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A Comparative Study of Serum Albumin Estimation: BCP and BCG Dye Binding Methods vs. Capillary Electrophoresis

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ABSTRACT

Dye binding methods remain popular for serum albumin estimation due to their practicality and affordability. BCG and BCP methods, the two dye binding techniques are known to yield discordant results and there is a lack of consensus in the literature necessitating further investigation and attention. To compare the BCG and BCP methods, a method comparison study was carried out against the CZE method serving as the reference. The albumin concentration of 47 blood samples was measured using the three methods. One-way ANOVA test followed by a post-hoc Tukey HSD test for pairwise discrepancies, independent sample t-test, Pearson Correlation, and Bland-Altman plots were employed to compare and evaluate the correlation and agreement between the methods. No significant difference was observed in measurements between the CZE (M = 35.72 g/L, SD = 7.12 g/L) and BCP (M = 36.4 g/L, SD = 7.08 g/L); t (88) = -450, p = 0.654 (p>0.05) methods. Mean difference between BCG (M = 40.28 g/L, SD = 5.53 g/L) and CZE methods was 4.56 g/L, demonstrating significance; t (88) = -3.39, p = 0.001 (p<0.05). Both BCG (r=0.875, p<0.001) and BCP (r= 0.910, p<0.001) methods showed a significant positive correlation with the CZE method while the BCP method had the strongest. Bland Altman analysis revealed a bias of 0.67 g/L, (95% limits of agreement, -5.00 g/L to 6.56 g/L) between the BCP and CZE methods. The BCG method showed a bias of 4.56 g/L (95% limits of agreement, -2.34 g/L to 11.46 g/L) versus the CZE method. This study concludes that the BCP method agrees more closely with the CZE method. The BCG method overestimates albumin in serum in comparison to the CZE method (positive bias), more so than observed with the BCP method.

KEYWORDS: *Albumin, HSA, Dye-binding, BCG, BCP, CZE*

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1 INTRODUCTION

Albumin is the most copious human plasma protein accounting for 60% of total plasma proteins. The plasma contains 60% of total albumin and the rest is found in extravascular spaces (Colombo et al. 2012). It is a monomeric, non-glycosylated, highly water soluble protein having a molecular weight of 66,438 Da (Turell et al. 2021). The levels of albumin present in serum depend on the balance between synthesis, secretion, and degradation of albumin within the human body. According to Rothschild, Oratz, and Schreiber, the liver synthesizes 12 g to 14 g of albumin in a 70 kg human being per day. The normal concentration of serum albumin ranges between 35 and 50 g/L in normal healthy individuals (Rabbani & Ahn 2019).

Albumin serves an extensive variety of functions. Albumin contributes to the maintenance of colloid osmotic pressure and regulation of capillary membrane permeability. Apart from that albumin is responsible for binding and transporting of ligands, free radical elimination, and also for antioxidant activity and circulatory protective properties (Fanali et al. 2012).

The endogenous ligands transported by albumin include fatty acids, metal ions (Cu, Zn, Ca, Mg, Fe), thyroxine, and bilirubin while multi-binding properties of albumin allow the transportation of exogenous compounds such as various drugs and toxic metal ions (Fatullayeva et al. 2021).

The existing scientific evidence clearly shows the diagnostic and prognostic significance of albumin measurement in numerous diseases. Such instances include diagnosis and assessment of nephrosis, nutritional status assessment, as an indicator of chronic liver disease, evaluation of generalized edema, and calculation of albumin dosage and therapy monitoring in patients undergoing a replacement albumin therapy (Infusino & Panteghini 2013).

