SERUM PROTEINS ANALYSIS BY CAPILLARY ELECTROPHORESIS

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ABSTRACT

The purpose of this study was to evaluate the efficacy of multi-capillary electrophoresis instrument in clinical laboratory. An automated clinical capillary electrophoresis system was evaluated for performing serum proteins electrophoresis and immuno-fixation electrophoresis by subtraction. In this study the performance of capillary electrophoresis was compared with the cellulose acetate membrane electrophoresis and agarose gel immunofixation electrophoresis for serum proteins. The results of capillary electrophoresis and cellulose acetate membrane electrophoresis were good ($r = 0.89 \sim 0.97$) for protein fractions and A/G ratio except for β -globulin fraction ($r = 0.60$). Both within-run and day to day precisions (CVs) of assay results for 5 main fractions and A/G ratio ($n = 10$) were between 0.3~6.3 %. The reference ranges of serum protein fractions obtained from 200 healthy individuals by cellulose acetate membrane electrophoresis were almost equal to that of capillary electrophoresis except for α -1 globulin fraction. No significant difference of electropherograms between cellulose acetate electrophoresis and capillary electrophoresis was observed in the abnormal serum such as presence of bilirubin (< 20 mg/dl), hemoglobin (< 300 mg/dl), lipid (Intralipos $< 1\%$) and samples from patients with acute phase response, liver injury, polyclonal hyper gammaglobulinemia or M-proteinemia. The method of capillary immuno-fixation electrophoresis by subtraction showed good agreement with agarose gel immuno-fixation electrophoresis by subtraction identifying 30 monoclonal gammopathy patient samples.

KEY WORDS: Capillary electrophoresis, capillary immunofixation electrophoresis, serum proteins, M-protein

INTRODUCTION

The proportions and the quantification of the individual serum protein fractions changes in a variety of diseases is of considerable value in clinical diagnosis. The fractionation of serum protein mostly is performed with cellulose acetate membrane or agarose gel electrophoresis (1-2). Recently evaluated capillary electrophoresis (CE) could be a new feasible technique for the analysis of serum proteins because of its high separation efficiency, on line data analysis, quick separation and its easy utility (3-4). CE depends

on two main principles in the separation of proteins. The first, alkaline pH, which was created by a strong electroosmotic flow (EOF). The EOF is overall fluid movement of positively charged buffer ions carrying fluid toward the cathode. The second, the electrophoretic migration of the individual proteins is based on their charge to mass ratio at specific voltage, electrolyte composition and pH conditions. CE by immunofixation electrophoresis/subtraction systems (CZE/IFE/s) has been recently developed (5), which involves incubation of human serum with sepharose beads to which a specific binder for a heavy (IgG, IgA, or IgM) or a light (κ and λ) chain is attached. During the incubation period the proteins that bind specially to the bead-linked antibodies are retained by the solid phase. After the beads settle, CE is performed on the supernatant. The monoclonal protein underlying the

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abnormal peak is identified by comparing the subtraction on capillary electropherogram with the control electropherogram (incubated with solid phase without linked antibody). The specificity of the immunospecific binder that caused the disappearance of the monoclonal peak identifies the paraprotein. Paragon CZE 2000, an automated capillary electrophoresis system (Beckman Coulter Inc. Brea CA, USA) is for human serum proteins fractions and also designed for the high performance separation with an automated IFE/s. The purpose of this study is to evaluate the efficacy of this multi-capillary electrophoresis instrument on the clinical laboratory use.

MATERIALS AND METHODS

Human serum protein fractions (albumin, α -1-globulin, α -2 globulin, β -globulin, γ -globulin and A/G ratio) were separated and quantified by using CE with the Paragon CZE 2000 system (Beckman Coulter Inc., Brea, CA, USA). Proteins were measured by direct absorption at 214 nm through a small optical window in the capillary. The length of capillary was 20 cm, and the i.d. was 20 μ m. Instrument settings for serum proteins electrophoresis were as follows: 1 min conditioning time, 1 second injection time (approximately 1 nL of 20 fold diluted serum samples), 4.3 min separation time (9000V at 24°C), 0.5 min wash time, 0.5 min rinse time. The running buffer used was borate buffer (pH 10.0), and the capillary was rinsed between samples with 10g/L NaOH cleaning solution (Beckman Coulter Instruments). The instrument selects pattern fractions using an involved software package. For conventional cellulose acetate electrophoresis, we used the automated cellulose acetate membrane electrophoresis system (Olympus AES 620, Olympus Co., Ltd, Tokyo, Japan) according to the manufacturer's instructions. The cellulose acetate membrane of Sartorius 11200 (Sartorius, Gottingen, Germany) was preequilibrated in veronal (barbital)-veronal sodium buffer, pH 8.6, approximately 1 μ L of sample added and carried out the electrophoresis at 220 V for 25 min in veronal-veronal sodium buffer, pH 8.6. Proteins were stained by Ponsou-S (Olympus instruments) for 10 min. The individual fractions were quantified by densitometry.

