

Differential Diagnosis Of Iron Deficiency Anemia And Beta Thalassemia Minor Via Different Discrimination Indexes In Patients Visiting Tertiary Care Hospitals Of Peshawar, Pakistan

Kifayat Ullah¹, Dr. Owais Ali², Inam Ullah¹, Wali Muhammad¹, Huzair Alam¹, Shahab Ahmed⁶, Faheem Usman¹, Naeem Ullah^{1,3}, Dr. Aamir Ali Khan^{4, 5, #}

¹Department of Pathology Northwest Institute of Health Sciences Peshawar, Pakistan

¹Email; kifayatullah7042@gmail.com

¹Email; inamkt66@gmail.com

¹Email; wali124513@gmail.com

¹Email; huzairalam6@gmail.com

²Northwest General Hospital & Research Center, Email: ali901456@yahoo.com

³Department of Medical Laboratory Sciences and Technology, University of Haripur, Pakistan

⁴Principal Medical Officer King Abdullah Teaching Hospital, Mansehra Pakistan

⁵Institute of Paramedical Sciences, Khyber Medical University, Peshawar, Pakistan.

⁶Faculty of Environment and life, College of life Sciences and Bioengineering, Beijing University of technology, china.

Correspondence:

Correspondence should be addressed to:

Dr. Aamir Ali Khan (PhD)

- Institute of Paramedical Sciences, Khyber Medical University, Peshawar, Pakistan.
- Faculty of Environment and life, College of life Sciences and Bioengineering, Beijing University of technology, china.

aamirkhan.research@outlook.com

aamir.lakki@kmu.edu.pk

DOI: 10.47750/pnr.2023.14.04.40

Abstract

Background:

Beta thalassemia minor and iron deficiency anemia both exhibit microcytic hypochromic anemia commonly. Different screening indexes have been introduced in order to differentiate between BTT and iron deficiency anemia which are easy to approach and convenient.

Objective:

Our study aimed to differentiate and screen the BTT and iron deficiency anemia on the basis of different discrimination indexes in order to evaluate the accuracy, sensitivity, and specificity of those indexes.

Results:

In this study, data from 201 people were collected, in which 175 cases were confirmed diagnosed with heterozygous beta thalassemia having HbA2 equal to or greater than 3.5%. 26 out of 201 were presenting iron deficiency anemia confirmed via serum ferritin. Both genders were included in this study. Upon applying eight different discrimination indexes, none of them showed 100% specificity and sensitivity. However, Shine & Lal index was more sensitive (93%) in diagnosing BTT as compared to others. 166(94%) out of 175 confirmed cases were diagnosed to have beta thalassemia trait via the S&L index followed by the Ricerca index that screened about 74% of total diagnosed cases of beta thalassemia trait. RDWI was also helpful in screening beta thalassemia traits and diagnosed about 73% of confirmed cases of BTT. The order of sensitivity of indexes in detecting BTT is S&L > RICERCA > RDWI > MCHD > MI > E&F > SRIVASTAV > MDHL. E&F index and MDHL screened 92% of confirmed IDA cases showing 88% of sensitivity and were helpful in diagnosing iron deficiency anemia. The order of accuracy of indexes in diagnosing IDA is E&F = MDHL > SRIVASTAV > MI > RDWI = RICERCA > S&L > MCHD

Conclusion:

Upon studying the sensitivity and specificity of different discrimination indexes regarding BTT and IDA, none of them seemed 100% authentic and accurate to be relied upon. Only RICERCA appears to be the most reliable index to discriminate between BTT and IDA. One should go for diagnostic lab tests after being screened with the discrimination index for getting correct and specific treatment and prevent any unpleasant condition upon relying and diagnosis on the basis of discrimination index. The discrimination index is helpful in screening some cases of BTT and IDA but it doesn't perfectly screen all cases.

Keywords: Beta thalassemia trait; Beta Thalassemia Minor; Iron deficiency anemia; discrimination index; RICERCA.

INTRODUCTION

The most commonly encountered disorders with mild microcytic anemia are iron deficiency anemia (IDA) and thalassemia trait (TT) (1). Insufficient iron for hemoglobin synthesis is the leading cause of anemia in infants and children. An estimated 30% of the world's population has iron deficiency anemia (IDA), with the vast majority residing in underdeveloped regions. Thalassemia causes microcytosis and hypochromia due to defective globin chain synthesis and reduced hemoglobin (Hb) synthesis; 1.5% of the global population carries genes for α -thalassemia (2). A recent report by the World Health Organization (WHO) showed that out of 2 billion anemia cases, 50% are IDA (3). Thalassemia was present in 0–12% of people in Sub-Saharan Africa, 0–11% of those in South and Southeast Asia, and 18% of those in the Eastern Mediterranean (4).

