Research article

Usefulness of serum globulin levels for discriminating patients with monoclonal gammopathies/ paraproteinemias

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ABSTRACT

Introduction and Aim: The confirmatory step in diagnosis of monoclonal gammopathies is bone marrow biopsy and presence of M-protein in serum protein electrophoresis. These tests are relatively expensive & invasive for screening and unavailable in low resource settings. Increased levels of serum globulin are clue to the diagnosis of monoclonal gammopathy. The aim of this study was to assess the relevance of serum globulin levels in discriminating between patients with & without monoclonal gammopathies/ paraproteinemia.

Materials and Methods: We retrospectively reviewed serum protein electrophoresis (SPE) and related investigations of patients suspected of monoclonal gammopathy. Reports with an M-band were considered as paraproteinemias, and those without as controls. ROC for sensitivities & specificities for serum globulin levels were computed.

Results: For the case-control study, median serum globulin values in cases were 4.4 (3.5-6.3) g/dL in males and 3.65 (3.33-5.0) g/dL in females. They were significantly higher than those with normal SPE pattern, with a $p < 0.001$. A cut-off value of 3.25 g/dL of globulin could distinguish between paraproteinemias and controls with a sensitivity of 82.1% and specificity of 85.4% in males; a sensitivity of 79.2%, a specificity of 76.7% for females. At a cut-off value of 3.4 g/dL, sensitivity was 77% and specificity 92.7% for males; sensitivity was 75% and specificity 83.7% for females. Alternatively, a cut-off value of 0.458 of globulin/total protein ratio could distinguish at a best sensitivity & specificity of 80% and 89% in males; 83.3% and 83.7% in females.

Conclusion: Serum globulin values and globulin/total protein ratio can reliably differentiate patients with paraproteinemias.

Keywords: Monoclonal gammopathies; paraproteinemia; serum globulin; M-protein, SPE.

INTRODUCTION

The 'paraprotein', synonymous with monoclonal component, M-protein or monoclonal protein, has been valued over a long period of time as crucial biomarker in monoclonal gammopathies/ paraproteinemias (1). Monoclonal gammopathies range from the benign to the malignant plasma cell dyscrasias (PCD) including monoclonal gammopathy of undetermined significance (MGUS), smouldering multiple myeloma (SMM) and multiple myeloma (MM) to name few (1).

Serum M-protein concentration <3 g/dL and plasma clonal cells of less than 10 % in the bone marrow are characteristic of MGUS. SMM is asymptomatic, identified as ≥3 g/dL of M-protein and/or 10–60 % infiltration of bone marrow by plasma cells. Both of them feature no myeloma-defining end organ damage (2).

Among haematological malignancies, multiple myeloma stands the 2nd most common in adults in the western population and accounts for nearly 1% of all cancers (3, 4). MGUS identified patients require monitoring, especially after the age of 50, since 0.5-1% of them progress to multiple myeloma per year (3, 5).

Serum protein electrophoresis (SPE) is recommended in patients presenting with indistinct clinical symptoms like anaemia, weakness and malaise, with or without bony pain, to detect and quantify monoclonal component (5-7). However, due to its asymptomatic nature, MGUS has been a challenge to diagnose, and screening for the same is not a common clinical practice(5). The higher cost of electrophoresis testing discourages clinicians from using it as a screening tool in the absence of a strong clinical suspicion for paraproteinemia/ monoclonal gammopathies.

The non-albumin fraction in serum total protein is comprised of the globulin proteins (8, 9). Also denoted as 'gamma gap', when elevated, is seen in monoclonal gammopathies as well in several acute infections and chronic inflammatory conditions. It remains a worthwhile screening tool in the clinical setting as it enables their early diagnosis (8, 10).

Multiple myeloma can present with a wide array of symptoms including hypercalcemia, non- iron deficiency anaemia, renal impairment, bony pain, etc.

