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Original article

Value of Serum Protein Electrophoresis and its Relation to Treatment Response in Adult Egyptian Chronic Idiopathic Thrombocytopenic Purpura Patients

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Abstract

Background: Idiopathic thrombocytopenic purpura (ITP) is isolated thrombocytopenia with normal bone marrow, in absence of other causes of thrombocytopenia. **Methods:** 100 patients with idiopathic thrombocytopenia were admitted according to **inclusion criteria:** any sex, >18 years, with chronic idiopathic thrombocytopenic purpura while **exclusion criteria:** Patients with secondary idiopathic thrombocytopenic purpura or other hematological-disorders or lymphoma or auto-immune diseases or drug/ infection-induced thrombocytopenia or consumptive thrombocytopenia like DIC. All patients were assessed by demographic data collection, detailed-history, complete clinical examination, laboratory investigations (platelet and total leukocyte count, serum protein electrophoresis (SPE) for α1, α2, β1, β2, γ1, albumin % and response to steroid therapy. **Results:** serum protein electrophoresis α1 was associated with 2.6 times increased risk of non-response (OR = 2.617, 95% CI = 1.567 - 4.371, P = <0.001). Serum protein electrophoresis α2 was associated with about 3 times increased risk of iron deficiency anemia (OR = 2.894, 95 CI% = 1.089 – 7.695, P = 0.033). Serum protein electrophoresis β2 was associated with about 2 times increased risk of non-response (OR = 2.241, 95 CI% = 1.509 - 3.327, P = <0.001). Serum protein electrophoresis albumin % was associated with an increased risk for non-response to steroids (OR = 1.128, 95 CI% = 11.054 - 1.207, P = <0.001). **Conclusion:** serum protein electrophoresis profiles, particularly α1, α2, β2 fractions and albumin percentage, are significant predictors of steroid response in chronic idiopathic thrombocytopenic purpura. Lower levels of α1, α2, β2, and albumin were associated with a better response to corticosteroids.

Keywords: Idiopathic thrombocytopenic purpura; Serum-protein electrophoresis; Steroid

Introduction

Idiopathic thrombocytopenic purpura (ITP), also known as primary immune thrombocytopenic purpura is defined as isolated thrombocytopenia with normal bone marrow and in the absence of other causes of thrombocytopenia (1).

Immune thrombocytopenia is a bleeding autoimmune disease due to decreased platelet production as well as accelerated platelet destruction mediated in part by autoantibody-based destruction mechanisms (2) attacking platelet surface glycoproteins (GP), such as GPIIb/IIIa and GPIb/IX complexes that play major roles in both platelet destruction and impaired platelet production (3).

Immune dysregulation, as represented by elevated or decreased serum immunoglobulin (Ig) levels, may increase disease severity as represented by failure to respond to treatment. These alterations in Ig levels may represent an inflammatory or activated immune state that makes the disease more difficult to control with specific treatment (4).

Although, it is unclear which immune factors related to disease predisposition, severity, and especially response to treatment, certain patients with known immunologic disorders have an increased the risk of ITP supporting the concept that immune dysregulation may contribute to the development of ITP (5).
The purpose of this study was to assess serum protein electrophoresis (SPE) in chronic ITP and its relation to response to treatment.

Patients and methods

This prospective observational study included 100 patients with ITP at Hematology Unit of Internal Medicine Department, Benha University Hospital during the period from December 2023 to December 2024.

An informed written consent was obtained from the patients. Every patient received an explanation of the purpose of the study and had a secret code number. The study was done after being approved by the Research Ethics Committee, Faculty of Medicine, Benha University.

- **Inclusion criteria** were adult patients (>18 years), both sexes, patients diagnosed with chronic ITP (Platelet count less than 100,000/ml, for more than 6 months when other causes of thrombocytopenia (such as infections, medications, leukemia, or other autoimmune disorders) are ruled out. This requires a comprehensive evaluation (6).

- **Exclusion criteria** were patients with secondary ITP or other hematological disorders, lymphoma, auto immune diseases, drug induced thrombocytopenia and infection induced thrombocytopenia or consumptive thrombocytopenia like DIC.

All studied cases were subjected to the following: Detailed history taking, including [Demographic information: Age, gender, and occupation, medical history: Presence of

comorbidities such as hypertension, diabetes mellitus, ischemic heart disease, or other chronic conditions, surgical history: Prior surgeries or interventions, medication history: Current and past medications, including compliance and side effects, family history: Hereditary conditions or similar complaints in family members, lifestyle factors: smoking status, alcohol consumption, physical activity, and dietary habits, symptom's review: Onset, duration, and progression of current symptoms, including chest pain, dyspnea, fatigue, or palpitations].

Full clinical examination: General examination including [Body mass index (BMI) and nutritional status assessment, signs of pallor, cyanosis, jaundice, or edema, examination for any signs of dehydration or lymphadenopathy, vital signs including: [Heart rate, rhythm, and regularity, blood pressure: Systolic and diastolic measurements in both arms if required, respiratory rate and pattern, body temperature]. **Routine laboratory investigations [**Platelet count, total leukocyte count, SPE parameters (α1, α2, β1, β2, γ1, albumin %)].

