ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 17, Issue 9, 2024

Online - 2455-3891
Print - 0974-2441

Review Article

# BISALBUMINEMIA: STILL UNREVEALED FOR CLINICIANS AND RESEARCHERS – A SYSTEMIC REVIEW

**SNEHA WADALKAR[1]*, SHALINI MAKSANE[2], KAVITA MORE[2], KSHAMA PIMPALGOANKAR**

**[1]Department of Biochemistry, MGM Medical College and Hospital, Navi Mumbai, Maharashtra, India. [2]Department of Biochemistry, MGM Medical College, Navi Mumbai, Maharashtra, India. [3]Department, Biochemistry, Immunology and Special Chemistry, HN Reliance Hospital, Mumbai, Maharashtra, India.**

***Corresponding author: Sneha Wadalkar; Email: sneha.wadalkar@gmail.com**

***Received: 30 May 2024, Revised and Accepted: 06 July 2024***

**ABSTRACT**

Bisalbuminemia, a rare finding on serum protein electrophoresis, presents as a double band in the albumin (ALB) region. Inherited bisalbuminemia is a benign condition with autosomal dominant inheritance whereas acquired bisalbuminemia can be associated with various conditions such as diabetes, pancreatitis, and myeloma. Capillary electrophoresis is the preferred method for the diagnosis due to its superior resolution compared to agarose gel electrophoresis. Bisalbuminemia itself has no clinical significance, but acquired forms warrant further investigation for underlying diseases. Future research focuses on the functional consequences of ALB mutations and potential disease associations. This review summarizes the current knowledge on bisalbuminemia, covering its types, causes, clinical significance, and diagnosis.

**Keywords:** Bisalbuminemia, Allo-albuminemia, Albumin, Electrophoresis.

 DOI: http://dx.doi.org/10.22159/ajpcr.2024v17i9.51644. Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

## INTRODUCTION

Bisalbuminemia, also known as "alloalbuminemia," is a rare protein abnormality encountered accidentally on serum electrophoresis performed as a routine investigation mainly for the detection of monoclonal gammopathies [1,2]. This phenomenon was first described in the year 1955 by Scheurlen in a diabetic German patient but this band was transient [2,3]. Stable heterozygotic bands were reported shortly thereafter by Knedel, 1957; Nennstiel and Becht, 1957; Earle *et al*., 1958; and Wuhrmann, 1959 [4-8].

Over the years, with advancements in electrophoresis techniques, bisalbuminemias are more frequently encountered with the development of capillary electrophoresis (CZE), because of its better resolution power [2,9].

Bisalbuminemia, with an incidence of around 1:1000–1:3000 (higher in some North American tribes; 1:100) [2,3], is characterized by a bifid electrophoretic pattern, appearing as two distinct bands in albumin (ALB) fraction on agarose gel electrophoresis (AGE) or a single widened ALB band in some cases [10]. Two separate Allo-ALB peaks are because of dissimilar mobilities of two ALB variants on electrophoresis where the fast type variant has increased electrophoretic mobility and the slow type variant has decreased mobility [1,11].

Despite better techniques available for SPE, bisalbuminemia is still an infrequent finding among Indians [1]. Bagade *et al*., 2023 from Hyderabad, observed the 0.06% prevalence of bisalbuminemia (two cases out of 3360 serum samples run for SPE, during the past 5 years) [2]. Another study from India conducted by Kapatia *et al*., 2021, at Chandigarh where they observed 40 cases of bisalbuminemia out of 39,900 SPEs performed over a span of 8 years with an incidence rate of 0.01% (1:1,000). Out of 40 bisalbuminemia cases, hypergammaglobulinemia was seen in 18 and the other 18 cases had normal electrophoretic patterns [3]. Both studies showcased the rarity of the scenario.

Bisalbuminemia can be inherited or acquired. Hereditary bisalbuminemia is caused by single-point mutation of the human serum ALB gene [12]. The prevalence of acquired forms is unknown, but its presence has been described in various pathological conditions including diabetes mellitus, Waldenstrom's macroglobulinemia, multiple myeloma (MM), sarcoidosis, Alzheimer's disease, pancreatic pseudocyst, nephrotic syndrome, chronic kidney disease, and also in patients receiving high doses of penicillin [1,13-16].

The genetic variants of human serum ALB have been studied to define their molecular defects and to correlate them to the functional properties and stability of the molecule. Most of the ALB variants have no proven pathological consequences, but it should be noted that some ALB mutated variants have been shown to have decreased binding to warfarin, bilirubin, Ni2+, orCu2+, or increased binding to prostaglandins, thyroid hormones, or fatty acids [17].

Although these findings cannot be directly detected by SPE, if Bisalbuminemia is identified, the alteration in ALB binding properties can be explored and may provide insight into the pathophysiology of diseases associated with abnormal binding of these ligands to ALB [17]. It is still unclear that whether these variants represent disease state or not, which makes it an area of interest for investigations and also provide area of research to explore and understand the phenomena with altered lipid, hormones and ions level in patients with other diseased conditions [2,9].

In most of the published data, bisalbuminemia is not found to be associated with monoclonal gammopathies but can cause difficulty in SPE reporting in various diseased conditions such as MM and plasma cell dyscrasia [2,18]. It may interfere with the diagnosis of such conditions and can be misinterpreted as an abnormal globulin peak [2]. Hence, it is very important to acknowledge its presence during serum SPE reporting.

The detection of hereditary-type alloalbumins may represent an innovative tool to track millennial human migration and can provide data on protein evolution and the molecular structure and characteristics of the ALB molecule.[17]

Plenty of individual case reports are available in the literature providing information about a single or few aspects details of the reported

cases from 1993 to 2023 with their unique clinical findings has been mentioned in Table 2. We found four review articles covering molecular and epidemiological aspects of bisalbuminemia [10,17,19] but there is a scarcity of detailed scientific scripts which provides collective information about this condition.

In the present review article, an attempt has been made to join the different dots of structure, genetic variation, clinical, pathophysiological, and practical aspects of bisalbuminemia to construct a frame to convey the most relevant and necessary information available yet at one platform. This information shall help clinicians, laboratory personnel, immunopathologists, and researchers in better interpretation of SEP reports while seeing suspected or confirmed cases of monoclonal gammopathies and a better understanding of the nature of other diseases related to atypical binding of various hormones, drugs, and metals with mutated ALB variants.