The serum albumin level is a frequently utilized parameter that aids in clinical decision making. In the current clinical setup albumin quantification is achieved via several methods which include dye colorimetric assays based on dye binding, immunological determination with the use of nephelometry or turbidimetry, and capillary zone electrophoresis (CZE) (Infusino & Panteghini 2013)

According to the database of the Joint Committee for Traceability in Laboratory Medicine (JTCLM), optimized immunoturbidimetry and immunonephelometry are listed as reference measurement methods for albumin in serum. As albumin constitutes a larger portion of serum, a substantial number of dilutions is required when quantification is done via immunoassays. If not, high concentrations of the analyte cause an underestimation of true values which is referred to as the 'Hooks effect' (Brinkman et al. 2004). This scenario has limited the use of immunoassays in estimation of serum albumin levels, but it is a popular method in detection of

microalbuminuria and albumin estimation of body fluids other than serum or plasma. Other reasons that make immunoassays less frequently used for serum albumin quantification include higher costs, requirement for sophisticated instruments, and qualified staff (Kumar & Banerjee 2017).

The use of electrophoresis for serum albumin separation and quantification was started and became more frequent after the Nobel Prize awarded research by Tiselius. This project developed an electrophoretic apparatus and it was the very first time that human serum albumin was separated using electrophoresis (Sastre Toraño et al. 2019). Electrophoresis utilizes the principle that different molecules in a solution have different mobilities in an electrical field depending on their net charge, so they separate accordingly (Kumar & Banerjee 2017). Capillary zone electrophoresis is the status quo electrophoretic method used in serum albumin quantification. The application of a higher voltage within a narrow bore capillary result in the separation of proteins in serum in a liquid medium. According to the electrophoretic mobility joined with electroendosmotic flow of proteins, the migrations occur and the separated fractions are directly detected by measuring absorbance at 214nm and the percent concentrations of each variate (albumin, α1, α2, β, and γ) are obtained (Duly et al. 2003). These percent values are then converted to concentrations using serum total protein concentrations determined by another assay (Padelli et al. 2019). The Biuret method is the most well-known spectrophotometric method of estimating total protein concentration in serum and is the method of choice for hyperproteinemic samples (5–160 g/L) (Chutipongtanate et al. 2012). The Biuret method exhibits minimal interference aside from intriguing instances of monoclonal protein interference (Tichy et al. 2009) and bilirubin interference (Ji & Meng 2011). Capillary zone electrophoresis has become a quite famous alternative to other techniques due to the advantages over other methods including low sample consumption, economic efficiency, high degree of automation, and the production of more precise, reproducible, efficient results (Tan et al. 2014).

1.1 Dye binding assays

These techniques remain the most popular and frequently utilized methods for serum albumin measurement in clinical biochemistry laboratories hitherto. The innovation of dye binding methods has been a breakthrough as it enabled albumin measurement rapid and convenient. Various types of dyes are used to quantify albumin such as methyl-orange, phenol red 1-anilinonaphthalene, and benzoic acid, however the two phthalein dyes bromocresol purple (BCP) and bromocresol green (BCG) are the current interest (Kumar & Banerjee 2017).

The bromocresol green (BCG) dye for albumin measurement was first described by Rodkey (1964) and it remains the most dominant method until today (Kumar & Banerjee 2017). BCG dye binds with the binding site I of the albumin molecule at pH 4 forming the BCG-

albumin complex. The concentration of albumin in the sample is directly proportional to the absorbance of the complex at 625 nm (Buzanovskii 2017). Even though this method is simple, rapid, and convenient its specificity is considered questionable mainly due to the non-specific interactions of the dye with acute phase reactants and serum globulins (Lopukhin et al. 2000). In consequence, BCG yields higher results in low concentrations of albumin and lower results in higher concentrations (Vanessa et al. 2018).

BCP method for serum albumin estimation was first discovered in 1968 by Louderback (Kumar & Banerjee 2017). BCP and BCG dye molecules are structurally similar and BCP also attaches to the binding site I of albumin molecule via hydrophobic interactions. The characteristic color change measured occurs due to the pH-dependent proton transfer reaction between BCP molecule and the carboxyl or amino group of human serum albumin (HSA). Spectrophotometric measurements at 590 nm can be utilized to determine the HSA concentration by assessing the absorbance of HSA-BCP complex (Ito & Yamamoto 2010). In contrast to BCG, BCP exhibits greater specificity for HSA and is free from precipitation effects. Several researchers state that BCP method has no interference by globulins, transferrin, and salicylate (Kumar & Banerjee 2017). Some investigators have stated that BCP is the choice of method for albumin estimation in serum and plasma and have recommended avoiding the use of BCG (Koerbin et al. 2019).