Steroid therapy:

Each patient received steroid therapy as the initial treatment in the form of oral prednisone at 0.5–2 mg/kg/day for 2–3 weeks, tapered to be stopped by 6–8 weeks. Complete Response (CR): Platelet count would be ≥ 100,000/μL. without bleeding symptoms, while Partial Response (PR): Platelet count would be ≥ 30,000/μL but < 100,000/μL, Improved bleeding symptoms while Failure to Respond: Platelet count < 30,000/μL after a full course of steroid therapy and No improvement in bleeding symptoms or clinical condition (7). If there was no response, second-, third-, and fourth-line treatments were introduced sequentially. Patients were followed up over 3 months to monitor their response to therapy and adjust the treatment plan accordingly. Treatment response was defined by clinical improvements, specifically in platelet count or overall clinical symptoms. Patients were categorized as responders (Those who showed significant clinical improvement) or non-responders (Those who required additional therapeutic interventions) based on their reaction to initial steroid therapy.

SPE Assessment Steps:

Sample Collection: A blood sample was collected from the patient, typically from the median cubital vein in the arm. The blood was allowed to clot and then centrifuged to separate the serum from the cellular components.

Preparation: The serum sample was applied onto a gel or another supporting medium designed to separate proteins based on their size and charge.

Electrophoresis: The gel was placed in an electrophoresis apparatus. An electric current was applied to the gel, causing proteins in the serum to migrate through the medium at different rates, depending on their size and charge.

Staining: After electrophoresis, the gel was stained to visualize the protein bands. Common stains used included Coomassie Brilliant Blue or silver stain.

Analysis: The stained gel was analyzed using densitometry to quantify the separated protein bands. The major protein fractions identified included albumin, alpha-1 globulins, alpha-2 globulins, beta globulins, and gamma globulins.

Approval code: MS 4-1-2023

Statistical analysis

Data management and statistical analysis were done using SPSS version 28 (IBM, Armonk, New York, United States). Quantitative data were assessed for normality using the Shapiro-Wilk test and direct data visualization methods. According to normality, quantitative data were summarized as means and standard deviations for age and heartbeat or medians and ranges for other variables. Categorical data were summarized as numbers and percentages. Quantitative data were compared according to response to steroids using the independent t-test or Mann-Whitney U test for normally and non-normally distributed quantitative variables, respectively. ROC analyses were done for SPE to predict response to steroids. The area under the curve with its 95% confidence intervals, best cutoff point, and diagnostic indices were calculated. Correlations between SPE and other parameters were done using Spearman's correlation. Univariate and multivariate logistic regression analyses were done to predict non-response to steroids using serum proteins. Odds ratio (OR) with 95% confidence intervals were calculated. All statistical tests were two-sided. P values less than 0.05 were considered significant (8).

Results

Table 1 showed age, sex and comorbid conditions. Platelet counts exhibited a wide range, with a median of 22. Total leukocyte counts also varied, with a median of 6.7 (The analysis of SPE in the studied cohort revealed the following median values for the measured fractions (g/dL): SPE α1 was 4.6, SPE α2 was 9.8, SPE β1 was 5.9, SPE β2 was 4.2, and SPE γ1 was 14.7. Additionally, the median percentage of SPE albumin was 59.4%.

Of the total cohort, 19 patients (19%) showed a response to initial steroid therapy. A significant proportion, 81 patients (81%), required second-line treatment, while 15 patients (15%) progressed to third-line therapy. Additionally, 3 patients (3%) required a fourth-line therapeutic approach.

A- *First line*:

1- Corticosteroids. (Dexamethasone, Prednisolone)

2- I.VIG. (For acute bleeding.).

B-*Second line:*

1- TPO receptor agonists. (Eltrombopag, Romiblostim, Avatrombopag).

C- *Third line:*

1- Rituximab.

2- Fostamatinib.

3- Other immunosuppressant.

D- *Fourth line:*

Splenectomy.

Patients who responded to steroids were significantly younger, with a mean age of 28 ± 12 years compared to 36 ± 13 years in non-responders (P = 0.018). Additionally, responders had a significantly lower median platelet count of 16 × 10^3/μL (range: 3–64) compared to 29 × 10^3/μL (range: 2–205) in non-responders (P = 0.013). Other variables, including sex (P = 0.397), comorbidities (P = 1), heart rate (P = 0.216), and total leukocyte count (P = 0.49), were not significantly associated with response to steroids. **Table 1**