## METHODS

The PubMed database was searched on March 18, 2024, at 1:00 pm. The search terms used in PubMed were (bisalbuminemia) OR (alloalbuminemia) for duration: 2001 to till date (2024). A total of 53 articles were found which included article types such as full text, case reports and series, original articles, abstracts, review articles, and epidemiological studies. Four records were removed due to foreign language. All the records were reviewed for relevance toward the topic. At the end, a total of 56 articles were used for review writing. Some cross references of articles were also used for review writing which may and may not be indexed with PubMed. As bisalbuminemia is a rare phenomenon, some old and relevant articles were also referred.

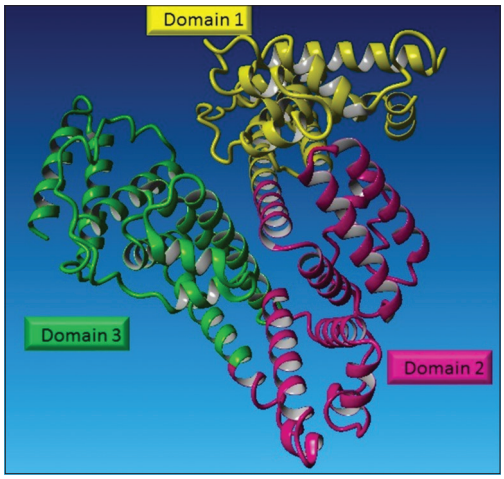


**Fig. 1: Structure of human serum albumin. (Picture source: Raoufinia R, Mota A, Keyhanvar N, Safari F, Shamekhi S, Abdolalizadeh J. Overview of Albumin and Its Purification Methods. Adv Pharm Bull. 2016 Dec;6(4):495-507.) [22]**

### ALB-Know me better

ALB is the vital and major constituent of human plasma, synthesized in the liver and constantly released in an amount of 14 g/day in healthy adult subjects. It has a half-life of approximately 19 days [17,20]. Besides being essential, ALB constitutes 60–65% of total plasma proteins. The normal concentration of ALB is 3.5–5 g/dL in healthy adults and 2.9–5.5 g/dL in children [21,22]. The major function is to maintain oncotic pressure along with the transportation of several endogenous and exogenous molecules. It acts as an antioxidant by binding to the major causative free radical produced, thus protecting the body from oxidative stress-mediated damage [2,3]. Human ALB also has interesting enzymatic properties including esterase activity, enolase activity, effects on eicosanoids, aryl acylamidase activity, stereospecificity, condensation reactions, and binding and activation of drug conjugates [22,23].

Serum ALB is a traditional biomarker for liver function and also for several diseases, such as inflammatory disorders, brain tumors, rheumatoid arthritis, myocardial ischemia, cancer, blood–brain barrier damage, kidney disease, cerebrovascular disease, and cardiovascular risk disease [22,24-28].

### Genetic roots and ALB sequencing

Human serum ALB is a single-chain polypeptide (molecular weight 66.5 kDa) with 585 amino acids and is secreted into plasma in non-glycosylated form [17,20]. The X-ray crystallographic studies revealed that ALB has 67%-helix content and no-sheet secondary structure,

**Table 1: Summary of acquired and inherited bisalbuminemia**

| Features | Inherited Bisalbuminemia | Acquired Bisalbuminemia |
|---|---|---|
| Cause | Genetic variation | Underlying medical condition |
| Inheritance | Autosomal dominant | Transient |
| Electrophoretic mobility | Slow or fast | Usually, faster |
| Clinical significance | Usually, benign | This may indicate underlying disease |

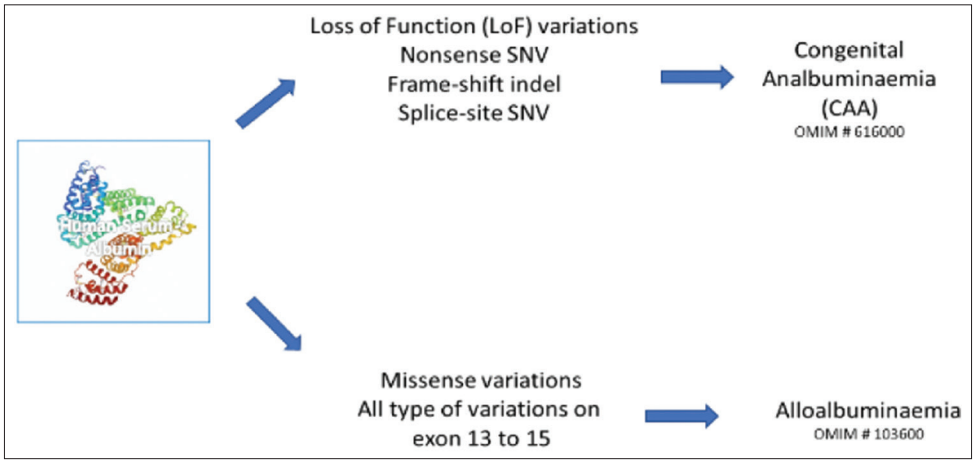


**Fig. 2: Effects of variants on albumin gene and their clinical consequences. (Source: Caridi G, Lugani F, Angeletti A, Campagnoli M, Galliano M, Minchiotti L. Variations in the Human Serum Albumin Gene: Molecular and Functional Aspects. Int J Mol Sci. 2022 Jan 21;23(3):1159.) [17]**

