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Role of Haemogram Parameters and RBC Indices in Screening and Diagnosis of Beta-Thalassemia Trait in Microcytic, Hypochromic Indian Children

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Abstract AIM: To assess the validity and discrimination power of haemogram parameters and RBC indices in diagnosing βTT in microcytic, hypochromic anaemia. MATERIAL AND METHODS: A prospective study was conducted on the microcytic, hypochromic anaemic OPD and indoor admitted patients in Umaid Hospital, Jodhpur measured with electronic cell counter (Sysmex K800). HbA2 was calculated on the cohort by the Hb variant [Bio-Rad, USA] system by using the HPLC technique. The Hb, MCV, MCH, TRBC and four RBC indices ,Mentzer, Srivastava, Shine and Lal and England and Frazer were assessed for the positives on the basis of their individual ROC cut offs obtained in the study. RESULTS: Out of 848 microcytic, hypochromic anaemic patients, 185 were diagnosed thalassemia minor. The various haemogram parameters and RBC indices were then analysed . CONCLUSION: MCV≤59.9 fL and Mentzer Index≤12.45 were the best discriminating indices obtained with high sensitivity, NPV, YI and ARUC. No test or parameter though, had a 100 % sensitivity or specificity.

Keywords: *βTT, haemogram, RBC INDICES, YI, ROC*

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1. Introduction

β- Thalassemia is one of the most common single gene disorders in India with an overall prevalence of 3-4 % [1].

The frequency of the β-thalassemia gene is population dependent. It is prevalent in a broad belt extending from the Mediterranean basin to Southeast Asia. It is estimated that 1.5% of the world's population carries β-thalassemia – that is, at least 80–90 million people with an estimated 60,000 new carriers are born each year [2].

A WHO update on β-thalassemia in India indicated a similar overall carrier frequency of 3–4%, which given the current national population would translate to between 35.6 and 47.5 million carriers of the disorder nation-wide [3]

Effective population screening of β thalassemia trait (βTT) can dramatically decrease the incidence of birth of a thalassemia major child. Like other recessive illnesses, it can be eliminated if the carriers of the disease are fully detected and treated. Through genetic counselling birth rate of β-thalassemia major can be reduced by as much as 90%.

The microcytic anaemias may be due to iron deficiency, thalassaemia, chronic diseases and sideroblastic anaemias. The red cell changes in homozygousbeta-thalassaemia are classical. It is the microcytosis due to βTT which needs to be distinguished from that due to non-thalassemic causes. The red cellindices have been reported to have a major role in this distinction [4].Among these, mean corpuscular haemoglobin (MCV) <76fL [5], mean corpuscular haemoglobin (MCH) <26pg. [5] and total red blood cell count(TRBC)>5m/mm³ [6] have been observed to be effectivescreening tests. There is controversy not only on the choice of red cell indices but also on the cut-off values to be used for distinguishing thalassemic from non-thalassemic microcytosis. The above cut-off values and interpretations are primarily based on western population studies. InIndia, iron deficiency is the commonest cause of non-thalassemic microcytosis [7]and may co-exist with thalassaemia minor, thus altering the red cell indices. Studies in India for finding new cut offs more suitable to our population have not been extensively done. To the best of our knowledge, besides a study by Kotwal et al [8], no other research has been done in the direction so far. In the present study, we used ROC curves to calculate the optimum cut-off values for various red cell indices in our population and to determine their utility.

2. Methods and Materials

All outdoor and admitted patients with Hb<12.0 gm/dl, MCV≤77fl, MCH≤27pg in the age group between 1

Month-18 years were included in the study. Diagnosed IDA (iron deficiency anaemia) or β thalassemia major were excluded. Those selected in the cohort were subjected to HbA2 by HPLC. Written consent was obtained from all the patients before collecting blood, which is a part of the procedure for obtaining ethical clearance from the institute.

The hematologic indices and parameters that were evaluated for each subject in the study groups were: Hb, MCV, MCH, TRBC, Mentzer Index (MI)- (MCV/RBC) [9], Srivastava Index (SI) (MCH/RBC) [10], Shine and Lal Index (S&L) (MCV × MCV × MCH/100) [11] and England and Frazer (E&F) (MCV-(5xHb)-TRBC-8.4) [12].

The sensitivity (Se), specificity (Sp), positive- and negative-predictive values (PPV and NPV), Youden index (YI) and area under ROC curve (ARUC) were calculated for the haemogram parameters and RBC indices.

3. Results

Out of 848 microcytic, hypochromic anaemic patients, 185 had HBA2 ≥3.5. Through the ROC curve, the following criterion was obtained: Hb≥6.82 g/dl, MCV≤59.9 fL., MCH≤16.81 pg., TRBC≥4.75 million/ cumm. At those cut offs, Hb was most sensitive but least specific at 91.89% and 22.32% respectively. MCV was most specific with 88.69% and had high NPV with 93.8% but low PPV. TRBC had highest NPV with 95.7%. Youden Index was in the following order: MCV>MCH>TRBC>Hb. It was maximum for MCV with 0.67, then 0.5 for MCH, 0.44 for TRBC and lowest for Hb with 0.14. Area under the curve showed that MCV had the maximum area with 0.86 followed by MCH and TRBC both with 0.78 and least of Hb with 0.55.

The cut offs for different RBC indices obtained through ROC curve were as follows: MI≤12.45, S.I≤3.3, S&L≤595 and E&F≤-1.39. We found that with the new criterion, MI had the highest sensitivity and negative predictive value (NPV) with 85.41% and 95.3% respectively. SI had 78% sensitivity and specificity and 92% NPV. S&L had 87.63% specificity and 93.4% NPV. E&F had highest specificity with 95.63% but lowest sensitivity of 40%. Youden Index (Y.I) was highest for M.I with 0.671 followed by S&L at 0.654, then Srivastava and least of E&F at 0.523.ARUC saw the same order; M.I had the maximum area with 0.879 followed by S&L,S.I and least of E &F with 0.815.

