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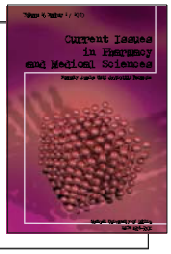

Analysis of serum proteins by agarose gel and capillary electrophoresis

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ABSTRACT

Electrophoresis is a basic technique used to identify disorders of blood serum protein fractions. Agarose gel is the most frequently used medium for routine protein separations. However, capillary electrophoresis seems to be an attractive alternative to gel electrophoresis. The article presents the results of comparative analysis of two systems (Sebia): Hydrasys designed for electrophoretic separations on agarose gel and Minicap for capillary electrophoresis. The purpose of study was to evaluate comparatively the concentrations of each serum protein fraction obtained by gel and capillary electrophoresis and to analyze the correlations between the results obtained by those two systems depending on the concentrations of each protein fraction. The study was carried out in the group of 98 patients, 46 females and 52 males. Despite slight quantitative differences in certain fractions obtained by both methods, capillary electrophoresis offers a fully automatic process of analysis, high speed and efficiency which proves that capillary electrophoresis is appropriate alternative to gel electrophoresis.

Keywords: Agarose gel electrophoresis (AGE), Capillary electrophoresis (CE), Serum proteins

INTRODUCTION

Electrophoresis has been a recognized technique to identify disorders of blood serum protein fractions. Abnormal electrophoretic separations can help detect diseases of the kidneys, liver, autoimmune problems and acute and chronic infections [13,16]. The most characteristic and diagnostically useful are the separations observed in the course of monoclonal gammopathies [13,18].

Electrophoresis is a process that uses protein migration in an electric field. Proteins are separated as the molecules move with various speed, depending on the charge-to-mass ratio, physical shape and size [1,7].

Agarose gel is the most frequently used medium for routine protein separations. Agarose gel electrophoresis (AGE) is used to separate five protein fractions: albumins, alpha-1-globulins, alpha-2-globulins, beta-globulins and gamma-globulins. After the separation, the gel is fixed, stained and densytometrically scanned to measure the concentrations of particular protein fractions. However, the technique of electrophoresis is work-consuming despite available ready-made gels, reagent kits or semi-automatic systems for electrophoresis [1,15,18].

Capillary electrophoresis (CE) seems to be an alternative to traditional manual or semi-automatic gel electrophoresis [18]. Recently, capillary electrophoresis has become a fully automatic system for the separation of proteins and other biopolymers, including blood serum fractions. Ionized molecules are placed in thin silicate capillaries less than 100 μm in diameter and high-voltage electrical power is applied to initiate the migration and separation of the molecules towards the destination capillary. The output is read directly at the cathode end of the destination capillary by spectrophotometry within the wave range of ca. 200 nm. The advantages of capillary electrophoresis include high accuracy and resolution, small amounts of analyte samples and reagents needed, short time of analysis and full automation of the process [2-5].

The purpose of study was to comparatively evaluate particular protein fraction concentrations obtained by gel and capillary electrophoresis and to analyze correlations between those two systems and to present graphically the differences in the results obtained by two systems of electrophoresis depending on mean concentrations of the fractions.

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MATERIAL AND METHOD

The study was conducted in the group of 98 subjects. Among the enrolled subjects there were 46 women of the mean age 65±10 years and 52 men of the mean age 61±8 years. The patients were treated at the Clinic of Hematology and Bone Marrow Transplantation, SPSK-1 in Lublin.

Blood was drawn from the cubital vein for clotting into tubes without anticoagulant. After 20-30 min of clotting, the tubes were centrifuged for 10 min at 2,500 rpm. The serum obtained this way, was separated into clean tubes and analyzed. The measurements were carried out in the Department of Laboratory Diagnostics, SPSK-1 in Lublin.

In all subjects, determination of total protein and protein electrophoresis tests were performed in blood serum.