Some aspects of albumin estimation using BCG and BCP methods have been reviewed in the past. There are studies that have shown that BCG non-specifically binds with globulins in serum resulting overestimation of albumin values (Vanessa et al. 2018). As BCG overestimates albumin measurements in serum, recommendations have been made for replacing this method by BCP (Meola & Brown 1979, Duggan & Duggan 1982), while some investigators stated that there is no apparent superiority of the BCP method when in comparison with the BCG method (McGinlay & Payne 1988, Selvarajah et al. 2017).

The chemical pathology laboratory of Karapitiya Teaching Hospital uses both BCG and CZE methods for serum albumin estimation. So, it is conceivable that successive serum samples for a single patient will be estimated by various methods of albumin measurement (BCG and CZE). If discrepancies are observed between results, it would be an issue. Consequently, when utmost precision in determining serum albumin concentration is essential, having a thorough grasp of the chosen technique and the proficiency to convert between methods becomes invaluable. Hence, the core objective of this study is to quantify the degree of any discrepancies. This research compares the results of BCG and BCP methods using CZE as a reference method and the goal is to circumvent the issues by finding the most appropriate method. While it has remained a long-standing query within the literature, it will be useful to get a better awareness of these discrepancies, and this information which is

investigated will support the clinicians to obtain a better and more accurate clinical picture.

1.2 Samples and stability of the analyte

The biological half-life of albumin is three weeks. Serum samples collected into dry containers are the recommended samples. Stability in blood at room temperature is 6 days and when stored at 2-6 °C, albumin is stable for up to 14 days. After serum separation, the stability of albumin is (-20) °C for 4 months, 4-8 °C for 5 months, and 20-25 °C for 2-5 months. It should be ensured that blood is collected avoiding hemolysis and with a minimum of venous stasis. The known interfering substances for serum albumin measurement include bilirubin, lipids, salicylates, and haemoglobin. Therefore icteric, hemolysed, and lipemic samples are usually included in the rejection criteria for the serum albumin measurement (WHO 2002).

2 RESEARCH METHODOLOGY

A method comparison study was designed and performed using the facilities of chemical pathology laboratories in the Teaching Hospital of Karapitiya, and District General Hospital, Negombo, Sri Lanka. Ethical approval was obtained from the Ethics Review Committee, Faculty of Allied Health Sciences, University of Ruhuna.

2.1 Patient Samples

The aim was to get a significant result ($p < 0.05$) with sufficient power (80%) to detect at least the correlation coefficient of 0.4. Therefore, the minimum required sample size for this study was 47. The formula for calculation is based on a two-tailed test (Guenther 1977).

The residual of blood samples (n=47) received by the Biochemistry and Chemical Pathology laboratory, Teaching Hospital, Karapitiya for protein electrophoresis were selected based according to an inclusion and exclusion criterion. The blood samples selected for the study were non-hemolyzed, non-turbid, and non-lipemic with adequate volume (approximately 200µl of serum). Hemolyzed, icteric, turbid, lipemic, and low volume blood samples and samples showing monoclonal gammopathy were excluded.

2.2 Sample Analysis

The Capillary Zone Electrophoresis method was performed with Sebia MINICAP Automated Capillary Electrophoresis System and the total protein concentration was estimated using BS 800M Biochemistry Analyzer which utilizes the Biuret method for protein estimation. When the total protein values were entered into the CZE system, it calculated the concentration of albumin present in the sample using the electrophoretic percentage of albumin fraction.

The aliquots of collected serum samples were separately analyzed to measure albumin concentration by BCG and BCP methods. The BCP method was performed using the Dimension Clinical Chemistry System analyzer. The BCG method was performed using the Mindray BS-480 chemistry analyzer.

2.3 Quality Control

Two levels of internal quality controls (IQCs) provided by the manufacturer (Sebia Hypergamma Control Serum and Sebia Normal Control Serum) were run prior to the protein electrophoresis.

