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Bisalbuminemia: Lack of Association with Monoclonal Gammopathy and Value-Justification for Reporting in Serum Protein Electrophoresis

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Abstract

Objective: Serum protein electrophoresis (SPE) is commonly used to detect and quantify monoclonal immunoglobulins/components (MC). SPE resolves serum proteins into 5 or 6 major fractions, including albumin. A split or double albumin band in SPE is called bisalbuminemia or alloalbuminemia, a condition caused by genetic or acquired changes. Although isolated cases of bisalbuminemia have been reported in patients with monoclonal gammopathy (MG), there has been no study linking the two statistically or pathophysiologically. The objective of this study is, thus, to determine if bisalbuminemia is significantly associated with MG, and hence to provide value justification for its reporting in SPE.

Methods: We conducted a retrospective study reviewing over 55,800 consecutive serum protein electrophoretograms for bisalbuminemia between June 2005 and October 2013. After exclusion of repeats, 33,512 electrophoretograms were available for analysis. MG was confirmed by immunofixation electrophoresis (IFE) and its positivity rate determined in a smaller sub-group with 3974 paired IFE and SPE results. SPE and IFE were performed on the Sebia Capillarys^{TM}2 and Sebia Phoresis^{TM} Electrophoresis Systems respectively.

Results: 9 persistent cases with clear double albumin spikes over time (pattern A) and 10 transient cases with a partial albumin split (pattern B) were identified. The prevalence of pattern A, pattern B and pattern A+B were 0.027%, 0.030% and 0.057% (19/33512), respectively. IFE positivity rate was 32.1% (1276/3874). The odds ratios (95% confidence interval) for pattern A, pattern B and patterns A+B bisalbuminemia over MG were 0.604 (0.125-2.91), 0.101 (0.006-1.72) and 0.249 (0.057-1.08) respectively. Chi-square test for independence (association) was not significant in all 3 scenarios ($p>0.05$).

Conclusion: Bisalbuminemia, genetic or acquired, is a rare incidental SPE finding that is not associated with MG. The extremely low prevalence and a general lack of association with diseases confer little or no clinical utility, nor value for its reporting in SPE.

Keywords: Bisalbuminemia; Alloalbuminemia; Albumin variants; Serum protein electrophoresis; Monoclonal gammopathy; Multiple myeloma

Introduction

Serum protein electrophoresis (SPE) is a crucial biochemical technique used for the investigation of monoclonal gammopathies (MG). It resolves serum proteins into 5 or 6 major protein fractions, namely albumin, alpha1, alpha2, beta1, beta2 and gamma (Figure 1a), and allows for the detection and quantification of monoclonal immunoglobulins or their components (MC) [1,2]. MC is the hallmark of a wide spectrum of conditions ranging from the clinically occult and stable (pre-malignant) Monoclonal Gammopathy of Undetermined Significance (MGUS) to the clinically overt and progressive (malignant) Multiple Myeloma, Waldenstrom Macroglobulinemia, AL-amyloidosis, etc. Changes in MC concentration often reflect the underlying tumour activity. The prevalence of MG in the age >50 years population is approximately 3-5% and increases as age increases. In individuals of African descent, the prevalence can be more than doubled.

A split or double albumin band in SPE is a rare finding and indicates the presence of bisalbuminemia, a condition that may be caused by a genetic change in the albumin gene [3,4] or is simply acquired (and thus transient) due to the binding of molecules such as aminoglycosides or pigments [4-6]. Moreover, not all albumin genetic variants present as bisalbuminemia which has an estimated prevalence between 1:10,000 to 1:1,000. While isolated cases of bisalbuminemia have been reported in patients with MG [7-11] and other diseases, there has been no systematic study demonstrating statistically significant associations or pathophysiologic links between bisalbuminemia and MG. Thus, the clinical significance of a bisalbuminemia finding in MG patients is unclear. Nevertheless, the Australian/New Zealand group recommended special commenting of bisalbuminemia in SPE [12], and 92% of Canadian clinical laboratories indicated in a survey that they would comment on the finding of bisalbuminemia in SPE patient reports [13]. Interpretive reporting of

bisalbuminemia can be alarming to some physicians, and lead to unnecessary follow-up testing and referrals.

The objective of this study is, thus, to determine if bisalbuminemia is significantly associated with MG, and hence to provide value justification, if any, for reporting in SPE. The work presented herein represents the first systemic study examining the relationship between bisalbuminemia and MG in a sizeable patient population.