Human sera from healthy individuals and patients with acute phase response, liver injury, polyclonal hyper gamma globulinemia or M-proteinemia were studied. General evaluation, consisted of evaluating reproducibility using human serum and ID zone control serum (Beckman Coulter Inc.), interference and reference ranges (200 healthy individuals). Confirmation of the validity of identifying the proteins fractionated was by employing affinity column chromatography separation (Seikagaku Kogyo, Tokyo, Japan) and antibody (Dako Japan, Kyoto, Japan) identification procedure. Concordance studies were performed using class and type of monoclonal immunoglobulins (IgG, IgM, IgA, κ and λ) in serum between the Paragon CZE 2000 system and agarose gel immuno fixation electrophoresis kit (Beckman Coulter Inc.) (6) with samples from patients exhibiting gammopathy.

RESULTS AND DISCUSSION

Separation of serum proteins

Human serum proteins were separated into five fractions by Paragon CZE 2000 (Figure 1) in accordance with those obtained by cellulose acetate membrane electrophoresis system (AES 620). We also confirmed separation of serum proteins into approximately 10 peaks employing affinity column chromatography and antibody identification procedure (Figure 2). From early migration time, we confirmed immunoglobulins (peak 1), C3 complement (peak 2), transferrin, (peak 3), α -2 microglobulin (peak 6A), haptoglobin (peak 6B), α -1 acid glycoprotein (Peak 7) α 1-antitrypsin (peak 8), albumin (peak 9) and prealbumin (peak 10). However, we could not settle 2 small peaks in the electropherogram (peaks 4 and 5) (Figure 2). These electropherograms of proteins were located by the migration pattern reported with cellulose acetate electrophoresis (1).

Method comparison study

To compare Paragon CZE 2000 (Y) with cellulose acetate membrane electrophoresis of AES 620 (X), 100 patients samples were analyzed with serum protein fractions. Good linear correlation was obtained between the methods for albumin ($Y=0.855 X+12.7$,

Fig. 1. Electropherogram of serum protein fractions

Fig. 2. Peak identification of normal human serum. 1. immunoglobulins; 2. C3 complement; 3. transferrin 4. unknown; 5. unknown, 6A. α 2-macroglobulin 6B, haptoglobin 7. α 1-antitrypsin 8. α 1-acid glycoprotein; 9. albumin 10. prealbumin

$r=0.98$), α -1globulin ($Y=0.48X+0.35$, $r=0.89$), α -2 globulin ($Y=0.82X+1.39$, $r=0.91$), γ -globulin ($Y=0.95X-0.095$, $r=0.99$) and A/G ratio ($Y=1.04X+0.22$, $r=0.97$). However, poor linear correlation was found on the β -globulin between Paragon CZE 2000 and Olympus AES 620 ($r=0.60$). These results may be due to differences of detection for transferrin in serum between the two methods (Figure 3).

Precision

Within-run and day to day precisions (CVs) with normal and abnormal controls (I.D. zone, Beckman Coulter Inc.) for Paragon CZE 2000 are shown in Table 1. Paragon CZE 2000 was mounted with seven capillaries in the system. We also confirmed precision of each of the seven capillaries. The 5 main fractions and A/G ratio ($n=10$) (Tables 2 and 3) gave the CV 0.3–6.3%. The CV values obtained with Paragon CZE 2000 are clearly better than the reported CV values for cellulose acetate electrophoresis (7).

Interferences

We investigated the effects of hemoglobin (<300 mg/dl), lipid (< 1% Intralipos), conjugated and unconjugated bilirubin (< 20 mg/dl) on Paragon CZE 2000 and AES 620 system by adding various concentrations to normal serum. No differences were observed between the two methods (data not shown).

Reference range

Reference intervals for the five serum protein fractions were determined by Paragon CZE 2000 and AES 620 from 200 healthy adults sera. Results of all fractions and A/G ratio of Paragon CZE values are statistically different ($P<0.01$) from values of AES 620 (Table 4). In particular, Paragon CZE 2000 of α -1 globulin fraction values were higher than values of AES620. Differences in the α -1 globulin are attributed to the direct UV detection of α -1 acid glycoprotein by Paragon CZE 2000. However, high sialic acid content of α -1 acid glycoprotein interferes with the binding of dyes used to quantitate the protein fractions such as AES 620 (8).