Two levels of commercial, assayed IQCs (Biorad Liquid Assay Multiqual) were used before the commencement of the analysis of collected samples by the three biochemistry analyzers for total protein estimation and albumin concentration.

2.4 Statistical Analysis

Version 26.0 of Statistical Package for the Social Sciences (SPSS) software was used to statistically analyze and evaluate the test results. Analyzed data was presented as mean (SD) or percentage, as applicable. To compare BCG, BCP and CZE methods, a one-way ANOVA followed by a post-hoc Tukey HSD test was performed. The independent t-testing was utilized to identify significant differences between the means of the 2 groups. Low p values (less than 0.05) were considered significant. Pearson correlation and Bland-Altman plots were employed to investigate the correlation and agreement between serum albumin levels, as measured by BCP, BCG, and CZE methods.

3 RESULTS

The statistical parameters of the three methods (CZE, BCG, and BCP) were calculated and are summarized in Table 1.

3.1 Comparison of BCG, BCP and CZE Albumin Estimation Methods

In order to assess the difference in albumin measures derived from three distinct techniques CZE, BCP, and BCG, we performed a one-way ANOVA followed by a post-hoc Tukey HSD test for pairwise discrepancies.

The one-way ANOVA test revealed a statistically significant difference in albumin levels among the three methods ($F = 6.51$, $p = 0.002$($p<0.05$). The post-hoc analysis revealed that BCG method consistently reported significantly higher albumin levels compared to both the CZE and BCP methods. Also, there was no statistically significant difference between the CZE and BCP methods as shown in the Table 2.

3.2 Comparing Means of Albumin Concentration by BCP and BCG Methods with CZE Method

To compare the albumin concentrations by the two dye binding methods with the CZE method, an independent-sample t-test was carried out. As indicated by the results there was no statistically significant difference in mean albumin concentration measurements between CZE method ($M = 35.72$ g/L, $SD = 7.12$ g/L) and BCP method ($M = 36.4$ g/L, $SD = 7.08$ g/L); $t(88) = (-450)$, $p = 0.654$ ($p>0.05$). Mean difference between BCG method ($M = 40.28$ g/L, $SD = 5.53$ g/L) and CZE method ($M = 35.72$ g/L, $SD = 7.12$ g/L) were 4.56 g/L, which is statistically significant $t(88) = (-3.39)$, $p = 0.001$ ($p<0.05$).

3.3 Correlational Studies Between Albumin Measurement by CZE, BCP, and BCG Methods

The correlation of albumin concentration measurements using CZE, BCP, and BCG methods was evaluated using the Pearson correlation coefficient (r-value) as shown in Table 3.

According to the statistical analysis, the measurements of BCP assay showed a strong positive correlation with the measurements obtained by CZE technique ($r= 0.910$, $p<0.001$). A strong positive correlation was demonstrated between CZE and BCG methods as well ($r=0.875$, $p<0.001$) which was statistically significant and less than the correlation between CZE and BCP methods. The correlation between BCG and BCP methods was strong, positive, and statistically significant ($r=0.903$, $p<0.001$) (Figures 1, 2, and 3)

3.4 The Degree of Agreement between Albumin Measurement by CZE, BCP, and CZE, BCG Methods

As correlation reveals the strength of association between measures and not the agreement between them, Bland- Altman plots were used. Bland Altman statistical technique calculates the mean and 95% range of the differences which represent the upper and lower limits of agreements, between the data points obtained from the two methods comparing each other.

CZE and BCP Methods

The Bland Altman plot for the degree of agreement between CZE and BCP methods gives a bias of 0.67 g/L, a lower limit of agreement of -5.00 g/L, and an upper limit of agreement of 6.56 g/L. The BCP method overestimates the concentration of albumin in comparison to the CZE method (positive bias) (figure 4).

CZE and BCG Methods

The plot gives a bias of 4.56 g/L, a lower limit of agreement of -2.34 g/L, and an upper limit of agreement of 11.46 g/L. The BCG method exhibits a greater overestimation of albumin when compared with the results of the CZE technique (positive bias) in contrast to the BCP method (figure 5).

