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Validity of Shine and Lal Index {MCV2XMCHX0.01} in Predicting Iron Deficiency Anemia

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ABSTRACT

Objective: To determine the diagnostic accuracy of the Shine and Lal index in detecting the presence of iron deficiency anaemia, keeping serum ferritin levels and serum transferrin saturation as the gold standard.

Study Design: Cross-sectional validation study.

Place and Duration of Study: Department of Paediatrics, Pak-Emirates Military Hospital, Rawalpindi Pakistan, Nov 2021 to May 2022.

Methodology: We studied a total of 113 patients who presented with microcytic anaemia were included for study. Patients diagnosed as cases of thalassemia, haemolytic anaemia, iron deficiency anaemia, had received blood transfusions within the past three months, or were receiving iron therapy within the six months were excluded. The diagnosis of iron deficiency was based on a serum ferritin level of less than 14 ng/mL and/or a transferrin saturation of less than 20%. A Shine and Lal index greater than 1530 was considered diagnostic for iron deficiency anaemia.

Results: The mean age of our sample was 6.64±2.89 years, 52.2% of whom were male. The Shine and Lal index carried a sensitivity of 34.8%, a specificity of 74.6% and a diagnostic accuracy of 58.4% in predicting the presence of iron deficiency anaemia, using a cut-off level greater than 1530. A total of 9.7% of patients tested positive for β-thalassemia trait.

Conclusion: The Shine and Lal index can be employed as a useful, rapid screening test performed on indices readily available as a standard part of complete blood counts but should not be used in isolation.

Keywords: Haemolytic anaemia, Iron deficiency anaemia, Shine and lal index, Thalassemia.

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INTRODUCTION

The prevalence of iron deficiency anaemia varies between regions, with some parts of the world having between 3% and 48% of the paediatric age group affected by the disorder.[1] Developing countries are estimated to account for the vast majority of the global iron deficiency disability burden.[2] In Pakistan, an estimated 26.7% of all children are thought to be suffering from the disorder.[3]

Iron deficiency manifests as a microcytic anaemia on complete blood counts, however, this finding in itself is insufficient as a screening tool for this disorder as a major differential includes β-thalassemia, especially in patients who have only the trait, a condition that is especially common in South Asia, due to the high occurrence of consanguineous marriages.[4,5] Thus, the diagnosis of iron deficiency requires further testing including serum ferritin levels and, in cases where ferritin levels are not able to establish a clear diagnosis, transferrin saturation, which is calculated using the serum iron and total iron binding capacity levels.[6] In addition, β-thalassemia requires screening in these patients, which relies on the use of more expensive and sometimes not readily available, techniques such as High Performance Liquid Chromatography (HPLC) or Polymerase Chain Reaction (PCR) tests.[7,8] In order to differentiate between patients who have a higher suspicion for the presence of iron deficiency anaemia versus β-thalassemia or vice versa, indices based on blood count parameters, such as the Mentzer index and the Shine and Lal index, have been developed as screening tools.[9,10]

We conducted this study to determine the diagnostic accuracy of the Shine and Lal index in detecting iron deficiency anaemia, using this index as a screening test. The rationale behind this objective was reducing the volume of HPLC and/or PCR prescriptions to rule out β-thalassemia trait in patients presenting with microcytic anaemia. If found to be a useful test, using the Shine and Lal index in patients with microcytic anaemia would reduce the number of patients prescribed these sometimes-unaffordable tests and reduce the requirement for follow-up visits.

METHODOLOGY

The cross-sectional validation study was conducted from November 2021 to May 2022 at the

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Department of Paediatrics, Pak-Emirates Military Hospital, Rawalpindi Pakistan. The WHO sample size calculator was used to calculate the sample size, keeping an expected sensitivity of 80.0%, expected specificity of 23.7%, expected prevalence of 92.1%.[11]

Inclusion Criteria: Patients of either gender aged 2-12 years, with a mean corpuscular volume (MCV) of less than 78 fL, were included in the study.

Exclusion Criteria: Patients who were diagnosed with cases of thalassemia, haemolytic anaemia, iron deficiency anaemia, had received blood transfusions within the past three months, or were receiving iron therapy within the six months were excluded.