Clinical impressions

We compared electropherograms with samples from patients for acute phase response, liver injury, polyclonal hyper gamma globulinemia or M-proteinemia (Figure 4). Each electropherogram was very similar with two methods.

Concordance studies for serum immuno proteins

Serum samples of 30 patients with monoclonal gammopathy were compared between Paragon CZE

Fig. 3. Correlation between cellulose acetate electrophoresis (Olympus AES 620) and capillary electrophoresis with human serum protein fractions.

Table 1. Reproducibility

Within run precision (N=69) I.D. Zone normal (%)						
	Alb	Alpha 1	Alpha 2	Beta	Gamma	A/G
Mean	61.68	5.38	9.41	11.49	12.04	1.61
SD	0.4	0.15	0.18	0.34	0.43	0.03
CV	0.65	2.87	1.92	2.95	3.54	1.66
Max	62.4	6	9.9	11.9	13.5	1.66
Min	59.7	5.1	8.9	10.7	11.3	1.48
Range	2.7	0.9	1	1.2	2.2	0.18
Day to day precision (N=10) I.D. Zone normal (%)						
	Alb	Alpha 1	Alpha 2	Beta	Gamma	A/G
Mean	62.18	5.25	9.18	11.45	11.93	1.64
SD	0.2	0.15	0.13	0.34	0.31	0.01
CV	0.33	2.86	1.46	2.93	2.64	0.84
Max	62.5	5.5	9.4	12	12.2	1.67
Min	61.9	5	9	11.1	11.3	1.63
Range	0.6	0.5	0.4	0.9	0.9	0.04
Within run precision (N=69) I.D. Zone abnormal (%)						
	Alb	Alpha 1	Alpha 2	Beta	Gamma	A/G
Mean	34.6	3	4.9	7.4	50.1	0.53
SD	0.32	0.13	0.14	0.23	0.48	0.01
CV	0.9	4.4	2.9	3.1	1	1.6
Max	35.8	3.5	5.3	8.2	50.9	0.56
Min	34	2.8	4.7	6.9	48.2	0.52
Range	1.8	0.7	0.6	1.3	2.7	0.04
Within run precision (N=10) I.D. Zone abnormal (%)						
	Alb	Alpha 1	Alpha 2	Beta	Gamma	A/G
Mean	34.47	3.06	4.98	7.55	50.04	0.52
SD	0.21	0.12	0.09	0.3	0.35	0.01
CV	0.61	4	1.88	3.97	0.7	1.23
Max	34.8	3.2	5.1	8.3	50.6	0.53
Min	34.2	2.8	4.8	7.1	49.4	0.51
Range	0.6	0.4	0.3	1.2	1.2	0.02

Fig. 4. Comparison of cellulose acetate electrophoresis (CA) and capillary electrophoresis of various patients' sera 1. acute phase proteins 2. liver injury 3. polyclonal hypergamma-globulinemia 4. M-proteinemia (IgA-K) 5. M-proteinemia (IgG-λ) 6. M-proteinemia (IgG-λ). The upper left shows cellulose acetate membrane electrophoretic pattern. The profiles represent capillary electropherograms of the sample

Table 2. Performance of 7 different capillaries

		Alb	Alpha 1	Alpha 2	Beta	Gamma	A/G
No. 1	Mean	62.1	5.4	9.3	11.7	11.6	1.6
	SD	0.13	0.11	0.1	0.1	0.23	0.01
	CV	0.2	2.1	1.9	0.9	2	0.4
No. 2	Mean	61.8	5.3	9.4	11.8	11.7	1.6
	SD	0.12	0.1	0.16	0.07	0.19	0.01
	CV	0.2	2	1.7	0.6	1.6	0.6
No. 3	Mean	61.5	5.5	9.4	11.6	12	1.6
	SD	0.67	0.24	0.12	0.18	0.55	0.04
	CV	1.1	4.4	1.3	1.5	4.6	2.8
No. 4	Mean	61.2	5.4	9.5	11.6	12.3	1.6
	SD	0.08	0.16	0.15	0.25	0.31	0.01
	CV	0.1	2.9	1.6	2.2	2.5	0.3
No. 5	Mean	61.4	5.4	9.6	11.3	12.4	1.6
	SD	0.18	0.06	0.09	0.32	0.32	0.01
	CV	0.3	1.2	1	2.8	2.6	0.7
No. 6	Mean	62	5.3	9.3	11.2	12.1	1.6
	SD	0.23	0.13	0.16	0.36	0.28	0.01
	CV	0.4	2.4	1.7	3.2	2.3	0.9
No. 7	Mean	61.7	5.4	9.5	11.3	12.1	1.6
	SD	0.21	0.1	0.23	0.31	0.26	0.02
	CV	0.3	2	2.4	2.7	2.2	1