Table 2 showed ROC analysis that was done for SPE α1 to predict response to steroids. It revealed that the AUC was 0.667, with a 95% confidence interval of 0.524 - 0.810. The best cutoff point was ≤3, at which sensitivity, specificity, PPV, and NPV were 36.84%, 96.30%, 70%, and 86.7%, respectively as in **figure 1**. ROC analysis was done for SPE α2 to predict response to steroids. It revealed that the AUC was 0.705, with a 95% confidence interval of 0.541 - 0.868. The best cutoff point was ≤7.8, at which sensitivity, specificity, PPV, and NPV were 52.63%, 92.59%, 62.5%, and 89.3%, respectively as in **figure 2**. ROC analysis was done for SPE β2 to predict response to steroids. It revealed that the AUC was 0.76, with a 95% confidence interval of 0.622 - 0.898. The best cutoff point was ≤2.1, at which sensitivity, specificity, PPV, and NPV were 52.63%, 96.30%, 76.9%, and 89.7%, respectively as in **figure 3**. ROC analysis was done for SPE albumin% to predict response to steroids. It revealed that the AUC was 0.693, with a 95% confidence interval of 0.533 - 0.853. The best cutoff point was ≤53.5, at which sensitivity, specificity, PPV, and NPV were 52.63%, 92.59%, 62.5%, and 89.3%, respectively as in **figure 4**. **Table 2**

Table 3 showed the correlation analysis between the different types of SPE revealed several significant associations. SPE α1 showed a significant positive correlation with SPE α2 (r = 0.458, P < 0.001), while SPE α2 was positively correlated with SPE β1 (r = 0.423, P < 0.001) and SPE β2 (r = 0.204, P = 0.042). SPE β1 demonstrated a positive correlation with SPE γ1 (r = 0.284, P = 0.004), and SPE β2 was significantly correlated with SPE γ1 (r = 0.265, P = 0.008). SPE γ1 also showed a significant negative correlation with SPE albumin% (r = -0.338, P = 0.001). Other correlations, including those between SPE α1 and SPE β1 (P = 0.557), SPE α1 and SPE β2 (P = 0.094), SPE α1 and SPE γ1 (P = 0.411), as well as between SPE β1 and SPE β2 (P = 0.301), were not significant. Similarly, the correlations between SPE α2 and SPE albumin% (P = 0.955), SPE β1 and SPE albumin% (P = 0.447), and SPE β2 and SPE albumin% (P = 0.437) were not significant. **Table 3**

Table 4 showed the correlation analysis between the different types of SPE and other variables revealed several significant associations. SPE β2 showed a significant positive correlation with age (r = 0.251, P = 0.013). SPE α2 demonstrated a significant negative correlation with total leukocyte count (r = -0.260, P = 0.009). Hemoglobin levels showed significant positive correlations with both SPE α2 (r = 0.346, P < 0.001) and SPE β2 (r = 0.351, P < 0.001). Other correlations, including those between

SPE α1 and age (P = 0.085), SPE α1 and hemoglobin (P = 0.151), SPE α1 and platelet count (P = 0.163), SPE α1 and total leukocyte count (P = 0.726), SPE β1 and age (P = 0.766), SPE β1 and hemoglobin (P = 0.679), SPE β1 and platelet count (P = 0.181), SPE β1 and total leukocyte count (P = 0.828), as well as correlations with SPE γ1 (P > 0.05 in all cases), were not significant. **Table 4**

Univariate and multivariate logistic regressions were done to predict non-response to steroids using SPEs controlling for age and gender. The model revealed that SPE α1 was associated with 2.6 times increased risk of non-response (OR = 2.617, 95% CI = 1.567 - 4.371, P = <0.001). Also, SPE α2 was associated with about 3 times increased risk of iron deficiency anemia (OR = 2.894, 95 CI% = 1.089 – 7.695, P = 0.033). Additionally, SPE β2 was associated with about 2 times increased risk of non-response (OR = 2.241, 95 CI% = 1.509 - 3.327, P = <0.001). Furthermore, SPE albumin % was associated with an increased risk for non-response to steroids (OR = 1.128, 95 CI% = 11.054 - 1.207, P = <0.001). **Table 4**

Table 1: Demographics, clinical, laboratory characteristics, serum protein electrophoresis and treatment of the studied cases

		Patients (n=100)		
Age (years)		35 ±13		
Sex	**Male**	3 (3%)		
	Female	97 (97%)		
Comorbidity		15 (15%)		
Heartbeat (bpm)		10.8 ±1.6		
Platelet (*10³/µL)		22 (2 - 205)		
Total leukocyte (*10³/µL)		6.7 (3.1 - 17)		
SPE	**α1**	4.6 (0.25 - 8.6)		
	α2	9.8 (0.65 - 14)		
	β1	5.9 (0.3 - 11.2)		
	β2	4.2 (0 - 6)		
	γ1	14.7 (0.77 - 20.9)		
	Albumin%	59.4 (3.9 - 73.7)		
Treatment	**Response to steroid**	19 (19%)		
	Need for 2nd line	81 (81%)		
	Need for 3rd line	15 (15%)		
	Need for 4th line	3 (3%)		
		Response to steroid		P-value
		Yes (n=19)	**No (n=81)**	
Age (years)		28 ±12	36 ±13	**0.018***
Sex	**Male**	0 (0)	3 (3.7)	0.397
	Female	19 (100)	78 (96.3)	
Comorbidity		3 (15.8)	12 (14.8)	1
Heartbeat (bpm)		10.3 ±1.6	10.8 ±1.6	0.216
Platelet (10^3/µL)		16 (3 - 64)	29 (2 - 205)	**0.013***
Total leukocyte (10^3/µL)		6.8 (3.1 - 11)	6.4 (4 - 17)	0.49
SPE	**α1**	4.5 (0.25 - 6.4)	4.6 (3 - 8.6)	**0.024***
	α2	7.8 (0.65 - 14)	9.9 (5.8 - 12.9)	**0.006***
	β1	4.9 (0.3 - 11.2)	5.9 (3.8 - 7.7)	0.096
	β2	0.45 (0 - 5.4)	4.3 (2.1 - 6)	**<0.001***
	γ1	14.8 (0.77 - 20.8)	14.2 (5.5 - 20.9)	0.501
	Albumin%	53.5 (3.9 - 65.2)	60 (51.1 - 73.7)	**0.009***

