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Normal Electrophoretic Values for Human Serum Proteins in University Students by Densitometric Scanning of Cellulose Acetate Membrane

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INTRODUCTION

Electrophoretic studies of serum proteins have become routine in most clinical laboratories throughout the world. In order to evaluate quantitative variations of the serum protein fractions obtained with electrophoresis, a thorough knowledge of the normal values and patterns is essential.

The world literature abounds in reports on the electrophoretic procedures for the quantitative determination of serum proteins and the normal values. (Oreskes and Corey, 1956; Wurm and Epstein, 1956; Graham, 1960; Colehour, 1960; Kohn, 1957; Aronson and Gronwall, 1957; Vebster, 1965; Samnons and Whitehead, 1963). Dozens of new instruments have been introduced to perform the actual fractionation and quantitation. The quality of the technical analysis has been greatly improved and the procedure simplified. But the normal values of serum protein fractions are variable in accor dance with the methods of fractionation, staining and quantitation.

The purpose of this study is to establish the normal values of serum protein fractions in Korean population for use in clinical laboratory by the method of microzone electrophoresis on cellulose acetate membranes which is being used in many clinical laboratories.

The statistical evaluation has been made on the sera from 58 healthy young persons (18 females and 40 males between twenty one and thirty years old). The comparisons between the females and males have been presented and the normal values have been compared with those of others obtained by various methods.

Staining and quantitation of the protein fractions were performed using Ponceau S stain and densitometric scanning equipment.

MATERIAL AND METHODS

1. Subjects

The subjects of this study were 58 healthy university students (18 females and 40 males between twenty-one and thirty years old)

2. Reagent and Instruments

(1) Technicon Autoanalyzer MTII System

(2) Beckman Model R-101 Microzone Electrophoresis Cell and Microzone Accessory Kit.

(3) Beckman Model R-112 Scanning Densitometer.

(4) Beckman Model RD-2 Duostat Power Supply.

(5) Cellulose Acetate Membranes, 5.8-by 14.5cm, Beckman Part No. 324330

(6) Barbital Buffer Solution, Beckman B-2 Buffer, Part No. 320024

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(7) Ponceau S Staining Solution, Beckman Fixative Dye, Part No. 324340

(8) Acetic Acid, Cyclohexanone, and Alcohols (analytical grade).

3. Determination of total protein.

The concentrations of total proteins were determined by a modified biuret method (Skeggs & Hochstrasser, 1964) using Technicon Autoanalyzer MT II system.

4. Electrophoretic procedure (Kaplan & Savory, 1970)

Separation of the protein fractions was perfo rmed on 5.8-by 14.5cm. cellulose acetate mem branes. using the pH 8.6 barbital buffer solution of 0.075 ionic strength. Electrophoretic separations were made for 18 to 20 minutes in the Beckman Model R-101 Microzone cell at a con stant voltage of 250 V (3.5 to 5.8 ma. per strip) supplied by a Beckman Model RD-2 Duostat.

Protein was stained with 0.2% Ponceau S fixative dye in aqueous solution containing 3% trichloroacetic acid and sulfosalicylic acid.

After dyeing is complete, back ground stain was removed by 3 successive washes in 5% aqueous acetic acid.

The membranes were dried by draining off excess liquid and dehydrated by immersing it in alcohol rinse soution (denatured alcohol) for one minute, and then immersed in clearing solution (30% cyclohexanone solution in denatured ethanol) for exactly 60 seconds.

The membrane was positioned on a glass plate in the clearing solution and removed with the plate and then heated in an oven for 15 to 20 minutes at 70° to 80°C.

Membrane was peeled from the glass plate and mounted in a plastic envelope. The quantation of the protein fractions was performed by Beckman Model R-112 Scanning Densitometer with a intergrator using a 520nm filter.

The percenrage of each protein fraction was calculated and the concentration of each fraction was obtained by multiplying its percentage by the total protein concentration, a figure obtained by a separate analysis.

RESULTS AND DISCUSSION

Analytical results of serum protein fractions from 58 healthy young university students are shown in Table 1.

The normal distribution pattern was obtained for each of the measurements of serum protein in which approximately 68 percent of the values are no more than 1 standard deviation from the mean, and 95 percent are within 2 standard deviations of the mean.

The concentrations of the protein fractions were expressed in terms of absolute concentration and relative concentration (percentage of the total protein).

The mean concentrations of total protein of the females (7.88 ± 0.50 g/100ml) was slightly higher than those of males (7.77 ± 0.30 g/100 ml). The values of albumin, α_1-, α_2-, and β-globulin did not show noticeable difference between the two groups. But the γ-globulin value of females was higher than the value of males.