These symptoms are evaluated for myeloma if they cannot be explained otherwise (11). While an increased globulin level is consistently observed in multiple myeloma, and a study on an association of globulin levels at diagnosis with the treatment response and overall survival in patients with MM is reported (12), data available on the clinical utility of globulin values for distinguishing the risk of presence of paraproteinemias is insufficient. The aim and objectives of this present study was to assess whether serum globulin values can be used as an inexpensive tool in the discriminating between patients with and without paraproteinemia, especially when clinical features are inconclusive; to help identify such patients for reflex testing and establishing evidence with SPE and bone marrow biopsy.

MATERIALS AND METHODS

Study design

The Institutional Scientific Committee approved the study protocol. The results of serum protein electrophoresis reports of all patients, referred for investigation of suspected monoclonal gammopathy, were retrospectively reviewed between August 2017 and August 2019 in the clinical biochemistry laboratory at Kasturba Medical College Hospital, Mangalore. SPE reports of patients showing one/multiple sharp M-band/spike in any of the globulin regions were corroborated with bone marrow biopsy reports, and those reported as suggestive of paraproteinemia were defined as cases. SPE reports without any sharp M-bands showing normal patterns were included as controls. Patients undergoing treatment for monoclonal gammopathies at the time of the test, patterns with diffuse globulin increase, and repeat tests were excluded from the study.

Data Collection

The data related to the gender; age; total protein, albumin, globulin levels and bone marrow biopsy reports were recorded from electronic records of the biochemistry department. Their medical, drug and treatment history were extracted from medical records of their outpatient and inpatient files.

Laboratory tests

Serum was separated from venous blood samples of subjects. Laboratory testing was performed at the hospital central laboratory within 1 h after collection. Biochemical indexes, such as total protein (TP), albumin (ALB), were measured using an automated chemical analyser (COBAS 6000 c501, Roche, Germany) and associated reagents with biuret and BCG method, respectively, were used. Serum globulin (G) was calculated as Total protein–Albumin (9). Serum globulin/Total protein values were calculated as a ratio. The SPE was run using a manual agarose gel electrophoresis system (SAS-MX, Helena BioSciences, UK) according to the manufacturer's instructions. The relative percentage of each protein fraction was calculated automatically by a densitometry. M protein was quantitated by the perpendicular drop method (7).

Statistical Analysis

SPSS 20.0 for Windows software (SPSS, Chicago, IL) was used to perform statistical analyses. Normality of data distribution was analysed using Shapiro-Wilk Test. Data were represented as median and percentiles wherever appropriate. Mann-Whitney U test was used for comparison of continuous data between patient groups. Receiver operating characteristic (ROC) curve analysis was done for evaluating the diagnostic performance of the test. Sensitivity and specificity were reported using optimally selected cut-off values. P values <0.05 were considered as statistically significant.

RESULTS

This study included 147 patients who had a complete disease evaluation at the time of diagnosis. SPE reports of 63 patients showed a presence of M-band of which 39 were males and 24 females. Of the 84 patients with normal SPE pattern, 41 were males and 43 females. Summary of age distribution and biochemical characteristics of patients are presented in fig. 1 and table 1 respectively.

Table 1: Salient descriptive characteristics in patients with & without paraproteinemias.

Variable	Gender	M-band on SPE	Normal SPE pattern	P value
Total protein (g/dL)	M	7.7 (7.3-9.2)	7.0 (6.4-7.4)	<0.001
	F	7.5 (6.83-8.45)	7.2 (6.7-7.4)	0.074
Albumin (g/dL)	M	3.3 (2.6-4.0)	3.9 (3.65-4.5)	<0.001
	F	3.55 (3.03-3.88)	4.1 (3.7-4.4)	<0.001
Globulin (g/dL)	M	4.4 (3.5-6.3)	2.9 (2.6-3.1)	<0.001
	F	3.65 (3.33-5.0)	3.1 (2.8-3.2)	<0.001
Globulin/ Total Protein ratio (G/TP)	M	0.59 (0.47-0.71)	0.42 (0.39-0.44)	<0.001
	F	0.52 (0.46-0.61)	0.42 (0.41-0.45)	<0.001

Data presented as Median (25th-75th percentile)
P <0.05 significant; M- Male; F- Female
Non-parametric Mann Whitney U test for comparison of median values

