Measurement of Serum Albumin by Three Different Methods

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Abstract

In this study ,serum albumin level was determined in 61 blood samples using three different methods ,cellulose acetate electrophoresis (CAE), salt fractionation and bromocresolgreen(BCG) . The values of serum albumin (mean (95% confidence interval)) measured in gm/L by the above methods were 38.95 (1.64) , 38.13 (1.76) and 38.11 (1.8) respectively .There were no significant differences in the level of serum albumin determined by these methods ($P \geq 0.05$). Also, these methods showed statistically significant differences in the level of serum albumin determined by these methods ($P \leq 0.001$) ,As the BCG method give a relatively inaccurate albumin levels in abnormal sera therefore ,it is recommended that the BCG is to be used only as a screening method for serum albumin measurement for its simplicity and rapidity

قياس مستوى الالبومين في الدم باستخدام ثلاث طرق مختلفة

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كلمات مفتاحية :الترحيل الكهربائي ، الترسيب التجزيئي ، وطريقة البروموكريسول كرين لقياسمستوى الالبومين في الدم

الخلاصة

في هذه الدراسة ،تم قياسمستوى الالبومين في الدم في 61 نموذج با ستخدام ثلاث طرق مختلفة الترحيل الكهربائي ، الترسيب التجزيئي ، وطريقة البروموكريسول كرين (BCG)،وكانت قيمالالبومين[(95 CL%)]Xمقاسا ب غم/لترللطرق الثلاثة هي38.95(1.64)،38.13(1.76)38.11((1.8) على التوالي .

لم يكن هناك ةاختلاف احصائي مهم في مستوى الالبومين مقاسا بالطرق الثلاثة ($p \geq 0.05$). أظهرت الدراسة وجود ترابط موجب مهم احصائيا بين الطرق الثلاثة($0.001P \leq$) ونتيجة لكون طريقة(BCG) تعطي نتائج البومين غير دقيقة نسبيا في النماذج غيرالطبيعية، لذلك نوصي باستعمالها فقط كطريقة مسح لقياس مستوى الالبومين وذلك لبساطتها وسرعتها.

Introduction

Albuminare a polymers of amino acid that are linked to each other by peptide bonds.The function of albumin in plasma includes regulation of the distribution of extracellular fluid by its effect on the oncotic pressureand as a constitutes part of amino acids pool, also a transporting agent for a wide range of naturally occurring substances and drugs [1-3].

A number of different methods have been used for the routine determination of serum albumin. Salt fractionation, electrophoresis and dye-binding techniques have in recent years become the most commonly used procedures [4, 5].

Salt fractionation method used 27.8% of sodium sulphate-sulphite solution to precipitate the globulin,and then copper in alkaline solution reacts with the peptide linkage of amino acids in protein producing a violet colour. This method is satisfactory with normal sera but less so with abnormal ones. Also in this method the globulin remain in supernatant, leading to underestimation of globulin and overestimation of albumin [(2)].

Cellulose acetate electrophoresis (CAE) method which depends on the movement of a charged particle, in electric field, can give misleading results particularly if albumin quantitation is made by determining the proportion of total dye on the strip. Staining is often not uniform at such high protein concentration making the response non-linear, in addition bands are less accurately assessed particularly with reflectance scanners, leading to underestimation of globulin and consequently overestimating thealbumin[(2)].

Dye-binding techniques have been more recently used bromocresolgreen (BCG) method. The measurmentof serum albumin is on its quantitative binding to the indicator 3,3,5,5 – tetra bromo-m-cresol sulphonphthalein (bromocresolgreenBCG) without deproteinization. The albumin –BCG complex absorbs maximally at630 nm.

The main advantage of BCG method would appear to be simple, rapidand one it is also in dealing more convenient in dealing with other methods, however, this technique has been shown to overestimation, albumin level in sera with high globulin level in jaundice samples .

Aim of study:-This work was undertaken to established the accuracy of these three methods was assessed by comparison with values obtained to prove useful for the detection of chronic liver ,kidneydiseas . This measurement appears to be a sensitive index for the presence of hepatic and renal dysfunction

Materials And Methods

This study was conducted among salahalddin Teaching Hospital in Tikrit cityfrom March 2010 to December 2010 .The samples were collected from clinical laboratories; the serum was used for determination the albumin by different methods.