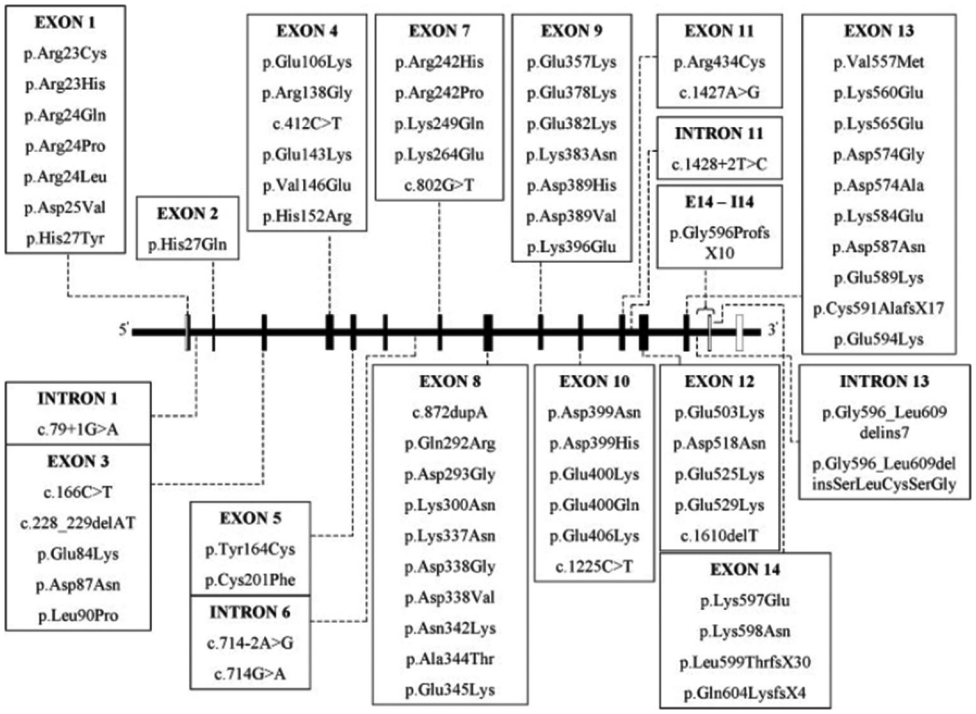


**Fig. 3: Distribution of mutations associated with bisalbuminemia and analbuminemia. (Picture source: Minchiotti L, Galliano M, Kragh-Hansen U, Peters T Jr. Mutations and polymorphisms of the gene of the major human blood protein, serum albumin. Hum Mutat. 2008 Aug;29(8):1007-16.) [4]**

folded into a heart-shaped molecule comprising three homologous domains (I–III) (Figure 1) [17,29]. The structure includes domains I in residues 1–195, domain II in residues 196–383, and domain III in residues 384–585. Each domain is composed of two subdomains (A and B), with different helical folding patterns linked by flexible loops [22,30].

**ALB gene: Mutation and polymorphism**

ALB belongs to the ALB superfamily, including others such as alpha-fetoprotein (AFP), Vitamin D-binding protein (Gc-globulin), and afamin. The single-copy genes of ALB are localized in chromosome 4 at position 4q13.3, near the centromere. The ALB gene (ALB; NCBI Genomic Sequence: NC_00004.12) consists of 15 exons, the last of which is untranslated, and 14 intervening introns that span 16,961 nucleotides from the first poly(A) addition site to the putative "cap" site [17,23,31].

The ALB gene has a notable level of DNA variation. The single nucleotide polymorphisms (SNP) data bank at NCBI currently contains reports on 91 SNPs that have no known clinical or biological impact [4]. Seventy-seven other mutations causing the presence of two circulating forms of the protein (bisalbuminemia or alloalbuminemia) or the virtual absence of the protein from the blood (analbuminemia) have been documented (Figures 2 and 3). Out of 77 mutations, 65 lead to bisalbuminemia [4].

Among the mutations, the vast majority reflect single-base changes in the structural gene, and the recurring alloalbumins are associated with mutations in hypermutable CpG dinucleotides. Because a single copy gene that is co-dominantly expressed controls protein synthesis, heterozygous patients with point mutations often have a 1:1 ratio between the normal and variant proteins. The variant with the widest geographical distribution is reported to be ALB Coari-1 (p. Glu382Lys), found broadly in Brazil, India, the United Kingdom, and Canada. ALB Oliphant, or B (p. Glu594Lys) is the form most often reported in Caucasians in India, Japan, Cambodia, and six or more European nationalities [4,32,33].

Allo-albuminemia is characterized by 74 different mutations, resulting in 71 distinct genetic variants of ALB and pro-ALB. All the 14 translated exons, as well as all the regions encompassing the five ends of exon 14 and the three ends of the following intron, are involved in determining the 74 ALB variations [17,34]. On the other hand, most of the amino acid changes are clustered in three regions of the protein. Out of these, one is located in the propeptide region and two in the amino terminus (residues 313–382 corresponding approximately to subdomain IIB in the crystal structure and residues 501–575 of the mature protein corresponding to subdomain IIIB) [17,34,35]. This distribution suggests that the frequency of allo-ALBs is probably not related to the variation sites in the gene but rather to the position of the mutated residue in the protein. ALB variants are not associated with any disease [17]. Genetic variants with known mutations can also provide valuable molecular information about ALB binding sites, antioxidant, and enzymatic properties, as well as stability. A better understanding of ALB binding sites, with the innovative aim of genetic manipulation, may be useful in different clinical contexts such as in regulating the clearance of fatty acids, through mutation at position 218 or for the presence of additional SH groups and high-affinity mutants may have an application in the emergency serological accumulation of endogenous substances, such as drug overdose; in dialysis, which is an extracorporeal treatment for patients with end-stage renal disease [17,36].

**Hereditary versus acquired bisalbuminemia**

The hereditary bisalbuminemia is permanent and reflects the coexistence of two types of ALBs, following the autosomal dominant trait [3]. One can demonstrate either slow (with less negative net charge) or fast (with more negative net charge) electrophoretic mobility [3]. Inherited forms may show single point mutation such as arginyl-ALB resulting from the substitution of arginine in place of the normal amino-terminal aspartic acid moiety or chain-termination mutations, for example, mutation in the amino-terminal Arg-Arg pro-peptide sequence required for post-transcriptional processing of ALB [3]. Bisalbuminemia is not a product of any disease but a chance finding which could be attributed to possibly hereditary type [37]. In terms of morbidity, very rare consequences are observed but some variants may have a different affinity from that of ALB to normal

