AFRICAN JOURNAL OF MEDICINE
and medical sciences

VOLUME 22, NUMBER 3, SEPTEMBER 1993

EDITOR: B.O. ONADEKO
ASSISTANT EDITORS:
B.O. OSOTIMEHIN and A.O. UWAIFO

SPECTRUM BOOKS LIMITED
Ibadan • Owerri • Kaduna • Lagos

ISSN 1116–4077

Afr. J. Med. med. Sci. (1993) **22**, 45-48

Problems associated with plasma albumin estimation in nephrotic syndrome using the bromocresol green method

E. O. AGBEDANA*, J. O. OSHOTA and G. OLADUNNI TAYLOR
Department of Chemical Pathology, College of Medicine, University of Ibadan, Ibadan, Nigeria.

Summary

Evaluation of albumin estimation by bromocresol green (BCG) method was carried out in sixty nephrotics and twenty control subjects. In nephrotic syndrome, ∝, 2-globulin and total cholesterol concentrations were significantly increased, while the mean albumin level was significantly reduced when compared with the corresponding control values. In both control and nephrotics, the determination of serum albumin by the BCG method showed good correlations with values obtained by cellulose acetate electrophoresis using the biuret method to determine the total protein, but the mean value for the nephrotics was higher by an average of 0.4g/100ml. Interference with the BCG reaction by an increased ∝, 2-globulin level was suggested as a possible explanation for the higher mean albumin level obtained by the BCG method in the nephrotics. Inclusion of 0.8M NaCl in the BCG assay system did not prevent the interference by other proteins. However, this interference could to a large extent, be offset by calibrating with a pool of fresh sera previously determined by electrophoresis.

Résumé

Nous avons effectué, par le test du bromocrésol vert (BCV), une évaluation du taux d'albumine chez soixante sujets néphrotiques et vingt sujets normaux. Chez les sujets néphrotiques, les concentrations d'alpha-2-globuline et de cholestérol augmentent de manière significative, tandis que le taux moyen d'albumine est nettement réduit par rapport aux niveaux correspondants chez les sujets normaux. Aussi bien chez les sujets normaux que chez les sujets néphrotiques, le calcul du taux de sérum-albumine par le test du BCV donne une bonne corrélation avec les valeurs obtenues par électrophorèse d'acétate de cellulose, en utilisant la réaction du biuret pour obtenir le taux total de protéine, mais la valeur moyenne chez les sujets néphrotiques est supérieurs par environ 0,4g/100ml. Nous avons suggéré qu'une interférence du niveau accru d'alpha-2-globuline avec le test du BCV pourrait expliquer l'accroissement du taux moyen d'albumine chez les sujets néphrotiques obtenu par cette méthode du BCV. L'inclusion de O, 8M de NaCl dans le dosage du BCV n'empêche pas l'interférence par d'autres protéines. Néanmoins, il est possible de compenser cette interférence en calibrant avec du sérum frais obtenu au préalable par électrophorèse.

Introduction

Severe modification of the metabolism of proteins are common during the nephrotic syndrome[1]. Thus when investigating cases of nephrotic syndrome, it is customary to estimate plasma albumin concentration using simple routine biochemical methods sufficiently precise and reliable to detect minor changes in albumin concentrations.

Of the many procedures described for assay of plasma albumin[2,3], the bromocresol green (BCG) dye-binding method[4] has obvious advantages. It is extremely simple to perform, requiring only the addition of sample to the reagent.

However, the recently reported interference by electrophoretically pure globulin fractions in the BCG method for serum albumin[5] estimation led us to investigate the possibility of an over-estimation of serum albumin concentration in nephrotic patients. The effects of addition of salt (0.8M NaCl) and calibration with a standard fresh human pooled serum were evaluated.

Methods

Sixty patients diagnosed as suffering from nephrotic syndrome and who were attending the Nephrology Outpatient Clinic of the University College Hospital, Ibadan were studied. Twenty normal age and sex-matched subjects served as the control group.

* To whom correspondence should be addressed

Venous blood samples were collected into heparinised bottles and plasma separated for biochemical estimations. The plasma albumin concentration was determined using the Bromocresol Green manual method of Doumas *et al*[4]. The separation of plasma protein fractions was carried out using the cellulose acetate electrophoretic method of Russel and John[2]. Albumin and ∝, 2-globulin components were eluted from the strips of cellulose acetate and total protein concentration of each fraction determined using the biuret reaction method of Reinhold[6]. Human plasma albumin and pooled fresh human sera electrophoretically determined albumin served as standard proteins in the calibration.