1-Cellulose acetate electrophoresis (CAE) To observe the presence of specific protein band, cellulose acetate paper was used in horizontal electrophoresis apparatuse(Fisher Scientific USA) with using Barbitol buffer pH8.6

- Sample apply through a micropipette (0.01 ml) on the cellulose acetate paper then cover the apparatuse Apply electricity power of 230Volts and 0.5 millampers per 1cm width of the strip and run for 45 min .
- Staining solution The dyePonCean's was dissolved in(3% Trichloroacetic acid and water)The dye solution was then filtered throughwhatman filter papers.
- The cellulose paper was removed from electrophoresisapparatuseand placed it in a container that containing the stain solution for 45 minuts.
- Thepaper was removed from the staining solution and fully submerged in the destaining solution(5%acetic acid and water)a few times until protein bands werevisualized.
- The visualized protein bands weredetermined byscanning to evaluate the individual protein components[(2)] .

2-Salt fractionation method used 27.8% of sodium sulphate-sulphite solution to precipitate the globulin then added(1-3)mls of ether,stopper thetube and invert it gently20 times.

Remove the stopper and centrifuge for 10 minutes.The ether causes the globulin precipitate to separateas a disc at the ether –water interface.Introduce pipette down the side of the tube pushing aside the globulin disc gently ,and withdraw of the supernatant layer into a test tube marked "A".

Take 3mls of standard protein solution into a test tube marked "S."

Take 3mls of distilled water into test tube marked" B".

Added 5mls of biuret reagent to each of the above tubes ,mix ,place in water bath at 37℃ for 10minutes .Read at 540nm[2].

Calculation:-

$$\text{Plasma Albumin (g /100ml)} = \frac{A - B}{S - B} \times \text{Standard concentration (6gm/L)}$$

3-Bromocresolgreen (BCG) method using albumin kit (Ref AB 361) from Randox (U.K). The measurmentof serum albumin is on its quantitative binding to the indicator 3,3,5,5 – tetra bromo-m-cresol sulphonphthalein (bromocresolgreen BCG) without deproteinization. The albumin –BCG complex absorbs maximally at630 nm[5].

Take 10µL of serum into 2ml of BCG in a test tube marked "T."

Take 10µL of standard protein solution into 2ml of BCG in a test tube marked "S."

Take 10µL of distilled water into2ml of BCG test tube marked" B".

Calculation:-

$$\text{Plasma Albumin (g /100ml)} = \frac{A}{S} \times \text{Standard concentration (5gm/L)}$$

Sixty one (61) blood samples were included in this study. Sera were separated and either analyzed immediately or stored frozen at -20℃ for later analysis, then serum albumin was measured in gm/L by the above methods, statistical analysis using least – square analysis was also performed .$P \leq 0.05$ was considered to be statistically significant.

RESULS:

The values of serum albumin ($\bar{X} \pm$ SD) determined by the three methods are presented in Table 1.

Table 1

Serum albumin concentrations (g/L) determined by three different methods ($\bar{X}$ ± SD).

	Methods		
	BCG	CAE	Salt fractionation
No. of sample	61	61	61
Serum albumin (gm/L)	38.11±7.07	38.95±6.4	38.13±6.9
95% CL	1.8	1.64	1.76

P≥ 0.05

There were no significant differences in the concentration of serum albumin determined by these methods (P≥0.05).

Table (2) presents the levels of serum albumin in patients with renal failure in addition to haemolysed, lipaemic and icteric samples. There were significant differences in serum albumin levels estimated by the three methods (P≤0.01) being higher by the BCG method compared to the other two methods.

Table 2 Abnormal albumin sera estimated by three different methods($\bar{X}$ ± SD).

	BCG	CAE	Salt fractionation
No. of sample	14	14	14
Serum albumin(gm/L)	42.28±3.55	38.95±3.15	38.13±2.91

P≤ 0.01

Correlation and regression analysis revealed that the BCG method showed statistically significant positive correlation with salt fractionation (r=0.972, P≤ 0.001) and cellulose acetate electrophoresis (r=0.962, P≤0.001) methods in addition salt fractionation method also showed statistically significant positive correlation with cellulose acetate electrophoresis (r=0.976, P≤ 0.001), Table 3, Fig. 1, 2 and 3.

Table 3

Correlation of results for albumin concentration (g/L)determined by three different method($\bar{X}$ ± SD).

Number	Method	M Slop	C Intercept	R Correlation	X Mean	Y Mean	T Significant difference
61	**BCG/Salt**	0.996	0.129	0.972	38.11	38.13	31.6
61	**BCG/CAE**	1.062	3.2	0.962	38.11	38.95	27.08
61	**CAE/Salt**	1.907	4.36	0.976	38.95	38.13	34.8

The standard dilution curve of a purified human serum albumin monomer was linear. For the day to day precision, aliquots of the pools were frozen and analyzed on 15 consecutive days, as demonstrated in Table 4.

