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Detection of erythrocyte membrane proteins by ELISA method in children with hereditary spherocytosis

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Abstract

The diagnosis of hereditary spherocytosis (HS) relies on a combination of clinical manifestations, hematologic parameters, and, where available, specialized laboratory techniques. In many settings, however, standard diagnostic tools such as eosin-5-maleimide (EMA) binding or osmotic gradient ektacytometry are not easily accessible, and practical quantitative approaches may be useful. In this study, erythrocyte membrane protein levels were measured in 20 children with HS and 20 healthy controls using enzyme-linked immunosorbent assay (ELISA) for ankyrin, α-spectrin, β-spectrin, band 3, and protein 4.2. Levels of α-spectrin, β-spectrin, and protein 4.2 were significantly lower in HS patients compared with controls (p=0.014, 0.008, and 0.034, respectively), whereas ankyrin and band 3 showed no significant differences. Discriminant analysis yielded 75% sensitivity and 75% specificity for distinguishing HS from controls. ELISA demonstrated group-level differences in multiple erythrocyte membrane proteins in pediatric HS. While not intended to replace guideline-endorsed methods, this practical and quantitative approach may be valuable in settings where standard diagnostic tools are not easily available. Further studies with larger cohorts are warranted to clarify its diagnostic contribution.

Keywords: Enzyme-linked immunosorbent assay (ELISA), hereditary spherocytosis, pediatrics

Introduction

Hereditary spherocytosis (HS) is one of the most common causes of hereditary hemolytic anemia among erythrocyte membrane disorders. Its symptoms typically include anemia, jaundice, and splenomegaly [1,2]. The clinical severity of this disease varies widely, ranging from asymptomatic carriers to patients with severe hemolysis. The need for blood transfusions in patients also varies according to these differences in severity. Some patients present with jaundice in the neonatal period and require exchange transfusion [3]. The frequency of HS ranges between 1:2000 and 1:5000 and can be observed in all racial and ethnic groups [1-4].

The primary pathophysiological defect in HS involves a loss of surface area in erythrocytes due to primary and secondary abnormalities in erythrocyte membrane proteins, particularly ankyrin, spectrin (α- and β-spectrin), band 3, and protein 4.2. This leads to the spherical shape of erythrocytes and a reduction in their ability to deform. Increased erythrocyte fragility, membrane loss, and poor deformability result in the rapid destruction of these cells in the spleen, leading to hemolysis [1,5]. This pathology is associated with mutations in the ANK1, SPTB, SPTA1, EPB4, and SLC4A1 genes [6]. Approximately 75% of cases show autosomal dominant inheritance, while the remaining cases arise from autosomal recessive inheritance or de novo mutations [1,2,5,7].

Despite the well-characterized pathogenesis, the diagnosis of HS remains challenging, especially in atypical or mild cases. Conventional diagnostic tools include the osmotic fragility (OF) test, acid glycerol lysis time (AGLT), and the eosin-5'-maleimide (EMA) binding test via flow cytometry. While EMA

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binding is currently one of the most widely used methods due to its high sensitivity and specificity, it predominantly detects band 3 deficiencies and may miss other membrane defects [8,9]. More advanced methods such as osmotic gradient ektacytometry and sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) are used in specialized centers but are time-consuming, expensive, and impractical for routine clinical use [8-10].

In this context, the enzyme-linked immunosorbent assay (ELISA) may represent a potentially useful adjunctive approach. ELISA allows for quantitative measurement of individual erythrocyte membrane proteins, is relatively low-cost, does not require radioactive materials, and can be performed in standard laboratory settings. Although its use for HS diagnosis was initially explored in the early 1990s, particularly in relation to spectrin and ankyrin deficiencies [11,12], there has been a striking absence of follow-up studies in the subsequent decades. The lack of adoption may reflect the historical emergence of flow cytometry techniques rather than intrinsic limitations of ELISA itself.

Given the increasing need for accessible, quantitative, and reproducible diagnostic tools—particularly in resource-limited settings—it is timely to revisit ELISA as a potential method for assessing erythrocyte membrane protein deficiencies in HS.

This study aimed to evaluate the levels of erythrocyte membrane proteins including α- and β-spectrin, ankyrin, band 3, and protein 4.2 in pediatric patients with HS using ELISA, and to explore whether this method could serve as a practical adjunct in the diagnostic evaluation of HS.