**Table 2: Summary of cases of bisalbuminemia reported from 1999 to 2023 with their highlights**

| S. No | Year of Reporting | Authors | No. of cases reported | Findings |
|---|---|---|---|---|
| 1 | 2023 | Bagade and Anjum [2] | 02 | • Two cases were reported out of 3360 patients screened for a duration of 5 years<br>• Both the patients were known cases of multiple myeloma on treatment for the same.<br>• No genetic study was performed in our cases to ascertain the genetic causes of bisalbuminemia.<br>• Bisalbuminemia is not associated with monoclonal gammopathies |
| 2 | 2022 | Ogawa *et al*., [45] | 01 | • Reported case of transient bisalbuminemia in a patient with nephrotic syndrome.<br>• Resolved 5 days after initiating glucocorticoid treatment, |
| 3 | 2021 | Kapatia *et al*., [3] | | • A total of 40 cases of bisalbuminemia were detected out of 39,900 patients over 8 years.<br>• It is an overtly benign condition and infrequent in the Indian population although not rare.<br>• It is associated with several clinical disorders such as DM, Waldenstrom macroglobulinemia, multiple myeloma, sarcoidosis, pancreatic pseudocyst, nephrotic syndrome, CKD, and in patients on high doses of penicillin; however, the association seems to be plausibly coincidental. |
| 4 | 2020 | Zoulati *et al*., [43] | 01 | • Reported a case of bisalbuminemia associated with non-steroidal anti-inflammatory drug-induced nephrotic syndrome.<br>• The condition resolved after antiproteinuric treatment with corticosteroids and diuretics was started |
| 5 | 2018 | Agarwal *et al*., [54] | 01 | • Incidental finding.<br>• No other comorbidities were found in the patient.<br>• The diagnostic electrophoresis was not available to provide information about whether the disease was acquired or hereditary. |
| 6 | 2015 | Shetty JK *et al*., [16] | 01 | • Bisalbuminemia may indicate an altered affinity for steroid hormones, thyroxine, or drugs in such a person. |
| 7 | 2013 | Chhabra *et al*. [1] | 03 | • Incidental findings<br>• Two patients were diabetic with chronic inflammation. 3rd patient was of arthralgia.<br>• One case also presented with bisalbuminuria.<br>• Hereditary bisalbuminemia, transmitted as an autosomal codominant character, is a relatively rare genetic disorder |
| 8 | 2012 | Neild *et al*., [55] | 01 | • Incidental finding.<br>• Newly diagnosed case of CKD with acute exacerbation of COPA.<br>• No interference with the course of disease or treatment. |
| 9 | 2010 | Boujelbene *et al*. [47] | 01 | • Reported bisalbuminemia in case of pancreatic tumor.<br>• Pseudobisalbuminemia by interference with AFP in electrophoretic mobility.<br>• Capillary protein electrophoresis enables a reliable and reproducible analysis of serum samples. |
| 10 | 2007 | Shetty and Prakash [16] | 01 | • Reported case of bisalbuminemia with Alzheimer's. |
| 11 | 2004 | Ejaz *et al*., [14] | 01 | • Reported in patients with chronic kidney disease.<br>• Some physiologic or pharmacologic substances may not bind to abnormal albumin variants also they bind to normal albumin and should not be discounted.<br>• The diagnosis, course, or prognosis of chronic renal disease was unaffected by the discovery of bisalbuminemia.<br>• It is unclear how bisalbuminemia affects renal disease. |
| 12 | 1999 | Hoang *et al*., [46] | 01 | • Reported bisalbuminemia with bisalbuminuria |
| 13 | 1993 | Winter *et al*., [56] | 01 | • Reported a case of acquired bisalbuminemia in staphylococcal endocarditis treated with dicloxacillin |

hormones, metal ions such as Zn, fatty acids, or hydrophobic anionic drugs [3].

The effects of mutations on ligand-binding by variants are generally small and probably not clinically significant. Kim *et al*., 2010, analyzed the clinical manifestations, genetic variations, and ALB-binding characteristics in two Korean patients with bisalbuminemia. A genetic variant known as Nagasaki-1 (Asp293Gly) was observed in one case, whereas a hitherto unknown missense mutation (c.593A>T; Lys198Ile) was observed in the other. Both times, the ALB mutation sites were not found at the protein-binding loci; hence, the ALB binding affinity and serum concentrations were unaffected [38].

Exceptions are three mutations (p. Leu90Pro; p. Arg242His; and p. Arg242Pro), which form strong binding sites for triiodothyronine (T3) Orthyroxine (T4). The former variant gives rise to the syndrome familial dysalbuminemic hypertriiodothyroninemia, and the presence of one of the two latter results in familial dysalbuminemic hyperthyroxinemia. The latter syndrome is the most common cause of inherited euthyroid hyperthyroxinemia in Caucasian populations [39,40].

These mutations would not be expected to be detected upon electrophoresis and were discovered by the large increase in total T3 or T4 found in serum, a source of confusion to endocrinologists until the variants were recognized [14,17]. This altered affinity (due to mutation) of ALB for thyroxine (T4), combined with assay interference as an artifact, can result in falsely elevated T4 levels. This misinterpretation may lead to misdiagnosis and potential overtreatment. The only potential clinical concern is confusion with hyperthyroidism or thyroid hormone resistance syndromes, which might lead to unnecessary

treatment [19,39,41]. Another example is conjugation of growth hormone with ALB variant was linked to enhanced pharmacokinetics of growth hormone therapy [19,42]. Thus, a deeper comprehension of ALB variations may result in the creation of possibly novel therapeutic strategies [19].

The acquired form of bisalbuminemia is usually transient and results from structural changes in some of the circulating ALB either by subtraction or by addition of material. With the exception of that linked to a pancreatic pseudocyst, it typically has little pathogenic relevance. Regardless of the cause, hyperproteinemia or hyperalbuminemia is not caused by the presence of bisalbuminemia. It generally shows more anodic/faster mobility toward the anode as compared with normal ALB. This form displays the presence of ALB dimers an extra band in the immediate post-ALB region signifying 10 to 15% of the total ALB fraction [3,14].

The appearance of two peaks in antibiotic therapy is explained by the binding of antibiotics to a part of the ALB, and the opening of the beta-lactam ring is followed by the bond between the carbamyl group of this ring and the amino group of a lysine of the ALB. Because the modified ALB migrates differently from regular ALB, it results in a second peak on electrophoresis. This peak is especially noticeable when the antibiotic dose is  The summary of hereditory and acquired Bisalbuminemia and is mention in Table 1 [43,44].

