveira VN. Malnutrition in chronic kidney failure: what is the best diagnostic method to assess? Braz J Nephrol 2010;32:57–70. https://doi.org/10.1590/S0101-28002010000100011.
[20] Comolli J, Divers S, Lock B, Camus MS. COMPARISON OF PROTEIN ELECTROPHORESIS AND BIOCHEMICAL ANALYSIS FOR THE QUANTIFICATION OF PLASMA ALBUMIN IN HEALTHY BEARDED DRAGONS (POGONA VITTICEPS). J Zoo Wildl Med Off Publ Am Assoc Zoo Vet 2021;52:253–8. https://doi.org/10.1638/2019-0218.
[21] Brackeen GL, Dover JS, Long CL. Serum albumin. Differences in assay specificity. Nutr Clin Pract Off Publ Am Soc Parenter Enter Nutr 1989;4:203–5. https://doi.org/10.1177/0115426589004006203.
[22] Carrero JJ, Stenvinkel P, Cuppari L, Ikizler TA, Kalantar-Zadeh K, Kaysen G, et al. Etiology of the protein-energy wasting syndrome in chronic kidney disease: a consensus statement from the International Society of Renal Nutrition and Metabolism (ISRNM). J Ren Nutr Off J Counc Ren Nutr Natl Kidney Found 2013;23:77–90. https://doi.org/10.1053/j.jrn.2013.01.001.

[23] Sabatino A, Cuppari L, Stenvinkel P, Lindholm B, Avesani CM. Sarcopenia in chronic kidney disease: what have we learned so far? J Nephrol 2021;34:1347–72. https://doi.org/10.1007/s40620-020-00840-y.
[24] Suryantoro SD, Ardhany AR, Basoeki W, Thaha M, Mardiana N, Tjempakasari A, et al. Dietary management of haemodialysis patients with chronic kidney disease and malnourishment. Asia Pac J Clin Nutr 2021;30:579–87. https://doi.org/10.6133/apjcn.202112_30(4).0004.
[25] Omar MN, Tashkandy MA, Tonsy AHE. Liver Enzymes and Protein Electrophoretic Patterns in Hemodialysis Patients with Antibodies Against the Hepatitis C Virus. Saudi J Kidney Dis Transplant 1995;6:163.