AGE was performed using Sebia Hydragel Proteine 15/30 kit on Hydrasys (Sebia, France) according to the manufacturer's directions. The serum was applied to 0.8% agarose gel in appropriate Tris-barbital buffer (pH 9.2). After electrophoresis, the gels were stained with amido black and destained, and an electrophoretogram was generated by scanning densitometry using Phoresis 8.6.

CE was performed on Minicap with Minicap Protein 6 kit (Sebia, France) according to the manufacturer's directions. The kit is designed for the separation of six human serum proteins with alkaline buffer (pH 9.9). It is equipped with two parallel capillaries, which allows two analyzing processes to be performed simultaneously. Buffered samples are inserted into the capillary by aspiration at its anode end. Direct reading of the protein separation output is read at the cathode end at 200 nm wave length.

Quantitative results for each fraction of the AGE or CE electrophoretogram were expressed in g/dl by multiplying the percentage of total area under the curve by total serum protein concentration which was measured on the biochemical autoanalyser Cobas Integra 400 (Roche, Basel, Switzerland).

STATISTICAL ANALYSIS

The results were analyzed statistically by STATISTICA 10.0 (StatSoft) and MedCalc 10.2. The values of parameters (albumins, alpha-1-globulins, alpha-2-globulins, beta-globulins, gamma-globulins) determined by AGE and CE were expressed descriptively as arithmetic mean (X), standard deviation (SD), median (Me) and percentile range 25-75%.

Electrophoretogram produced by AGE showed conventional separation into five regions, so quantitative results for beta-1 and beta-2 fractions obtained by CE were considered as a unique fraction.

Distribution of the results was analyzed by Shapiro-Wilk test and differences between variables were evaluated by Wilcoxon signed rank test; correlations were determined by Spearman's rank correlation test. To present the character of differences between the results Bland-Altman plot and Mountain plot was applied. Statistical significance was assumed at $p<0.05$.

RESULTS

In the examined group, total protein concentration ranged from 3.48 g/dl to 11.89 g/dl, mean concentration of total protein was 6.95±1.26 g/dl.

Table 1 presents the concentrations of each protein fraction determined by AGE and CE.

In the examined group mean serum albumin concentration obtained by AGE was 4.02±0.77 g/dl and was statistically significantly higher ($p<0.0001$) than the value determined by CE (3.87±0.66 g/dl). Mean alpha-1-globulin concentration on AGE (0.22±0.07 g/dl) was statistically significantly lower ($p<0.0001$) compared to the concentrations measured by CE (0.33±0.10 g/dl). The concentrations of alpha-2-globulins on AGE (0.76±0.15 g/dl) were statistically significantly higher ($p<0.0001$) in comparison to the values on CE (0.72±0.16 g/dl). The concentrations of gamma-globulins on AGE (1.26±1.19 g/dl) were statistically significantly lower ($p<0.0001$) than the values on CE (1.30±1.15 g/dl). There were no statistically significant differences between beta-globulin concentrations determined by AGE and CE.

Table 2 presents the correlations between each protein fraction concentration determined by AGE and CE.

The results were highly correlated ($p<0.0001$). The highest coefficient $r=0.933$ was noted for albumin fraction. The correlation coefficient for beta-globulin fractions was $r=0.561$ and it reached the lowest value ($p<0.0001$).

Figure 1 illustrates graphically the results obtained by Bland-Atman plot and Mountain plot.

Table 1. Descriptive statistics of each protein fraction concentration

Parameters	Agarose gel electrophoresis				Capillary electrophoresis			
	n	X±SD	Me	25-75%	n	X±SD	Me	25-75%
Albumin [g/dl]	98	4.02±0.77*	4.21	3.72-4.48	98	3.87±0.66	4.02	3.52-4.33
Alpha-1-globulin [g/dl]	98	0.22±0.07*	0.20	0.18-0.26	98	0.33±0.10	0.31	0.27-0.36
Alpha-2-globulin [g/dl]	98	0.76±0.15*	0.74	0.64-0.82	98	0.72±0.16	0.70	0.62-0.80
Beta-globulin [g/dl]	98	0.70±0.12	0.69	0.61-0.77	98	0.74±0.37	0.68	0.57-0.78
Gamma-globulin [g/dl]	98	1.26±1.19*	0.95	0.68-1.31	98	1.30±1.15	0.99	0.70-1.46