Ogawa *et al*., 2022, observed transient bisalbuminemia in a 51-year-old newly diagnosed nephrotic syndrome patient on capillary SPE which resolved 5 days after initiating glucocorticoid treatment, although massive proteinuria and severe hypoalbuminemia remained [45].

Zoulati *et al*., 2020, also reported a case of bisalbuminemia associated with nonsteroidal anti-inflammatory drug-induced nephrotic syndrome in a 49-year-old woman which also resolved after antiproteinuric treatment with corticosteroids and diuretics was started [43].

Monoclonal gammopathies including myelomas may be randomly seen with bisalbuminemia [2]. Chan *et al*. found that bisalbuminemia is a rare incidental finding in monoclonal gammopathies but it is not associated with the same [18].

Waldenström macroglobulinemia, along with MM and monoclonal gammopathy of uncertain significance, is classified as a plasma cell dyscrasia. It is considered a low-grade non-Hodgkin's lymphoma (specifically, lymphoplasmacytic lymphoma) that leads to the secretion of IgM into the blood [39].

In these conditions, bisalbuminemia can result due to the binding of monoclonal immunoglobulin to ALB in subjects with myeloma, giving it slower electrophoretic properties in agarose. The most common kind of Ig that can readily form a complex with ALB is IgA; IgM is less common [43].

Fast Bis-ALB variants have been observed after intravenous administration of large amounts of penicillin, due to the binding of this drug to ALB, and they disappeared after the cessation of penicillin therapy [46].

In the case of pancreatic pseudocyst, protein lysis by pancreatic enzymes would be responsible for the double peak. In fact, acquired bisalbuminemia can appear after the rupture of a pancreatic cyst in a serous cavity, releasing pancreatic enzymes which will digest part of the ALB. Chymotrypsin and carboxypeptidase partially hydrolyze natural ALB, resulting in a modified ALB. On electrophoresis, the digested and undigested ALB fractions move differently, forming two separate peaks [14]. Pseudobisalbuminemia has been reported due to interference with AFP related to a pancreatic tumor [47]. Incidences of bisalbuminemia with diabetes and Alzheimer's have been reported but the exact cause is not known in both diseases [16].

The acquired form should arouse suspicion and make the clinician think if there is any underlying disease process going on which is not been diagnosed yet. Clinicians and laboratory physicians must keep this entity on their radar and interpret it accordingly with caution [3].

Congenital analbuminemia is very rare with the incidence of an autosomal recessive disorder characterized by the absence or a very low level, of ALB in serum, caused by variations in the ALB [17]. It has a prevalence of less than 1:1,000,000 [17,20]. According to a study by Ruiz *et al*. and Sunthornthepvarakul *et al*., 12 mutations have been identified that lead to a rare condition-analbuminemia, in which patients have serum ALB concentrations <0.1 g/dL (Figure 3). Given the fundamental roles of ALB in maintaining oncotic pressure and transporting lipophilic compounds, this was surprising that the disease results only in mild edema, mild fatigability, and hyperlipidemia [48,49]. In many cases, the disease is entirely asymptomatic [17].

**Identifying the doublet: Diagnosis of bisalbuminemia**

Serum protein electrophoresis (SPE) is an investigation performed routinely for the screening of monoclonal gammopathies using various techniques such as AGE, cellulose acetate electrophoresis, and CZE. [2] Electrophoresis pattern normally reveals ALB, the largest peak followed by the next five components of globulins labeled as alpha1, alpha2, beta1, beta2, and gamma (Fig. 4). The main emphasis of the interpretation of SPE is on the subsets of these proteins and their relative abundance. ALB usually appears as a tall, single, discrete peak on electrophoretogram, very rarely, it appears as two peaks in the ALB region, which could be completely distinct peaks or partial splitting of the ALB peak (Figs. 5a and b) [2,50].

CZE is the method of choice compared to AGE, giving more discrete bands due to its higher resolution [37]. AGE will not always produce a clear separation of ALB fractions, and sometimes, it may need an increased migration time and buffer pH [12,37,51]. Yang *et al*., 2016, observed bisalbuminemia accompanying bis-albuminuria detected in CZE but not in gel electrophoresis [52].

The difference in resolution is mainly due to the different analytical methods. In AGE, proteins migrate toward the anode in a solid phase and an alkaline buffer with low voltage whereas in CZE, proteins rapidly move in a liquid phase toward the cathode thanks to the high voltage applied. This allows a better separation of proteins with similar physicochemical characteristics, thus generating multiple narrower peaks [37,53].

With AGE, an ALB peak is apparently wider than normal, demonstrating as the visual interpretation of AGE profiles could lead to both false-negative and false-positive detection of bisalbuminemia [37]. CZE has

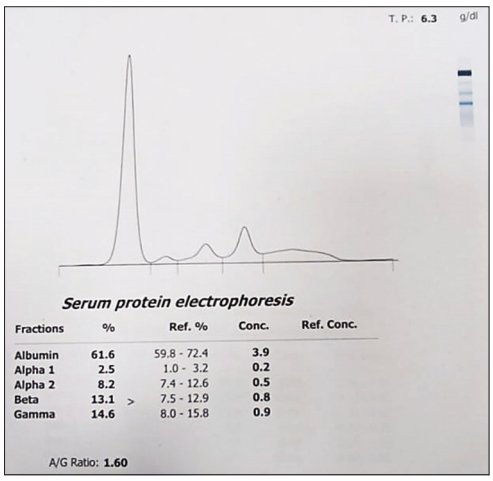


**Fig. 4: Normal serum electrophoresis (capillary method). (Picture source: Normal serum electrophoresis processed on Sebia Minicap in a global reference laboratory, Mumbai)**