I.D. Zone normal (n=10,%)

		Alb	Alpha 1	Alpha 2	Beta	Gamma	A/G
No. 1-7 (n=7)	Mean	61.68	5.38	9.41	11.49	12.04	1.61
	SD	0.28	0.07	0.09	0.23	0.28	0.02
	CV	0.4	1.2	0.9	2	2.3	1.2
	Max	62.06	5.52	9.95	11.84	12.42	1.64
	Min	61.24	5.31	9.31	11.2	11.61	1.58
	Range	0.82	0.21	0.24	0.64	0.81	0.06

2000 and agarose immunofixation method(Beckman Coulter Inc.). Concordance studies showed good agreement in identifying monoclonal hyper gammopathy patients samples. Typical electropherograms are shown in Figures 5-11. It is very important in the clinical diagnosis to look for small monoclonal components, polyclonal components, free monoclonal light chains, and/or other paraproteins in

samples by Paragon CZE 2000, which we will report in the next paper in near future.

Conclusions

We have shown the evaluation of capillary electrophoresis system by using Paragon CZE 2000. The Paragon CZE 2000 system provides a faster fractionation of serum proteins in a more

Table 3. Performance of 7 different capillaries

		Alb	Alpha 1	Alpha 2	Beta	Gamma	A/G
No. 1	Mean	34.9	3	5	7.6	49.5	0.54
	SD	0.3	0.18	0.14	0.29	0.31	0.008
	CV	0.9	6	2.8	3.8	0.6	1.5
No. 2	Mean	34.8	3	4.9	7.5	49.8	0.53
	SD	0.12	0.06	0.09	0.23	0.32	0.004
	CV	0.3	2.1	1.8	3.1	0.6	0.8
No. 3	Mean	34.4	3	5	7.5	50.2	0.52
	SD	0.4	3.5	2.6	1.5	0.6	0.9
	CV	1.1	4.4	1.3	1.5	4.6	2.8
No. 4	Mean	34.6	3	4.8	7.3	50.3	0.53
	SD	0.2	0.07	0.07	0.2	0.32	0.005
	CV	0.6	2.3	1.6	2.7	0.6	1
No. 5	Mean	34.2	3	5	7.5	50.3	0.52
	SD	0.14	0.08	0.09	0.11	0.21	0.003
	CV	0.4	2.7	1.8	1.5	0.4	0.6
No. 6	Mean	34.5	3	4.9	7.4	50.2	0.53
	SD	0.46	0.19	0.13	0.17	0.71	0.011
	CV	1.3	6.3	2.8	2.3	1.4	2.1
No. 7	Mean	34.7	3.1	4.9	7.2	50.2	0.53
	SD	0.22	0.13	0.17	0.15	0.28	0.006
	CV	0.6	4.2	3.4	2.1	0.6	1.2
I.D. Zone abnormal (n=10,%)							
		Alb	Alpha 1	Alpha 2	Beta	Gamma	A/G
No.	Mean	34.6	3	4.9	7.4	50.1	0.53
1-7	SD	0.2	0.04	0.08	0.13	0.29	0.005
(N=7)	CV	0.6	1.3	1.5	1.8	0.6	0.9
	Max	34.87	3.05	5.02	7.57	50.34	0.54
	Min	34.24	2.95	4.78	7.15	49.49	0.52
	Range	0.63	0.1	0.24	0.42	0.85	0.02

Table 4. Reference range of serum protein fractions (%)

	albumin	α 1-globulin	α 2-globulin	β -globulin	γ -globulin	A/G Ratio
CZE	56.4~68.3	3.2~5.6	4.8~8.6	7.8~12.3	11.5~22.5	1.32~2.16
CAE	62.4~74.4	1.8~2.6	4.7~7.1	6.4~10.3	10.3~20.5	1.62~2.82

CZE : Paragon CZE 2000 capillary electrophoresis, CAE : Olympus AES 620 cellulose acetate electrophoresis.

Fig. 5. Figs 5-11, Comparison of proposed capillary immunofixation electrophoresis and manual immunofixation electrophoresis with serum of various gammopathy patients.

Fig. 6.

Fig. 7.

Interpretation:
IgA-Lambda, Bclonal

	(mg/dL)
IgG	447
IgA	8024
IgM	13.1

Fig. 8.

Fig. 9.

Fig. 10.

Fig. 11.

convenient way and classify the type of monoclonal gammopathy more clearly than the conventional methods. Precision and correlation between the Paragon CZE 2000 method and cellulose acetate electrophoresis for serum protein fractions were

good. Paragon CZE 2000 is simple and can be automated. We conclude that the Paragon CZE 2000 system is a useful, rapid and suitable tool for the clinical laboratory.

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