Int J Adv Life Sci Res. Volume 9(3) 93-103
https://doi.org/10.31632/ijalsr.2026.v09i03.008

International Journal of Advancement in Life Sciences Research

Online ISSN: 2581-4877

Journal homepage http://ijalsr.org

Original Article

M-spike Frequency and Serum Fraction Changes in Multiple Myeloma Assessed by Capillary Serum Protein Electrophoresis in and around Chennai, Southern India

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Abstract

Introduction: Multiple myeloma is a clonal malignancy of plasma cells characterized by the proliferation of abnormal plasma cells in the bone marrow and the excessive production of monoclonal immunoglobulin. Incidence increases with age and is slightly more common in males. Serum protein electrophoresis is a crucial diagnostic method for detecting monoclonal proteins and evaluating protein fraction abnormalities in multiple myeloma. Objectives: The present study works to evaluate serum protein electrophoresis (SPE) patterns from patients investigated for multiple myeloma in a clinical laboratory reference setting. **Methods:** Serum protein electrophoresis supports detection of monoclonal proteins and other protein abnormalities relevant to multiple myeloma. The method applies an electric field to separate serum proteins according to electrophoretic mobility. Multiple myeloma represents a B-cell lineage neoplasm and accounts for ~10% of haematological malignancies. Serum proteins separate into albumin and globulin fractions; albumin forms the predominant fraction, while globulin fractions migrate closer to the negative electrode. **Results:** M-spikes were detected in the beta region in 3.80% of electrophoresis reports and in the gamma region in 20.95% of electrophoresis reports. M-spikes were absent in 75.23% of reports. Beta gamma bridging was observed in 7.61% of electrophoresis patterns. **Conclusion:** Accurate SPE interpretation supported identification of clinically relevant abnormal patterns. Higher occurrence was observed among men aged 50 to 70 years. Higher alpha-1 and lower beta-1 fractions were observed in women, while men showed stronger alpha-1 and lower beta-1 fractions.

Keywords: *Haematological Malignancy; Multiple Myeloma; Serum Protein Electrophoresis*

Introduction

Multiple myeloma represents a malignancy of plasma cells. Normal plasma cells reside in bone marrow and contribute to immune defense. Multiple myeloma arises from clonal plasma cells that expand in bone marrow and secrete monoclonal immunoglobulin, frequently accompanied by end-organ damage such as anemia, bone lesions, hypercalcemia, and renal dysfunction. These clinical

Received on :15th February 2026; Revised version received on :6th May 2026; Accepted: 13th May 2026

and pathological features support classification of plasma cell neoplasms and support distinction of active myeloma from precursor states and localized tumors (Fend *et al*., 2023).

Electrophoresis applies an electric field to a fluid containing charged particles and drives migration toward an oppositely charged electrode. Migration depends on mass, shape, and size (Kuhn & Hoffstetter-Kuhn, 2013). Plasma protein concentrations change predictably during acute inflammation, cancer, trauma, necrosis, infarction, burns, and chemical injury.

Monoclonal gammopathy typically produces a discrete spike-like peak in the gamma region (Bailey & Fitzgerald, 2024). The quantity of M-protein, bone marrow biopsy findings, and additional clinical features support differentiation of multiple myeloma from other causes of monoclonal gammopathy. Polyclonal gammopathy may arise during reactive or inflammatory activity (Tripathy, 2012). The present study aims to analyze serum protein electrophoresis patterns generated in a clinical diagnostic laboratory and assess their diagnostic significance in the detection of monoclonal gammopathies, particularly multiple myeloma and related plasma cell disorders. A total of 25 samples were collected from the laboratory and analyzed using serum protein electrophoresis and 105 previously recorded patient datasets were collected from the diagnostic laboratory and analyzed to evaluate the occurrence of myeloma with respect to age distribution and gender.

Materials and Methods

Sample Collection

Twenty-five available serum samples were collected from the laboratory between February and May 2025 and analyzed for serum protein electrophoresis. Previous serum protein electrophoresis data from 105 patients were collected and analyzed. This cross-sectional study contained all sequential serum samples acquired from male and female patients for standard diagnostic analysis during the study period. Serum protein electrophoresis was performed using capillary electrophoresis. No pre-determined inclusion or exclusion criteria were applied, and the dataset represents routine laboratory practice. Samples were transported at 2–8 °C and analyzed within 5 days to maintain integrity prior to analysis using capillary electrophoresis.

