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Specific protein pattern in adult healthy Nigerians

I. C. ONWUAMEZE

Department of Chemical Pathology, University of Nigeria, Enugu Campus, Anambra State, Nigeria

Summary

Serum concentrations of total protein, albumin, total globulins, pre-albumin, alpha-1-antitrypsin, caeruloplasmin, transferrin, immunoglobulins (IgG, IgA, IgM) and plasma fibrinogen were determined in 120 healthy adult Igbo-Nigerian subjects (60 males and 60 females). There was no sex or age related differences in the concentration of the various specific proteins, although a significant sex difference was observed in the mean level of plasma fibrinogen. Our values for caeruloplasmin, transferrin and IgM were significantly higher, although IgG, IgA and plasma fibrinogen were lower than reported in previous studies on other ethnic groups of Nigerians.

Résumé

Les concentrations sériques des protéines totales, albumine, globuline totale, pré-albumine, alpha-1-antitrypsine, caeruloplasmine, transferrine, immunoglobulines (IgG, IgA, IgM) et du fibrinogen de la plasma sont étudiés chez 120 sujets adultes sains du Nigéria, d'ethnie Igbo (60 mâles et 60 fémâles). Il n'y a pas eu des différences liées au sexe ou à l'age dans les concentrations des spécifiques et différentes protéines, bien qu'une différence importante liée au sexe a été remarquée dans le niveau moyen du fibrinogen de la plasma. Nos values pour le caeruloplasmine, le transferrine et l'IgM sont plus importantes que celles obtenues dans les études portant sur les autres groupes ethniques du Nigéria, alors que les concentrations de l'IgG, l'IgA et le fibrinogen de la plasma sont plus basses.

Introduction

Previous comparative studies of serum protein levels and patterns in Nigerians have demonstrated a lower serum albumin but a higher γ-globulin concentration than found in Caucasians [1–3]. The study of serum immunoglobulins in Nigerians has received a considerable amount more attention [4–7] than other specific proteins. In Nigerians the serum level of IgG has been shown to have a seasonal variation (higher in the rainy season than the dry season) while IgA, IgM and IgD mean levels have been reported to be similar in both Caucasians and Nigerians [7].

This study was undertaken to determine the levels and pattern of specific proteins in Igbo-Nigerians with a view to relating these specific proteins (namely pre-albumin, alpha-1-antitrypsin (AAT), caeruloplasmin, transferrin, immunoglobulins and fibrinogen) to the values previously reported in other Nigerians.

Subjects and methods

The subjects comprised healthy volunteers drawn from laboratory staff, blood donors, medical students, nursing staff and patients attending the infertility clinics at the University of Nigeria Teaching Hospital who were considered to be in good health. There were 60 males and 60 females whose ages ranged from 18–56 years. None of these selected subjects were pregnant or had malaria, liver disease or had any evidence of infection or inflammatory disease at the time of investigation.

Blood was collected from the antecubital fossa vein with minimum of occlusion. The blood was discharged into venoject tubes and allowed to clot for 2–3 h at room temperature. The sera obtained were stored at −20°C pending analysis. All the sera were analysed in batches within a fortnight of specimen collection.

Plasma for fibrinogen determination was obtained by placing nine parts of blood into one part of a 31.1 g/l tri-sodium citrate solution and

mixing. Subsequently the blood was centrifuged at 3000 r.p.m. for 10 min at laboratory temperature (27–28°C). Any haemolysed samples were discarded. Fibrinogen assays were usually performed 4–6 h after blood collection. The dilution of the blood using citrate was adjusted in the final fibrinogen concentration.

Total protein and albumin concentrations were determined using a modified Reinhold-biuret method as described by Varley [8]. The serum total globulins were obtained by finding the difference between the serum total protein and serum albumin.