A significant incidence of thalassemia had emerged in North America and Northern Europe, two places that were formerly thought to be free of the illness until massive waves of immigration from thalassemia-endemic areas (5). Ten percent of all thalassemia cases were found in the Middle East, compared to nine percent in South East Asia and eight percent in the Mediterranean. Homozygous thalassemia was one of the most frequent forms of hemoglobinopathy in Pakistan, affecting over 5,000 infants per year. And 5 percent of Pakistanis were heterozygous for the beta thalassemia gene (6). Microcytic hypochromic anemia was frequent in individuals with heterozygous BT and iron deficient anemia (7). Since IDA and BTT may seem quite similar in complete blood count records, it might be difficult to tell them apart (8). Efficient diagnosis of TT and IDA may be achieved with the use of several tests, such as serum Fe^{+3} , TIBC, and HbA₂ estimate (9). Beta thalassemia trait may be confirmed by the measurement of hemoglobin A₂ using either high-performance liquid chromatography or gel electrophoresis (10).

In areas where HPLC and Hb electrophoresis are not readily available, however, other studies have developed discrimination indices for distinguishing between BTT and IDA for diagnosis. These indices were implemented to speed up the diagnosis of IDA and BTT without resorting to more laborious and pricey methods. Hematological reports indicating low MCV, MCH, and high RDW can be used to diagnose beta thalassemia carriers and iron deficiency anemics, respectively (11). It is believed that the differential index with the highest specificity and sensitivity can identify the largest and most consequential number of BTT cases when IDA is present (12).

Numerous formulas have been put up over the past 40 years to distinguish between the two major causes of microcytic hypochromic anemia (13). These formulas, depending on H band RBC count, MCV and RDW were helpful in straight forward yet not in those with IDA and BTT, a prevalent occurrence on the Indian subcontinent (14). By computing sensitivity, specificity and youden index we find the validity of several discriminating indices to distinguish beta thalassemia from iron deficiency anemia (15).

Material and Methods

Study design and study setting

This retrospective cross-sectional study was conducted between March and September 2022 in Peshawar Khyber Pakhtunkhwa. Peshawar city is the capital and largest city by population of Khyber Pakhtunkhwa province of Pakistan.

IRB and Ethical Approval

Approval for data collection was taken from Director of Northwest General Hospital & Research Center. The whole data collected was kept confidential and only primary data collectors and supervisors had access. After approval from the (IRB) committee of Northwest Institute of Health Sciences (NWIHS).

Data collection

We retrospectively analyzed 201 patients that are suggested to undergo Hb study and serum ferritin test after being diagnosed with microcytic hypochromic anemia. Samples were obtained from 71 males and 130 females with no clinical symptoms of acute or chronic inflammation or infectious diseases. None of them had received a transfusion or had an acute bleeding episode in the previous month. The samples were obtained during the course of routine analysis and collected in EDTA anticoagulant tubes. complete blood parameters, including Hb, red blood cell (RBC) count and RBC indices, like MCV, MCHC, MCH, packed cell volume (PCV) and RDW, were assessed on a CEL DYN E RUBY Hematology Analyzer. While hemoglobin variant (HbA2) is evaluated via BIO RAD D-10 Hemoglobin Testing System. Serum ferritin level is evaluated by ALINITY c-i series.

Statistical analysis

Data were analyzed by using SPSS version 25. Descriptive statistics, frequencies and percentages have been calculated for nominal and ordinal data and explained via graphs and tables where necessary. Mean and standard deviation have also been calculated for continuous variables. Sensitivity, specificity was calculated using previously suggested formulae.