The BCP and BCG Methods

As illustrated in Figure 6, the plot between BCP and BCG methods gives a bias of 2.28 g/L, a lower limit of agreement of -1.17 g/L, and an upper limit of agreement of 5.73 g/L. The BCG method shows a positive bias by overestimating the concentration of albumin compared to the BCP method

Table 1. Descriptive statistics of the three methods of serum albumin measurement of the collected samples from THK

	Range	Minimum	Maximum	Mean	Std. Deviation	Variance
Albumin Concentration by CZE (g/L)	28.30	20.90	49.20	35.72	7.12	50.70
Albumin Concentration by BCP (g/L)	27.90	21.00	48.90	36.39	7.08	50.16
Albumin Concentration by BCG (g/L)	19.80	28.90	48.70	40.27	5.52	30.55

Table 2. Tukey HSD Post- Hoc Test

Group 1	Group 2	Mean Difference	p-value	Lower Bound	Upper Bound	Significance
BCG	BCP	-4.05	0.01	-7.33	-0.77	Significant
BCG	CZE	-4.56	0.00	-7.83	-1.28	Significant
BCP	CZE	-0.51	0.93	-3.79	2.77	Not Significant

Table 3. Pearson correlation between CZE, BCP, and BCG methods

Methods Compared	Pearson Correlation (r)	p-Value
BCG Method Vs CZE Method	0.91	<0.001
BCP Method Vs CZE Method	0.88	<0.001
BCG Method Vs BCP Method	0.90	<0.001

