



BETA THALASSEMIA TRAIT; DIAGNOSTIC IMPORTANCE OF HAEMATOLOGICAL INDICES IN DETECTING BETA THALASSEMIA TRAIT PATIENTS

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ABSTRACT... Objectives: To assess the sensitivity of the various discrimination indices as screening test in beta-thalassemia trait patients in our population. **Study design:** Cross sectional descriptive study. **Period:** 1-Sep 2012 to 31-Jan 2013 (5 months). **Settings:** Pathology Department, District Head Quarters (DHQ) Hospital, Rawalpindi. **Materials and Methods:** A total of 150 diagnosed cases of beta thalassemia trait were included in the study. Study was done from 1-Sep 2012 to 31-Jan 2013 in DHQ hospital, Rawalpindi. Four discrimination indices i.e. Mentzer, Shine & Lal, Srivastava, and Red cell Distribution Width Index were calculated for all the patients. The number of correctly identified cases were determined and sensitivity of each discrimination index was calculated accordingly. **Results:** The sensitivity in diagnosing beta thalassemia trait patients was highest for Shine and Lal index (95%), followed by Mentzer index (52% sensitivity), and then Srivastava index (46% sensitivity). Red cell distribution width index had poor sensitivity of 2% in our study. None of the discrimination indices showed 100% sensitivity. **Conclusion:** We concluded that Shine & Lal and Mentzer index are the most sensitive indices. They can be used for cheap and quick screening of beta-thalassemia trait patients in laboratories where advanced investigation tools are not available. Patients who give positive result for beta thalassemia trait with these indices may then be referred for further workup to confirm the diagnosis.

Key words: Beta-thalassemia Trait, Discrimination Index, Shine and Lal Index, Mentzer Index, Sensitivity, Srivastava Index.

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INTRODUCTION

Thalassemia is a genetic haematological disorder in which there is impaired synthesis of globin chain of haemoglobin.¹ It is inherited in an autosomal recessive pattern.¹ The word "Thalassemia" arises from two Greek words, i.e. "thalassa", which means "sea" and "mias", which means "blood".^{2,3} It was named so because its prevalence was higher in people living in Mediterranean countries.^{2,3} Thalassemia is divided into two subtypes, i.e. alpha thalassemia and beta thalassaemia.² Beta thalassaemia is genetic disorder in which there is impaired synthesis of beta globin chain.² It is caused due to mutation in beta globin gene located on chromosome 11.² Beta thalassemia is further divided into the beta thalassemia major and beta thalassaemia minor.² The mutations are homozygous in the former, and heterozygous in the latter.²

Beta Thalassemia is prevalent worldwide.¹ According to one estimate, about 60000 newborns are born with thalassemia every year all over the world.¹ About 79% of affected babies are born in the Asian countries.¹ The worldwide prevalence of Beta Thalassemia Trait varies from 1.7 - 9 %.² In Pakistan, the carrier rate of Beta Thalassemia Trait ranges from 1%- 7%.² Approximately, 5000 children are diagnosed with beta thalassemia each year in Pakistan.^{1,4,5} About 25,000 children are registered with thalassemia federation in Pakistan so far.¹ But the actual number of patients may be higher than this because a lot of people are living in villages and they are not registered with thalassemia centers.¹ This high prevalence of Thalassemia in Pakistani population is due to the trend of consanguinous marriages in families.¹

The patients with beta thalassemia trait are usually

asymptomatic.^{2,6} Such patients usually have features of mild anemia.² But they do not respond to iron supplements.^{2,6} When asked, such patients give positive history of consanguinous marriages in their family, and the parents of such patients are usually first or second cousins.²

The beta thalassemia trait patients come to notice by finding of microcytic hypochromic red blood cells on peripheral blood film.² The laboratory tests required for the diagnosis of beta thalassemia include hemoglobin concentration, red blood cell count and mean corpuscular volume, with addition of examination of peripheral blood smear.^{2,6,7} Usually, the complete blood counts show low hemoglobin, decreased MCV of less than 75 fL, low MCH of less than 25 pg and raised RBC count > 5.0 millions/cmm.² The final definitive diagnosis of beta thalassemia trait is made by Haemoglobin electrophoresis.² An elevated Hemoglobin A2 level of more than 3.5 % is diagnostic of beta thalassaemia trait.²

Microcytic hypochromic blood picture is seen in beta thalassemia trait as well as iron deficiency anemia.^{6,7,8} Serum ferritin, iron and HbA2 level are required to differentiate beta thalassemia trait from iron deficiency anemia.^{6,9} Beta thalassemia should be differentiated from iron deficiency anemia because hemoglobin level does not improve in the earlier and iron supplements in such cases may lead to iron overload.^{6,10} This causes increased morbidity and mortality in such patients.^{6,10} Also, if patient of beta thalassemia trait is misdiagnosed as iron deficiency, he or she may get married to a beta thalassemia trait patient, and this may result in homozygous or thalassemia major children in next generation.^{6,11}