Material and Methods

Participants

This cross-sectional study was conducted with the approval of the Non-Interventional Clinical Research Ethics Committee of Adnan Menderes University Medical Faculty, protocol number 2017/1256, dated 26.10.2017, and with the support of the Adnan Menderes University Scientific Research Projects Coordination Unit under project number TPF-17067.

All patients diagnosed with HS at the Pediatric Hematology Department of Adnan Menderes University Medical Faculty between 2004 and 2017 were included in the study.

The family history, clinical features (jaundice, pallor, splenomegaly), hemoglobin, mean corpuscular volume (MCV) and MCHC levels, reticulocyte count, presence of spherocytes in the peripheral smear, total and indirect bilirubin levels, lactate dehydrogenase (LDH) levels, glucose-6-phosphate dehydrogenase (G6PD) and pyruvate kinase (PK) levels, and direct Coombs test results were recorded from the patients' files.

The diagnosis of HS was based on a positive family history, MCHC > 35 g/dL, the presence of spherocytes in peripheral blood smears, reticulocytosis, elevated serum total and indirect bilirubin levels, a negative direct Coombs test, and a positive OF test [5]. Other potential causes of hemolytic anemia, such as PK and G6PD deficiencies, were excluded.

Patients were separated into three groups (mild, moderate, and severe) according to the severity of illnesses based on Eber's criteria [13].

The control group consisted of age- and sex-matched children who presented to the general pediatric outpatient clinic for non-hematologic complaints, had normal complete blood counts, and no clinical or laboratory signs suggestive of hemolytic anemia or membrane disorders. Both the patients and the healthy group's families were informed about the study's purpose, why blood samples were taken, and the reasons for the tests, and written informed consent was obtained.

Collection of Samples and Analysis by ELISA Method

Blood samples were collected into EDTA tubes, centrifuged at 3,000 rpm for 5 minutes, and after separating the plasma, all samples were stored at –80°C until analysis. All tests were performed using commercial ELISA kits according to the manufacturers' protocols. Plasma samples were placed into the wells of antigen-coated plates, incubated at 37°C, and then antibodies were added. After washing steps, a substrate solution was introduced, and the reaction was stopped once color developed. The absorbance was read at 450 nm using a microplate reader (ELX800). Concentrations were automatically calculated from the standard curves provided by the kits.

The levels of Human Erythrocyte Membrane Protein Band 4.2 were measured using the Bioassay Technology Laboratory kit (catalog number E4632Hu; detection range 0.015–3.2 ng/mL). Human Band 3 protein was determined with the Bioassay Technology Laboratory kit (catalog number E4634Hu; detection range 0.009–2.0 ng/mL). Human SPTAN1 (α-spectrin) was determined using the Elabscience kit (catalog number E-EL-H1450; detection range 0.16–10 ng/mL). Human SPTβN4 (β-spectrin) was measured with the Elabscience kit (catalog number E-EL-H2406; detection range 0.16–10 ng/mL). Human Ank-B (ankyrin β) was measured with the Elabscience kit (catalog number E-EL-H0341; detection range 0.16–10 ng/mL).

Assay validation and analytical considerations

All measurements were performed with commercially available ELISA kits and according to the manufacturers' protocols (catalogue numbers and detection ranges are detailed above). These kits are not clinically validated for quantifying red cell membrane skeletal proteins in HS, and performance characteristics for this specific matrix (erythrocyte membranes) have not been established. ELISA quantifies epitope-specific antigen abundance and cannot distinguish protein isoforms or detect qualitative/functional defects. Pediatric reference intervals are lacking; therefore, and strictly for exploratory purposes, values

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below the minimum observed in the control group were treated as "low." No external calibration against guideline-endorsed methods (e.g., EMA binding, osmotic gradient ektacytometry) or genetic confirmation was performed in this study.

Statistical Analysis

Categorical variables were described using count (n) and percentage (%). The normality of continuous variables was assessed with the Kolmogorov-Smirnov test, and descriptive statistics were expressed as median (25th-75th percentiles). A p-value of <0.05 was considered statistically significant. The normality of erythrocyte membrane proteins analyzed by ELISA was also assessed using the Kolmogorov-Smirnov test, and all variables followed a normal distribution. Thus, descriptive statistics were presented as mean ± standard deviation. For normally distributed membrane protein levels, comparisons between HS patients and controls were made using the independent samples t-test. Due to the lack of established reference ranges for erythrocyte membrane proteins in children, values below the minimum level observed in the control group were pragmatically considered low for analytical purposes. Discriminant analysis identified proteins distinguishing patients from healthy individuals. Statistical analyses were performed using IBM SPSS Version 21 (IBM Corp., Armonk, NY).

