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Comparative analysis and predictive modeling of hematologic β-thalassemia minor in Pakistan: a cross-sectional study from 2019-2021

Aftab Ahmad Khan[1], Rizwan Uppal[2], Gul E Rehan[3], Wardah Aslam[4], Muhammad Rehan Uppal[5], and Umar Saeed[6*]

[1, 2, 3,4,5,6] Department of Pathology and Research, Islamabad Diagnostic Center (IDC), Islamabad Pakistan

[6] Clinical and Biomedical Research Center (CBRC), Foundation University School of Health Sciences (FUSH), International Center of Medical Sciences Research (ICMSR), Islamabad

ABSTRACT

Background: Hypo chromic microcytic anemia happens to be the commonest form of anemia in Pakistan. Iron deficiency anemia (IDA) and Beta Thalassemia Trait (BTT) are its most common causes. Both are having similar clinical and laboratory findings. It is necessary to establish relevant cost-effective screening methods based on blood complete picture (CP) to predict BTT in order to reduce burden of Beta Thalassemia Major in Pakistani population.

Methods: A cross sectional study was conducted from 2019 to 2021. 10,739 BTT or IDA suspected subjects were recruited. Blood CP was done for each patient to analyze different red cell parameters like total red blood cell count (TRBC), hemoglobin (Hb), mean cell volume (MCV), mean cell hemoglobin(MCH), mean cell hemoglobin concentration(MCHC) and red cell distribution width(RDW). For serum ferritin level, serum was collected after centrifugation and analyzed by using Abbott Alinity chemistry analyzer.

Results: 1044 individuals had BTT and 270 were diagnosed as having IDA. Their RBC indices were analyzed using 25 formulae to differentiate between both conditions. Matos and Carvalho index and Pornprasert discriminative index appeared to be the most reliable indices with sensitivity, specificity, positive and negative predictive values, more than 98%.

Conclusion: By utilizing Matos and Carvalho index and Pornprasert index, we can cost effectively screen huge population for BTT.

Key words: Red cell parameters, Beta-thalassemia trait, Discriminative indices

Introduction

Anaemia, a global health problem affects one third of the world population including both developed and under developed countries. It has a direct impact on social and economic development of any country. Iron deficiency anemia comprises 50 percent of total cases of anaemia worldwide and is the leading cause of microcytic hypochromic anaemia. Similarly the prevalence of this disease in Pakistan, where large part of the population lives below the poverty line, is >50% in all age groups.[1]

The second most common cause of microcytic hypo chromic anaemia in our population is BTT which has a prevalence rate of 5-7%, as against a global prevalence of 1.5%. This reflects the importance of screening for BTT in Pakistan due to its high disease burden.[2]

The real danger of undiagnosed BTT carriers getting married is a potential homozygous offspring having Beta Thalassemia Major. It is important to screen for and reliably diagnose BTT so as to reduce this burden of Beta Thalassemia Major Patients in Pakistan. These Beta Thalassemia Major patients happen to be the biggest burden on our blood banks because of their regular blood transfusion requirements.[3] Furthermore an unknown number of misdiagnosed thalassemia may be unnecessarily treated with iron. Hence the appropriate screening and correct diagnosis is extremely essential in all cases of hypochromic microcytic anemia.[4]

The confirmatory diagnosis of BTT needs HbA2 quantification either by capillary electrophoresis or by high performance liquid chromatography.[5] These tests are costly and not available in every hospital in Pakistan. Previously multiple formulae have been

CORRESPONDENCE AUTHOR

Dr. Umar Saeed *

Islamabad Diagnostic Center (IDC), F-8 Markaz

Islamabad (44000), Pakistan

Email: umarsaeed15@yahoo.com

proposed to predict BTT or IDA in various geographical regions. These discrimination indices are derived from various basic haematological parameters and assessed statistically. By using them, BTT and IDA can be differentiated. For different geographic areas, different discrimination indices are found effective in terms of high specificity, sensitivity and other statistically significant formulae.[6, 7]

Utilization of red cell indices and parameters in these discrimination indices are derived from blood complete picture using basic haematology analysers.[8] These analysers are easily available and are cost effective. So applying these formulae to all the suspected cases will streamline the need for expensive confirmatory tests for diagnosis of BTT, ultimately reducing the healthcare cost.[9, 10]

In this era of cost constraints, effective and accurate diagnostic strategies should be adopted so that expensive testing is minimized.

Methods

This single centre study was carried out between October 2019 and October 2021 at Islamabad Diagnostic Centre (IDC), Islamabad, after approval from IDC Ethical Committee (letter number: IDCERB06223190) dated 12-Sep -2019.

A total of 10,469 subjects were screened for BTT during these 02 years. Out of them, 1044 individuals (9.9%) were detected as having BTT. Further 270 patients, referred to the laboratory and diagnosed with iron deficiency anemia were also included in the study for comparison of RBC indices. Those having history of recent blood transfusion in the preceding eight weeks were excluded from the study. Blood samples were collected aseptically in EDTA tube and Plain Gel tube for Blood CP/Hb Electrophoresis and Serum Ferritin analysis respectively.