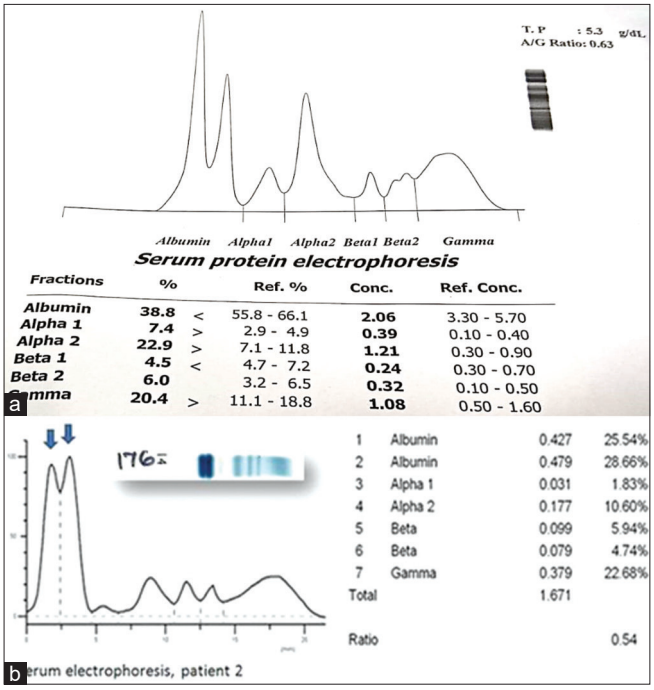


**Fig. 5: (a) Electrophoretogram suggesting bisalbuminemia. (Picture source: Bisalbuminemia on serum electrophoresis processed on Sebia Minicap in a global reference laboratory, Mumbai). (b) Electrophoretogram suggesting bisalbuminemia [1]. (Picture source: Chhabra S, Bansal F, Saikia B, Minz RW. Bisalbuminemia: A rarely encountered protein anomaly. J Lab Physicians. 2013 Jul;5(2):145-6 [1]**

completely replaced classical AGE by being the most sensitive method. The application of higher resolution techniques, such as CZE, can result in an increased number of abnormal profiles and open a gateway for a deeper knowledge of the clinical importance of these new profiles [37].

**Future directions: Beyond the double band**

Research on bisalbuminemia continues to explore the functional consequences of various ALB mutations. Genetic mutants with altered affinity for various ligands may hold therapeutic relevance. In addition, investigating the potential association between specific variants and certain diseases might hold future clinical relevance.

## CONCLUSION

Bisalbuminemia, though an extremely rare entity encountered on SPE, offers a unique window into ALB variations. Understanding the underlying genetics through ALB sequencing and the distinction between inherited and acquired forms is crucial for proper diagnosis and clinical evaluation. While typically benign, bisalbuminemia highlights the intricate relationship between gene sequence, protein structure, and potential health implications. Clinicians and laboratory physicians must keep their sensors active about this entity for thoughtfulness interpretation of SPE.

## AUTHORS CONTRIBUTION

Conceptualization, designing of the article, and data collection were done by Dr.Sneha Wadalkar. Data analysis and interpretation were done by Dr.Shalini Maksane. The drafting of the article was done by Dr. Sneha and Dr. Shalini. Critical reviewing and final approval were done by Dr. Kavita More and Dr.Kshama Pimpalgoankar. The manuscript has been read and approved by all the authors and the requirements for authorship as stated above have been met. Each author believes that the manuscript represents honest work.

## CONFLICTS OF INTERESTS

None.

## FUNDING

Self.

## REFERENCES

1. Chhabra S, Bansal F, Saikia B, Minz RW. Bisalbuminemia: A rarely encountered protein anomaly. J Lab Physicians. 2013 Jul;5(2):145-6. doi: 10.4103/0974-2727.119869, PMID: 24701116
2. Bagade S, Anjum A. Bisalbuminemia: A rare incidental finding in monoclonal gammopathy. EJIFCC. 2023 Dec 21;34(4):325-9. PMID: 38303749
3. Kapatia G, Wadhwa M, Malhotra P, Prakash G, Aggarwal R. Bisalbuminemia: A pathologist's insight of an uncommon phenomenon. J Lab Physicians. 2021 Sep;13(3):219-23. doi: 10.1055/s-0041-1730878, PMID: 34602785
4. Minchiotti L, Galliano M, Kragh-Hansen U, Peters T Jr. Mutations and polymorphisms of the gene of the major human blood protein, serum albumin. Hum Mutat. 2008 Aug;29(8):1007-16. doi: 10.1002/humu.20754, PMID: 18459107
5. Knedel M. Die Double albuminemia: A new hereditary protein anomaly. Blood. 1957;3(3):129-34. doi: 10.1007/BF01630687, PMID: 13446004
6. Nennstiel HJ, Becht T. On the Hereditary Occurrence of Albumin Fragmentation in the Electrophoresis Diagram. Clinical Weekly. 1957;35(13):689. doi: 10.1007/BF01481274, PMID: 13515039
7. Earle DP, Hutt MP, Schmid K, Gitlin D. A unique human serum albumin transmitted genetically. Trans Assoc Am Physicians. 1958;71:69-76. PMID: 13603504
8. Wuhrmann F. Albumin double peaks as an inherited blood protein anomaly. Swiss Medical Weekly. 1959;89(6):150-2. PMID: 13634937
9. Adioui T, Igorman H, Radouane I, Ouahid S, Berrag S, Tamzaourte M. A rare bisalbuminemia etiology: Cholestatic hepatitis case report. Asian J Res Rep Gastroenterol. 2023;7:1-5.
10. Faviou E, Nounopoulos C, Dionyssiou-Asteriou A. Bisalbuminemia from a clinical chemist's viewpoint: A case report and review of the recent literature. Minerva Med. 2006 Jun;97(3):287-93. PMID: 16855523
11. Kobayashi S, Okamura N, Kamoi K, Sugita O. Bisalbumin (fast and slow type) induced by human pancreatic juice. Ann Clin Biochem. 1995;32(1):63-7. doi: 10.1177/000456329503200105, PMID: 7762952
12. Angouridaki C, Papageorgiou V, Tsavdaridou V, Giannousis M, Alexiou-Daniel S. Detection of hereditary bisalbuminemia in a Greek family by capillary zone electrophoresis. Hippokratia. 2008 Apr;12(2):119-21. PMID: 18923658
13. Simundić AM, Miler M, Nikolac N, Topić E, Čaržavec D, Milanović B, *et al*. Bisalbuminemia in a male croatian patient with sarcoidosis. Biochem Med. 2009;19:95-100. doi: 10.11613/BM.2009.010
14. Ejaz AA, Krishna M, Wasiluk A, Knight JD. Bisalbuminemia in chronic kidney disease. Clin Exp Nephrol. 2004 Sep;8(3):270-3. doi: 10.1007/s10157-004-0291-1, PMID: 15480907
15. Kalambokis G, Kitsanou M, Kalogera C, Kolios G, Seferiadis K, Tsianos E. Inherited bisalbuminemia with benign monoclonal gammopathy detected by capillary but not agarose gel electrophoresis. Clin Chem. 2002;48(11):2076-7. doi: 10.1093/clinchem/48.11.2076, PMID: 12407005
16. Shetty JK, Prakash M, Gopalakrishna K. Bisalbuminemia in an adult male with Alzheimer's disease. Indian J Med Sci. 2007 July;61(6):356-7.
17. Caridi G, Lugani F, Angeletti A, Campagnoli M, Galliano M, Minchiotti L. Variations in the human serum albumin gene: Molecular and functional aspects. Int J Mol Sci. 2022 Jan 21;23(3):1159. doi: 10.3390/ijms23031159, PMID: 35163085
18. Chan PC, Yasodhara A, Truong D. Bisalbuminemia: Lack of association with monoclonal gammopathy and value-justification for reporting in serum protein electrophoresis. J Clin Chem Lab Med. 2017 Dec;1(1):108.
19. Wu JR, Lin M, Lin F, Zhan XF, Wang JL, Yang H, *et al*. Human serum albumin variants in China: A molecular epidemiological investigation and literature review. J Int Med Res. 2021 Dec;49(12):1-11. doi: 10.1177/03000605211064225, PMID: 34919010
20. Peters T. All about Albumin: Biochemistry, Genetics, and Medical Applications. San Diego, CA, USA: Academic Press; 1996.
21. Farrugia A. Albumin usage in clinical medicine: Tradition or therapeutic? Transfus Med Rev. 2010;24(1):53-63. doi: 10.1016/j.