A post-hoc power analysis was conducted to evaluate the adequacy of the sample size based on the difference in β-spectrin levels between the patient and control groups. Among the evaluated membrane proteins, β-spectrin demonstrated the largest between-group difference, and was therefore selected as the reference parameter for this analysis. Using the observed effect size (Cohen's d = 3.17), with 20 participants per group and a significance level of 0.05, the calculated statistical power was 1.0. This result indicates that the sample size was sufficient to detect this difference with high confidence.

Results

Demographic and Clinical Characteristics of Participants

Table 1 shows the demographic, clinical, and laboratory findings of the patient and healthy groups. A total of 40 children, including 20 patients and 20 healthy controls, participated in the study. Of the patients, 55% (n=11) were female, and 65% (n=13) of the healthy group were female (p=0.531). The median age of both the patient and healthy groups was eight years (IQR: 6.0-14.0) (p=0.951). The median age at HS diagnosis was found to be three years (IQR: 2.0-5.0). When the symptoms and findings at the time of diagnosis were evaluated, 5% (n=1) of the patients had fatigue, 80% (n=16) had pallor, 10% (n=2) had anemia, 15% (n=3) had neonatal jaundice, 60% (n=12) had jaundice, 10% (n=2) had gallstones, and 45% (n=9) had splenomegaly. Fifteen percent (n=3) of the patients were investigated due to a family history. A history of neonatal jaundice within the first 24 hours was present in 15% (n=3) of the patients. Exchange transfusion was performed in 5% (n=1) of the patients. Fifty percent (n=10) of the patients had a family history of HS, 40% (n=8) had a history of splenectomy, and 25% (n=5) had early-onset cholelithiasis in their families. Gallstones were present in 25% (n=5) of the patients. Fifteen percent (n=3) of the patients had undergone cholecystectomy and splenectomy. Fifty percent (n=10) of the patients had received at least one red blood cell transfusion. Seventy percent (n=14) were regularly taking folic acid. While 20% (n=4) of the patients were receiving regular red blood cell transfusions, 50% (n=2) of them no longer required transfusions after the age of two.

The mean hemoglobin level at presentation was 8.7±2.5 g/dL in patients with HS and 12.4±0.7 g/dL in the healthy group (p<0.001). MCV and MCHC at presentation were 79±7.0 fL and 34.3±1.8 g/dL in the patient group, and 80.9±4.9 fL and 33.9±0.8 g/dL in the healthy group, respectively (p=0.337 and p=0.322). When classified according to the hemoglobin levels at diagnosis, 45% (n=9) of the patients were assessed as having severe HS, 40% (n=8) as moderate, and 15% (n=3) as mild HS. All of the patients with severe HS had received at least one red blood cell transfusion. In 22.22% (n=2) of severe HS cases, there was a history of cholelithiasis, and in 33.33% (n=3), there was a history of splenectomy and cholecystectomy. The frequency of red blood cell transfusions and the rates of splenectomy and cholecystectomy were significantly higher in patients with severe HS compared to those with mild and moderate HS (p<0.001 for each). At the time of presentation, 90% (n=18) of the HS patients had spherocytes on peripheral smear, and 65% (n=13) had signs of hemolysis. The peripheral smears of the healthy group were normal (p<0.001). At diagnosis, the median corrected reticulocyte percentage of the patients was 5% (IQR: 0.9-10.3), the median total bilirubin level was 3.7 mg/dL (IQR: 0.6-6.4), the median indirect bilirubin level was 3.3 mg/dL (IQR: 0.4-5.8), and the median LDH level was 400 IU (IQR: 332-628.8). The direct agglutination test was negative in all patients. G6PD and PK levels were within normal ranges.

ELISA Test Results

Table 2 shows the erythrocyte membrane protein levels in the healthy group. In the healthy group, the lowest ankyrin level was found to be 2.689 ng/mL, with 30% (n=6) of the patients having ankyrin levels below this value. The lowest spectrin α level in the healthy group was 2.055 ng/mL, with 10% (n=2) of the patients having levels below this value. The lowest spectrin β level in the healthy group was 2.43 ng/mL, with 25% (n=5) of the patients having levels below this value. The lowest band 3 level in the healthy group was 0.533 ng/mL, with 10% (n=2) of the patients having levels below this value. The lowest protein 4.2 level in the healthy group was 0.656 ng/mL, with 45% (n=9) of the patients having levels below this value.