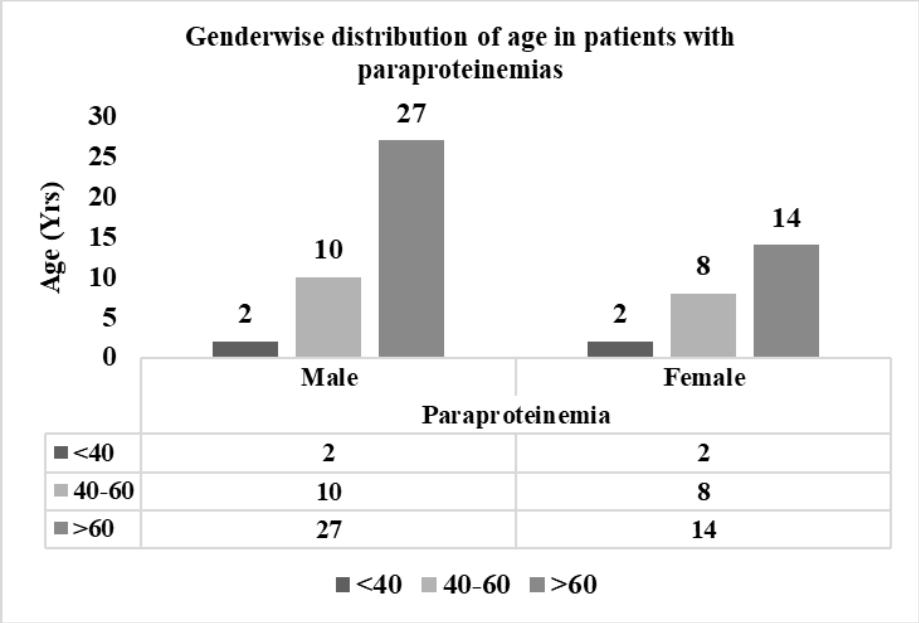

Fig. 1: Bar diagram of distribution of cases in different categories of age

Table 2: Descriptive characteristics in patients with paraproteinemias grouped by monoclonal protein concentration levels.

Variable	Gender	M-Protein <3g/dL (n=26)	M-Protein >3g/dL (n=11)	P value
Total protein (g/dL)	M	7.55 (1.1)	10.60 (4)	<0.001
	F	7.2 (1.5)	9.5 (2)	<0.05
Albumin (g/dL)	M	3.55 (1.1)	2.80 (1.4)	<0.05
	F	3.55 (0.9)	3.45 (1.1)	0.27
Globulin (g/dL)	M	3.35 (1.7)	7.5 (5.5)	<0.001
	F	3.5 (1.1)	6.2 (1.6)	<0.005
Globulin/ Total Protein ratio (G/TP)	M	0.53 (0.18)	0.74 (0.182)	<0.001
	F	0.49 (0.08)	0.65 (0.11)	<0.05

Data presented as Median (Interquartile range)
P <0.05 significant; M- Male; F- Female
Non-parametric Mann Whitney U test for comparison of median values

Median serum globulin values in patients with paraproteinemias were significantly higher (p <0.001) than those with normal SPE pattern, in both males and females (test statistic U=192, U=213, respectively; Fig 2).