Results and Discussion

A total data of 201 peoples were collected, in which 175 cases were confirmed and diagnosed with heterozygous beta thalassemia having HbA2 equal to or greater than 3.5%. All these patients were first subjected to complete blood count (CBC) to analyze their Hb, MCV, MCH, MCHC and RDW (Table 1). Out 201 patients 26 were presenting iron deficiency anemia confirmed via serum ferritin. Both genders were included in this study. Out of 175 BTT cases 68 were male and 107 were female while in IDA 23 were female and 3 were male (Table 2) Upon applying eight different discrimination indexes, none of them showed 100% specificity and sensitivity. However, Shine & Lal index was more sensitive (93%) in diagnosing BTT as compare to others. 166 (94%) out of 175 confirmed cases were diagnosed to have beta thalassemia trait via S&L index followed by Ricerca index that screened about 74% of total diagnosed cases of beta thalassemia trait. (Table 4)

RDWI was also helpful in screening beta thalassemia trait and diagnosed about 73% of confirmed cases of BTT. The order of sensitivity of indexes in detecting BTT is S&L > RICERCA > RDWI > MCHD > MI > E&F > SRIVASTAV > MDHL. E&F index and MDHL screened 92% of confirmed IDA cases showing 88% of sensitivity and was helpful in diagnosing iron deficiency anemia. The order of accuracy of indexes in diagnosing IDA is E&F = MDHL > SRIVASTAV > MI > RDWI= RICERCA > S&L > MCHD (Table 3 & 4) (Figure 1 & 2).

Table 1: Shows the hematological parameters of diagnosed patients with IDA/BTT

RBC indices	N	Minimum	Maximum	Mean	Std. Deviation
Hb	201	3.60	17.12	9.9694	2.02090
TRbc	201	2.660	7.300	5.01281	.883044
MCV	201	48.60	102.00	64.8585	8.48460

MCH	201	11.00	34.60	20.0020	3.44806
MCHC	201	2.40	37.82	30.4699	3.13147
RDW	201	7.56	42.00	14.4176	4.10181

Youden's index (YI) calculate the overall ability of diagnostic test. YI of RICERCA was found 50.0, which is good a reliable discriminator between β TT and IDA (Table-3.4).

Table 2: Shows the gender distribution of diagnosed patients

Diagnosis	Male(n)	Female(n)	TOTAL
IDA	3	23	26
BTT	68	107	175

Table 3: Shows the differential values of each discrimination index and correctly identified number of patients.

Discrimination indexes	Beta Thalassemia Trait N=175	Iron Deficiency anemia N=26	Totally correctly identified cases	Percentage
Shine & Lal Index				
BTT<1530	164 out of 175	02 out of 26	166 out of 201	82%
IDA>1530				
Ricerca Index				
BTT<3.3	135 out of 175	19 out of 26	154 out of 201	76%
IDA>3.3				
Red Cell Distribution Width Index				
BTT<220	133 out of 175	19 out of 26	152 out of 201	75%
IDA>220				
Mean Cell Hb Density				
BTT>0.3	118 out of 175	07 out of 26	125 out of 201	62%
IDA<0.3				
Mentzer Index				
BTT>13	104 out of 175	19 out of 26	123 out of 201	61%
IDA<13				
England & Fraser Index				
BTT<0	92 out of 175	23 out 26	115 out of 201	57%
IDA>0				
Srivastav Index				
BTT<3.8	88 out of 175	21 out of 26	109 out of 201	54%
IDA>3,8				

Mean Density of Hb/L Of Blood				
BTT>1.63	76 out of 175	23 out of 26	99 out of 201	49%
IDA<1.63				

Table 4: Shows the sensitivity, specificity and Youden's Index of each discriminant function used in differentiation between IDA and b-thalassemia

Discrimination indexes	SENSITIVITY	SPECIFICITY	YOUDEN'S INDEX
Shine & Lal Index			
BTT	93%	07%	00
IDA	07%	93%	
Ricerca Index			
BTT	77%	73%	50
IDA	73%	77%	
Red Cell Distribution Width Index			
BTT	76%	73%	49
IDA	73%	76%	
Mean Cell Hb Density			
BTT	67%	27%	-04
IDA	27%	67%	
Mentzer Index			
BTT	59%	73%	32
IDA	73%	59%	
England & Freser Index			
BTT	53%	88%	41
IDA	88%	53%	
Srivastav Index			
BTT	50%	80%	30
IDA	80%	50%	
Mean Density of Hb/L of Blood			
BTT	43%	88%	23
IDA	88%	43%	