tmrv.2009.09.005, PMID: 19962575
22. Raoufinia R, Mota A, Keyhanvar N, Safari F, Shamekhi S, Abdolalizadeh J. Overview of albumin and its purification methods. Adv Pharm Bull. 2016 Dec;6(4):495-507. doi: 10.15171/apb.2016.063, PMID: 28101456
23. Kragh-Hansen U, Minchiotti L, Galliano M, Peters T Jr. Human serum albumin isoforms: Genetic and molecular aspects and functional consequences. Biochim Biophys Acta. 2013;1830(12):5405-17. doi: 10.1016/j.bbagen.2013.03.026, PMID: 23558059
24. Gupta D, Lis CG. Pretreatment serum albumin as a predictor of cancer survival: A systematic review of the epidemiological literature. Nutr J. 2010;9:69. doi: 10.1186/1475-2891-9-69, PMID: 21176210
25. Apple FS, Wu AH, Mair J, Ravkilde J, Panteghini M, Tate J, *et al*. Future biomarkers for detection of ischemia and risk stratification in acute coronary syndrome. Clin Chem. 2005;51(5):810-24. doi: 10.1373/clinchem.2004.046292, PMID: 15774573
26. Tibbling G, Link H, Ohman S. Principles of albumin and igg analyses in neurological disorders. I. Establishment of reference values. Scand J Clin Lab Investig. 1977;37(5):385-90. doi: 10.1080/00365517709091496, PMID: 337459
27. Fouque D, Kalantar-Zadeh K, Kopple J, Cano N, Chauveau P, Cuppari L, *et al*. A proposed nomenclature and diagnostic criteria for protein-energy wasting in acute and chronic kidney disease. Kidney Int. 2008;73(4):391-8. doi: 10.1038/sj.ki.5002585, PMID: 18094682
28. Blennow K, Wallin A, Fredman P, Karlsson I, Gottfries CG, Svennerholm L. Blood-brain barrier disturbance in patients with Alzheimer's disease is related to vascular factors. Acta Neurol Scand. 1990;81(4):323-6. doi: 10.1111/j.1600-0404.1990.tb01563.x, PMID: 2360400
29. Olivieri JR, Craievich AF. The subdomain structure of human serum albumin in solution under different pH conditions studied by small angle X-ray scattering. Eur Biophys J. 1995;24(2):77-84. doi: 10.1007/BF00211402, PMID: 8582321
30. Fanali G, di Masi A, Trezza V, Marino M, Fasano M, Ascenzi P. Human serum albumin: From bench to bedside. Mol Aspects Med. 2012;33(3):209-90. doi: 10.1016/j.mam.2011.12.002, PMID: 22230555
31. Minghetti PP, Ruffner DE, Kuang WJ, Dennison OE, Hawkins JW, Beattie WG, *et al*. Molecular structure of the human albumin gene is revealed by nucleotide sequence within q11-22 of chromosome 4. J Biol Chem. 1986;261(15):6747-57. doi: 10.1016/S0021-9258(19)62680-3, PMID: 3009475
32. Brennan SO, Arai K, Madison J, Laurell CB, Galliano M, Watkins S, *et al*. Hypermutability of CpG dinucleotides in the propeptide-encoding sequence of the human albumin gene. Proc Natl Acad Sci U S A. 1990a;87(10):3909-13. doi: 10.1073/pnas.87.10.3909, PMID: 2339130
33. Galliano M, Kragh-Hansen U, Taárnoky AL, Chapman JC, Campagnoli M, Minchiotti L. Genetic variants showing apparent hot-spots in the human serum albumin gene. Clin Chim Acta. 1999;289(1-2):45-55. doi: 10.1016/s0009-8981(99)00166-7, PMID: 10556652
34. Kragh-Hansen U, Chuang VT, Otagiri M. Practical aspects of the ligand-binding and enzymatic properties of human serum albumin. Biol Pharm Bull. 2002;25(6):695-704. doi: 10.1248/bpb.25.695, PMID: 12081132
35. Ghuman J, Zunszain PA, Petitpas I, Bhattacharya AA, Otagiri M, Curry S. Structural basis of the drug-binding specificity of human serum albumin. J Mol Biol. 2005;353(1):38-52. doi: 10.1016/j.jmb.2005.07.075, PMID: 16169013
36. Mitzner SR. Extracorporeal liver support-albumin dialysis with the Molecular Adsorbent Recirculating System (MARS). Ann Hepatol. 2011;10(Suppl 1):S21-8. doi: 10.1016/S1665-2681(19)31602-3, PMID: 21566251
37. Gili C, Bonsembiante F, Bonanni R, Giordano A, Ledda S, Beffagna G, *et al*. Detection of hereditary bisalbuminemia in bottlenose dolphins (*Tursiops truncatus*, Montagu 1821): Comparison between capillary zone and agarose gel electrophoresis. BMC Vet Res. 2016 Aug 20;12(1):172. doi: 10.1186/s12917-016-0801-x, PMID: 27544582