Capillary Electrophoresis Platform

Human serum proteins were separated using capillary electrophoresis under alkaline buffer conditions (pH 9.9). Normal serum separation produced six major fractions. Serum preparation was performed using the Capillarys/MiniCap PROTEIN(E) kit, followed by separation into five zones. Automated sequencing generated protein profiles for qualitative and quantitative analysis (Navarro *et al*., 2022).

Instrumentation and Detection

Analysis was performed using the minicap flex piercing system (Sebia). Proteins were separated in silica capillaries and measured at 200 nm. Electropherograms were reviewed visually to detect abnormal patterns, and direct detection enabled quantification of individual protein fractions (Jenkins *et al*., 1997).

Test Principle

Serum protein electrophoresis provides a well-established clinical approach for detecting protein abnormalities. The capillary's protein 6 system enabled automated, high-resolution separation (Landers, 1995). Separation was performed in alkaline buffer (pH 9.9 ± 0.5), and migration reflected electrophoretic mobility and electroosmotic flow (Oda *et al*., 1997). Eight capillaries enabled simultaneous analysis of eight samples. Samples were aspirated at the anodic end, proteins were separated under high voltage, and detection at 200 nm was performed at the cathodic end. Capillaries were cleaned automatically between analyses. The system resolved albumin, α1-globulins, α2-globulins, β1-globulins, β2-globulins, and γ-globulins within defined zones.

Reagents and Materials

The kit supplied buffer, wash solutions, dilution segments, and disposable filters. The buffer maintained alkaline pH and included stabilizing additives. Wash solution (pH ~12 after dilution) was applied after each separation. Disposable filters-maintained buffer quality and reduced contamination risk.

Sample Handling and Acceptance Criteria

The undiluted serum samples were analyzed. Hemolyzed, lipemic, or improperly stored sera were excluded because artifacts can distort fractions, particularly in the β2 and γ zones. Samples were transported at 2–8 °C and analyzed within 5 days or stored frozen for longer preservation. Visual inspection assessed turbidity, hemolysis, and cryoglobulin formation before analysis. Protein fraction quantification was generated automatically, and electropherograms were reviewed for abnormal patterns consistent with monoclonal components. Confirmatory immunofixation was recommended when monoclonal components were suspected (Oda *et al*., 1997; Chartier *et al*., 2011).

Interpretation Support and Confirmation Step

C-reactive protein may migrate in the β2 region, and complement C4 may migrate between β1 and β2 zones (Sjöholm & Laurell, 1973). A relative β2 increase without clinical context for inflammation was treated as a cautionary finding requiring further evaluation (Prisi *et al.,* 2022). Suspected monoclonal components were verified using reduction with beta-mercaptoethanol followed by repeat analysis. Reduction protocol: 1% beta-mercaptoethanol in Fluidil; 100 µl reducing solution added to 300 µl neat serum; vortex; incubate ≤15 minutes; proceed with routine analysis (Chan, 2018).

Interference and Limitations

High concentrations of lipoproteins, triglycerides, or biliary pigments can mimic bis-albumin patterns (Nikolac, 2014). Carry-over after high-concentration monoclonal samples may occur rarely; repeat analysis or additional wash programs were recommended in such situations. Resolution limitations may prevent detection of all monoclonal components, particularly when monoclonal immunoglobulins remain concealed within polyclonal backgrounds. Clinical context and immunotyping remained essential, and agarose gel immunofixation was recommended when uncertainty persisted (Fadili *et al.,* 2025).

Data Analysis

Data analysis was performed using Microsoft Excel to evaluate myeloma based on age, as well as serum protein levels in men and women. Pearson's chi-square analysis was conducted using SPSS software version 16.

Ethical Approval

The ethical approval has been issued by Saveetha Institute of Basic Medical Sciences, India with reference number 024/02/2025/IEC/FSR/SIBMS dated on 24thFebruary 2025.