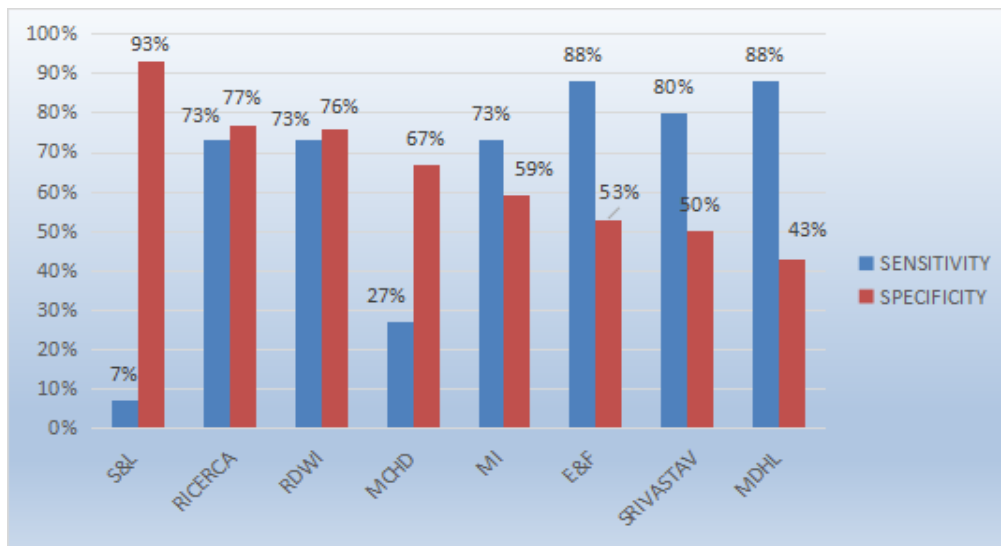


Figure 1: Bar chart shows the Sensitivity and specificity of Iron deficiency anemia cases

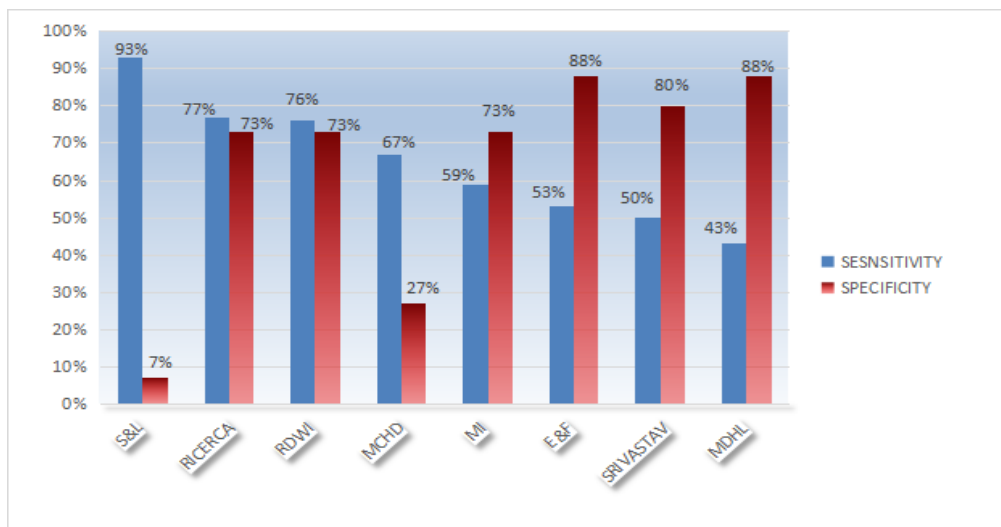


Figure 2: Bar chart shows the Sensitivity and specificity of Beta thalassemia minor cases

In conclusion by studying the sensitivity and specificity of different discrimination indexes regarding BTT and IDA, none of them seemed 100% authentic and accurate to be relied upon. Only RICERCA appears to be the most reliable index to discriminate between BTT and IDA. One should go for diagnostic lab tests after being screened with the discrimination index for getting correct and specific treatment and prevent any unpleasant condition upon relying and diagnosis on the basis of discrimination index. The discrimination index is helpful in screening some cases of BTT and IDA but it doesn't perfectly screen all cases.

DISCUSSION

The agricultural nation of Pakistan is fighting a losing battle against IDA, the world's most common nutritional deficit. Females of reproductive age in Pakistan have a prevalence of IDA of 50%, while males have a prevalence of 65-78% and females of adolescence have a prevalence of 39% (16).