Using automated haematology analyzer (Mindray BC-6200), complete blood parameters, including Hb, RBC count and RBC indices, like MCV, MCH, MCHC, RDW were performed. For HbA2 level estimation, automated capillary electrophoresis was done (Sebia Capillarys 3 OCTA analyzer). Serum was collected after centrifugation at 6000 RPM for 10minutes for serum ferritin estimation which was analyzed by using Abbott Alinity chemistry analyzer.

Various formulae (Table1) were utilized for differentiating BTT and IDA using accuracy measures like positive (PPV) and negative (NPV) predictive value, True Positive Rate (TPR) or sensitivity, True Negative Rate (TNR) or specificity, false positive (FPR) and negative (FNR) rate. P value of 0.05 or less was considered as statistically significant. Microsoft Excel Sheet 2016 (v16.0) was utilized for statistical analysis of the data.

Table 1: Established discriminant formulae for differentiating IDA and BTT

Discriminant Formula	Calculation	Cut-off BTT	Cut-off IDA
Bessman	RDW	<14	>14
Bordbar	(80−MCV)×(27−MCH)	>44.76	<44.76
CRUISE	MCHC+0.603 RBC+0.523 RDW	≥42.63	<42.63
Ehsani	MCV − (10 RBC)	<15	>15
England and Fraser (E&F)	MCV − RBC − (5 HB) − 3.4	<0	>0
Green and King (G&K)	$(MCV^2 \times RDW)/(100\ HB)$	<65	>65
Huber– Herklotz	(MCH×RDW/10 RBC)+RDW	<20	>20
Jayabose (RDWI)	(MCV×RDW)/RBC	<220	>220
Keikhaei	(HB×RDW×100)/ (RBC2×MCHC)	<21	>21
Kerman I	(MCV×MCH)/RBC	<300	300-400
Kerman II	(MCV×MCH×10)/ (RBC×MCHC)	<85	85-105
Matos and Carvalho (MC)	1.91 RBC+0.44 MCHC	>23.85	<23.85
Mentzer	MCV/RBC	<13	>13
Nishad	0.615 MCV+0.518 MCH+0.446 RDW	<59	>59
Pornprasert	MCHC	<31	>31
RBC	RBC	>5	<5
Ricerca	RDW/RBC	<4.4	>4.4
Sehgal	MCV^2 /RBC	<972	>972
Shine and Lal (S&L)	$(MCV)^2 \times MCH/100$	<1530	>1530
Sirachainan	1.5 HB – 0.05 MCV	>14	<14
Sirdah	MCV − RBC − (3 Hb)	<27	>27
Srivastava	MCH/RBC	<3.8	>3.8
Telmissani – MCHD	MCH/MCV	<0.34	>0.34
Telmissani – MDHL	(MCH×RBC)/MCV	>1.75	<1.75
Wongprachum	(MCV×RDW/RBC) – 10 HB	<104	>104

Results

Out of the total of 10,469 subjects screened for BTT, 1044 individuals (9.97%) were detected as having BTT. Another 270 patients, diagnosed as having IDA were included in the study for comparison purpose. The diagnostic performance of 25 discrimination indices used was calculated and summarized (Table 2 and 3).

Table 2. TPR, TNR, FNR and FPR, PPV and NPV of each discrimination index for differentiating BTT (n=1044) from IDA (n=270)

Discriminant Formula	TPR (%)	TNR (%)	FNR (%)	FPR (%)	PPV (%)	NPV (%)
Bessman	4.89	27.04	95.11	72.96	20.56	6.85
Bordbar	95.11	40.37	4.89	59.63	86.05	68.13
CRUISE	0.86	1.11	99.14	98.89	3.26	0.29
Ehsani	74.71	21.11	25.29	78.89	78.55	17.76
England and Fraser (E&F)	55.94	7.78	44.06	92.22	70.11	4.37
Green and King (G&K)	67.15	17.04	32.85	82.96	75.78	11.83
Huber– Herklotz	84	83.7	16	16.3	95.22	57.51
Jayabose (RDWI)	74.23	24.07	25.77	75.93	79.08	19.46
Keikhaei	0.96	0	99.04	100	3.57	0
Kerman I	84.29	30.37	15.71	69.63	82.4	33.33
Kerman II	6.42	0.74	93.58	99.26	20	0.2
Matos and Carvalho	99.81	100	0.19	0	100	99.26
Mentzer	73.28	20	26.72	80	77.98	16.22
Nishad	83.05	25.56	16.95	74.44	81.18	28.05
Pornprasert	99.81	98.89	0.19	1.11	99.71	99.26
RBC	66.38	23.33	33.62	76.67	77	15.22
Ricerca	89.94	81.11	10.06	18.89	94.85	67.59
Sehgal	87.07	30	12.93	70	82.79	37.5
Shine and Lal (S&L)	98.85	68.15	1.15	31.85	92.31	93.88
Sirachainan	29.79	22.22	70.21	77.78	59.69	7.57
Sirdah	68.3	13.33	31.7	86.67	75.29	9.81
Srivastava	62.55	15.19	37.45	84.81	74.04	9.49
Telmissani–MCHD	92.91	83.33	7.09	16.67	95.57	75.25
Telmissani–MDHL	48.37	92.96	51.63	7.04	96.37	31.77
Wongprachum	64.08	17.04	35.92	82.96	74.92	10.93