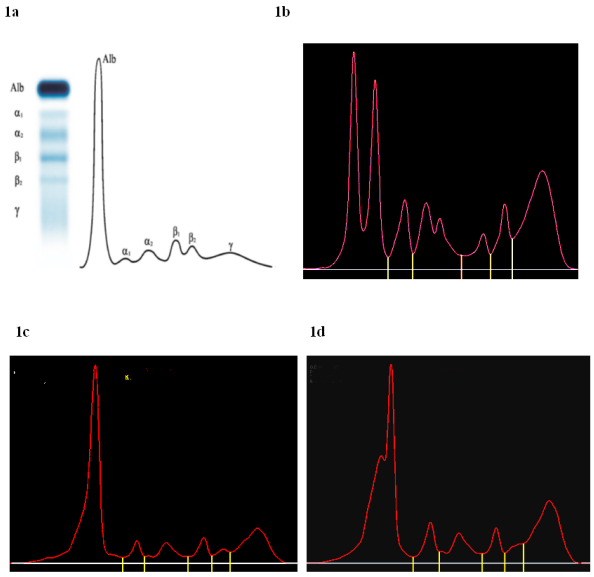

Figure 1: Serum protein electrophoretograms: Figures 1a and 1c show the single-spike wild-type albumin that is seen in most individuals; Figures 1b and 1d show bisalbuminemia of pattern A (likely genetic) and pattern B (likely acquired) respectively. Figures 1c and 1d were from the same patient but three months apart.

Methods

We conducted a retrospective study reviewing over 55,800 consecutive serum protein electrophoretograms between June 2005 and October 2013 at our institution, a 1200-bed tertiary care facility with Oncology as one of the 6 focus programs. SPEs from repeat episodes of the same patient were excluded, and for those with bisalbuminemia, only the first episodes positive for bisalbuminemia were included. After exclusion, a total of 33,512 patient-electrophoretograms were available for statistical analysis. To estimate the prevalence of monoclonal gammopathy in this patient population, a subset of the patients who had both SPE and Immunofixation Electrophoresis (IFE) available at the same time (over 95% were requested together and the rest due to reflex testing) during the period between January 2010 and June 2013 was analyzed. This smaller cohort has been described in another study elsewhere [14]. The IFE positivity rate for this smaller cohort was taken as representative of the study population and was used in the construction of a 2×2 contingency table for odds ratios analysis (see below).

SPE and IFE techniques were performed on the Sebia Capillarys^{TM}2 and Sebia Phoresis^{TM} Electrophoresis Systems respectively. All SPE patterns and IFE were reviewed by two independent reviewers. Bisalbuminemia was detected, enumerated and sub-categorized based on observations of split albumin peak shapes and recurrence. No genetic study was performed to ascertain the genetic causes of bisalbuminemia. To determine if there is an association between bisalbuminemia and MG, the odds ratio was calculated using GraphPad Instat, with significance of association tested by a 2-tail Chi square test with Yates correction. Statistical significance was accepted at $p<0.05$.

Results

Of the 33512 electrophoretograms, 19 cases of bisalbuminemia were identified and subsequently sub-categorized into two arbitrary bisalbuminemia patterns: 9 with clear double albumin spike (pattern A) (Figure 1b) and 10 with a partial split (pattern B) (Figure 1d). If repeat episodes were counted, there were a total of 14 and 17 episodes of patterns A and B bisalbuminemia respectively. For pattern A, there was no change in albumin peak shape over time, but for pattern B patients, 4 out of 10 exhibited different albumin peak shapes over time, presenting with a normal single albumin spike (Figure 1c) or tricuspid peak shape [13] in at least one other episode. This transient nature of pattern B suggested that this type of bisalbuminemia was acquired rather than genetic.

The overall prevalence of bisalbuminemia (both patterns A and B) was determined to be 0.057% (19/33512), while the prevalence for pattern A and pattern B were 0.027% and 0.030% respectively. Reduced SPE serum albumin concentration (reference interval: 34-53 g/L) was more common among pattern B patients (7/10) than pattern A patients (2/9 or 22%), with a mean ± SD of 27.7 ± 10.7 (n=10) versus 38.4 ± 8.9 (n=9) g/L (unpaired t-test, $p<0.05$).