Data are presented as mean ± SD or frequency (%), SPE; Serum protein electrophoresis., bpm: beats per minute, *Significant p-value ≤ 0.05.Data are presented as mean ± SD or frequency (%), SPE: Serum protein electrophoresis, bpm: beats per minute, *Significant p-value ≤ 0.05.

Table 2: ROC analysis of SPE α1, SPE α2, SPE β2 and SPE albumin% to predict response to steroids

	AUC (95%CI)	Best cutoff	Sensitivity	Specificity	PPV	NPV	P-value
SPE α1	0.667 (0.524 - 0.810)	≤3	36.84%	96.30%	70%	86.7%	**0.024***
SPE α2	0.705 (0.541 - 0.868)	≤7.8	52.63%	92.59%	62.5%	89.3%	**0.006***
SPE β2	0.76	≤2.1	52.63%	96.30%	76.9%	89.7%	**< 0.001***
SPE albumin%	0.693 (0.533 - 0.853)	≤53.5	52.63%	92.59%	62.5%	89.3%	**0.009***

Data are presented as mean ± SD or frequency (%), ROC: Receiver operating curve, CI: Confidence Interval, SPE: Serum protein electrophoresis, AUC: Area under the curve, PPV: Positive predictive value, NPV; Negative predictive value, *Significant p-value ≤ 0.05.

Table 3: Correlation between the different types of serum protein electrophoresis and between the different types of serum protein electrophoresis and other variables

	α1		α2		β1		β2		γ1	
	r	**P**	**r**	**P**	**r**	**P**	**r**	**P**	**r**	**P**
SPE α2	0.458	**<0.001***								
SPE β1	0.059	0.557	0.423	**<0.001***						
SPE β2	0.168	0.094	0.204	0.042	0.104	0.301				
SPE γ1	0.083	0.411	0.1	0.324	0.284	0.004	0.265	**0.008***		
SPE albumin%	0.071	0.484	0.006	0.955	-0.077	0.447	-0.079	0.437	-0.338	**0.001***

	Age (years)		Heartbeat		Platelet		Total leukocyte	
	r	**P**	**r**	**P**	**r**	**P**	**r**	**P**
α1	-0.176	0.085	0.145	0.151	-0.143	0.163	-0.035	0.726
α2	-0.031	0.766	0.346	**<0.001***	-0.137	0.181	-0.260	**0.009***
β1	-0.015	0.886	0.042	0.679	0.039	0.161	0.022	0.828
β2	0.251	**0.013***	0.351	**<0.001***	0.116	0.024	0.061	0.544
γ1	0.05	0.628	0.087	0.389	0.008	0.935	0.14	0.166
Albumin %	-0.072	0.484	-0.093	0.359	0.008	0.935	0.096	0.344

Data are presented as mean ± SD or frequency (%), SPE: Serum protein electrophoresis, *Significant p value ≤ 0.05.

Table 4: Univariate and multivariate logistic regression analyses to predict non-response to steroids using the different SPE.

	Univariate		Multivariate	
	COR (95% CI)	**P value**	**AOR (95%CI)** †	**P value**
α1	0.673 (1.355 - 2.836)	**<0.001***	2.617 (1.567 - 4.371)	**<0.001***
α2	0.345 (1.169 - 1.707)	**<0.001***	1.528 (1.222 - 1.911)	**<0.001***
β2	0.834 (1.579 - 3.355)	**<0.001***	2.241 (1.509 - 3.327)	**<0.001***
Albumin %	0.093 (1.03 - 1.169)	**0.004***	1.128 (1.054 - 1.207)	**0.001***

Data are presented as mean ± SD or frequency (%), SPE: Serum protein electrophoresis, COR: Crude Odds Ratio, AOR: Adjusted Odds Ratio, †: Adjusted for age and gender. *Significant p-value ≤ 0.05