Table 4

Precision within day and day assay of S. albumin g/L by the BCG

	1	2	3
Within day			
No. assay	15	15	15
Mean± SD	43± 0.5	26± 0.7	38± 0.6
CV%	1.16	2.69	1.57
Between days assay			
No. assay	15	15	15
Mean± SD	43.6± 0.6	26.4± 0.8	38.5± 0.7
CV%	1.37	3.03	1.8

On the other hand quality control methods used to monitor the precision and accuracy of analytical results obtained on specimens, a bach of commercially available control serum . (CAT NO. UN1557,lot NO. 126 UN) analyzed by BCG method is reported to have 40gm/L with range 34 – 46 gm/L in this communication the albumin level of the same serum was 38 gm/L.

Discussion

More attention was focused on BCG method for serum albumin measurement for its simplicity and feasibility, by using this method one can deal easily with large number of samples. The results indicated that serum albumin measured by BCG method did not significantly differs with the other two methods, However evaluation of these three methods using several haemolysed , lipaemic and icteric samples revealed that albumin concentration in the high normal level tends to be underestimated by the BCGmethod, due to the competition for the binding sites on albumin between the indicator dye and other plasma compounds e.g. bilirubin[6], on the other hand, there is overestimation of serum albumin by BCG method at low albumin concentration as a result of the binding of the indicator dye to plasma proteins other than

It has been reported that S-albumin concentration is overestimated BCG method particularly in the presence of an abnormally high globulin fraction [7, 8].

Serum albumin concentration in patients with nephrotic syndrome has been found to be directly proportional to two globulins and total cholesterol [9,10], therefore, the mean value of serum albumin in these patients tends to be higher due to such interference, so the BCG method seems suitable as a screening method, and abnormal sera should be analyzed by cellulose acetate electrophoresis. However, the electrophoretic technique is not free from disadvantages as it is not suitable for dealing with large number of samples in short time, the need for scanning to determine the individual proteins, and other problem is that the dye uptake by albumin and globulin is not always similar. On the other hand, electrophoresis has the advantage that on assessment, visual or quantitative can be made on the globulin of all abnormal sera [6].

In the BCG method, the reaction has been shown to be almost immediate [11]. However, there are several commercial instruments now availablethat are suitable for routine use and allow the immediate measurement of absorbance after undiluted serum samples are mixed with the reagents [4, 11, and 12] thus it is now possible to be reliably determine serum albumin automatically at low reagent labor cost by simple and rapid BCG method.

Acknowledgenent

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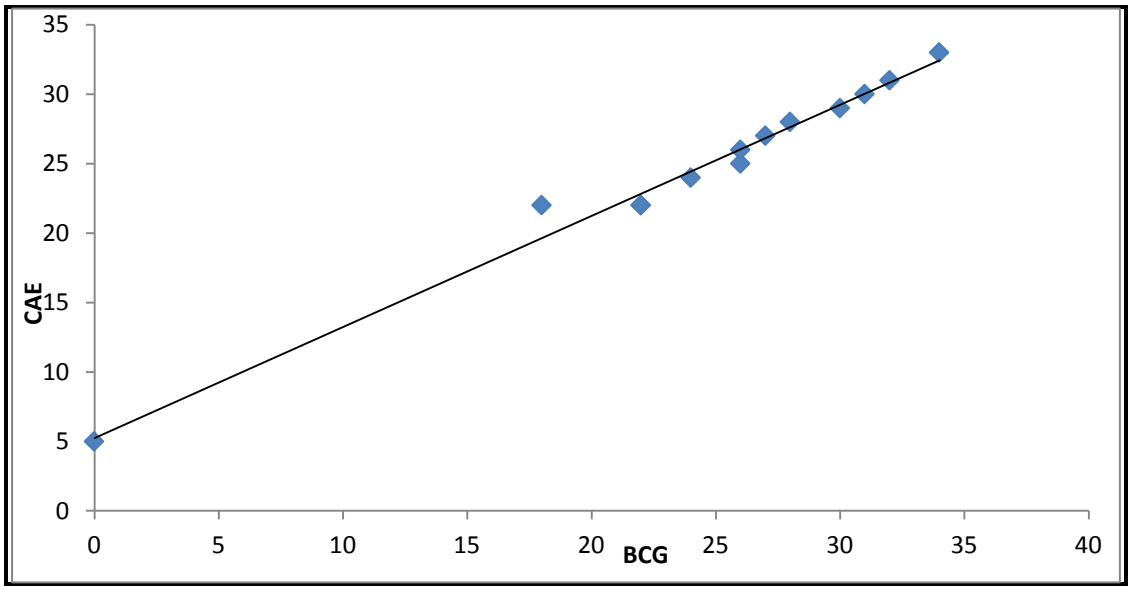