Thalassemia and iron deficiency anemia are two of the most common underlying conditions that manifest clinically as mild microcytic hypochromic anemia. Identifying thalassemia carriers and preventing the premature birth of thalassemia patients requires discriminating between the two. From IDA, TT must be performed. To try to restore the MCV of a thalassemia heterozygote by giving them iron is a waste of time and resources. The presence of is diagnostic of -TT-typical RBC microcytosis and elevated HbA2 levels (17).

Although several RBC indexes and formulas had some degree of success, none of them were perfect. This research aimed to develop a formula with the maximum sensitivity and specificity for distinguishing BTT from IDA. This formula would aid in dividing individuals with hypochromic microcytic anemia into two main groups, allowing for more targeted testing (either ferritin or Hb electrophoresis). Time will be saved, and money will be saved on diagnostics (18).

. Red blood cell indices calculated from a routine complete blood count are used in faster but less accurate methods to create discrimination indices. Additional studies have shown that only 61%-91% of people with microcytic anemia due to Thalassemia trait or IDA were correctly identified by the various indexes' described. All of the indices showed overlapping values in patients with both TT and IDA, making it impossible to definitively distinguish between the two diseases (19).

Although Ferrara et al. (2010) found that the RDWI was most sensitive (78.1%), the England and Fraser index was most specific (98.1%), the Youden's index was most efficient (80.2%), and the Green and King index was most efficient (80.2%) in a study of 458 children aged 1.8 to 7.5 years with mild microcytic anemia, this study suggests that the shin and lal index is the most sensitive index to find the BTT. When trying to differentiate between BTT and IDA, research is the second most sensitive measure (20). In our study, the order of sensitivity of indexes in detecting BTT is S&L > RICERCA > RDWI > MCHD > MI > E&F > SRIVASTAV > MDHL. The order of accuracy of indexes in diagnosing IDA is E&F = MDHL > SRIVASTAV > MI > RDWI = RICERCA > S&L > MCHD and for IDA.

In patients with hypochromic microcytic anemia, Ullah et al. (2015) propose the use of RDWI as the most effective formula for distinguishing BTT from IDA. This formula can be used in places where Hb electrophoresis is unavailable for large-scale screening of microcytic hypochromic anemia (12). In our study Ricerca is the most effective formula to discriminate IDA from BTT

Hypochromic and microcytic anemia can be classified as either BTT or IDA using these formulas; however, studies show that they are only 61-91% accurate. The outcomes from each formula were similar (21). In our study we also apply eight different discrimination indexes but none of them showed 100% specificity and sensitivity.

In comparison to other indices and formulas, Wickramaratne KA at el recommends using the Shine and Lal index while conducting BTT screening programs. Additional research on other ethnic groups in Sri Lanka is encouraged to validate the results of this one (22). Our study also showed that the shine and Lal index was more sensitive (93%) in diagnosing BTT from other.

Conflict of Interest

The authors declared that they have no conflict of interest.

Author contribution

The idea was conceived by kifayat ullah, Dr. Owais Ali and Dr. Aamir Ali Khan. Data collection and analysis were performed by Inam Ullah, Wali Muhammad and Huzair Alam. Patients were interviewed and history were taken by Naeem Ullah and Faheem Usman. All authors were involved in the writing of the manuscript. Dr. Aamir Ali Khan edit and approved the final draft of the manuscript and provides the overall supervision in the current research.

Acknowledgment

The publication charges for this article are partially/fully borne from Khyber Medical University Publication Fund (No. DIR/ORIC/Ref/23/00026).