Total cholesterol concentration was estimated using the method Zlatkis *et al*[7]. For each assay a commercial quality control sample (Well control, Wellcome Reagents Ltd) was always included.

Results

As shown in Table 1, the mean plasma total cholesterol and ∝, 2-globulin levels in nephrotic syndrome were significantly higher than the corresponding values in the control group. On the other hand, both total protein and albumin levels in nephrotic syndrome were significantly reduced ($P < 0.05$).

Table 1: Biochemical characteristics of nephrotic syndrome (Mean ± S.D)

Parameters	Control	Nephrotic Syndrome
	$n = 20$	$n = 60$
Total Protein[a] (g/100ml)	7.5 ± 0.6 (6.6 - 8.7)	5.2 ± 1.0 (3.8 - 7.6)
Albumin[b]	3.9 ± 0.3 (3.1 - 4.4)	1.7 ± 0.8 (0.6 - 4.0)
∝, 2-Globulin[b] (g/100ml)	0.4 ± 0.2 (0.2 - 0.8)	1.4 ± 0.6 (0.8 - 2.7)
Total Cholesterol (mg/100ml)	193 ± 66 (107 - 320)	478 ± 218 (116 - 853)

a = Quantitation using biuret method
b = Quantitation using cellulose acetate electrophoresis
( ) = Range of values
n = Number of subjects
S.D = Standard Deviation

Figure 1 shows that the plasma albumin results given by the BCG method were positively correlated with those given by the electrophoretic method in controls ($r = 0.98$, $P < 0.001$) and nephrotic syndrome ($r = 0.96$, $P < 0.001$) respectively.

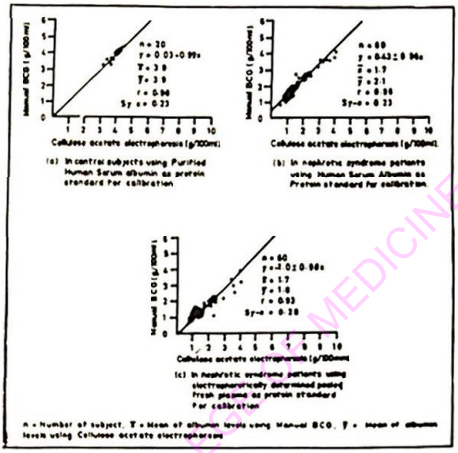

Fig. 1: Correlation between albumin levels in control and nephrotic syndrome measured by cellulose acetate electrophoresis (X) and by the manual BCG (Y) methods.

As shown in Table 2, in control subjects the mean plasma albumin results given by the BCG method were similar to those of the electrophoretic method irrespective of the standard calibration protein used. Also the addition of 0.8M NaCl had no effect on the results. On the other hand in nephrotic syndrome, the BCG method gave significantly higher ($P < 0.001$) albumin values than the electrophoretic method in the presence or absence of 0.8M NaCl when purified human serum albumin was used as the standard protein. However, when pooled plasma was used as the standard calibration protein, the mean plasma albumin values obtained by both methods were similar.

Table 2: Plasma albumin concentration ($\bar{X}$ ± S.D) in nephrotic syndrome

	Control	Nephrotic Syndrome
	($n = 20$)	($n = 60$)
Albumin (BCG) g/100ml[a]	3.9 ± 0.3	2.1 ± 0.7
Albumin (BCG) g/100ml[b]	3.8 ± 0.3	1.6 ± 0.3
Albumin + 0.8M NaCl[a]	3.9 ± 0.3	2.1 ± 0.8
Albumin (Electrophoresis) g/100ml	3.9 ± 0.3	1.7 ± 0.8

a = Purified human serum albumin as standard
b = Electrophoretically determined pooled serum as standard
n = Number of subjects

Discussion

In the present study the mean plasma cholesterol and ∝, 2-globulin levels were significantly elevated in nephrotic syndrome, while the total protein and albumin levels were reduced. Our results confirm some of the specific biochemical changes commonly associated with nephrotic syndrome[8].