In the sub-cohort of 22,900 consecutive SPEs between 2010 and 2013, 3974 had paired IFE results, of which 1276 (32.1%) were IFE positive. Among the bisalbuminemic patients, the IFE positivity rates for pattern A and B were 2/9 (22.2%) and 0/10 (0%) respectively. Combining with the IFE positivity rate of the study population, the odds ratios (95% confidence interval) for pattern A, pattern B and patterns A+B bisalbuminemia were 0.604 (0.125-2.91), 0.101 (0.006-1.72) and 0.249 (0.057-1.08) respectively. None of the odds ratios reached statistical significance at $p<0.05$. Results are summarized in Tables 1 and 2.

Bisalbuminemia		MG+	MG-	Total
Pattern A	Bisalb+	2	7	9
	Bisalb-	10758	22745	33503
Pattern B	Bisalb+	0	10	10
	Bisalb-	110760	22742	33502
Pattern A & B	Bisalb+	2	17	19
	Bisalb-	10758	22735	33493

Bisalb: Bisalbuminemia, MG: Monoclonal Gammopathy, "+"=positive, "-"=negative

Table 1: Number of cases with Bisalbuminemia with or without Monoclonal Gammopathy (MG).

Bisalbuminemia	Odds Ratio	95% CI	p
Pattern A	0.604	0.125-2.91	0.781
Pattern B	0.101*	0.006-1.72	0.066
Patterns A and B	0.249	0.057-1.08	0.077

*Since one of the values was zero (Table 1), 0.5 was added to each value in the 2 × 2 contingency table to make the calculations possible.

Table 2: Odds ratios for Bisalbuminemia in patients with Monoclonal Gammopathy.

Discussion and Conclusion

Bisalbuminemia (patterns A+B) on SPE is a rare incidental finding with a low frequency of 0.057% or 1:1,754 (1:3,703 for pattern A alone) in the current study population. This frequency of occurrence is quite comparable to the reported prevalence of between 1:1,000 and 1:10,000 for hereditary bisalbuminemia [15,16]. While we did not confirm the genetic causes for pattern A bisalbuminemia with molecular analysis, its stability over time did seem to support its hereditary nature and was in direct contrast to the transient nature of pattern B bisalbuminemia. While hereditary bisalbuminemia is a result of genetic variations or mutations in the albumin gene, not all genetic variants give rise to bisalbuminemia. Examples of the latter may include mutations that are responsible for analbuminemia or familial dysalbuminemic hyperthyroxinemia and familial dysalbuminemic hypertriiodothyroninemia [3,17,18]. Moreover, the reporting of bisalbuminemia in individual patients with diseases such as multiple myeloma [8,9], MGUS [7,10], Waldenstrom Macroglobulinemia [11], chronic kidney disease [19], nephrotic syndrome [20,21], diabetes [22,23], Alzheimer's disease [24], and others [25-27] has sometimes been misconstrued as a significant association of bisalbuminemia with these conditions [28]. To date, no known statistical or causal connection between hereditary bisalbuminemia and disease has been demonstrated, whereas acquired transient bisalbuminemia can result from pancreatitis and beta-lactam therapy [5,29,30]. In the current study, we examined possible associations between bisalbuminemia as detected by SPE and the presence of MG in a large cohort of patients with a variable degree of increased suspicion for MG. The finding could have important implications as SPE is the routine biochemical technique used for MC detection and quantification that also detects both hereditary and acquired bisalbuminemia. A significant association with MG may justify the reporting of bisalbuminemia in SPE even in the absence of a demonstrable MC.

To assess possible association between bisalbuminemia and MG, we calculated the odds ratio for pattern A (likely hereditary), pattern B (acquired transient) or patterns A+B bisalbuminemia which were 0.604 (95% CI: 0.125-2.91), 0.101 (95% CI: 0.006-1.72) and 0.249 (95% CI: 0.057-1.08) respectively. Overall, none of these odds ratios was greater than one, indicating that the probability of finding bisalbuminemia in patients with MG is much lower than the probability of finding it in individuals without MG. Chi square test for association between bisalbuminemia and MG did not reach significance either ($p>0.05$ in all 3 scenarios). Our finding is in line with a lack of reported pathophysiologic mechanisms that link the two conditions. Since low albumin concentration is related to poor prognosis in multiple myeloma patients [31] and that transient bisalbuminemia (pattern B) tended to have a lower albumin concentration as shown in this study, one wonders if bisalbuminemia may mediate its association, if any, with diseases such as myeloma through the effect of reduced albumin. Clearly, our results did not support such proposition as there was not a single pattern B bisalbuminemic patient with MG. In another unpublished observation over 70 consecutive myeloma patients presented at our institution, we did not observe any cases of bisalbuminemia. It is most likely that previously reported cases of bisalbuminemia and MG were coincidental findings and that the two conditions were not related at all.