Results

The graphs below represent the normal report pattern and myeloma pattern respectively**.**

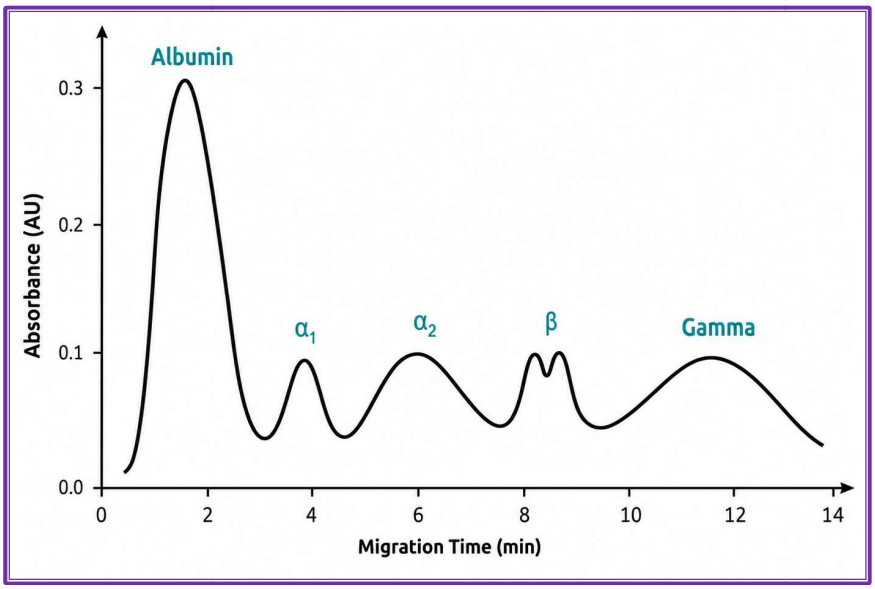

***Figure 1**: Represents The Serum Protein Electrophoresis Pattern of a Normal Patient*

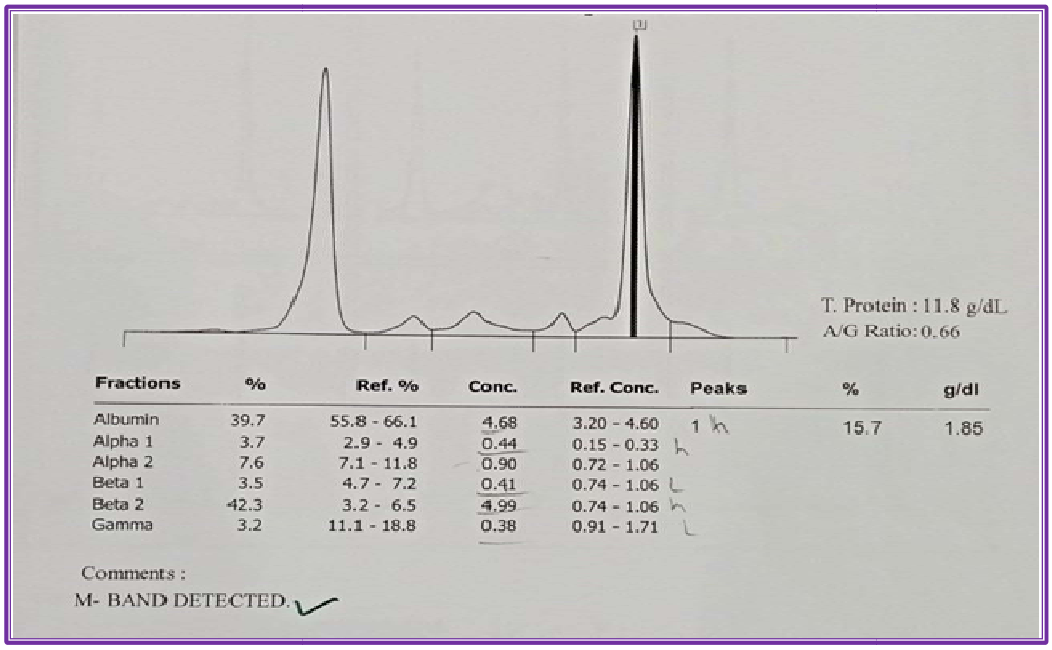

***Figure 2:** Represents the Serum Protein Electrophoresis Pattern with M-Band*

Analysis the Protein Level for Men

The results of total serum protein analysis showed that albumin levels were low in 18.09% of patients. The alpha-1 fraction was low in 1.90% of patients, the alpha-2 fraction was low in 13.33%, the beta-1 fraction was low in 25.71%, the beta-2 fraction was low in 22.85%, and the gamma fraction was low in 11.42% of patients. In contrast, albumin levels were high in 1.90% of patients. The alpha-1 fraction was high in 36.19% of patients, the alpha-2 fraction was high in 15.23%, the beta-1 fraction was high in 2.85%, the beta-2 fraction was high in 8.57%, and the gamma fraction was high in 14.28% of patients (Figure 3).