The values of serum protein fractions between the females and males were statistically compared, and the results of the t-test and the p values were shownin Table 2.

α_2-globulin values exhibited a high degree of identity ($t<0.64$, $p>0.50$) and total protein, albumin, β-globulin values and A/G ratio were at variance within experimental limits ($t<1.47$, $p>0.10$).

Highly significant difference between the female and male groups, greater than 5% level ($t>2.93$, $p<0.05$), was found in the γ-globulin values. Statistical comparison with absolute con-

centration of α_1-globulin shows significant difference ($t=2.64$, $p<0.02$).

We compared the results of present study with those of others obtained by similar or different analytical procedures (Table 3).

The mean value of total protein determined by biuret method using Technicon Autoanalyzer MT II system were higher than the normal values reported by others (Kaplan & Savory, 1965; Moon et al., 1965) and it seems to be due to the analytical method and instrument employed. But this values are similar to the value reported by Shepherd and Mason (1965) using a biuret method.

The mean concentrations of albumin were slightly high on comparison with the normal values of Korean population reported by Moon et al(1965), and Shim(1957) using a paper electrophoresis technique but similar to the values reported by Shepherd and Mason (1965) using a cellulose acetate membrane.

In the case of α_1-globulin, the normal values of present study was lower than the values of Korean population using paper strips (Moon et al., 1965) and the normal values reported by Wurm and Epstein (1956), and Corey (1956)

Table 1. Normal values for serum protein fractions separated by microzone cellulose acetate electrophoresis. Ponceau S staining. Evaluation by densitometric scanning. Specimens were obtained from 58 healthy medical students (18 females and 40 males between 21 and 30 years old)

Protein Fraction	Female (n=18)						Male (n=40)					
	Percent of total protein			Amount (g/100ml)			Percent of total protein			Amount (g/100ml)		
	mean	SD	95% range	mean	SD	95% range	mean	SD	95% range	mean	SD	95% range
total protein				7.88	0.50	6.88-8.88				7.77	0.30	7.17-8.37
albumin	60.9	3.4	54.1-67.7	4.79	0.33	4.13-5.45	62.2	2.9	56.4-67.9	4.83	0.26	4.31-5.35
α_1-globulin	2.5	0.5	1.5- 3.4	0.19	0.04	0.11-0.27	2.8	0.6	1.7- 3.9	0.22	0.04	0.14-0.30
α_2-globulin	7.2	1.0	5.3- 9.2	0.57	0.08	0.41-0.73	7.4	1.3	4.7-10.1	0.57	0.11	0.35-0.79
β-globulin	9.5	1.5	6.5-12.6	0.75	0.14	0.47-1.03	10.1	1.3	7.3-12.7	0.78	0.11	0.56-1.00
γ-globulin	19.9	2.5	15.0-24.8	1.57	0.24	1.09-2.05	17.8	2.4	12.9-22.6	1.38	0.20	0.98-1.78
A/G ratio				1.57	0.23	1.11-2.03				1.66	0.20	1.26-2.00

Table 2. The result of the t-test with the mean values of the serum protein fractions between the female and male groups.

Protein fractions	t value		p value (df*=56)	
	absolute concentration	percent of total protein	absolute concentration	percent of total protein
protein total	1.10	—	$0.30>p>0.25$	—
albumin	0.36	1.41	$0.80>p>0.70$	$0.20>p>0.10$
α_1-globulin	2.64	1.98	$0.02>p>0.01^{+}$	$0.10>p>0.05$
α_2-globulin	0.00	0.64	$p>0.9$	$0.60>p>0.50$
β-globulin	0.80	1.47	$0.50>p>0.40$	$0.20>p>0.10$
γ-globulin	2.93	3.00	$0.005>p>0.001^{++}$	$0.005>p>0.001^{++}$
albumin:globulin ratio	1.43	—	$0.20>p>0.10$	—

* df=degree of freedom

\+ The difference is significant.

++ The difference is highly significant.

Table 3. The comparisons of normal values of serum protein fractions obtained in this study with the values reported by others.