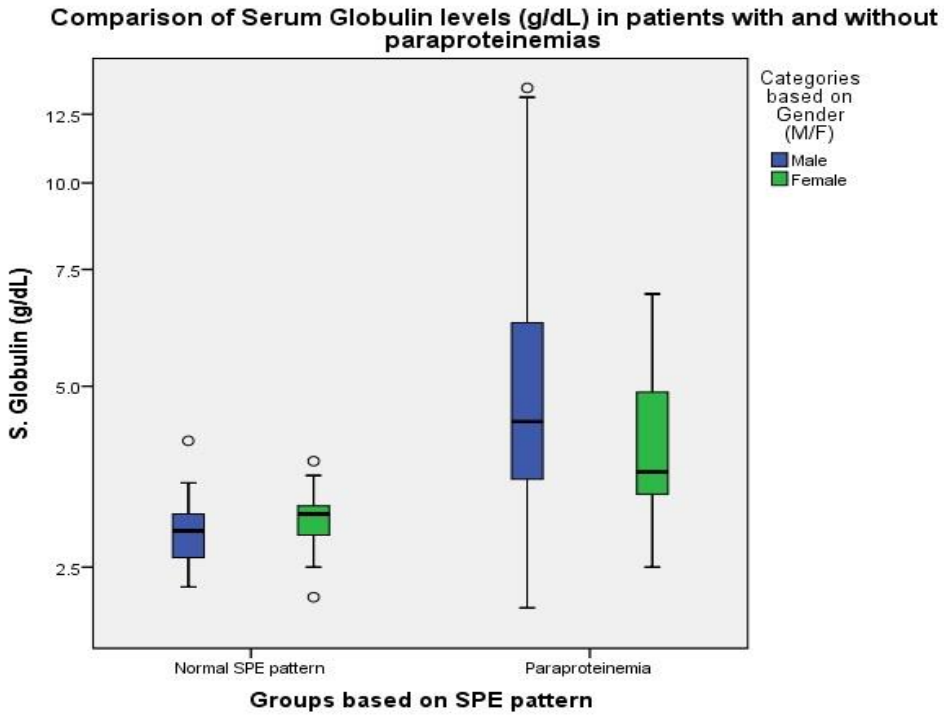

Fig. 2: Boxplot showing median serum globulin values (g/dL) in patients with and without paraproteinemias.

At an optimal cut-off value of 3.25 g/dL, serum globulin levels could distinguish between paraproteinemias and normal patients with a sensitivity of 82.1% and specificity of 85.4% in males; a sensitivity of 79.2%, a specificity of 76.7% in females (Fig. 3). At another cut-off value of 3.4 g/dL, serum globulin levels could distinguish paraproteinemias with a sensitivity was 77% and specificity 92.7% for males; sensitivity was 75% and specificity 83.7% for females. (AUC=0.848, CI 95%: 0.775-0.920).

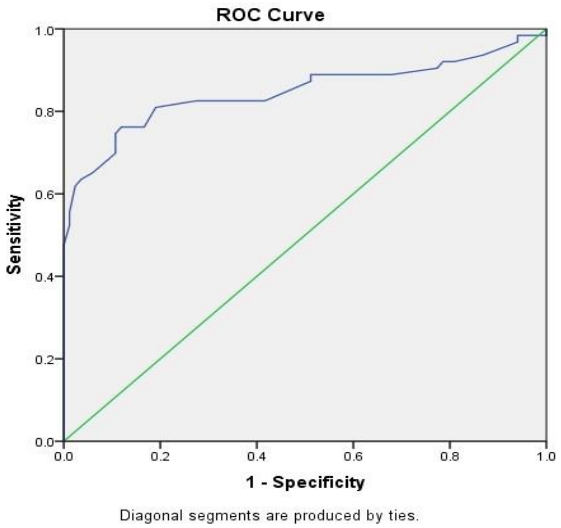

Fig. 3: ROC curve analysis of serum globulin values in patients with and without paraproteinemias

Alternatively, a cut-off value of 0.458 of globulin/total protein ratio could distinguish between paraproteinemias and normal patients at a best sensitivity and specificity of 80% and 89% in males; 83.3% and 83.7% in females. (AUC= 0.857, CI 95%: 0.790-0.924) (Fig. 4).

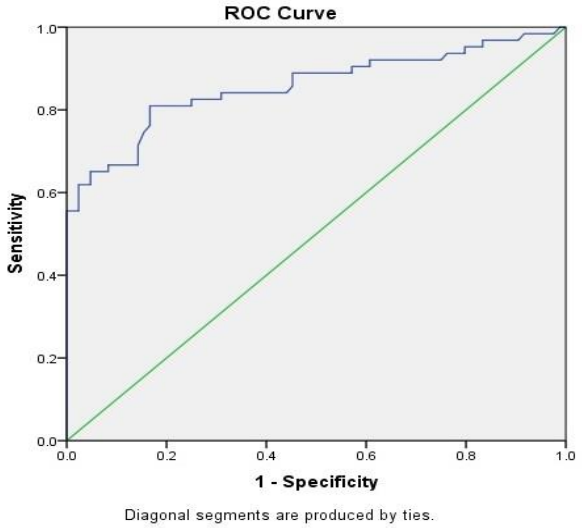

Fig. 4: ROC curve analysis of globulin/total protein ratio in patients with and without paraproteinemias