Matos and Carvalho index showed the highest sensitivity and specificity of 99.8% and 100% respectively. This index also gives 100% positive predictive value and 99.2% negative predictive value. Another significant discriminative index is the Pornprasert which also gives sensitivity, specificity, PPV, NPV of all more than 98%. Moreover it is based on just one parameter and that is MCHC. Both these discriminative indices have a p value of 0.05.

Table-3: Based on TPR, TNR, PPV and NPV and accuracy, ranking of diagnostic performance of discriminant formulae for differentiating BTT (n=1044) from IDA (n=270).

Discriminant Formula	TPR	TNR	PPV	NPV	Accuracy
Bessman	24	11	23	21	22
Bordbar	4	8	8	5	7
CRUISE	25	23	25	23	24
Ehsani	11	16	13	13	12
England and Fraser (E&F)	19	22	20	22	20
Green and King (G&K)	15	18	16	16	17
Huber– Herklotz	9	4	5	7	6
Jayabose (RDWI)	12	13	12	12	11
Keikhaei	23	25	24	25	25
Kerman I	8	9	10	9	9
Kerman II	22	24	23	24	23
Matos and Carvalho	1	1	1	1	1
Mentzer	13	17	14	14	13
Nishad	10	12	11	11	10
Pornprasert	2	2	2	2	2
RBC	16	14	15	15	15
Ricerca	6	6	6	6	5
Sehgal	7	10	9	8	8
Shine and Lal (S&L)	3	7	7	3	3
Sirachainan	21	15	21	20	21
Sirdah	14	21	17	18	16
Srivastava	18	20	19	19	19
Telmissani–MCHD	5	5	4	4	4
Telmissani–MDHL	20	3	3	10	14
Wongprachum	17	19	18	17	18

Discussion

The commonest etiology of hypochromic microcytic anemia in Pakistan population is IDA and BTT. [11] These typically have similar clinical presentation. Spotting the exact difference between the two hematological disorders is necessary for correct

treatment of IDA. Identification of BTT and proper family screening helps in reducing the risk of having Thalassemia Major affected children [1, 12, 13] who not only happen to be a big challenge for their parents but are also a huge burden on national blood banking system because of their regular transfusion requirements.[14]

Hb electrophoresis is performed to diagnose BTT based on the increased HbA2 level [15,] whereas for diagnosis of IDA, principal methods used are TIBC, serum iron, serum ferritin and transferrin saturation.[16] Several different formulae with high performance index, have been used in various large studies to differentiate between these two disorders based on basic red blood cell parameters which are part of routine Blood CP, without using expensive tests.[17, 18]

In this study, 25 discriminant formulae were applied for evaluating the differences between BTT and IDA. We found out that Matos and Carvalho index has the highest TPR, TNR, PPV and NPV which concurs with a study conducted in Brazil in 2016.[18] Another study conducted by S. Faraj et al also shows good performance value of this index.[19] However, in current study this index is found to be even more effective than the earlier studies.

We found out that Pornprasert index also has effectivity of more than 98% by all the considered statistical parameters. However a study conducted on Iranian population, didn't find it to be as effective in screening.[5]

Another index, Shine and Lal, performed the third best in current study with TPR, PPV and NPV all above 92%. A recent study conducted in Sri Lanka showed that Shine and Lal index is most beneficial for their population.[20] Likewise in another study conducted in Bengal this index was found to be of high diagnostic significance [21] which concurs with our study.

Telmissani –MCHD index performed the fourth best in current study with TPR, TNR and PPV above 83%. Mina Jehangiri et al analysed several formulae on Iranian population for the same purpose but did not find Telmissani-MCHD as effective.[5]

Conclusion

Matos and Carvalho index and Pornprasert index performances remained excellent. Among these two, Pornprasert index has the advantage of being solely dependent on MCHC which is easily obtained from simple hematology analyzer. Therefore these two indices may be of great value for mass screening in areas having higher burden of the disease in underdeveloped countries. A combined approach of the two best performing indices of Matos and Carvalho index and Pornprasert can further enhance the efficacy of screening and channelizing the meager resources in the right direction. However, studies on larger scale in Pakistani population are required for further validation.

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CONTRIBUTION OF AUTHORS	
AUTHOR	**CONTRIBUTION**
Aftab Ahmad Khan	A,B,C
Rizwan Uppal	A,B,C
Gul E Rehan	A,B,C
Wardah Aslam	B,C
Muhammad Rehan Uppal	B,C
Umar Saeed	A,B,C