Fig(1): Correlation between BCG and CAE methods

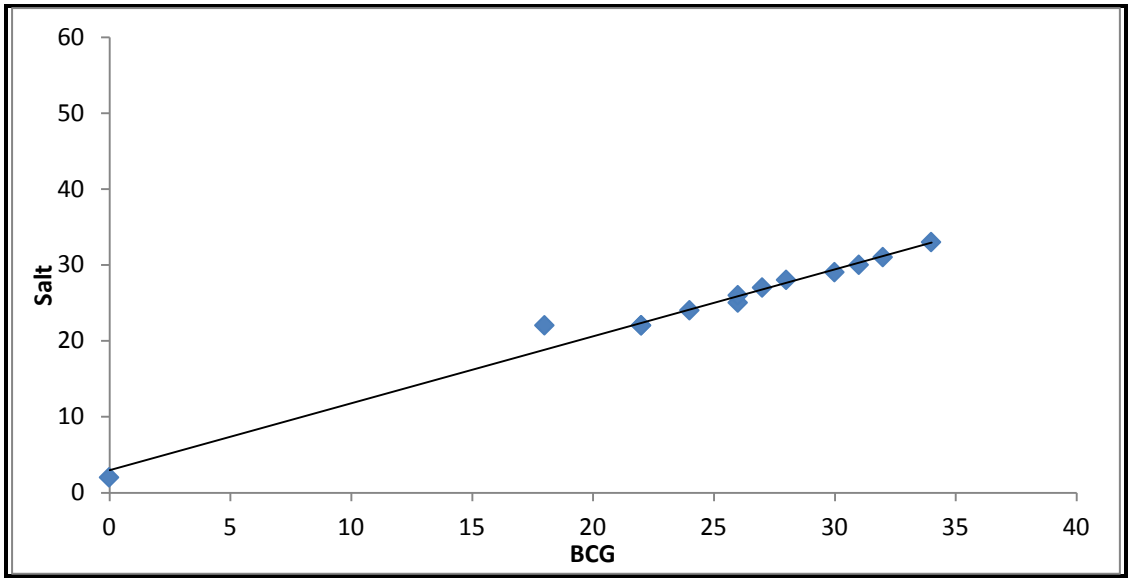

Fig (2) :Correlation between BCG and Salt fractionationmethods

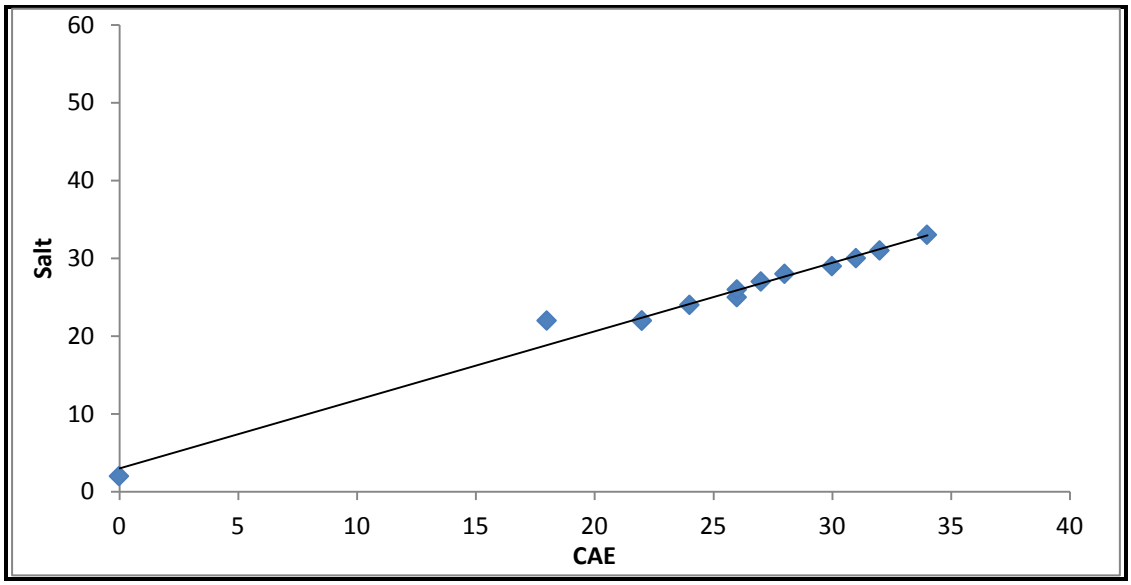

Fig (3): Correlation between CAE and Salt Fractionation methods