Reference	Method		T.P. (g/100ml)	Alb. (g/100ml)	α_1 (g/100ml)	α_2 (g/100ml)	β (g/100ml)	γ (g/100ml)	A/G ratio
Present study	CAM Ponceau S Densitometric scanning	female	7.88±0.50	4.79±0.33 (60.9±3.4)	0.19±0.04 (2.5±0.5)	0.57±0.08 (7.2±1.0)	0.75±0.14 (9.5±1.5)	1.57±0.24 (19.9±2.5)	1.57±0.32
		male	7.77±0.30	4.83±0.26 (62.2±2.9)	0.22±0.04 (2.8±0.6)	0.57±0.11 (7.4±1.3)	0.78±0.11 (10.1±1.3)	1.38±0.20 (17.8±2.4)	1.66±0.20
Moon et al	Paper Bromophenol blue	female	6.77±0.50	(63.5±3.9)	(3.8±0.2)	(6.7±1.2)	(8.9±1.1)	(17.5±2.6)	1.77±0.29
		male	7.07±0.46	(58.9±3.9)	(3.9±0.2)	(6.9±1.4)	(11.0±1.9)	(19.5±2.3)	1.46±0.26
Shim	Paper		7.35	(58.6±3.9)	(3.4±0.8)	(6.4±1.3)	(11.6±1.7)	(19.8)	1.44±0.22
Shepherd and Mason	CAM, Ponceau S Extraction with 0.1N NaOH		7.68±0.59	4.99±0.47	0.25±0.08	0.62±0.17	0.68±0.16	1.14±0.34	1.91±0.39
Kaplan and Savory	CAM, Ponceau S Densitometric scanning		7.50	4.47	0.25	0.75	0.9	1.11	—
Kohn	CAM, Ponceau S Densitometric scanning		—	4.3	0.2	0.6	0.7	0.9	—
Wurm and Epstein	Paper, Bromophenol blue, Densitometric scanning		—	(60.1)	(3.8)	(8.1)	(12.6)	(15.4)	—
Oreskes and Corey	Moving boundary		—	(58.9)	(5.4)	(9.3)	(13.4)	(13.2)	—
Colehour	Density gradient		—	(55.3)		(9.0)	(16.7)	(15.3)	—

Figures in parentheses are percent of total protein.

using a paper and moving boundary eleotrophoresis, but similar to the values reported by Shepherd and Mason,(1965), Kaplan and Savory, (1965) and Kohn (1960) using a cellulose acetate electrophoresis technique.

The mean value of α_2-globulin was lower than the normal values of Caucasian population but slightly higher than the normal values obtained by paper electrophoresis technique.

The mean value of β-globulin was lower than those of Caucasian population but similar to the results of electrophoresis on Korean population.

The normal values of γ-globulin was similar to those of Korean population obtained by paper electrophoresis but very higher than those of caucasian population obtained by cellulose acetate electrophoresis.

SUMMARY

The normal values of serum protein fractions by cellulose acetate electrophoresis had been determined from the sera of 58 healthy university students and a statistical evaluation of the data has been presented.

The normal distribution patternhad had been obtained for each of the measurements and on the comparison between the female and male groups, highly significant difference has been found in the γ-globulin values.

It seems that γ-globulin values of Korean population are higher than those of Caucasian population regardless of analytical methods employed.

》국문 초록《

Cellulose Acetate 전기영동법에 의한 한국인 혈청단백분획의 정상치

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Microzone cellulose acetate 전기영동법에 의한 한국인 혈청단백 분획의 정상치를 얻기 위하여 21~30세 사이의 건강한 대학생 58명(여자 18명, 남자 40명)을 대상으로 본 실험을 행하여 다음과 같은 결과를 얻었다.

1. Technicon사 자동분석기 MT II system을 사용하여 biuret법으로 측정한 혈청총단백질의 정상치는 여자 7.88±0.50g/100ml이고 남자는 7.77±0.30g/100ml이였다

2. 혈청 albumin, α_1—, α_2—, β, 및 γ-globulin 분획의 정상치는, 여자의 경우 각기 4.79±0.33g/100ml (60.9±3.4%), 0.19±0.04g/100ml (2.5±0.5%), 0.57±0.08g/100ml(7.2±1.0%), 0.75±0.14g/100ml (9.5±1.5%) 및 1.57±0.24g/100ml(19.9±2.5%)이고 남자의 경우는 각기 4.83±0.26g/100ml(62.2±2.9%), 0.22±0.04g/100ml(2.8±0.6%), 0.57±0.11g/100ml (7.4±1.3%), 0.78±0.11g/100ml(10.1±1.3%), 및 1.38±0.20g/100ml(17.8±2.4%)이였다.

3. 혈청 albumin/globutin ratio의 평균치는 여자 1.57±0.23, 남자는 1.66±0.20이였다.

4. 남여 혈청단백분획의 정상치를비교 해보면 α_1—, 과 γ-globulin의 경우 남녀간의 차가 통계학적으로 유의성이있으며 ($P<0.02$, $P<0.005$), 다른 단백분획에 있어서는 유의성이 없었다.

5. 여지 전기영동법에 의한 한국인 정상치와의 비교에 있어선 α_1-globlin 분획을 제외하고는 큰 차이가 없었으며, 사용되어진 전기영동 방법에는 무관하게, 한국인의 평균 γ-globlin 치가 외국인 정상치보다 큰것으로 나타났다.

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