38. Kim YH, Lee YW, Jeon BR, Lee YK, Shin HB, Kang DH, *et al*. Clinical characteristics and ALB gene mutation analysis of Korean patients with bisalbuminemia. Korean J Lab Med. 2010 Jun;30(3):307-11. doi: 10.3343/kjlm.2010.30.3.307, PMID: 20603593
39. Valasek MA, Hopley RT, Wians FH Jr. Resolving the baneful and banal: Bisalbuminemia in an adult with waldenström macroglobulinemia. Lab Med. 2008 Dec;39(12):723-26. doi: 10.1309/LMD51RR5FXHKDHRX
40. Kragh-Hansen U, Galliano M, Minchiotti L. Clinical, genetic, and protein structural aspects of familial dysalbuminemic hyperthyroxinemia and hypertriiodothyroninemia. Front Endocrinol (Lausanne). 2017;8:297. doi: 10.3389/fendo.2017.00297, PMID: 29163366
41. Heufelder AE, Klee GG, Wynne AG, Gharib H. Familial dysalbuminemic hyperthyroxinemia: Cumulative experience in 29 consecutive patients. Endocr Pract. 1995;1(1):4-8. doi: 10.4158/EP.1.1.4, PMID: 15251607
42. Dabboubi R, Amri Y, Sahli C, Fredj SH, Essaddam L, Zoghlami A, *et al*. Inherited bisalbuminemia with growth hormone deficiency. Clin Chem Lab Med. 2019;57(9):e226-9. doi: 10.1515/cclm-2018-1133, PMID: 30763259
43. Zoulati G, El Boukhrissi F, Maïga RY, Bamou Y, El Baaj M, Balouch H. A case of bisalbuminemia associated with nonsteroidal anti-inflammatory drug-induced nephrotic syndrome. Adv Biochem Biotechnol. 2020 Dec 11;5(4):10104.
44. Tance Y, Deneuville T, Tiev KP, Tolédano C, Gain M, Josselin-Mahr L, *et al*. A transient peak in serum protein electrophoresis. Rev Med Interne. 2009;30:436-37.
45. Ogawa M, Toda N, Kurahashi S, Fujita K, Tanigaki K, Hirashima H, *et al*. Bisalbuminemia in nephrotic syndrome. Kidney Int. 2022 Oct;102(4):943. doi: 10.1016/j.kint.2022.04.039, PMID: 36150771
46. Hoang MP, Baskin LB, Wians FH Jr. Bisalbuminuria in an adult with bisalbuminemia and nephrotic syndrome. Clin Chim Acta. 1999 Jun 15;284(1):101-7. doi: 10.1016/s0009-8981(99)00054-6, PMID: 10437648
47. Boujelbene A, Hellara I, Neffati F, Najjar MF. False bisalbuminemia and hyperalphafetoproteinemia. Gastroenterol Clin Biol. 2010 Nov;34(11):636-8. doi: 10.1016/j.gcb.2010.08.006, PMID: 20832217
48. Ruiz M, Rajatanavin R, Young RA, Taylor C, Brown R, Braverman LE, *et al*. Familial dysalbuminemic hyperthyroxinemia: A syndrome that can be confused with thyrotoxicosis. N Engl J Med. 1982 Jan;306(11):635-9. doi: 10.1056/NEJM198203183061103, PMID: 6173750
49. Sunthornthepvarakul T, Likitmaskul S, Ngowngarmratana S, Angsusingha K, Kitvitayasak S, Scherberg NH, *et al*. Familial dysalbuminemic hypertriiodothyroninemia: A new, dominantly inherited albumin defect. J Clin Endocrinol Metab. 1998 May;83(5):1448-54. doi: 10.1210/jcem.83.5.4815, PMID: 9589637
50. McPherson RA, Pincus MR. Henry's Clinical Diagnosis and Management by Laboratory Methods. St. Louis, Missouri: Elsevier; 2017.
51. Dodig S, Cepelak I, Benko B, Raos M, Branovic K. Bisalbuminemia in two croatian families. Arch Med Res. 2000 Nov-Dec;31(6):608-12. doi: 10.1016/s0188-4409(00)00251-4, PMID: 11257330
52. Yang HS, Cho SY, Kim YJ, Park TS, Lee HJ. Bisalbuminemia accompanying bisalbuminuria detected in capillary electrophoresis, not in gel electrophoresis. Clin Chem Lab Med. 2016 Jul 1;54(7):e191-3. doi: 10.1515/cclm-2015-0850, PMID: 26684351
53. Bossuyt X, Lissoir B, Mariën G, Maisin D, Vunckx J, Blanckaert N, *et al*. Automated serum protein electrophoresis by Capillarys. Clin Chem Lab Med. 2003 May;41(5):704-10. doi: 10.1515/CCLM.2003.107, PMID: 12812271
54. Agarwal P, Parkash A, Tejwani N, Mehta A. Bisalbuminemia: A rare finding on serum electrophoresis. Indian J Hematol Blood Transfus. 2018 Jul;34(3):558-9. doi: 10.1007/s12288-017-0911-z, PMID: 30127575
55. Neild GH, Chakraborty S, Sural S, Sen S. Serum electrophoresis with a bifid albumin peak. Clin Kidney J. 2012 Dec;5(6):607. doi: 10.1093/ckj/sfs136, PMID: 26069814
56. Winter V, Rosenvall A, Heikkilä S, von Essen R. Acquired Bisalbuminemia in staphylococcal endocarditis treated with dicloxacillin. Scand J Infect Dis. 1992 Jul;24(6):811-4. doi: 10.3109/00365549209062470, PMID: 1287818