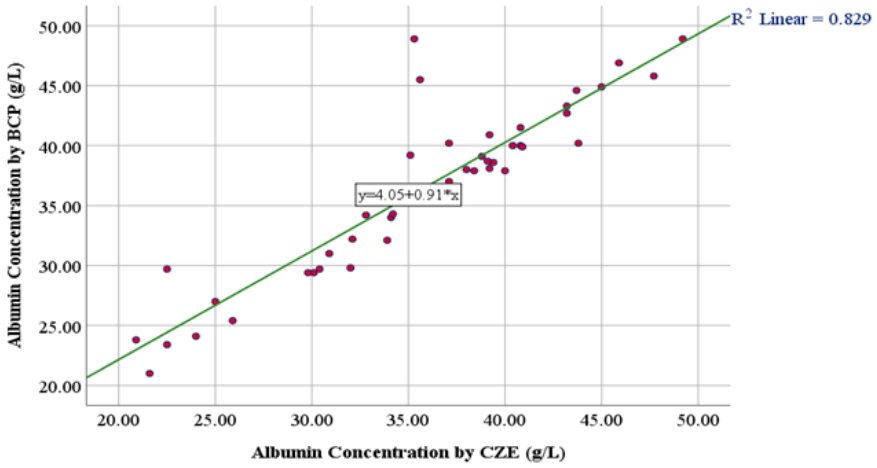

Figure 1. Graph of correlation between BCP method and CZE methods

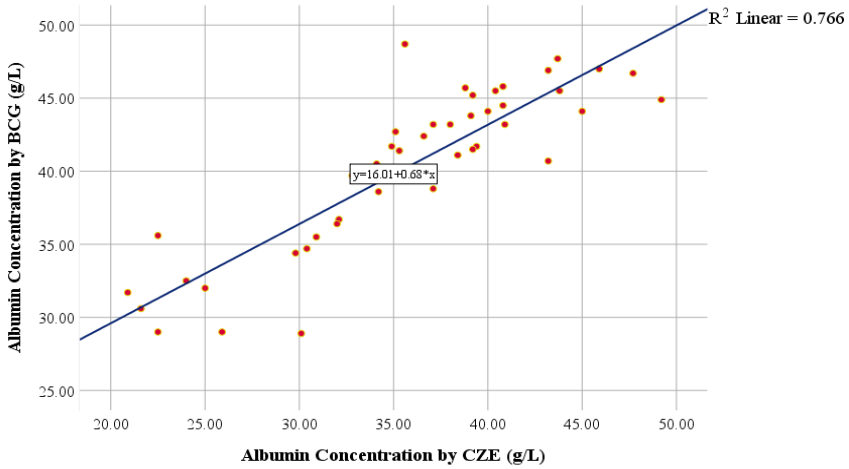

Figure 2. Graph of correlation between BCG method and CZE methods

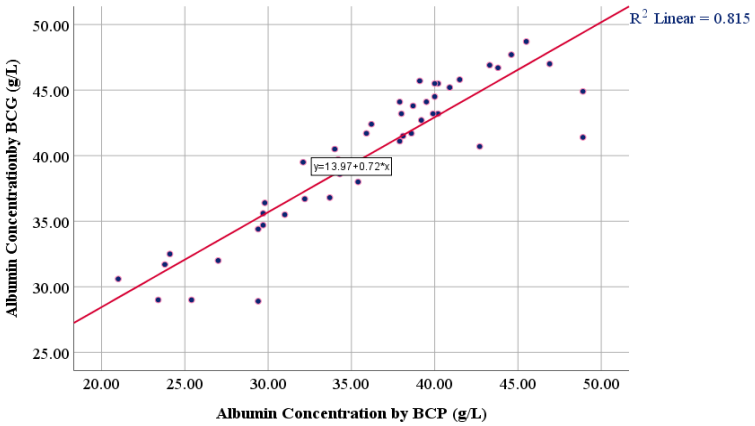

Figure 3. Graph of correlation between BCG method and BCP methods

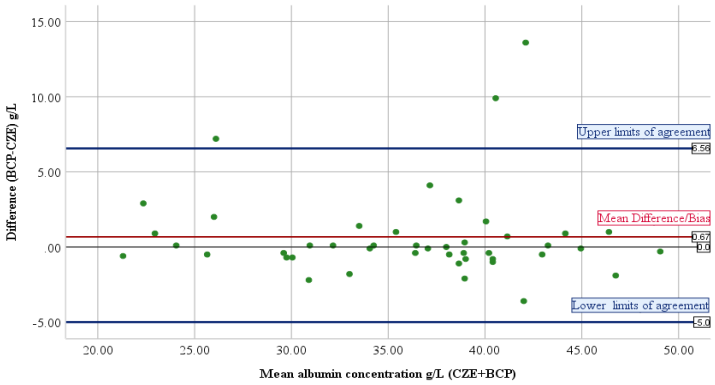

Figure 4. Bland Altman plot for the degree of agreement between CZE method and BCP method

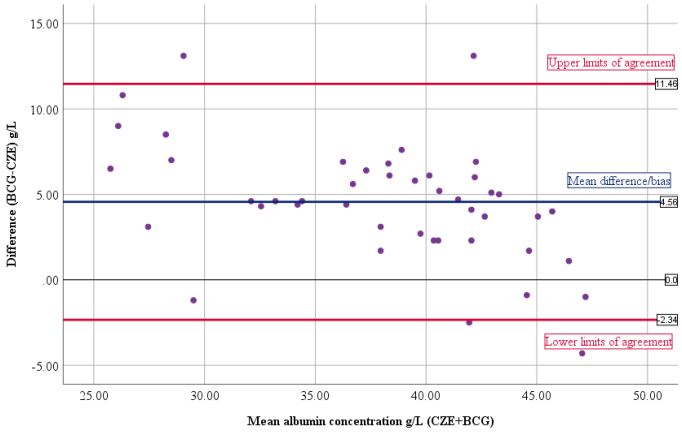

Figure 5. Bland Altman plot for the degree of agreement between CZE method and BCG method