As with most retrospective studies, the current one is not without limitations. For example, the prevalence of MG reported in this group is likely higher than that of the general population. However, this is exactly what SPE is supposed to be used for i.e. to detect MC in patients with an increased clinical suspicion for MG. If bisalbuminemia is associated with MG, an increase in MG frequency will also increase that of bisalbuminemia. To convince ourselves that the high MG positivity rate would not have affected our conclusion, we repeated the contingency table analysis with an IFE positivity rate of 5%. Reanalysis with a 5% IFE positivity rate resulted in an overall odds ratio for bisalbuminemia that was still not significantly increased (OR: 2.24, 95% CI: 0.52-9.69), and the Chi square test for association was not significant at $p>0.05$, supporting our conclusion.

The lack of association between bisalbuminemia and MG brings up the question of the value of commenting bisalbuminemia on SPE reports. Best practice guidelines on interpretive reporting [32,33] stipulate that comments should be relevant and add clinical value to the investigation. With an extremely low prevalence and a lack of association with MG and possibly other conditions, bisalbuminemia does not provide any value justification for reporting in SPE. For laboratories that routinely include SPE tracing or electrophoretogram in patient reports, the unusual looking bisalbuminemic pattern may cause concern and lead to unnecessary repeat testing or even referral to specialists. If this becomes a concern, a comment indicating "There is no known association between a bisalbuminemic pattern and increased odds for monoclonal gammopathy. Further testing is not warranted unless clinical suspicion for MG remains high." may be used.

In conclusion, bisalbuminemia, genetic or acquired, is a rare incidental SPE finding that is not associated with MG. The extremely low prevalence and a general lack of association with any significant diseases confer little or no clinical utility, and thus no value, in the reporting of bisalbuminemia during the investigation of MG or other diseases.

References

1. NICE NG35 Guideline, UK.
2. Dimopoulos MA, Sonneveld P, Leung N, Merlini G, Ludwig H, et al. (2016) International Myeloma Working Group Recommendations for the Diagnosis and Management of Myeloma-Related Renal Impairment. J Clin Oncol 34: 1544-1557.
3. Minchiotti L, Galliano M, Kragh-Hansen U, Peters T Jr (2008) Mutations and Polymorphisms of the Gene of the Major Human Blood Protein, Serum Albumin. Hum Mutat 29: 1007-1016.
4. Kragh-Hansen U, Minchiotti L, Galliano M, Peters T Jr (2013) Human serum albumin isoforms: genetic and molecular aspects and functional consequences. Biochim Biophys Acta1830: 5405-5417.
5. Rocha J, Bohner J, Kompf J (1995) Transient bisalbuminemia: separation by isoelectric focusing of human albumin fractions linked to different numbers of benzylpenicilloyl groups. Electrophoresis 16: 1031-1033.