n – number of examined; * $p<0.0001$

Table 2. Spearman rank correlation between the results of AGE and CE

Parameters	n	Correlation coefficient (r)	Level of significance (p)
Albumin on AGE vs. Albumin on CE	98	0.933	0.0001
Alpha-1-globulin on AGE vs. Alpha-1-globulin on CE	98	0.825	0.0001
Alpha-2-globulin on AGE vs. Alpha-2-globulin on CE	98	0.771	0.0001
Beta-globulin on AGE vs. Beta-globulin on CE	98	0.561	0.0001
Gamma-globulin on AGE vs. Gamma-globulin on CE	98	0.867	0.0001

n – number of examined; AGE – agarose gel electrophoresis; CE – capillary electrophoresis

Bland-Altman plot graph (Fig. 1 A1) presents the correlations between the differences in the results of albumin concentrations obtained by both methods (AGE and CE) and the range of albumin concentrations; gradient coefficient was 0.157 and it was statistically significantly different from zero (Fig. 1 A1). Percentile distribution of differences between the results of AGE and CE determined by Mountain plot was slightly left-wise inclined at the median of differences Me=0.195 g/dl, 95% percentile range of differences was from -0.71 to 0.73 g/dl (Fig. 1 A2).

Bland-Altman plot (Fig.1 B1) illustrates significant differences between the results of alfa-1-globulins depending on the determined concentration range; gradient coefficient was -0.287 and it was significantly different from zero ($p<0.0001$). 95% range of differences was from -0,26 to -0,02 g/dl at median Me=-0,10 g/dl; percentile distribution of differences between the results was markedly left-wise inclined (Fig. 1 B2).

Graphic illustration according to Bland and Altman (Fig.1 C1) found no differences between the values of alfa-2-globulin depending on the concentration determined, gradient coefficient (-0.027) was not significantly different from zero ($p=0.6464$). The range of differences assessed by Mountain plot was symmetrical and ranged from -0.12 to 0.49 g/dl at median of differences Me=0.04 g/dl close to zero (Fig.1 C2).

The analysis of beta-globulin fraction revealed significant differences between the results depending on the range of concentrations determined; gradient coefficient was 1.414 and it was significantly ($p<0.0001$) different from zero (Fig.1 D1). Percentile distribution of beta-globulin differences obtained by AGE and CE assessed by Mountain plot was markedly left-wise inclined; 95% percentile range of differences was wide from -2.44 to 0.39 at the median close to zero Me=0.01 g/dl. (Fig.1 D2).

Bland-Altman graph (Fig.1 E1) did not find any significant differences ($p=0.227$) between the concentrations of gamma-globulins depending on the concentration ranges. Gradient coefficient (0.032) was close to zero (Fig.1 E1). Percentile distribution of differences between AGE and CE results on Mountain plot was slightly right-wise inclined at the median Me=-0.10 g/dl; 95% percentile range of differences was from -0.44 to 1.71 g/dl (Fig.1 E2).

DISCUSSION

The article presents the results of comparative analysis of two electrophoresis systems (Sebia): Hydrasys designed for electrophoretic separation on agarose gel and Minicap for capillary electrophoresis. We assessed practical usefulness of both systems for diagnostic routine investigations. Therefore we examined correlations between separations obtained on both systems and looked for differences between the results.

Mean concentrations of alpha-1-globulins and gamma-globulins determined by CE were statistically significantly higher compared to AGE results. The fractions of albumins and alpha-2-globulins were higher when determined by AGE.

Chartier et al. [4] obtained similar results: albumins and alpha-2-globulins concentrations were higher on AGE and the concentrations other protein fractions were higher on CE when Hydrasys apparatus was compared to AGE and two commercial systems for capillary electrophoresis, i.e. Capillarys 2 (Sebia) and V8 (Helena).