The specific proteins pre-albumin, AAT, caeruloplasmin, transferrin, immunoglobulins (IgG, IgA, IgM) and fibrinogen were determined using Partigen and Nor-partigen plates obtained from Hoechst (Nigeria) Limited. This employed the single radial immunodiffusion technique using a Behring precision dispenser. Five microlitres of patient's serum or plasma, standards and controls were placed in separate wells as described in the respective kit method. After 48 h incubation at laboratory temperature the resulting precipitin rings were measured with a special Behring immunodiffusion viewer. Specimens for the determination of AAT, IgG and fibrinogen levels were diluted in saline 1 in 3, 1 in 20 and 1 in 3 respectively before assay. All other specific proteins were analysed using neat sera.

Protein fractionation involved separation of the serum protein on cellulose acetate membrane using a barbiturate buffer, pH 8.6 and a constant current of 0.4 mA per cm width of strip. After the 45-min run the strips were stained for 10 min in Ponceau S stain which contains 3% trichloroacetic acid. The strips were then washed in three changes of 5% acetic acid and then dried between filter papers. The sections representing each fraction were cut and eluted in 2 ml of 0.1 N sodium hydroxide. The absorbances were measured at 546 nm in a 4010 photometer supplied by Boehringer Corporation.

Statistical analysis for the significance of the

Table 1. Precision of assay methods for proteins (n = 20 in each case)

Protein	Precision	Concentration (g/l) $\bar{x}$ ± s.d.	CV (%)
Total protein	Within batch	74.4 ± 3.50	4.70
	Between batch	75.0 ± 4.80	6.40
Albumin	Within batch	43.6 ± 0.90	2.06
	Between batch	37.0 ± 3.20	8.60
Total globulins	Within batch	30.8 ± 1.0	3.25
	Between batch	40.0 ± 2.5	6.25
Pre-albumin	Within batch	0.21 ± 0.01	4.76
	Between batch	0.30 ± 0.02	7.82
Alpha-1-antitrypsin	Within batch	2.84 ± 0.12	4.25
	Between batch	2.80 ± 0.18	6.43
Caeruloplasmin	Within batch	0.35 ± 0.02	5.71
	Between batch	0.35 ± 0.03	6.80
Transferrin	Within batch	2.96 ± 0.14	4.73
	Between batch	2.80 ± 0.16	5.71
IgG	Within batch	30.60 ± 1.25	4.08
	Between batch	28.80 ± 1.30	4.51
IgA	Within batch	3.39 ± 0.10	3.03
	Between batch	3.00 ± 0.10	3.33
IgM	Within batch	3.94 ± 0.13	3.30
	Between batch	3.50 ± 0.16	4.57
Fibrinogen	Within batch	2.84 ± 0.07	2.46
	Between batch	1.50 ± 0.10	6.36

differences between the various groups was evaluated by Student's *t*-test.

Results

Within batch precision of the assay methods was determined using 20 replicate determinations of a pool of normal sera. Table 1 shows the mean value, standard deviation and coefficient of variation of each protein determined using the pool sera. The values obtained in this study were similar to previous published data [9].

Table 2 summarizes the result of protein concentration in both male and female adult Igbo Nigerians. With the exception of fibrinogen which showed a lower mean plasma level for males there were no significant differences for sex in mean serum levels. The levels of individual proteins when grouped according to the decade of age showed no significant difference and so the results were treated together.

Table 3 shows the result of the protein fractions in 20 male and 20 female subjects. The level of the protein fractions obtained by this method were generally slightly lower than those obtained by direct biuret or immunochemical quantitation methods. The mean serum level of α-1-globulins, α-2-globulins, β-globulins and γ-globulins obtained were higher than previously reported [1,2,10] although this agrees with previous reports in Igbos [11].