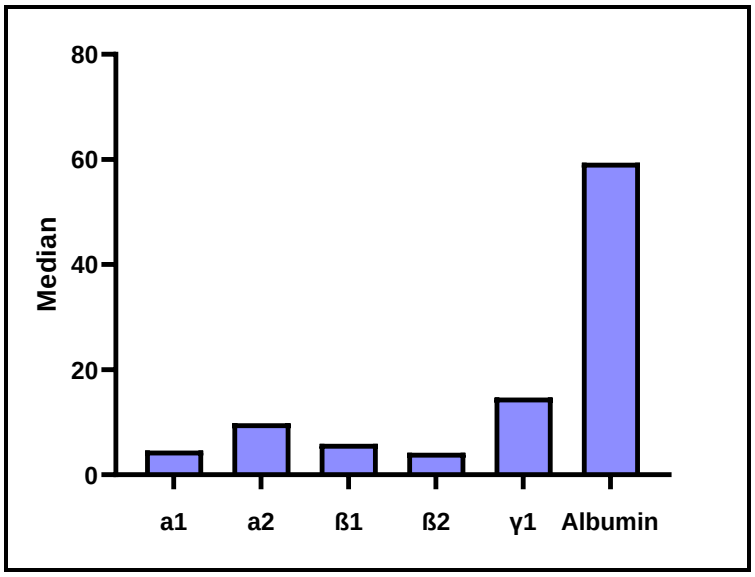

Figure 1: Serum protein electrophoresis of the studied cases

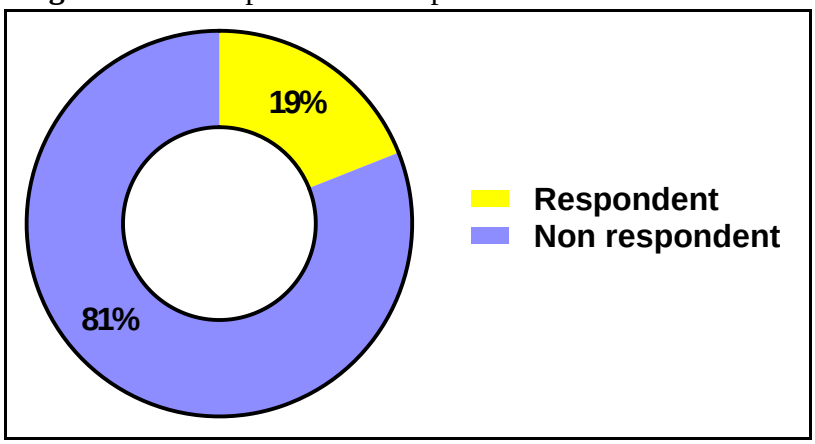

Figure 2: Response to steroid in the studied patients

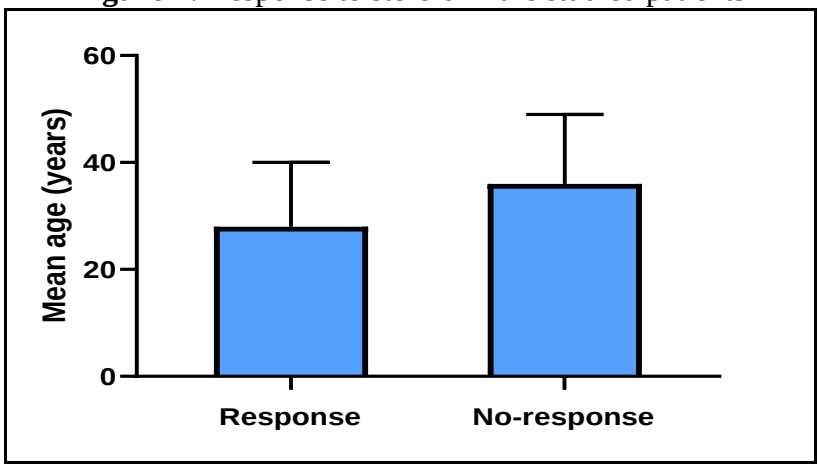

Figure 3: Age of the studied cases according to response to steroids

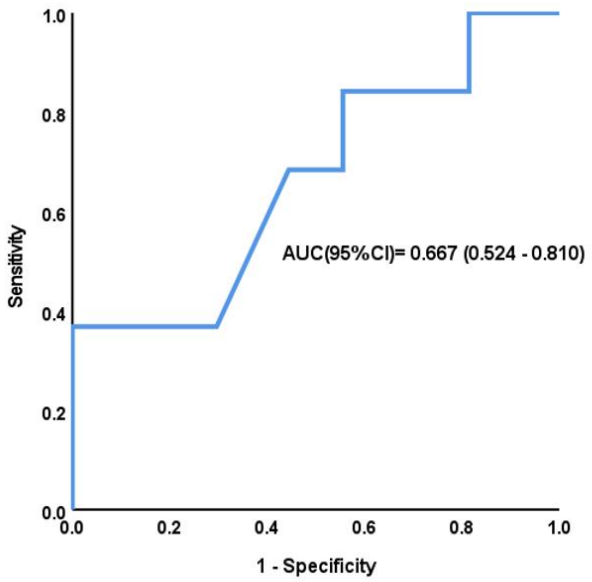

Figure 4: ROC analysis of SPE α1 to predict response to steroids.