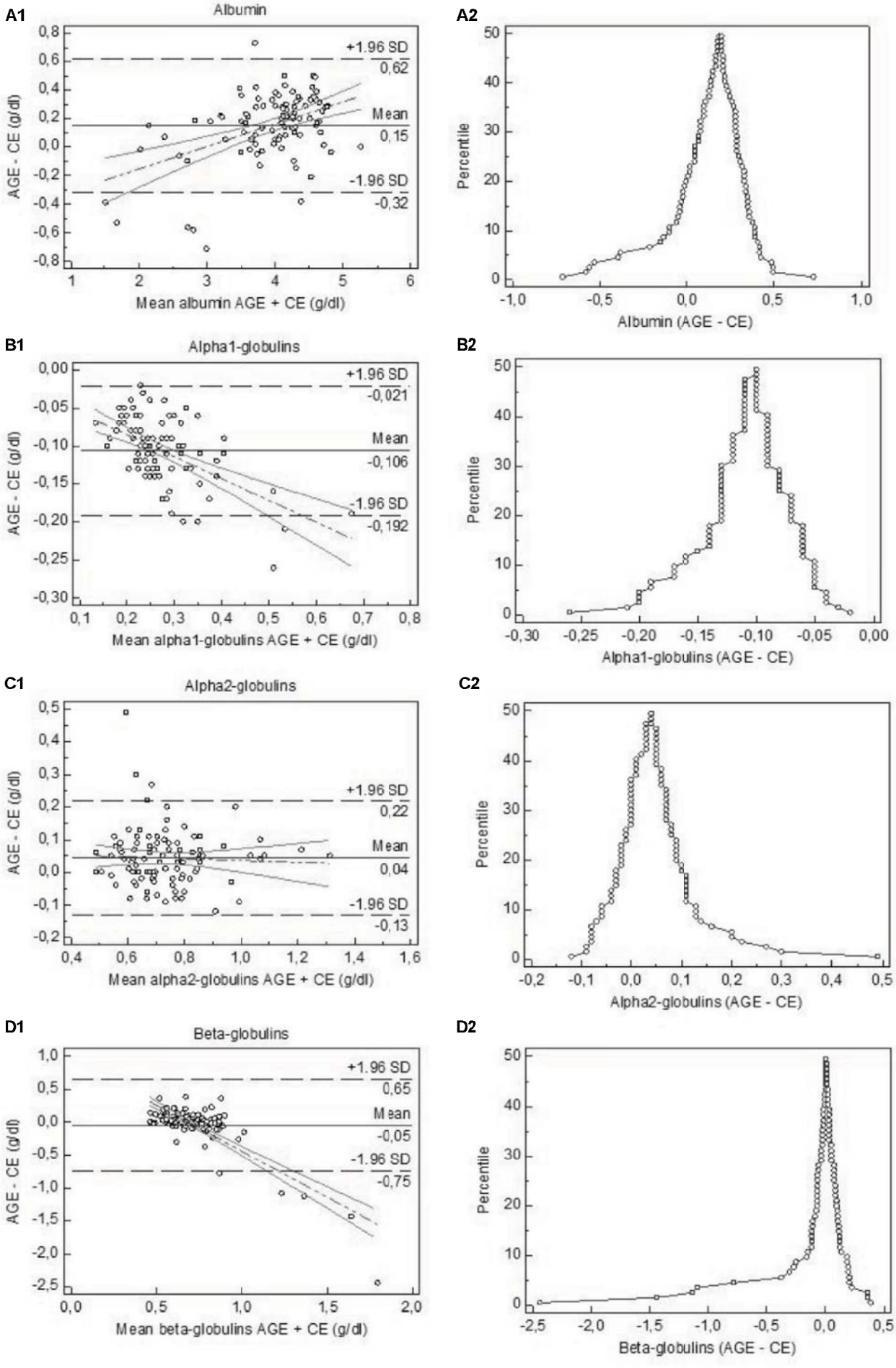
Bossuyt et al. [2] assessed three Beckman systems for electrophoresis. CE produced significantly higher concentrations for alpha-1-globulins and lower concentrations were determined for albumins, alpha-2-globulins and beta-globulins compared to electrophoresis on agarose and cellulose gels. Our results were consistent with those cited. The authors did not find significant differences in the fractions of albumins, alpha-1-globulins and alpha-2-globulins obtained on agarose and cellulose gels.