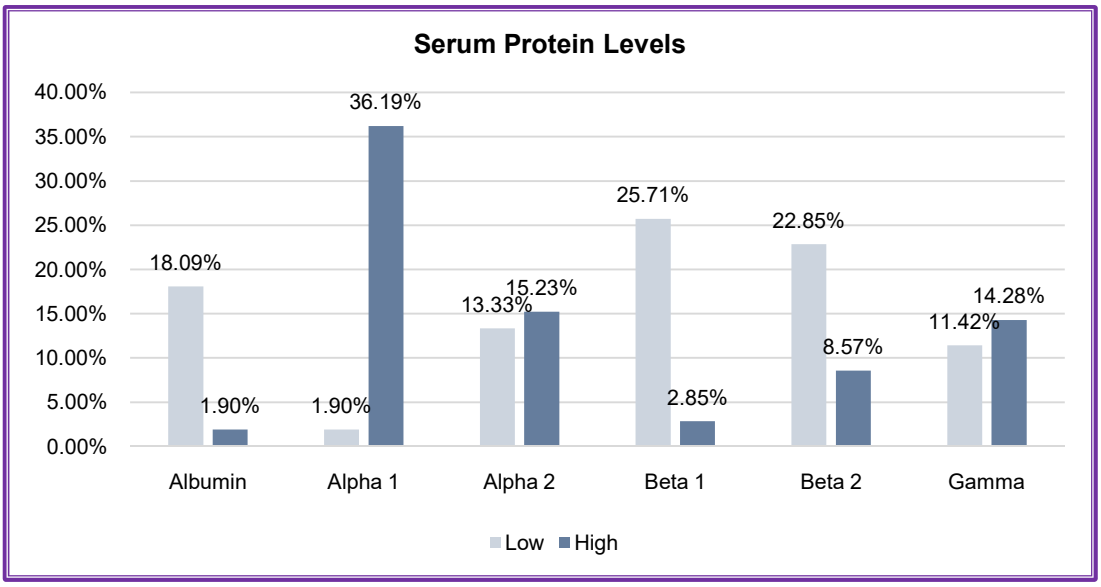

Figure 3: *The Graph Shows That; Most Males Have Low Levels of Beta 1 and High Levels of Alpha 1*

Analysis the Protein Level for Women

The results of total serum protein analysis showed that albumin levels were low in 9.52% of patients. The alpha-1 fraction was low in 0.95% of patients, the alpha-2 fraction was low in 0.90%, the beta-1 fraction was low in 17.14%, the beta-2 fraction was low in 13.33%, and the gamma fraction was low in 3.80% of patients. In contrast, albumin levels were high in 5.71% of patients. The alpha-1 fraction was high in 24.76% of patients, the alpha-2 fraction was high in 13.3%, the beta-1 fraction was high in 0%, the beta-2 fraction was high in 4.76%, and the gamma fraction was high in 11.42% of patients (Figure 4).