REFERENCES

1. Rahim F, Keikhaei B. Better differential diagnosis of iron deficiency anemia from beta-thalassemia trait. *Turk J Hematol.* 2009 Sep 1;26(3):138-45.
2. Vehapoglu A, Ozgurhan G, Demir AD, Uzuner S, Nursoy MA, Turkmen S, Kacan A. Hematological indices for differential diagnosis of Beta thalassemia trait and iron deficiency anemia. *Anemia.* 2014 Apr 10;2014
3. Joint World Health Organization/Centers for Disease Control and Prevention Technical Consultation on the Assessment of Iron Status at the Population Level (2004) Geneva, Switzerland). (2007). Assessing the iron status of populations: including literature reviews: report of a Joint World Health Organization/Centers for Disease Control and Prevention Technical Consultation on the Assessment of Iron Status at the Population Level, Geneva, Switzerland, 6-8 April 2004, 2nd ed. World Health Organization. <https://apps.who.int/iris/handle/10665/75368>
4. Rund D, Rachmilewitz E. β -Thalassemia. *New England Journal of Medicine.* 2005 Sep 15;353(11):1135-46.
5. Weatherall DJ, Clegg JB. Inherited haemoglobin disorders: an increasing global health problem. *Bulletin of the World Health Organization.* 2001;79(8):704-12.
6. Khattak MF, Saleem M. Prevalence of heterozygous β -thalassaemia in the northern areas of Pakistan. *J Pak Med Assoc.* 1992 Feb;42(1):32-4.
7. Ansari SH, Shamsi TS, Bohray M, Khan MT, Farzana T, Perveen K, Erum S. Molecular epidemiology of β -thalassemia in Pakistan: far reaching implications.
8. Jameel T, Baig M, Ahmed I, Hussain MB, bin DoghaimAlkhamaly M. Differentiation of beta thalassemia trait from iron deficiency anemia by hematological indices. *Pakistan journal of medical sciences.* 2017 May;33(3):665.
9. Al-Dabbagh B, Shawqi S, Yasin J, Al Essa A, Nagelkerke N, Denic S. Half of the Emirati population has abnormal red cell parameters: challenges for standards and screening guidelines. *Hemoglobin.* 2014 Feb 1;38(1):56-9.
10. Mosca A, Paleari R, Ivaldi G, Galanello R, Giordano PC. The role of haemoglobin A2 testing in the diagnosis of thalassaemias and related haemoglobinopathies. *Journal of Clinical Pathology.* 2009 Jan 1;62(1):13-7.
11. Rahim F, Keikhaei B. Better differential diagnosis of iron deficiency anemia from beta-thalassemia trait. *Turk J Hematol.* 2009 Sep 1;26(3):138-45.
12. Ullah Z, Khattak AA, Ali SA, Hussain J, Noor B, Bano R, Mahsud MA. Evaluation of five discriminating indexes to distinguish Beta-Thalassemia Trait from Iron Deficiency Anaemia. *J Pak Med Assoc.* 2016 Dec 1;66(12):1627-31.
13. Jayabose S, Giavanelli J, Levendoglu-Tugal O, Sandoval C, Ozkaynak F, VisintainerP.H Differentiating iron deficiency anemia from thalassemia minor by using by an RDW-based index. *J Pediatr Hematol* 1999; 21: 314
14. Bain BJ. Haemoglobinopathy diagnosis.
15. Sadagheyani HE, Sharafkhani R, Sakhaei S, Jafaralilou H, Shahmirzalou P. The Evaluation of Results of Twenty Common Equations for Differentiation of Beta Thalassemia Trait from Iron Deficiency Anemia: A Cross-Sectional Study. *Iranian Journal of Public Health.* 2022 Apr 14;51(4):929-38.
16. Ansari SH, Shamsi TS, Bohray M, Khan MT, Farzana T, Perveen K, Erum S. Molecular epidemiology of β -thalassemia in Pakistan: far reaching implications.
17. Olivieri NF. MEDICAL PROGRESS: THE BETA THALASSAEMIA.
18. Miri-Moghaddam E, Sargolzaie N. Cut off determination of discrimination indices in differential diagnosis between iron deficiency anemia and β -thalassemia minor. *International journal of hematology-oncology and stem cell research.* 2014;8(2):27.
19. Raper AB. Differentiation of iron-deficiency anemia from thalassemia trait. *The Lancet.* 1973 Apr 7;301(7806):778. Raper AB. Differentiation of iron-deficiency anemia from thalassemia trait. *The Lancet.* 1973 Apr 7;301(7806):778.
20. Ferrara M, Capozzi L, Russo R, Bertocco F, Ferrara D. Reliability of red blood cell indices and formulas to discriminate between β thalassemia trait and iron deficiency in children. *Hematology.* 2010 Apr 1;15(2):112-5.
21. Miri-Moghaddam E, Sargolzaie N. Cut off determination of discrimination indices in differential diagnosis between iron deficiency anemia and β -thalassemia minor. *International journal of hematology-oncology and stem cell research.* 2014;8(2):27.
22. Wickramaratne KA, Wijewickrama DC. Screening for beta-thalassemia trait; applicability of red cell indices and parameters—A study in Sri Lanka. *International Journal of Health Sciences.* 2021 Jan;15(1):29.