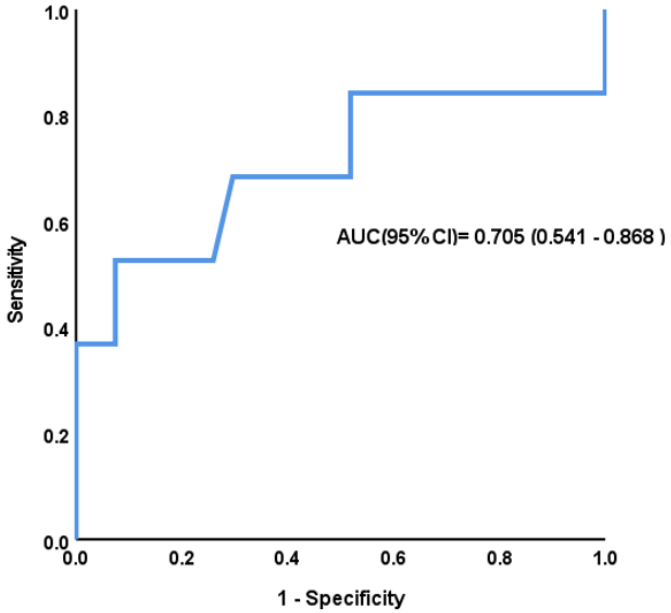

Figure 5: ROC analysis of SPE α2 to predict response to steroids.

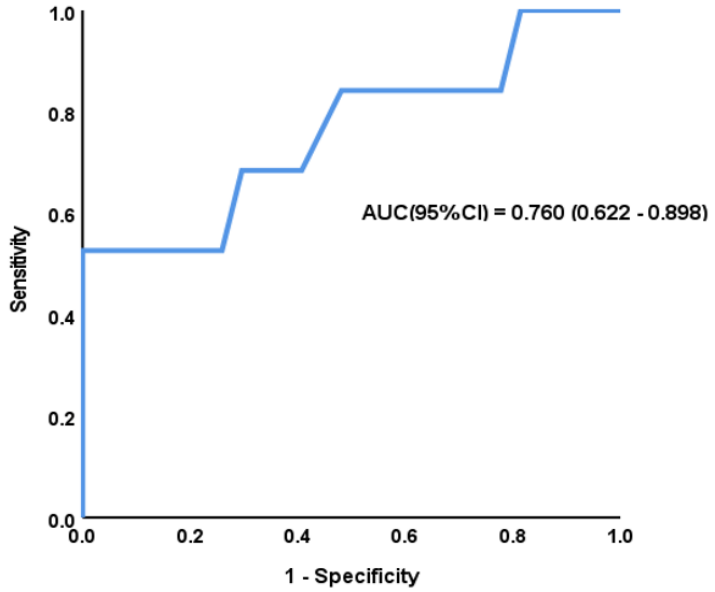

Figure 6: ROC analysis of SPE β2 to predict response to steroids.

Discussion

ITP, also known as primary immune thrombocytopenia, is a chronic autoimmune disorder characterized by isolated thrombocytopenia, defined as a platelet count below 100,000/μL, in the absence of other identifiable causes of thrombocytopenia and with a normal bone marrow examination (9). SPE is a diagnostic technique that separates serum proteins into fractions (albumin, α1, α2, β, and γ globulins), providing insights into immune activity and systemic inflammation. Elevated levels of certain fractions, such as γ-globulins, are indicative of hypergammaglobulinemia, a marker of immune activation. Conversely, reductions in albumin levels may reflect a pro-inflammatory state associated with chronic disease. In the context of ITP, abnormalities in SPE profiles could serve as biomarkers for immune dysregulation and offer predictive value regarding treatment response (10).

The study included 100 patients with a mean age of 35 ± 13 years, predominantly female (97%), with 15% having comorbidities. Steroid response was achieved in 19% of patients, while 81% required second-line therapies.

In a stud by Ellithy et al. (11) found that age of the patients ranged from 14 to 60 years, with a mean age of 30.6±11.12 years, 100 % of the patients were treated with corticosteroids and 0 % showed complete response.

In the present study, SPE revealed median values for α1, α2, β1, β2, and γ1 fractions as

4.6, 9.8, 5.9, 4.2, and 14.7 g/dL, respectively, with albumin percentage at 59.4%.

SPE separates serum proteins into distinct fractions, each with specific physiological ranges. Albumin, the most abundant protein, typically constitutes 55–65% of total serum protein, with normal levels ranging from 3.5 to 5.0 g/dL. The α1-globulin fraction accounts for approximately 2–5% (0.1–0.3 g/dL), while the α2-globulin fraction comprises 7–13% (0.6–1.0 g/dL). The β-globulin fraction is divided into β1 (5–7%) and β2 (3–6%), with total β levels ranging from 0.7 to 1.1 g/dL. The γ-globulin fraction, representing immunoglobulins, constitutes 10–20% (0.8–1.6 g/dL) of total protein. Deviations from these ranges often indicate systemic inflammation, immune dysregulation, or other pathological processes (12).