The participants underwent a clinical history and examination on inclusion. A reconfirmation of low MCV followed this via phlebotomy and testing of the blood sample to obtain full blood counts (Sysmex XW-100 Haematology Analyzer). Once microcytosis was identified, the patient was tested for serum ferritin levels (Eurolyser Smart 700/546 Assay). Iron deficiency was a serum ferritin level of less than 14 ng/mL. Patients also underwent testing for serum iron and total iron binding capacity (Dimension EXL 200 Integrated Chemistry System), considering the possibility of iron deficiency anaemia with high serum ferritin levels (e.g., due to an acute inflammatory state). Patients with a transferrin saturation of less than 20% were considered iron deficient. Any one of low serum ferritin or transferrin saturation was considered sufficient in diagnosing iron deficiency. A Shine and Lal index of greater than 1530 indicated iron deficiency anaemia, while a figure less than that was considered to be associated with the thalassemia trait. Patients subsequently underwent testing using haemoglobin electrophoresis.

Statistical Package for Social Sciences (SPSS) version 23.0 was used for the data analysis. Quantitative variables were expressed as Mean±SD and qualitative variables were expressed as frequency and percentages. Chi-square test was applied to explore the inferential statistics. The *p*-value of ≤0.05 was considered statistically significant. Diagnostic parameters were calculated using a 2x2 table. Sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy were determined by using the standard formulae.

RESULTS

We studied 113 patients with the mean age of 6.64±2.89 years. The mean haemoglobin level was 9.22±1.11 g/dL. The mean value of the Shine and Lal index for the entire sample was 1375.39±214.90. According to the Shine and Lal index, 33(29.2%) patients were iron deficient. The mean serum ferritin level of the sample was 37.41±33.02 ng/mL, while the mean transferrin saturation was 33.72±24.80%. Lastly, 11 (9.7%) patients tested positive for the β-thalassemia trait, one of whom was also iron deficient. The patient characteristics and results according to gender are displayed in Table-I. We constructed a 2 x 2 contigency table, shown as Table-II, to determine the various test parameters, such as sensitivity and specificity of the Shine and Lal Index, in predicting the presence of iron deficiency anaemia. The characteristics of the Shine and Lal index in predicting the presence of iron deficiency anaemia are shown in Table-III. We found it to have a sensitivity of 34.8%, a specificity of 74.6% and a diagnostic accuracy of 58.4%, using a cut-off level of greater than 1530 to indicate the presence of iron deficiency.

Table-I: Study characteristics according to gender (n=113)

Variables	Male	Female	*p*-value
Gender	59(52.2%)	54(47.8%)	-
Age (years)	6.81±2.89	6.44±2.89	0.499
Weight (kg)	25.78±10.88	25.55±12.50	0.917
Haemoglobin Level (g/dL)	9.27±1.11	9.17±1.12	0.620
Mean Corpuscular Volume (fL)	70.22±5.37	72.95±2.69	0.003
Mean orpuscular Haemoglobin (pg)	26.53±1.68	26.92±1.57	0.174
Shine and Lal Index	1319.62±250.49	1436.31±147.25	0.004
Iron Deficiency Anaemia according to Shine and Lal Index			
Yes	17(28.8%)	16(29.6%)	0.924
No	42(71.2%)	38(70.4%)	
Serum Ferritin Level (ng/mL)	40.85±36.02	33.65±29.27	0.249
Transferrin Saturation	33.54±25.56	33.91±24.17	0.938
Iron Deficiency Anaemia according to Serum Ferritin Level/Transferrin Saturation			
Yes	23(38.9%)	23(42.6%)	0.696
No	36(61.1%)	31(57.4%)	
β-Thalassemia Trait	4(6.8%)	7(12.9%)	0.268

Table-II: Contingency table for shine and lal index (n=113)

		Presence of Iron Deficiency Anaemia According to Serum Ferritin Level/Transferrin Saturation	
		Yes	No
Prediction of Iron Deficiency according to Shine and Lal Index	Yes	True Positive: 50(44.2%)	False Positive: 30(26.5%)
	No	False Negative: 17(15.1%)	True Negative: 16(14.2%)