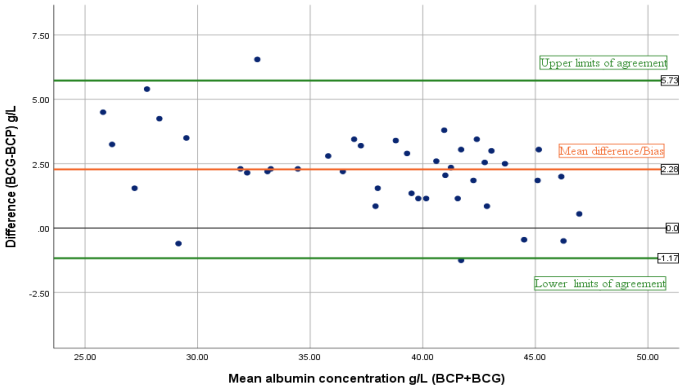

Figure 6. Bland Altman plot for the degree of agreement between BCG method and BCP method

4 DISCUSSION

The measurement of serum albumin, one of the most frequent investigations done in clinical biochemistry, possesses a noteworthy significance as it is used as a diagnostic and prognostic marker of many diseases that have been discussed previously. There is a significant interest in the methodological aspects of albumin assessment. The dye binding The present study mainly focused on investigating the correlation and agreement of albumin measurements obtained using the BCG method and capillary electrophoresis method and BCP method and capillary electrophoresis method. The primary objective of this research was to compare the results of BCG and BCP methods using CZE as a reference method and the goal was to circumvent the issues by finding the most appropriate method.

Based on the statistical analyses conducted, a strong correlation and a good agreement were demonstrated between the CZE and BCP methods. The BCP method exhibited a greater correlation to CZE method ($r=0.910$; $p<0.01$) showing a mean difference of less than 1 g/L. In contrast, the mean difference between BCG and CZE methods was larger (4.56g/L) despite the strong correlation between the methods ($r=0.875$; $p<0.01$). The BCP method agrees more closely with the CZE method and the BCG method overestimates the serum albumin measurement compared to the CZE method. An overestimation of albumin concentration was observed with both dye binding methods when compared to the CZE method with the BCG method exhibiting the highest degree of overestimation.

methods especially BCG and BCP remain the most adapted procedures over several decades (Xu et al. 2022). Out of these two, the BCG method is the dominant method in Sri Lanka. As far as we could know, this subject has not been investigated locally and not much information exists about the agreement between the albumin measurement using BCG, BCP, and CZE.

Similar results have been obtained by several studies carried out to compare these methods. According to a study done by(Vanessa et al. 2018), the mean difference between CZE and BCP methods was smaller, indicating a good agreement between the two methods (mean difference, 0.97 g/L; 95% CI, 0.56-1.38). The BCG method showed a positive bias in contrast to the CZE method (3.54 g/L). Another research designed to compare albumin estimation by several methods including BCP, BCG, CZE, and high specific array immunoassay techniques, concludes that the results have shown a positive bias of BCG with CZE (4.51 g/L; 95% limits of agreement, 3.77 to 5.26) and BCP (3.85 g/L; 95% limits of agreement, -1.42 to 9.12). CZE and BCP have demonstrated a closer agreement (0.67 g/L; 95% limits of agreement, -4.39 to 3.06). They conclude that BCP method is superior to BCG method for estimating albumin (Duly et al. 2003).

Even though this study has used the CZE method as the reference method, it has its limitations too. Determining the albumin

fraction of serum by CZE method requires the quantification of total protein concentration by another approach. The Biuret method employed in this investigation is the most popular spectrophotometric approach for estimating the total protein concentration in serum. According to the literature, this method is interfered by bilirubin (Ji & Meng 2011) and monoclonal proteins (Tichy et al. 2009). In addition to this, it is a widely known factor that monoclonal gammopathy interferes with serum protein electrophoresis (Snozek et al. 2007). As we have excluded all samples with such conditions, those drawbacks of CZE method were kept to a minimum throughout the investigation. Apart from that, quality controls were run daily and the laboratory participated in external quality assurance programs for both analyzers (Sebia MINICAP and BS 800M biochemistry analyzer) and as a result, the accuracy of total protein concentration and CZE results from the analyzer could be verified to be accurate, and precise.