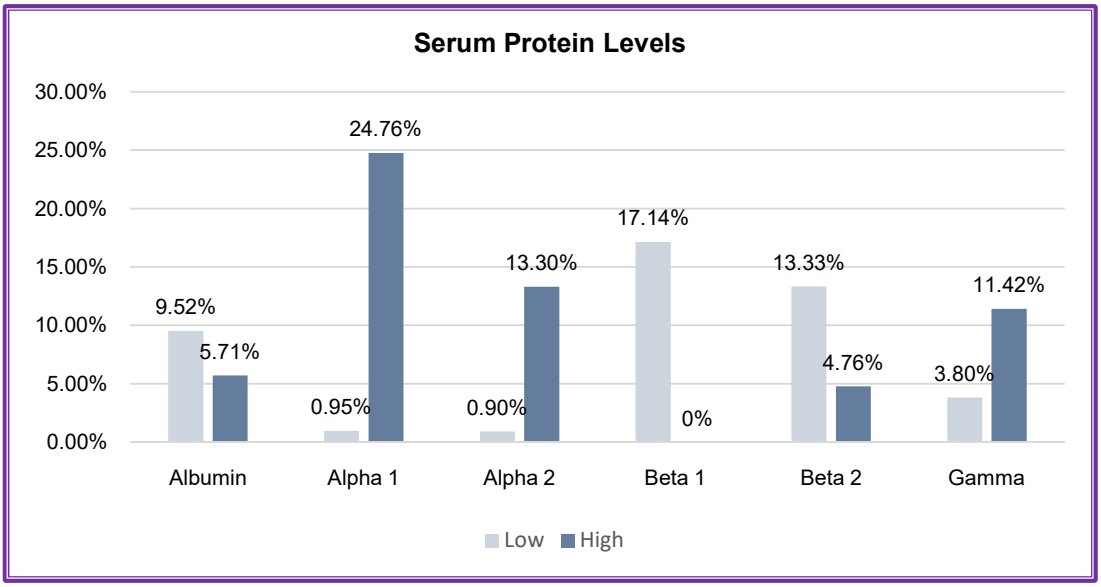

Figure 4: *The Graph Shows That; Most Females Also Have an Excess of Alpha 1 and Deficient of Beta 1*

Age Distribution

Age distribution showed the highest proportion of cases in the 51–60 and 61–70-year groups and age distribution showed the lowest proportion of cases in the 11-to-30-year groups (Figure 5).

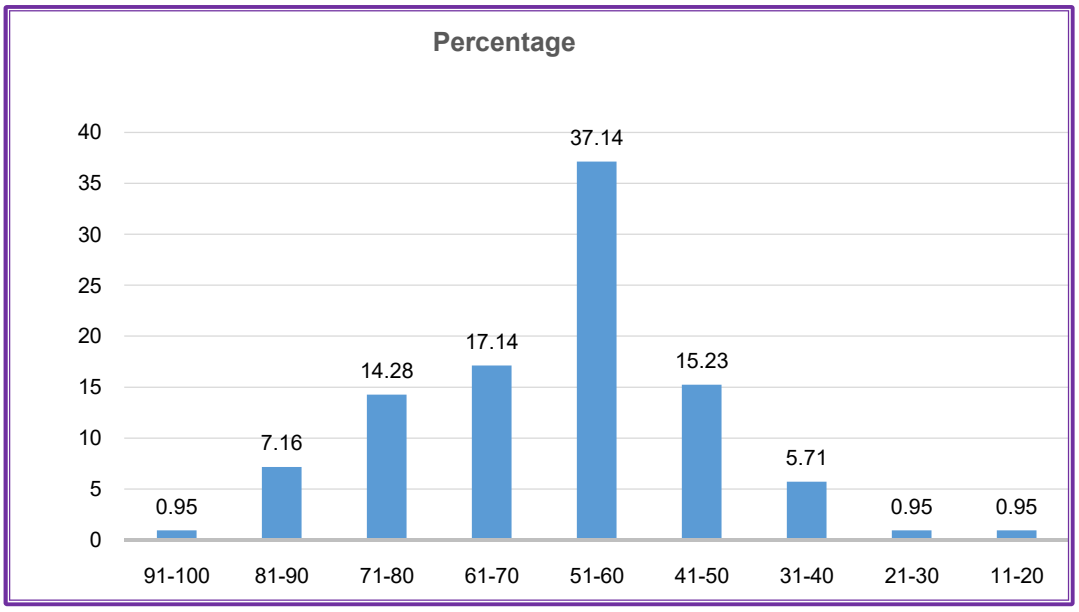

***Figure 5: The** Graph Shows the Age-Based Distribution of Myeloma Cases*

Discussion

Alpha-1-antitrypsin is a type of acute phase reactant. This means that it will be raised in both acute and chronic inflammatory diseases, infections, and some cancer types. Alpha-1-Antitrypsin levels may also rise in response to oral contraception, pregnancy, or stress. In those with mild to moderate Alpha-1-Antitrypsin insufficiency, these brief or chronic increases may make levels appear normal.