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Table 1. Demographic, clinical and laboratory findings of the participants

Variable	Patient group (n=20)	Healthy group (n=20)	p-value
Median age of the participants (years)	8.0 (IQR: 6.0-14.0)	8.0 (IQR: 6.0-14.0)	0.951
Gender (female)	65% (n=13)	55% (n=11)	0.531
Median age at diagnosis (years)	3.0 (IQR: 2.0-5.0)		
Patients' symptoms and findings at the time of diagnosis			
Fatigue	5% (n=1)		
Pallor	80% (n=16)		
Anemia	10% (n=2)		
Neonatal jaundice	15% (n=3)		
Jaundice	60% (n=12)		
Gallstones	10% (n=2)		
Splenomegaly	45% (n=9)		
Family history	15% (n=3)		
General characteristics of the patients			
Severe HS at the time of diagnosis	45% (n=9)		
Moderate HS at the time of diagnosis	40% (n=8)		
Mild HS at the time of diagnosis	15% (n=3)		
Family history of HS	50% (n=10)		
Family history of early-onset gallstones	25% (n=5)		
Family history of splenectomy	40% (n=8)		
At least one red blood cell transfusion	50% (n=10)		
Regular red blood cell transfusion	10% (n=2)		
Folic acid usage	70% (n=14)		
Gallstones	25% (n=5)		
Cholecystectomy	15% (n=3)		
Splenectomy	15% (n=3)		
Laboratory findings of the participants			
Mean hemoglobin level at presentation (g/dL)	8.7±2.5	12.4±0.7	<0.001
MCV at presentation (fL)	79.0±7.0	80.9±4.9	0.337
MCHC at presentation (gr/dL)	34.3±1.8	33.9±0.8	0.322
Spherocytes on peripheral smear at presentation	90% (n=18)	0% (n=0)	<0.001
Signs of hemolysis on peripheral smear at presentation	65% (n=13)	0% (n=0)	<0.001
Median corrected reticulocyte percentage at the time of diagnosis	5% (IQR: 0.9-10.3)		
Median total bilirubin level at the time of diagnosis (mg/dL)	3.7 (IQR: 0.6-6.4)		
Median indirect bilirubin level at the time of diagnosis (mg/dL)	3.3 (IQR: 0.4-5.8)		
Median LDH level at the time of diagnosis (IU)	400 (IQR: 332-628.8)		
Direct Coombs test negativity	100% (n=20)		

n: number of individuals; IQR: interquartile range; HS: hereditary spherocytosis; g/dL: gram/deciliter; MCV: mean corpuscular volume; fL: femtoliter; MCHC: mean corpuscular hemoglobin concentration; mg/dL: milligram/deciliter; LDH: lactate dehydrogenase; IU: International unit

In 30% (n=6) of the patients, erythrocyte membrane proteins were found to be normal. Of these patients, 50% (n=3) had severe HS, with two of them having undergone splenectomy and cholecystectomy, while the other patient was receiving regular transfusions. The remaining 50% (n=3) were diagnosed with mild HS. All three of these patients had a family history of HS. Two of them had no clinical symptoms, while one patient had gallstones. In our study, 35% (n=7) of the patients were found to have combined protein deficiencies, involving at least two protein deficiencies. Among these patients, 57.14% (n=4) had two protein deficiencies, and 42.86% (n=3) had three protein deficiencies. The distribution of combined protein deficiencies is shown in Table 3. Of the patients with combined protein deficiencies, 71.42% (n=5) had severe HS, 14.29% (n=1) had

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moderate HS, and 14.29% (n=1) had mild HS, with one patient being dependent on red blood cell transfusions. Additionally, three of these patients had a family history of HS, and one had undergone splenectomy and cholecystectomy. When erythrocyte membrane protein levels were evaluated separately, no statistically significant difference was found between the ankyrin levels of the patient and healthy groups (p=0.162). The spectrin α level in the patients was significantly lower than in the healthy group (p=0.014). The most significant difference between the patient and healthy groups was in spectrin β. Spectrin β was the lowest protein detected in the patient group compared to the healthy group (p=0.008). When looking at band 3 levels, there was no statistically significant difference between the patient and healthy groups (p=0.362). The protein 4.2 level was significantly lower in the patient group compared to the healthy group (p=0.034). The comparison of erythrocyte membrane protein levels between the patient and healthy groups is presented in Table 4. As a result of the discriminant analysis, the sensitivity and specificity of the ELISA test in diagnosing HS were found to be 75%. The most effective proteins in achieving this were spectrin β and protein 4.2.