Discussion

The main factors contributing to variations in

Table 2. Specific protein levels in Igbo Nigerians (concentration in g/l; mean ± s.d. and range)

Protein	Males and females (n = 120)	Males (n = 60)	Females (n = 60)
Total protein	73.81 ± 5.97 (61.90–85.80)	74.42 ± 6.62 (61.20–87.70)	73.65 ± 6.00 (61.70–85.70)
Albumin	41.70 ± 5.80 (31.20–52.20)	41.92 ± 5.38 (31.20–52.70)	41.48 ± 5.19 (31.10–51.90)
Total globulins	32.10 ± 5.80 (20.40–43.70)	32.30 ± 6.20 (19.90–44.70)	31.82 ± 5.42 (20.90–42.70)
Pre-albumin	0.30 ± 0.10 (0.12–0.50)	0.31 ± 0.09 (0.15–0.50)	0.30 ± 0.09 (0.12–0.48)
Alpha-1-antitrypsin	2.66 ± 0.51 (1.80–3.89)	2.64 ± 0.49 (1.90–3.60)	2.69 ± 0.52 (1.80–3.90)
Caeruloplasmin	0.36 ± 0.06 (0.19–0.45)	0.36 ± 0.06 (0.23–0.43)	0.35 ± 0.07 (0.90–4.30)
Transferrin	3.23 ± 0.64 (1.90–4.70)	3.21 ± 0.64 (1.90–4.70)	3.24 ± 0.14 (1.90–4.30)
IgG	21.10 ± 4.10 (15.90–32.70)	20.70 ± 3.80 (16.80–30.90)	21.50 ± 4.40 (15.90–32.70)
IgA	2.00 ± 0.71 (1.20–4.30)	2.20 ± 0.81 (1.30–3.40)	1.90 ± 0.60 (1.00–3.20)
IgM	1.62 ± 0.62 (0.50–3.40)	1.60 ± 0.70 (0.50–3.40)	1.70 ± 0.63 (1.00–2.80)
Fibrinogen*	2.80 ± 0.65 (1.30–4.10)	2.63 ± 0.61 (1.40–3.90)	3.20 ± 0.54 (2.10–4.30)

*Significant difference between males and females ($P < 0.001$). Figures in brackets indicate the range found in this study.

Table 3. Serum protein fractions in Igbo Nigerians* (concentration in g/l)

Protein	Mean ± s.d.	Range
Albumin	36.20 ± 6.27	28.60–46.70
Alpha-1-globulin	3.24 ± 1.72	3.10–7.10
Alpha-2-globulin	6.83 ± 1.79	3.20–10.60
Beta-globulin	8.67 ± 2.64	4.80–12.90
Gamma-globulin	18.87 ± 4.34	12.20–29.80

*n = 40 (20 males and 20 females).

the level and pattern of serum proteins and specific proteins include socio-economic and cultural factors such as ignorance, malnutrition, dietary habits and taboos, hook-worm infestation and malaria. Two decades ago, Edozien [1,2] working at Ibadan showed that there was an elevated serum γ-globulin in predominantly Yoruba Nigerian population. This finding has been confirmed in other major ethnic groups [10–12]. The elevated γ-globulin was attributed to the prevalence of malaria and other microbial infections.

The pattern of serum protein obtained in this study is similar to those reported on Igbo Nigerians by Reid & Chukwudebelu [11] and Isichei [12]. The serum level of pre-albumin and AAT in our adult healthy subjects are similar to those reported elsewhere in other Nigerians [13–15]. The main roles of pre-albumin and AAT are in the binding of thyroxine and retinol, and in the protection through antiproteolytic activity. Our data, however, differ widely from those previously reported on Nigerians in that a significantly elevated caeruloplasmin, transferrin and IgM but a significantly reduced IgG, IgA and fibrinogen were observed. These differences are difficult to explain. It cannot however be attributed to technical error in the methods used since our precision compared well with those previously reported at other centres [7,9,10]. Reid & Onwuameze [16] have shown a good correlation between single radial immunodiffusion technique and the clot weight method for the determination of plasma fibrinogen. The concentration of plasma fibrinogen obtained in this study is similar to those reported by Reid & Onwuameze [16], and Reid [17].

The circulating levels of some serum or plasma specific proteins have been shown to be influenced by such factors such as age, seasonal variations, immune response, environment, social customs, nutritional status and genetic factors [7,18,19]. In our opinion the differences may be due to variations in nutritional status or socio-economic status and possibly genetic factors. The phenotyping of these proteins may help in explaining the differences.

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