A low amount of Beta-1-Globulin in the serum, which is commonly found as part of a blood protein profile, can have a variety of consequences. Beta-1-Globulin is one of the serum protein fractions, and its low levels may signal a variety of underlying medical issues. To determine the specific cause, the larger clinical context must be considered, as well as other diagnostic procedures. Possible causes of a low Beta-1-Globulin level include liver disease, kidney disease, malnutrition, and certain genetic abnormalities that impact protein production. As a result, a healthcare provider will often interpret this result in conjunction with other clinical and laboratory results to make an accurate diagnosis and, if necessary, provide appropriate treatment (Abedi *et al*., 2024).

Alpha-1-antitrypsin is a type of acute phase reactant. This means that it will be raised in both acute and chronic inflammatory diseases, infections, and some cancer types. Alpha-1-Antitrypsin levels may also rise in response to oral contraception, pregnancy, or stress. In those with mild to moderate Alpha-1-Antitrypsin insufficiency, these brief or chronic increases may make levels appear normal.

Changes in plasma protein levels are very predictable in response to burns, acute inflammation, cancer, trauma, necrosis, infarction, and chemical harm. The CRP, alpha1-antitrypsin, haptoglobin, ceruloplasmin, fibrinogen, complement's C3 segment, and alpha1 acid glycoprotein are increased in this so-called "acute-reaction protein pattern." Lower levels of albumin and transferrin are frequently observed in conjunction with this. The gamma region, which is primarily made up of IgG type antibodies, receives most of the attention when interpreting serum protein electrophoresis. In agammaglobulinemia and hypogammaglobulinemia, the gamma-globulin zone is reduced. The following illnesses cause an elevation in gamma-globulin levels: multiple myeloma, Waldenström's macroglobulinemia, amyloidosis, granulomatous diseases, connective tissue disorders, chronic lymphocytic leukemia, Hodgkin's disease, and malignant lymphoma (Tripathy, 2012).

A clinical investigation published in 2025 highlighted the practical value of serum protein electrophoresis (SPE) in recognizing individuals with diminished alpha-1 globulin fractions. The researchers observed that when the alpha-1 band measured below approximately 3% of total protein, a substantial proportion of patients were found to carry SERPINA1 variants such as PIMS,PIMZ, and

PISZ. These findings indicate that SPE can function as more than a descriptive laboratory method; it may serve as an effective preliminary screening approach for alpha-1-antitrypsin (AAT) deficiency, particularly in cases where acute-phase elevations could temporarily obscure reduced baseline AAT levels (Perales-Afán *et al.,* 2025).

In a separate 2025 proteomics analysis, investigators explored associations between circulating acute-phase proteins and metabolic disease. The study reported altered alpha-1-antitrypsin concentrations in individuals newly diagnosed with type 2 diabetes mellitus who also had obesity. The authors proposed that chronic low-grade inflammation and imbalance between proteases and antiproteases might contribute to these changes. Their results support the concept that AAT could have relevance as an indicator of metabolic inflammatory activity, extending its clinical significance beyond inherited deficiency or classical acute inflammatory states (Wang *et al*., 2025).

The Journal of Occupational and Environmental Medicine published research in 2025 that described additional advancements in laboratory interpretation. This work emphasized improvements in electrophoretic resolution, particularly within the β-globulin region. Enhanced differentiation of β-1 and β-2 subfractions was shown to improve detection of subtle abnormalities, including beta-region elevations related to monoclonal proteins or persistent inflammatory responses. Such refinements strengthen the diagnostic contribution of SPE in evaluating disorders like plasma cell dyscrasias and other chronic inflammatory conditions (Solanki *et al.,* 2025).

Age distribution in our data showed the greatest proportion of cases in the 51–60 and 61–70-year groups (Figure 5). This aligns with published epidemiological data demonstrating that incidence rises markedly after 50 years of age and is relatively uncommon below 40 years (Lee *et al*., 2021).