A number of studies show that red cell indices can be helpful in giving probable diagnosis of beta thalassemia trait.^{6,10,12,13} There are different discrimination indices that are used for this purpose ; i.e Srivastava index , Shine and Lal index, England and Fraser index, Mentzer index, Ricerca index, and Red cell Distribution Width index.^{14,15,16,17,18,19,20} Peripheral smear examination along with red blood cell indices are reliable and easy tool for initial screening of thalassemia trait

patients.² Screening should be done to identify carriers of beta thalassemia, and thus to reduce the rate of birth of affected infants.² Screening should be done for those who are candidates for marriage or at prenatal clinics.²

The present study was done to assess the usefulness of four discrimination factors i.e Shine and Lal index, Srivastava index, Mentzer index, and Red cell Distribution Width index by calculating their sensitivity in giving probable diagnosis of beta thalassemia trait patients. An ideal discrimination index is the one that has high sensitivity and is easy to calculate.

OBJECTIVE

The objective of the study was to assess the sensitivity of the various discrimination indices as screening test in beta-thalassemia trait patients in our population.

Setting

The study was done in Pathology Department, District Head Quarters (DHQ) Hospital, Rawalpindi.

Study Period

The study was conducted from 1-Sep 2012 to 31-Jan 2013 (5 months).

Sample Size

Blood samples of 150 patients already diagnosed for beta thalassemia trait were taken. Sample size was calculated based on estimated numbers of samples that need to be analyzed considering the estimated proportion of thalassemia, precision value 0.05 and confidence interval of 95%.

Sampling Technique

Non probability purposive sampling.

SAMPLE SELECTION

Inclusion Criteria

1. Patients with the family history of thalassemia.
2. Those who are not transfused.
3. The study subjects, who has hemoglobin <12 g/dl with increased RBC count.

Exclusion Criteria

1. Subjects having Hemoglobin > 13.5g/dl.
2. Patients having conditions such as acute and chronic inflammatory diseases, infections, hypothyroidism, acute bleeding and any kind of malignancy that may alter the level of ferritin and hemoglobin.
3. Patients who were transfused in last 3 months.

Study Design

It was a Cross sectional descriptive study.

Procedure

Venous blood samples were taken from all the candidates. The complete blood count and hemoglobin electrophoresis was done. HbA2 level of above 3.5% was considered as beta thalassemia trait.^{21,22} The value of four discriminatory indices i.e Shine and Lal, Mentzer, Srivastava and Red cell distribution width were

calculated from hematological parameters provided by the automated analyzer. The cutoff values of these discriminatory factors are shown in Table-I.

Data Analysis

Data was analyzed using a statistical tool SPSS 16. The qualitative data was analyzed in terms of frequency and percentages.

RESULTS

Total 150 diagnosed cases of beta thalassemia trait patients (N=150) were included in this study. Out of these, 50 patients (25%) were male and 100 patients (75%) were females.

The sensitivity of different discrimination indices for 150 cases of beta thalassemia trait are given in Table-II.

Hematological Index	Formula	Beta Thalassemia Trait	Iron Deficiency Anemia
Mentzer index ^[1,2,10]	MCV/RBC count	<13	>13
Srivastava index ^[1,3,11]	MCH/RBC count	<3.8	>3.8
Shine and Lal index ^[1,4,12]	MCV ² x MCH/100	<1530	>1530
Red cell Distribution Width index ^[1,2,3,4]	MCVxRDW/RBC	<220	>220

Table-I. Haematological indices to detect beta thalassemia trait, and differentiate it from iron deficiency anemia

Differentiation Index	Number of cases diagnosed as beta thalassemia trait by the differentiation factor (True positives)	Number of cases diagnosed as non-beta thalassemia trait i.e iron deficiency anemia by the Differentiation Factor (False negatives)	Sensitivity of the discrimination factor
Mentzer's index	78	72	52%
Srivastava index	69	81	46%
Shine and Lal index	142	8	95%
Red cell distribution width index	3	142	2%

Table-II. Sensitivity of different differentiation factors in diagnosed cases of beta thalassemia trait (N= 150)

DISCUSSION

Beta Thalassemia is a genetic disorder of blood, in which there is abnormal synthesis of beta globin chain.² Worldwide carrier rate of beta thalassemia trait ranges from 1.7% to 9 %.² In Pakistan, the carrier rate of beta thalassemia ranges from 5-8%.² Patients of beta thalassemia are usually asymptomatic.² The definitive diagnosis of beta thalassemia trait is made by estimation of Hb A2 levels by Hb electrophoresis.⁶ But it is expensive and time-consuming test, so there is a need to

use cheap, simple, quick and easily available test.