Table 2. Erythrocyte membrane protein levels in the healthy group (ng/mL)

Protein	Number of individuals (n)	Minimum value	Maximum value	Mean value	Standard Deviation
Band 3	20	0.533	1.998	1.070	0.407
Protein 4.2	20	0.656	1.867	1.067	0.345
Ankyrin	20	2.689	4.506	3.745	0.473
Spectrin α	20	2.055	4.954	3.456	0.988
Spectrin β	20	2.437	5.170	3.549	0.882

ng/mL: nanogram/milliliter

Table 3. Distribution of combined protein deficiencies

Protein deficiency	Patient group (n=20)
Ankyrin + Protein 4.2	10% (n=2)
Spectrin β + Protein 4.2	10% (n=2)
Ankyrin + Spectrin α + Spectrin β	5% (n=1)
Ankyrin + Spectrin α + Protein 4.2	5% (n=1)
Spectrin β + Band 3 + Protein 4.2	5% (n=1)

Table 4. Comparison of erythrocyte membrane protein levels between the patient and healthy groups

Protein	p-value (t-test)	Mean difference	Standard error of the difference
Band 3	0.362	0.115	0.125
Protein 4.2	0.034	0.262	0.119
Ankyrin	0.162	0.333	0.234
Spectrin α	0.014	0.702	0.269
Spectrin β	0.008	0.673	0.242

Discussion

In this study, erythrocyte membrane proteins were quantitatively evaluated using the ELISA method in pediatric patients diagnosed with HS.

Hereditary spherocytosis is a genetically heterogeneous red blood cell membrane disorder, most commonly involving variants in genes encoding key membrane and cytoskeletal proteins such as ANK1, SPTA1, SPTB, SLC4A1, and EPB41. Such alterations lead to reduced membrane surface area, impaired vertical or horizontal protein interactions, and increased membrane fragility, ultimately resulting in premature erythrocyte destruction. This genotypic diversity contributes to the heterogeneity of clinical manifestations and laboratory findings, and may also lead to variability in the results obtained from different diagnostic methods used to assess membrane integrity or protein expression. [2,7].

Although ELISA was first proposed as a diagnostic method for HS in the early 1990s, particularly by Pekrun et al. [12], it has not gained traction in clinical practice, and no subsequent studies have systematically re-evaluated its potential. This may be due in part to the growing adoption of flow cytometry–based EMA testing and the increased availability of osmotic gradient ektacytometry in specialized centers. However, such resources remain inaccessible in many clinical settings, especially in low-resource environments. Revisiting ELISA under these circumstances may help bridge