Literature data suggest that lower values for alpha-1-fraction on AGE result from big amounts of sialic acid radical in alpha-1 acidic glycoprotein, which is the member of the same fraction. As a result the protein poorly binds the stains used in AGE thus the concentrations obtained are lower [3,4,6,12]. It does not affect the results of CE, though.

The affinity of the reagents used to stain each fraction can induce incompatibilities observed in albumin fraction [4]. Data from literature have shown [11,14,18] that very strong affinity of albumin to stains can produce higher concentrations of albumins on AGE than on CE with UV detector. Our results confirm that observation too.

The discrepancy of results may also be explained by the presence of aromatic amino acids such as phenylalanine, tryptophan, tyrosine and histydine in the proteins. Those amino acids contain side chains that absorb light waves of 240-280 nm so they can impair the absorption of peptide binds at 214 nm wavelength and directly affect final UV reading on CE [2].

In the present study, there were no major differences in electrophoretograms obtained by AGE and CE. Similarly, Joliff et al. [9] have demonstrated 96% compatibility of separation in both methods in the group of 240 patients with dysproteinemias. Also Bossuyt et al. [3] confirmed


A1
Albumin
+1.96 SD
0,62
Mean
0,15
-1.96 SD
-0,32
AGE - CE (g/dl)
Mean albumin AGE + CE (g/dl)
A2
Percentile
Albumin (AGE - CE)
B1
Alpha1-globulins
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C1
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+1.96 SD
0,22
Mean
0,04
-1.96 SD
-0,13
AGE - CE (g/dl)
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C2
Percentile
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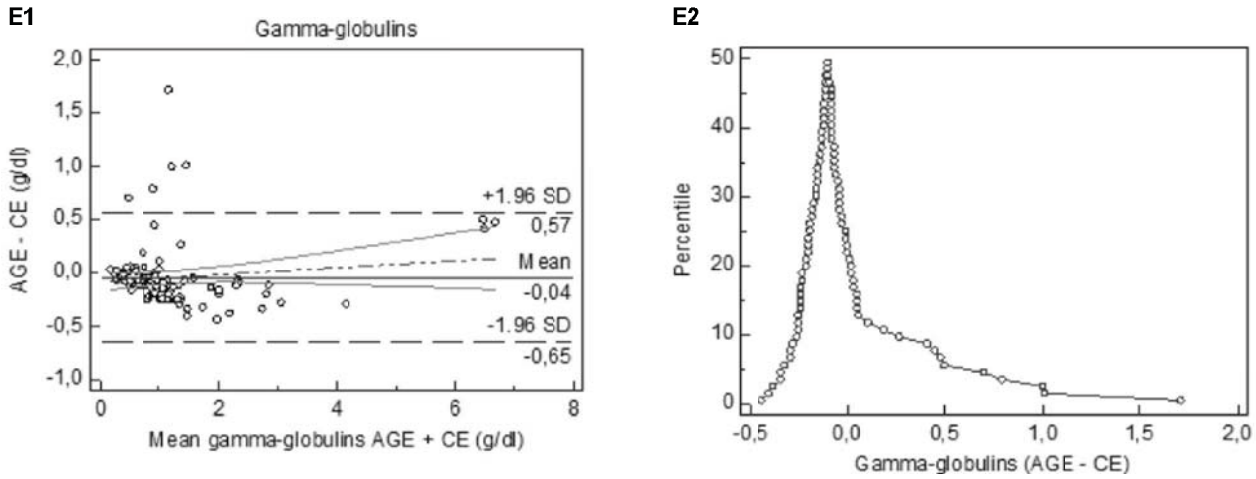