Conversely, reductions in albumin levels may reflect a systemic inflammatory state or increased vascular permeability. The α1 and α2 globulin fractions may also be elevated, indicating an acute phase response driven by chronic immune activation. These changes highlight the role of SPE in detecting underlying immune disturbances and its potential utility in predicting treatment responses in ITP (13).

In the current study, responders were significantly younger than non-responders and had lower platelet counts.

ITP impacts platelets count due to immune-mediated mechanisms. Autoantibodies directed against platelet surface glycoproteins lead to enhanced platelet destruction by macrophages in the spleen and liver, resulting in persistently low platelet levels. Additionally, these autoantibodies interfere with megakaryocyte maturation and platelet production, compounding the thrombocytopenia. In untreated ITP, platelet counts can range from severely low ($<10 \times 10^3/\mu L$) to moderately low levels depending on disease activity (14). Steroid treatment, the first-line therapy for ITP, works by suppressing immune activity and reducing autoantibody production. Corticosteroids also decrease macrophage-mediated platelet destruction and improve platelet survival. In responsive patients, platelet counts typically rise within days to weeks of initiating treatment, with levels stabilizing in the normal or near-normal range in many cases (15).

While in contrast, Seçkin and Ciftciler et al. (16) revealed that median platelet count was significantly higher in patients with complete response [197.0 (106–622)] than patients with no response [24.0 (3.0–578.0)] with no significant p value = 0.12. These differences may be due to the different population or methodology.

In the current study, SPE analysis showed responders had lower median levels of α1, α2, β2, and albumin. β1 and γ1 fractions did not differ significantly between groups.

Regarding SPE, alpha-1 globulin levels are elevated in cases of acute and chronic inflammatory disease, neoplasia, trauma, surgery or pregnancy. In cases of liver carcinoma, alpha-1 globulin levels can increase in response to an increase in alpha-fetoprotein levels. In general, however, liver diseases can cause a decrease in the overall

levels of alpha-1 globulins. Also, alpha-2 globulins include haptoglobin, alpha-2-macroglobulin and ceruloplasmin. These proteins are elevated in cases of adrenal insufficiency, steroid therapy, severe diabetes mellitus or nephritic syndrome, whereas they are reduced in cases of malnutrition, megaloblastic anemia, protein-losing enteropathy, severe liver disease or Wilson disease (17).

β-globulin has two peaks: beta-1, essentially composed by transferrin; and beta-2, composed by β-lipoproteins (for example, low density lipoprotein – LDL). C3 and other components of the complement system, β2-microglobulin, and antithrombin III are also found in this band (18). There is an increase in these globulins in the acute inflammatory process, hypothyroidism, iron deficiency anemia, malignant hypertension, obstructive jaundice, pregnancy, and some cases of diabetes mellitus. Diminished rates are found in cases of malnutrition (17).

Furthermore, Hanson et al. (19) found that administration of‘ corticosteroids induces an immunosuppressed state and augments hepatitis B virus replication; the abrupt withdrawal of this immunosuppressive agent may then induce a clearance of viral replication and an improvement in the chronic liver disease.

In the interpretation of SPE, most attention focuses on the gamma region, which is composed predominantly of antibodies of the IgG type. The gamma-globulin zone is decreased in hypogammaglobulinemia and agammaglobulinemia. Diseases that produce an increase in the gamma-globulin level include Hodgkin’s disease, malignant lymphoma, chronic lymphocytic leukemia, granulomatous diseases, connective tissue diseases, liver diseases, multiple myeloma, Waldenström’s macroglobulinemia, and amyloidosis (20, 21). Although many conditions can cause an increase in the gamma region, several disease states cause a homogeneous spike-like peak in a focal region of the gamma-globulin zone. These so-called “monoclonal gammopathies” constitute a group of disorders that are characterized by proliferation of a single clone of plasma cells that produce a homogeneous M protein (22).

The gamma globulin band is composed of immunoglobulins, especially immunoglobulin G. Immunoglobulins A, D, E and M as well as CRP are situated at the junction between the beta and gamma bands. The absence or decrease in the size of the gamma band indicates congenital or acquired immunodeficiency, while an increase suggests an elevation in gamma globulin levels that may be correlated with chronic inflammatory diseases (autoimmune or other), liver diseases or neoplasia, all of which exhibit a polyclonal pattern (17, 23).

A study by Settipane et al. (24), found that serum immunoglobulins were determined before corticosteroids were administered, an average of 15 days while on corticosteroids, and again an average of 22 days after corticosteroids were discontinued. While on corticosteroids (averaging 16.8 mg prednisone daily) for 15 days, mean serum IgG was significantly decreased (-22%, p less than or equal to 0.01).

Concerning the effect of steroids on serum globulins, Aun et al. (25) found that there was a 21.7-fold increased risk of hypogammaglobulinemia among OCS users. Fifty percent of OCS users (4 patients) had decreased in the IgG level.