6. Kobayashi S, Okamura N, Kamoi K, Sugita O (1995) Bisalbumin (fast, slow type) induced by human pancreatic juice. Ann Clin Biochem 32: 63-67.
7. Kalambokis G, Kitsanou M, Kalogera C, Kolios G, Seferiadis K, et al. (2002) Inherited bisalbuminemia with benign monoclonal gammopathy detected by capillary but not agarose gel electrophoresis. Clin Chem 48: 2076-2077.
8. Porta FA, Riches PG, Richardson D, Knox J, Hobbs JR, et al. (1983) Genetic bisalbuminemia occurring together with myeloma. Ann Clin Biochem 20: 90-92.
9. Pola V, Tichy M (1985) Bisalbuminemia: Critical review and report of a case of an acquired form in a myeloma patient. Folia Haematol Int Mag Klin Morphol Blutforsch 112: 208-218.
10. Dubost JJ, Deseubia T, Sauvezie B, Gentou C, Rampon S, et al. (1985) A tricuspid electrophoretic pattern: familial bisalbuminemia with monoclonal immunoglobulin. Pathol Biol 33: 837-838.
11. Valasek MA, Hopley RT, Wians FH Jr (2008) Resolving the Baneful and Banal: Bisalbuminemia in an Adult With Waldenström Macroglobulinemia. Lab Medicine 39: 723-726.
12. Tate J, Caldwell G, Daly J, Gillis D, Jenkins M, et al. (2012) Working Party on Standardised Reporting of Protein Recommendations for standardized reporting of protein electrophoresis in Australia and New Zealand. Ann Clin Biochem 49: 242-256.
13. Pak Cheung Chan, Yu Chen, Randell EW (2017) On the path to evidence-based reporting of serum protein electrophoresis patterns in the absence of a discernible monoclonal protein-A critical review of literature and practice suggestions. Clin Biochem 9120: 30873-30881.
14. Chan PC, Chen J (2015) Value of reflex testing based on hypogammaglobulinemia as demonstrated in serum protein electrophoresis. Clin Biochem 48: 674-678.
15. Fine JM, Marneux M, Rochu D (1987) Human albumin geneticvariants: an attempt at classification of European allotypes. Am J Hum Genet 40: 278-286.
16. Takahashi N, Takahashi Y, Isobe T, Putnam FW, FujitaM, et al. (1987) Some amino acid substitutions in inherited albumin variants from Amerindian and Japanese populations. Proc Natl Acad Sci USA 84: 8001-8005.
17. Sunthornthepvarakul T, Likitmaskul S, Ngowngarmratana S, Angsusingha K, Kitvitayasak S, et al. (1998) Familial dysalbuminemic hypertriiodothyroninemia: A new, dominantly inherited albumin defect. J Clin Endocrinol Metab 83: 1448-1454.
18. Heufelder AE, Klee GG, Wynne AG, Gharib H (1995) Familial dysalbuminemic hyperthyroxinemia: Cumulative experience in 29 consecutive patients. Endocr Pract 1: 4-8.
19. Ejaz AA, Krishna M, Wasiluk A, Knight JD (2004) Bisalbuminemia in chronic kidney disease. Clin Exp Nephrol 8: 270-273.
20. Hoang MP, Baskin LB, Wians FH Jr (1999) Bisalbuminuria in an adult with bisalbuminemia and nephrotic syndrome. Clin Chim Acta 284: 101-107.
21. Akhmouch I, Alayoud A, Bahadi A, Zajjari Y, Montasser D, et al. (2012) Bisalbuminemia during remission of nephrotic syndrome. Saudi J Kidney Dis Transpl 23: 1251-1253.
22. Bellomo A, Mancinella M, Troisi G, Marigliano V (2010) A rare familiar case of hereditary bisalbuminemia and diabetic predisposition: a possible predictive link?. Arch Gerontol Geriatr 50: 345-346.
23. Ouzzif Z, Derouiche M (2002) Hereditary bisalbuminemia in a man with diabetes mellitus and hypertension. Ann Biol Clin 60: 707-710.
24. Shetty JK, Prakash M, Gopalakrishna K (2007) Bisalbuminemia in an adult male with Alzheimer's disease. Indian J Med Sci 61: 356-357.
25. Pesántez D, Zamora C, Crespo J (2017) Bisalbuminemia in a patient with metastatic lung cancer. Med Clin 148: 145-146.
26. Hajoui FZ, Biaz A, Zouiten L, Mechtani S, Dami A, et al. (2015) A case of bisalbuminemia in a patient with digestive adenocarcinoma. Ann Biol Clin 73: 190-194.
27. Šimundić, A, Miler M, Nikolac N, Topic E, Čaržavec D, et al. (2009) Bisalbuminemia in a male Croatian patient with sarcoidosis. Biochemia medica 19: 95-100.
28. Oladipo J, Racco A, Simpkins H (2013) Is This Pathological?. Clini Chem 59: 448-449.
29. Faviou E, Nounopoulos C, Asteriou DA (2006) Bisalbuminemia from a clinical chemist's viewpoint: A case report and review of the recent literature. Minerva Med 97: 287-293.
30. Renversez JC, Roussel S, Rollux A (1984) Acquired bisalbuminemia in pancreatitis. Demonstration of a proteolytic process in ten patients. Ric Clin Lab 14: 417-423.
31. Greipp PR, San Miguel J, Durie BG, Crowley JJ, Barlogie B, et al. (2005) International staging system for multiple myeloma. J Clin Oncol 23: 3412-3420.
32. ES Kilpatrick (2014) Best practice when providing interpretative comments for laboratory medicine.
33. Vasikaran S, Sikaris K, Kilpatrick E, French J, Badrick T, et al. (2016) on behalf of the IFCC WG Harmonization of Quality Assessment of Interpretative Comments. Clin Chem Lab Med.