A recent 2025 study examined how age at diagnosis influences survival outcomes in multiple myeloma. The analysis revealed that individuals in the 50–59, 60–69, and over 70-year age brackets experienced progressively higher mortality risks compared with younger patients. Notably, patients over 70 had an estimated 3.3-fold increase in the risk of death, highlighting that advanced age not only correlates with greater incidence but also with poorer long-term prognosis (Lawanson *et al*., 2025).

In a separate 2025 global epidemiological assessment, researchers reported that the incidence and prevalence of multiple myeloma increase substantially with age, especially in adults above 40 years. The study emphasized that demographic aging contributes significantly to the overall disease burden and projected that the number of cases will continue to grow as populations become older. These findings help explain why multiple myeloma predominantly affects later decades of life and why cases in younger adults remain uncommon (Liu *et al*., 2025).

Association between Gender and Myeloma Occurrence

Pearson's chi-square test indicated a significant association between gender and occurrence in the analyzed datasets ($\chi^2 = 56.681$; *df*. = 5; table value = 11.070; 5% level). The result supported higher occurrence among men, consistent with reported epidemiology. Analysis was done using SPSS software 16.

Protein Fraction Changes by Gender

Men: Low beta-1 fractions and high alpha-1 fractions occurred frequently.Women: High alpha-1 fractions and low beta-1 fractions also occurred frequently (Table 1). Alpha-1-antitrypsin acts as an acute-phase reactant and increases during inflammatory conditions, infections, and several malignancies. Pregnancy, oral contraception, and physiological stress can also elevate alpha-1-antitrypsin, which can mask mild-to-moderate deficiency states. Low beta-1 globulin can reflect multiple underlying conditions, and interpretation requires clinical correlation and supportive laboratory findings (Health Matters link).

Table 1: *Serum Fraction Deviations in Men and Women*

Category	Albumin	Alpha-1	Alpha-2	Beta-1	Beta-2	Gamma
Men						
Low	18.09%	1.90%	13.33%	25.71%	22.85%	11.42%
High	1.90%	36.19%	15.23%	2.85%	8.57%	14.28%
Women						
Low	9.52%	0.95%	0.90%	17.14%	13.33%	3.80%
High	5.71%	24.76%	13.30%	0%	4.76%	11.42%

Perales-Afán *et al*. (2025) investigated the role of serum protein electrophoresis (SPE) in identifying reduced alpha-1 globulin levels and detecting possible serpina1 gene mutations. Their findings indicated that a diminished alpha-1 band on electrophoresis may be suggestive of an underlying genetic deficiency. Wang *et al*. (2025) described alpha-1-antitrypsin as a key acute-phase reactant that participates in immune regulation and inflammatory pathways. Their review explains why its concentration tends to rise in conditions such as inflammation, infection, and certain malignancies. Tran *et al*. (2025) reported that increased circulating alpha-1-antitrypsin levels are associated with systemic inflammatory activity in clinical populations, further supporting its clinical value as a marker of acute-phase response.

Abnormal Electrophoretic Patterns

M-spikes were detected in the gamma region in 20.95% of electrophoresis reports and in the beta region in 3.80% of reports. M-spikes were absent in 75.23% of reports. Beta–gamma bridging was observed in 7.61% of electrophoresis patterns.A monoclonal band typically appears as a discrete, sharp peak. Polyclonal gammopathy typically produces a broader increase involving multiple immunoglobulin components. Bone marrow biopsy remains essential for definitive diagnosis when monoclonal gammopathy is detected. Beta-gamma bridging reflects reduced separation between beta- and gamma fractions and is classically associated with chronic liver disease, although reports describe its occurrence in non-hepatic conditions as well (Camus *et al*., 2010).

Monoclonal bands may appear not only in the gamma region but also in the beta and even alpha regions on SPEP (Duggal *et al.,* 2025). Mefire *et al.* (2025) reported the presence of two monoclonal bands in the beta-2 and gamma regions on SPEP due to IgA polymerization. Since the present study used a cross-sectional design, no follow-up data were available to examine disease progression, treatment effects, or survival rates. This limits the ability to assess longitudinal clinical outcomes. Additionally, the limited resolution of capillary electrophoresis may hinder the detection of minimal levels of monoclonal proteins, particularly when they are obscured by the background of normal polyclonal immunoglobulins, potentially leading to underprediction in some cases.