There were good correlations for plasma albumin between the results given by the BCG and cellulose acetate electrophoresis methods for control and nephrotic syndrome respectively. This shows that both methods were equally capable of measuring plasma albumin quantitatively in the two groups.

However, the BCG method gave significantly higher albumin values than the electrophoretic method in the nephrotics but not in the controls when purified human serum albumin was used as the standard protein. The calculated percentage increase over and above the electrophoretically determined concentration was as high as 41% (7g/l) in some patients although the overall mean in the nephrotic group was 25.9 (4g/l). This probably suggests over estimation of plasma albumin by this procedure and could therefore explain the occasional normal or slightly raised albumin concentration observed in some nephrotics during routine determinations.

Over estimation of human serum albumin by the BCG method, because of dye binding of other plasma proteins has been reported as a draw back of this method[9]. Also shortening of the reaction time has been suggested as a way to reduce such interference by these workers. However, in the present study it was important that when we kept to the traditional reaction time of 10 minutes, the disparity between BCG and electrophoretic methods was exaggerated only in the nephrotic group, where the ∝, 2- globulin showed over a 3-fold increase. Similarly in vitro experiment[5] have indicated that interference by added pure ∝, 2-globulin with the albumin BCG reaction was the most pronounced when compared with other plasma protein fractions. Thus, the interference with the BCG reaction by an increased ∝, 2-globulin concentration in our nephrotic syndrome could be responsible for the higher albumin level obtained by the BCG method in that disease.

In an attempt to reduce the extent of interference by 'non- albumin' plasma components we included 0.8M NaCl solution as suggested by O'Donell and Lot[9]. Similar to the results of Rodinguez-Segade *et al*[5] inclusion of 0.8M NaCl solution in the BCG assay system did not prevent the interference by other proteins.

However, when a pool of fresh plasma previously determined by electrophoresis was used as the standard protein, the interference by other proteins in the BCG assay was offset to a large extent. It is customary to use readily procurable commercial standard human serum albumin as standard protein for the BCG method, especially in the developing countries where facilities for electrophoresis and other more sophisticated techniques are not readily available. There is considerable evidence to suggest that a few problems like over estimation of human serum albumin may arise in using the simple and rapid BCG dye-binding technique for albumin estimation because of dye binding of other plasma proteins especially ∝, 2-globulin fraction. However, because of the large number of samples encountered in diagnostic routine laboratories, the use of the suggested calibration procedure for this method instead of shortening of reaction time is advocated for accurate results.

Acknowledgements

We are grateful to Joyce Fatoke for Secretarial help and Mr. O. A. Sokoya of Medical Illustration Unit University College Hospital, Ibadan for the graphic illustrations.

References

1. Girot RF, Jaubert M, Leon B, *et al.* Albumin fibrinogen, prothrombin and antithrombin III. Variations in blood, urine and rat liver nephrotic syndrome (Heymann Nephritis). Thromb. Haemost. 1983; 49: 13-17.
2. Russel OB and John DM. Electrophoresis of serum protein with cellulose acetate. A method for quantitation. Amer. J. Clin. Path. 1964; 421: 547-551.
3. Sater L, Carter P, Hobbs M. Measurement of albumin in sera of patients. Ann. Clin. Biochem. 1975; 12: 33-40.
4. Doumas B, Watson W and Biggs H. Albumin standards and the measurement of sera albumin with BCG. Clin. Chim. Acta 1971; 31: 87-96.
5. Rodriquez-Segade S, Quton Caamono M, Tutor JC and Paz JM. The influence of globulins in the measurement of serum albumin with bromocresol green. Clin. Chem. Newsletter,

1987; 34- 38.
6. Reinhold (1953). In: standard methods in clinical chemistry. Reiner M. (ed) New York: Academic Press; Vol. 1, 1988 edition.
7. Zlatkis A, Zak B and Boyle AJ. A new method for the direct determination of cholesterol. J. Lab. Clin. med. 1953; 41: 486-492.
8. Smith FG Jr., Gonick H, Stanley TM and MeIntosh RM. The nephrotic syndrome: current concepts. Ann. Intern. Med. 1972; 76: 463-477.
9. O'Donell N and Lot J. Reducing interference from globulin in the BCG determination of serum albumin. Clin. Chem. 1978; 24: 1004-1005

(Accepted 10 September, 1990)