The automated analyzers easily provide hematological parameters like hemoglobin, RBC count, mean corpuscular hemoglobin (MCH), mean cell volume (MCV), and red cell distribution width.²³ These parameters can be used to derive hematological indices, which can give preliminary diagnosis of beta thalassemia trait.²³

Thus, automated cell counters can provide

a rapid, simple and cost effective method for screening beta thalassemia trait cases.^{2,6} There is only one obstacle that is proper calibration of the haematology analyzer. If the machine is properly calibrated, the results of hematological parameters are accurate, and then our carrier detection will be quite sensitive. Hundreds of blood counts are done in routine on daily basis. If we implement the carrier screening system on each sample of blood, majority of the carrier families will come to light. When a patient is turned out to be a thalassaemia trait, we can confirm it by Hb. Electrophoresis test for Hb A2 estimation.

Many formulae have been proposed for probable diagnosis of beta thalassemia trait.^{2,7} But in the present study, the only formula which gives above 90% sensitivity is shine and lal index. According to srivastava index, the value of less than 3.8 is indicative of beta thalassemia trait.⁷ In the present study, this index identified 46% of the confirmed beta thalassemia trait patients. This is not satisfactory index in the present study. According to Mentzer index, value of less than 13 is indicative of beta thalassemia trait.⁷ In the present study, this index identified 52% of the confirmed beta thalassemia trait patients. The RDW index describes value of less than 220 as beta thalassemia trait.⁷ In the present study, this index showed poor sensitivity as it was able to identify only 2% of beta thalassemia trait patients. The most sensitive and specific index is shine and lal index which identified 95% of beta thalassemia trait confirmed cases. This index states that value less than 1530 is diagnostic of beta thalassemia trait.⁷ So shine and lal index was the most sensitive index, followed by Mentzers index in the present study.

In one study done by Kumar et al in 2017, Shine and Lal index showed the highest sensitivity of 97% in cases of beta thallememia trait, followed by Srivastava index (79% sensitivity), Mentzer's index (76%) and Red cell distribution width index (67% sensitivity).⁷ This is in accordance with the present study.

In another study done by Bordbar E et al, Shine and Lal index had highest sensitivity of 87%,

followed by Mentzer index (sensitivity 75%), and Srivastava index (sensitivity 73%).¹² These findings are also consistent with the present study. Okan et al also reported that Shine & Lal is highly sensitive discriminatory index, with sensitivity of 97.7% in detecting beta thalassemia trait.²⁴

But according to a study done by Niazi M et al, it was found that Red cell Distribution Width Index has highest sensitivity of 91%, followed by Mentzer Index (89% sensitivity), Shine and Lal (72%) and srivastava index (61%).²⁵ DeMaeyer et al. observed the Srivastava index to be valuable.²⁶ In one study, highest sensitivity of 79.1% was found for srivastava index.⁷ Ehsani et al. and Batebi et al observed Mentzers Index as the best discriminatory index.^{27,28} These findings are not consistent with our study.

The sensitive hematological indices may be used for screening in areas of high prevalence of beta thalassemia trait. Prevention programs for beta thalassemia trait in Italy and Greece have relied on initial screening by hematological indices, followed by Hb A2 estimation.²⁹

Screening of population with hematological indices and peripheral smear examination in high prevalence areas is a cheap and easy way to bring to light the beta thalassemia trait patients, who can then be referred for HB electrophoresis for definitive diagnosis.² This will identify carriers, and hence prevent marrying of a carrier with another carrier and hence decrease the rate of birth of babies with thalassemia major.

CONCLUSION

We concluded that Shine and Lal, and Mentzer index have the highest sensitivity in detecting thalassemia trait patients in our population. They can be used as cheap and quick tests for initial screening of patients in high prevalence areas where either advanced investigation tool are not available, or in low socio economic families who cannot afford high cost investigations. The patients identified can then be referred for specialized tests.

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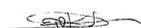
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Marriage is an adventure, like going to war.

– Gilbert K. Chesterton – ”

AUTHORSHIP AND CONTRIBUTION DECLARATION

Sr. #	Author-s Full Name	Contribution to the paper	Author=s Signature
1	M. Ihtesham Khan	Main Author, Main Idea, Result Compilation	
2	Hamid Nawaz Khan Muhammad Usman	Review of article, Literature Review.	
3		Proof reading	