Also, Bonnan et al. (26) revealed that IgG levels were significantly lower in MS patients exposed to CS infusion during the last 24 months. IgG levels were also lower in DMD-treated patients exposed to CS.

Albumin is the most abundant plasma protein, accounting for approximately 60% of the total protein concentration. It is synthesized exclusively by the liver. Albumin functions include the transport of different substances and the maintenance of plasma oncotic pressure. Albumin levels are reduced in cases of liver disease, malnutrition, chronic infections, hormone therapy, pregnancy or burns, and they can be elevated in dehydrated patients. Acute and chronic inflammatory diseases are the major causes of a decrease in the plasma concentrations of albumin. Several factors can lead to lower plasma concentrations of albumin, including hemodilution, increased vascular permeability (leading to plasma extravasation), increased local cell uptake and reduced synthesis due to cytokine inhibition (17, 27).

In a study by Steiner et al. (28) found that after corticosteroid therapy in patients with Crohn disease, the rates of appearance of phenylalanine (32%) and leucine (26%) increased significantly, reflecting increased protein breakdown, and the rate of appearance of urea also increased significantly (273%), reflecting increased protein loss. Whole body protein breakdown and loss increased significantly after 2 weeks of corticosteroid therapy in children with newly diagnosed Crohn disease, which may have profound effects on body composition.

In the current study, ROC analysis indicated that α1 (AUC = 0.667), α2 (AUC = 0.705), β2 (AUC = 0.76), and albumin (AUC = 0.693) were predictive of steroid response, with β2 being the strongest predictor at a cutoff of ≤2.1 (sensitivity = 52.63%, specificity = 96.30%).

A study by Rodriguez-Otero et al. (29) found that α1-antitrypsin has an AUC(95%CI) of 0.699 (0.577-0.822). Bazzi et al. (30) revealed α2m/C can diagnose ESRD with an AUC of 0.891, P value of (<0.0001), at Cutoff point of ≥4.79, sensitivity of 89%, and specificity of 86%.

In the present study, correlation analysis revealed significant correlations among SPE fractions, with β2 showing a positive correlation with age (r = 0.251, P = 0.013) and α2 correlating negatively with leukocyte count (r = -0.260, P = 0.009).

These correlations can be attributed to their shared origin and interconnected roles in the immune and inflammatory processes. Albumin and globulin fractions (α1, α2, β1, β2, and γ) are synthesized in the liver and immune cells, and their levels often fluctuate together in response to systemic inflammation, immune activation, or chronic disease states (10, 31).

Regarding the correlation between β2 and age, there is growing evidence that B2M

plays an important role in modulating inflammatory mediators, growth factors and hormones functions (32). In illness, increasing its levels contribute to cancer formation and metastasis, joint malfunction in hemodialytic patients and amyloid plaques formation (33) (34) (35). In a study by Althubiti et al. (36) found that B2M levels increased in plasma of old subjects compared to younger ones as there was a positive correlation between B2M levels and the age of participants ($p < 0.001$).

In our study, multivariate logistic regression identified higher α1 (OR = 2.617), α2 (OR = 1.528), β2 (OR = 2.241), and albumin percentage (OR = 1.128) as significant predictors of non-response to steroids, controlling for age and gender.

The study by Rodriguez-Otero et al. (29) revealed that α (1)-AT levels at the time of diagnosis are predictive for responses to treatment with an OR (95%CI) of 4.10 (0.98-17.1) and P value of 0.053. Bazzi et al. (30) found that ESRD can be predicted by α2m/C ($P< 0.0001$).

Jang et al. (37) found that pretreatment low serum albumin was a significant indicator of autoimmune encephalitis prognosis in the short-term and long-term with an OR (95% CI) of 0.13 (0.02–0.86) (P value = 0.034).

The limitations of the study were conducted at a single center, which may limit the generalizability of the findings to other populations. The relatively small sample size may affect the statistical power and robustness of the results. The follow-up period was limited to three months, potentially overlooking long-term treatment responses or relapses. Only corticosteroid therapy was assessed, excluding the evaluation of other treatment modalities. SPE was the sole immunological parameter studied, leaving other potential biomarkers unexamined.

Conclusion

SPE profiles, particularly α1, α2, β2 fractions, and albumin percentage, are significant predictors of steroid response in chronic ITP patients. Lower levels of α1, α2, β2, and albumin were associated with a better response to corticosteroids, highlighting their potential as biomarkers for treatment stratification.

Therefore, it is recommended that future studies include multicenter collaboration to enhance the generalizability of the results. A larger sample size should be considered to increase statistical power and validate findings. Long-term follow-up is suggested to assess sustained treatment responses and relapse rates. The inclusion of other therapeutic options, such as thrombopoietin receptor agonists or immunosuppressive agents, is recommended to provide a broader understanding of treatment responses. Additional immunological parameters and biomarkers should be investigated to further elucidate the pathophysiology and treatment predictors in ITP.

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