Table-III: Diagnostic Parameters (n=113)

Test	Sensitivity	Specificity	Positive Predictive Value	Negative Predictive Value	Diagnostic Accuracy
Shine and Lal Index	34.8%	74.6%	48.5%	62.5%	58.4%

DISCUSSION

Iron deficiency in Pakistani paediatric population is one of the most common causes of microcytic anaemia. However, the β-thalassemia trait represents a major differential diagnosis which is thought to occur in anywhere between 5-8% of the in this country.[12,13] To avoid burdening patients with costly investigations, most physicians opt to place patients on trials of treatment using iron therapy and to monitor for improvement, but this is not without risk as patients of β-thalassemia trait are at an increased risk of iron overload and iron supplements should be avoided.[14,15] Moreover, the existence of both conditions in a concurrent fashion further complicates matters.[16]

Various methods have been proposed to fulfil this role, some of which include the Mentzer index, the Shine and Lal index, Red Cell Distribution Width (RDW) and the England and Fraser index, among others, with varying degrees of success.[17,18] Our study looked at the Shine and Lal index in this capacity and found that it had a sensitivity of 34.8%, a specificity of 74.6%, a positive predictive value of 48.5%, a negative predictive value of 62.5% and a diagnostic accuracy of 58.4%. A total of 29.2% of patients were iron deficient, according to this index. Ullah *et al*. reported that this test was 100% sensitive but only 39% specific in differentiating the β-thalassemia trait from iron deficiency anaemia. However, they did not mention what cut-off value they used for the index.[19] Kar *et al*. in their study in a Turkish population, noted that the Shine and Lal index had a sensitivity of 100% and a specificity of 0%, at a cut-off of 1530 and suggested that a lower cut-off value of 571 would carry a sensitivity of 88.0% and a specificity of 38.0%,[20] while another Turkish study by Nalbantoğlu *et al*. also noted a similar sensitivity and specificity of 87.1% and 37.5%, respectively.[21]

There is a great degree of heterogeneity in the characteristics of the results quoted across the literature. We believe that several reasons are responsible for this variability: First, some studies have not described the cut-off level for Shine and Lal index, which naturally alters the sensitivity and specificity reported. Second, the gold standard for comparison to distinguish iron deficiency from the β-thalassemia trait has usually been haemoglobin electrophoresis, which, although an excellent test, is not completely sensitive and specific. In addition, different studies have used different cut-off values for haemoglobin A2, which may have produced a level of confounding. Third, there appears to be a variation in how effective the index is based on race/ethnicity, which requires further study. Lastly, there was no standardization in how the red blood cell parameters were obtained across different studies, which would also produce a variation in the results. Regardless of the issues mentioned above, it is quite clear that the degree of variability in the diagnostic accuracy of the Shine and Lal index in predicting the presence of iron deficiency anaemia precludes it as a diagnostic test. However, it may still have a role as a screening utility.

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LIMITATION OF STUDY

Our study was limited to a single centre, with a comparatively small sample size; Pakistan is an ethnically diverse country and determining whether our results apply to all ethnicities is an aspect this study is lacking. Lastly, the study would have been further strengthened if comparison could have been established with other indices.

CONCLUSION

The prescription of investigations in resource-poor areas such as Pakistan is dictated by the patient's finances. Therefore, performing expensive discriminatory tests to differentiate iron deficiency from the β-thalassemia trait is problematic. This is even more concerning, considering the high prevalence of the diseases mentioned above in our population. Surrogate indicators such as the Shine and Lal index have been developed to distinguish the two. However, it does not appear to be completely satisfactory in this role.

Conflict of Interest: None.

Author's Contribution

Following authors have made substantial contributions to the manuscript as under:

SH: & SZ: Data acquisition, data analysis, critical review, approval of the final version to be published.

ST: & HZ: Study design, drafting the manuscript, data interpretation, critical review, approval of the final version to be published.

AAR: & ANA: Concept, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of

any part of the work are appropriately investigated and resolved.

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