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the diagnostic gap. Compared to conventional approaches, ELISA offers several practical advantages. It allows for quantitative assessment of individual membrane proteins, is cost-effective, does not require specialized instrumentation, and is well-suited for standard laboratory conditions. In our study, ELISA demonstrated 75% sensitivity and specificity for diagnosing HS. While this performance is promising, it should be interpreted in context. The test identified membrane protein abnormalities in the majority of patients; however, six patients exhibited normal quantitative levels of all measured proteins. This may be due to biological variability, but it is also plausible that these individuals have qualitative defects—such as missense mutations or conformational changes—that do not affect protein abundance but impair function. Because ELISA relies on antigen-antibody binding, it cannot detect structural or functional anomalies beyond the targeted epitope, limiting its ability to detect all pathogenic variants. Current diagnostic standards for HS rely on a combination of clinical findings, laboratory abnormalities, and tests such as the OF test, AGLT, and the EMA binding test [14,15]. While OF has historically been used, its sensitivity is limited—68% in fresh blood and 81% in incubated samples [15]—and may be especially poor in compensated cases. Accordingly, guidelines by Bolton-Maggs et al. advise against routine OF testing, noting that a negative result does not rule out HS in the presence of suggestive clinical findings [16]. Several advanced diagnostic tools have been developed to overcome these limitations. The Pink test, AGLT, SDS-PAGE, cryohemolysis, EMA testing, and osmotic gradient ektacytometry offer increasingly refined measurements of red cell membrane stability and deformability [17]. The EMA test, in particular, has gained prominence for its high sensitivity and specificity, but it mainly detects band 3 deficiencies and may miss spectrin or ankyrin abnormalities [18-20]. SDS-PAGE provides semiquantitative information on protein content but is technically demanding and may overlook subtle or functional defects [21]. Osmotic gradient ektacytometry is currently considered the gold standard for assessing red cell deformability and membrane surface area; however, it is available only in specialized centers due to its complexity, cost, and need for technical expertise [9,22,23]. Spectrin and ankyrin proteins can also be measured using radioimmunoassay (RIA) or ELISA techniques, though these are not routinely employed in clinical laboratories [12,24]. Current guidelines recommend the EMA binding test and, where available, osmotic gradient ektacytometry as the most reliable diagnostic approaches for HS [20,23]. These methods provide high sensitivity and specificity but require specialized equipment and expertise. In many centers, such resources are not readily accessible. In this context, practical and quantitative methods such as ELISA may serve as a supplementary option rather than a replacement for guideline-endorsed tools. Our findings highlight that, although ELISA identified group-level differences in selected proteins, it should be regarded as an adjunctive method with potential value in resource-limited settings. Our findings align with prior reports in the literature. Ayhan et al. reported membrane protein abnormalities in only 42% of patients using SDS-PAGE, without correlation to hemoglobin levels [21]. In our study, while we did not perform statistical correlation between protein levels and clinical severity, this relationship warrants future investigation. Bianchi et al. observed that AGLT and Pink tests had sensitivities of 95% and 91%, respectively, while the EMA test demonstrated 93% sensitivity, independent of clinical phenotype [15]. Crisp et al. reported high specificities for cryohemolysis (95.2%) and EMA (95.9%), with spectrin and ankyrin defects detected in 72.3% of affected family members [25]. King et al. found that EMA had 92.7% sensitivity and 99.1% specificity and proposed it as a frontline diagnostic tool [17]. Girodon et al. similarly showed high performance of EMA even in compensated anemia [26]. Mariani et al. demonstrated that combining AGLT with OF testing increased sensitivity to 99% [27]. In a recent study, Beltrán et al. reported 100% sensitivity and 93.3% specificity for a flow cytometry–based HS diagnostic protocol in children [9].

One of the strengths of our study lies in its consistent application of ELISA across all samples, enabling standardized and simultaneous quantification of five major erythrocyte membrane proteins. To our knowledge, this is the first pediatric study to evaluate ELISA for this purpose using a multi-protein panel. The use of a practical, scalable method such as ELISA is particularly relevant for clinical settings that lack access to flow cytometry or mechanical ektacytometry devices.

This study has several limitations. Commercially available ELISA kits used here have not been clinically validated for red cell membrane protein quantification, and performance characteristics for this specific application remain uncertain. ELISA is epitope-based and cannot distinguish isoforms or detect qualitative/functional protein defects, which may explain the presence of clinically severe cases with normal quantitative results. The sample size was relatively small, and genetic confirmation of membrane defects was not performed. In addition, protein deficiencies were pragmatically defined as values below the minimum observed in the control group in the absence of established pediatric reference ranges. For these reasons, the results should be considered exploratory. Larger, genetically characterized cohorts are required to validate diagnostic thresholds and to better define the potential role of ELISA in clinical practice.

Conclusion

In conclusion, this study demonstrates that ELISA may serve as a feasible and informative method for quantifying erythrocyte membrane proteins in pediatric HS. While not intended to replace established diagnostic tools, its ease of use, low cost, and ability to evaluate multiple proteins simultaneously make it a promising adjunct, particularly in resource-limited settings. By revisiting a previously overlooked technique, our findings contribute to the ongoing search for accessible and quantitative diagnostic

approaches in HS. With further standardization, clearly defined diagnostic thresholds, and validation in larger cohorts, ELISA has the potential to complement existing diagnostic workflows and improve membrane protein assessment in clinical hematology.

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Conflict of Interests
The authors declare that there is no conflict of interest in the study.

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Ethical Approval
This cross-sectional study was conducted with the approval of the Non-Interventional Clinical Research Ethics Committee of Adnan Menderes University Medical Faculty, protocol number 2017/1256, dated 26.10.2017.

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