As there is still no gold standard approach available for albumin measurement it is a challenge to decide the most appropriate one to use in routine analysis. The common consideration is that the immunological methods are superior and they yield more precise and accurate results. There are studies that have compared dye binding methods with immunological methods. Such study conducted by Van de Logt et al. (2019) to evaluate BCP and BCG dye binding methods in comparison to immunoassay, demonstrated a higher mean bias for the BCG assay (6.2 g/L, with a standard deviation of 2.4 g/L) compared to a bias of 0.3 g/L (standard deviation 1.5 g/L) for the BCP assay. In the literature, there are other studies with similar results (Maguire & Price 1986, Clase et al. 2001).

The BCG method overestimates serum albumin measurements due to the longer reading time of analysis (Park et al. 2020). Apart from this at lower albumin concentrations BCG molecules nonspecifically bind with acute phase globulins (Alpha 1 and 2) causing overestimation of albumin values (Vanessa et al. 2018). Garcia Moreira et al. (2018) state that the bias observed with BCG assay and alpha 1-globulin concentrations exhibits a good correlation ($r = 0.758$); moderate and weak correlations with alpha-2-globulin ($r = 0.585$); while no correlation was observed with beta-globulin ($r = 0.120$) or gamma globulin ($r = –0.303$). Similar results have been obtained by (Xu et al. 2011).

As mentioned previously, the various methods of HSA concentration detection in recent years include dye-binding methods, capillary zone electrophoresis and immunochemical methods. Other methods such as chromatography, fluorescent probe methods and biosensor-based detection methods are hot topics in current literature, but they have a long way to go before they can be used for clinical detection (Xu et al. 2022). So that classical dye binding methods remain as the mainstay in the clinical laboratory setup. According to our knowledge, the BCG method is the most prominently used method for routine estimation of human serum albumin

in Sri Lanka due to the widespread availability of BCG kits imported by clinical reagent suppliers. The BCP method has a minimal implementation in clinical laboratories as most of the clinical reagent suppliers are solely marketing BCG kits. According to our knowledge, Bromocresol Purple (BCP) method has minimal implementation, with only two government sector laboratories currently using it.

Despite the excellent resolution and specificity, the CZE approach is frequently not appropriate for routine albumin quantification due to its higher cost, technical complexity, and lower throughput when compared to dye-binding methods(Luraschi et al. 2003). The cost for serum protein electrophoresis utilizing the CZE method costs around 2500 rupees which is higher compared to serum albumin estimation by dye binding methods which costs about 800 rupees (Sri Jayewardenepura General Hospital 2022). As discussed above, although earlier research has compared the correlation of the BCG method with CZE and reported notable mean differences alongside closer agreement, there is no literature evaluating the BCP technique in Sri Lankan contexts (Selvarajah et al. 2017, Harshanee et al. 2024).

Evaluating the outcomes of our study, we strongly recommend adopting the BCP method for routine albumin quantification because it demonstrates superior specificity and accuracy compared to BCG. Despite of the distinct advantages of the CZE method in resolving complex protein profiles, its cost and technical requirements make it less viable for widespread clinical use in Sri Lanka. Better diagnostic accuracy could be ensured in typical clinical settings by switching to BCP without causing major logistical or budgetary challenges.

In summary, our study signifies that BCP and BCG methods yield discrepant results and the BCG method overestimates the albumin values. As the BCG method is the most common method used in Sri Lanka, it is an important condition to address as important clinical decisions are made upon these results. One such example is the estimation of corrected calcium for patients with low hypoalbuminemia using an equation that requires serum albumin concentration. Adjusted results with analytically underperforming methods can cause administration of improper treatments and failure to address the patient's requirements.

5 CONCLUSION

The BCP method shows a closer agreement with the CZE method while the BCG method has a greater positive bias (overestimation of albumin concentration) compared to the CZE method than the BCP method.

6 RECOMMENDATIONS & LIMITATIONS

The reference assay (capillary zone electrophoresis) may be susceptible to bias (e.g., in serum from patients with multiple myeloma). Further studies with additional comparative techniques such as albumin

immunoassay and HPLC are recommended. It would be better to perform further studies using reagents from different manufacturers with different formulas, developed to combat the nonspecific binding of the dye.

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