A logistic regression was performed to ascertain the effects of age, gender, serum globulin on the likelihood that participants have paraproteinemia. In the model, only globulin (p= <0.05) added significantly to the model prediction, but the others did not add significantly to the model. The model explained 54.3% (Nagelkerke R2) of the variance in paraproteinemia and correctly classified 83.0% of cases. The variables in the equation output shows us that the regression equation is l n (ODDS) = −9.038+ 0.017Age -0.466Gender +2.313Globulin ratio.

For the present study, assuming an alpha level of 0.05, difference between means of 2.1, sigma difference of 2, power calculated for two tailed test was 0.9997.

DISCUSSION

The multiple myeloma incidence in India reportedly stands at 1.9 per 100,000 persons (13). However, the prevalence data of monoclonal gammopathies in India is insufficient, as well as inconsistent due to differential inclusion criteria. Several studies have reported varying data depending on the population that was evaluated on suspicion of monoclonal gammopathies. In 2010, Singh *et al.*, (14), observed monoclonal gammopathy in 7.5% of patients of the screened population. In 2018, Gupta *et al*., (13*)* published an overall prevalence of MGUS at 1.43%, lower than that reported for global population. In 2019, a study by Bora (15), reported 1916 of documented cases of MM as registered in the National Cancer Registry Programme reports, which accounted for 1.19% of all cancers. These findings indicate the need to invest in screening methods for monoclonal gammopathies in patients within appropriate age groups.

In the present study, SPE showing the presence of M-band were seen higher in men than women. This is consistent with an Indian study published by Shaft *et al.*, where male to female ratio of 1.4:1 was observed (16). However, other Indian studies have found monoclonal gammopathies higher in women (14).

In the present study, serum globulin values as well as globulin/total protein (G/TP) ratios in patients with paraproteinemias were significantly higher than those with normal SPE pattern. In the present study, cut-off values for best sensitivities & specificities of serum globulin levels (3.25-3.4 g/dL) demonstrated reliable discrimination between patients with and without paraproteinemias. This is similar to study by Thakkinstian *et al.*, (17), who in 2008, tried to develop a clinical decision rule with variables like age, gender, globulin, haemoglobin, etc in patients that were associated with presence of a paraprotein. The factor found most significant by them was globulin, with levels higher than 4.1g/dL having five-fold higher odds of having a paraprotein proposing that higher gamma gap was a strong predictor for reflex testing with electrophoresis. Mitchell *et al.*, reported an increased detection rate (4% more) of monoclonal gammopathies when patients with elevated plasma globulins were investigated further. Serum gamma globulins greater than 4 g/dl were associated with an 76% incidence of monoclonal gammopathies (18). In

2015, Juraschek *et al.,* also reported a gamma gap of 3.1 g/dl or more was associated with 30% higher risk of death (19). A study summarized risk stratification markers and tools for MM as recently as in 2020 (20).

CONCLUSION

The findings in the present study indicate serum globulin values and globulin/total protein ratio can differentiate between patients with and without paraproteinemias with moderate sensitivity and specificity. To conclude, in low resource settings, and for continuous monitoring of patients in the elder age group, elevated globulin levels can be a useful diagnostic tool to help identify at-risk individuals before they become "patients with bad prognosis", to help identify/ prioritize such patients for reflex testing and establishing evidence with SPE and bone marrow biopsy, especially in asymptomatic patients. Consequently, pro-actively screen patients for monoclonal gammopathies.

Limitations of the study

Study population could have been higher; due to missing tests, other biochemical tests that are known to be deranged in monoclonal gammopathies could not be analysed & assessed.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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