Recent evidence from 2026 studies further strengthens the understanding of age-related and biochemical variations in multiple myeloma. A large-scale clinical analysis demonstrated that advancing age remains a major determinant of disease incidence, with individuals over 50 years of age showing significantly higher susceptibility due to cumulative genetic alterations and immune senescence (Zhong *et al*., 2026). Another investigation emphasized the growing role of inflammatory biomarkers, including acute-phase proteins such as alpha-1-antitrypsin, in reflecting underlying disease activity and systemic inflammation, suggesting their potential utility in disease monitoring (Huang *et al*., 2026). Advances in electrophoretic and proteomic techniques have also improved the resolution of serum protein fractions, particularly within the beta and gamma regions, enabling better identification of subtle monoclonal components and complex patterns that may otherwise be overlooked (Kumar *et al*., 2026). Furthermore, recent diagnostic guidelines advocate for an integrated approach combining serum protein electrophoresis with immunofixation and serum free light chain assays to enhance early detection, especially in cases with minimal or atypical M-protein expression (Pang *et al*., 2025). Collectively, these findings reinforce the importance of continuous technological advancements and comprehensive diagnostic strategies in improving the accuracy, early diagnosis, and clinical management of multiple myeloma.

Limitations

This study is limited by the absence of standard confirmatory testing using serum free light chain assays for monoclonal bands detected by SPEP and serum immunofixation electrophoresis (IFE), which may affect the detection limit and discriminatory ability. Future investigations should address these limitations by including larger sample sizes across multiple centers to improve the generalizability of the findings. Incorporating advanced diagnostic approaches, such as immunofixation, free light chain assays, and molecular techniques, would enhance the accuracy of monoclonal protein detection.

Future Scope

Prospective studies with longitudinal follow-up are essential to better understand disease evolution, treatment effectiveness, and patient outcomes. Combining laboratory findings with clinical and imaging data could provide a more comprehensive assessment of the disease. Further research focusing on the identification of novel biomarkers may support early diagnosis and improved risk classification. In addition, the use of artificial intelligence in interpreting electrophoretic patterns could minimize observer-related variability and improve diagnostic consistency. Studies exploring variations based on age and gender, as well as evaluating the potential role of serum protein electrophoresis in screening high-risk individuals, may contribute to earlier detection and improved clinical management of multiple myeloma.

Conclusion

The present study highlights that multiple myeloma-related abnormalities were more frequently observed in individuals aged 50 to 70 years, with a higher prevalence among men compared to women. Serum protein fraction analysis demonstrated consistent alterations, including elevated alpha-1 fractions and reduced beta-1 fractions in both genders, with more pronounced alpha-1 elevation in males. Monoclonal protein peaks were predominantly identified in the gamma region, with fewer cases detected in the beta region, while a substantial proportion of electrophoresis reports did not exhibit detectable M-spikes. Beta–gamma bridging was observed in a smaller subset of cases, indicating additional complexity in electrophoretic patterns. The findings reinforce the importance of serum protein electrophoresis as a fundamental tool for detecting and characterizing abnormal protein patterns associated with multiple myeloma. Although M-proteins are most commonly detected in the gamma region, their presence in other regions reflects variability in electrophoretic migration and necessitates careful interpretation. Accurate analysis of electrophoretic patterns is crucial for differentiating monoclonal gammopathies from polyclonal ones and for identifying subtle abnormalities that might otherwise go unnoticed. Overall, serum protein electrophoresis remains a valuable and accessible diagnostic technique for the identification and monitoring of multiple myeloma. Its effective use, particularly in individuals over 50 years presenting with suggestive clinical features such as bone pain, anemia, recurrent infections, or renal dysfunction, can facilitate early detection, guide further diagnostic evaluation, and support improved patient management and clinical outcomes.

Conflict of Interest

The authors declare that no conflict of interest

Acknowledgement

The authors are grateful to the sample accession staff, laboratory technicians, pathologist, sample pickup riders, senior scientific officers for their kind support and assistance throughout this study.

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