Fig. 1. Comparison of each protein fraction obtained by AGE and CE
A – albumin fraction, B – alfa-1-globulin fraction, C– alfa-2-globulin fraction, D – beta-globulin fraction, E – gamma-globulin fraction; graph 1 – differences determined by Bland-Altman plot (Center line represents the mean difference, and top and bottom lines represent the mean difference ±2SD); graph 2 – percentile distribution of ranked differences on Mountain plot.

that clinical data obtained by CE were comparable to the results obtained by agarose or cellulose gel electrophoresis. However, Jaeggi-Groisman et al. [8] and Kalambokis et al. [10] proposed interesting conclusions. They found that capillary electrophoresis was more sensitive to detect bisalbuminemia than gel electrophoresis.

The results of our research have shown highly significant correlations between the evaluated systems for electrophoresis in all protein fractions but beta-globulins where Spearman's coefficient was lower (0.561; $p<0.0001$). The other correlations ranged from 0.971 to 0.771 ($p<0.0001$).

Gay-Bellile et al. [6] evaluated Capillarys and Hydrasys systems in a group of 116 patients in whom the presence of monoclonal protein was excluded. They found a very good correlation between the results of albumins and gamma-globulins obtained by both systems ($r=0.99$; $p<0.001$), the correlation values of other fractions were lower.

Similar correlations were determined by Wijnen et al. [17]. They have demonstrated significant correlations for albumins, alfa-2-globulins and gamma-globulins ($r=0.95$, 0.99 and 0.97 respectively).

Contradictory results were obtained by Bossuyt et al. [3], who observed considerably lower correlations compared to our and other authors' results [6,17], that ranged from $r=0.41$ to 0.67.

The available literature [4,6,15] and our study, reported lower correlations for beta-globulins compared to other protein fractions. Lower correlation values may have resulted from the fact that β1 and β2-globulin fractions obtained on CE were added and expressed as the value of one fraction to be further referred to the results for beta-globulin obtained by gel electrophoresis. Hence the results are slightly diverse. Lower coefficient of linear regression for beta-globulins can partly result from the structure of buffer used to separate the fraction into β1 and β2 in CE. Such a low correlation for beta-globulin fractions may also result from a different migration of beta-lipoproteins in gel electrophoresis and capillary electrophoresis [12].

Moreover, we analyzed the correlations between the values of mean pairs of results for each fraction with reference to the differences in their concentrations obtained by both evaluated methods. Therefore we used Bland-Altman plot graphic analysis and the results revealed significant differences ($p<0.0001$) in measurements for the fractions of albumins, alpha-1-globulins and beta-globulins. The results of alpha-2-globulins and gamma-globulins were not statistically significant.

The results by Chartier et al. [4] were slightly different. Using Bland-Altman plot they found good compatibility of the results obtained by AGE and CE. However the results of beta and gamma-globulin fractions were slightly dispersed (V8). They also noted that the values of differences between AGE and CE results were proportional to the concentrations of each fraction ($p<0.001$), excluding albumins (V8) and gamma-globulins (Capillarys 2).

Also Gay-Bellile et al. [6] found statistically significant differences ($p<0.001$) in Bland-Altman plot between AGE and CE which were proportional to fraction concentrations, excluding gamma-globulins.

CONCLUSIONS

1. The comparison of protein fraction concentrations obtained by gel and capillary electrophoresis revealed higher values of alpha-1 and gamma-globulins on CE and higher albumin and alpha-2-globulin fractions on AGE.
2. High correlation coefficients between the results of each protein fraction suggest that both systems are equally useful for the separation of blood serum protein fractions.

3. In comparison to AGE, capillary electrophoresis, despite slight quantitative differences noted for certain fractions, provides a fully automatic process of separation, works fast and is effective which proves that it is a good alternative to gel electrophoresis.

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