4. Discussion

Iron deficiency anaemia (IDA) and β-thalassemia trait (βTT) are the most common causes of microcytic anaemia. The differentiation between them has important clinical implications.

The presumptive identification of haemoglobin disorders must rely on inexpensive methods of detection, to allow an efficient use of the resources. Thus, this study intended to investigate the performance of haemogram and RBC indices in differential diagnosis of genetic and acquired microcytic anaemia. The diagnosis of βTT was established by HbA2≥3.5. [13]

The cut offs for different haemogram parameters - Hb, MCV, MCH, TRBC for the βTT obtained in this study through our own ROC were comparable to those found in the study of Kotwal et al at AIIMS on a sample size 573 patients. [8]Another Indian study [14] had cut-off values of MCV 78.0 fL. Or less, MCH 28 pg. or less, and HbA2 more than 3.8% for βTT diagnosis.

At the cut off values of Hb≥6.82 g/dl, MCV≤59.9 fL., MCH≤16.81 pg., TRBC≥4.75 million/ cumm, the sensitivity, specificity, positive predictive value and negative predictive value were calculated (Table 1).

Table 1. Comparison of haemogram parameters in the diagnosis of βTT

Parameter	Hb (≥6.82g/dl)	MCV (≤59.9fl)	MCH (≤16.81pg)	TRBC (≥4.75m/mm³)
Sensitivity (%)	91.89	78.92	75.14	85.95
Specificity (%)	22.32	88.69	75.26	58.52
Positive Predictive Value (%)	24.8	66.1	45.9	36.6
Negative Predictive Value (%)	90.8	93.8	91.6	95.7
Y.I	0.14	0.67	0.50	0.44
ARUC	0.55	0.86	0.78	0.775

In our study MCV was a good diagnostic tool for detection and exclusion of βTT as it has sensitivity, specificity, PPV and NPV of 78.92%, 88.69%, 66.1% and 93.8%. These results are comparable results were obtained by A Batebi et al [15] (81.3%, 81.7%, 81.1%, 94.9% respectively). Similar results were obtained by Parthasarathy et al (MCV's sensitivity highest at 94.9%) and Kotwal et al too. In another study by Soliman et al, a MCV less than 73 fL. was able to differentiate between the two groups with 91.7% sensitivity and 100% specificity [16].

MCH and TRBC emerged as good tests for exclusion of βTT with a high NPV at 91.6% and 95.7% respectively in this study. However, since their positive predictive value (PPV) were less than 50% (45.9%, 37% respectively), these parameters cannot be used as reliable diagnostic tests.

Thus, summing up the results above, one can deduce that while MCV is a good diagnostic test, Hb, MCH and TRBC are good tests for exclusion of βTT test.

In a study by Soliman et al TRBC count at value above 5.47 million/ mm3 can differentiate BTT from IDA with 100% sensitivity and 100% specificity [16].

Since a good screening test requires a high NPV, TRBC emerged as the best screening variable with the highest NPV (95.7%) at a cut off of ≥4.75m/mm³ followed by MCV (81.8%). The above results conclude that TRBC and MCV are among the most reliable haematological parameters for screening of βTT. These results match with the conclusions of the study by Eldibany et al [17] who found the same two screening variables as most effective. However, in the study by Sahli et al [18], TRBC exclusively had the highest values in all the screening

parameters including PPV establishing it as both a good diagnostic test and a reliable test for exclusion.

YI and ARUC were used to assess the discrimination of the variables and MCV had the best results. In the present study, MCV's YIwas 0.67, higher than 0.54 reported by Sahli et al [18].The YIfor TRBC was 0.44 in this study, which is lower than that those obtained by Aysel (0.65), Demir et al (0.82) [19] and Sahli [18]. In present study MCV had maximum ARUC (0.86) followed by MCH and TRBC (0.78). However, Sahli et al obtained the maximum ARUC for MCH(0.960) followed by TRBC and then MCV.

The ROC cut offs were calculated for four RBC indices: Mentzer Index (M.I), Srivastava Index (S.I), Shine and Lal (S&L) and England and Frazer (E&F). (Table 2).

Table 2. Comparison of RBC indices in the diagnosis of βTT

Parameter	Mentzer Index(≤12.45)	Srivastava Index (≤3.3)	Shine and Lal (≤595)	England and Frazer(≤-1.39)
Sensitivity (%)	85.41	78.38	77.84	40
Specificity (%)	81.75	78.58	87.63	95.63
Positive Predictive Value (%)	56.6	50.5	63.7	71.8
Negative Predictive Value (%)	95.3	92.9	93.4	85.1
Y.I	0.671	0.569	0.654	0.523
ARUC	0.879	0.83	0.872	0.815

In the present study, cut off value of M.I. at ≤12.54, was most sensitive, followed by S.I, S&L and E&F. However as far as specificity is concerned, E&F at a cut off of ≤-1.39 was most specific (95.63%) followed by S&L (87.63 %), M.I. (81.75%)and S.I (78.58%).This observation was similar to the observations of Sirdah et al [20]and much lower than that found by Shen et al [21].

Besides a high specificity, E&F also had the highest PPV (71.8%), followed by S&L (63.7 %.), M.I (56.6%) and S.I (50.5%).

NPV is the most important parameter for screening. In our study, M.I had the highest NPVvalue (95.3%) followed by S&L (93.4%), S.I (92.9%) and E&F (85.1%).

Thus above results conclude that M.I and S&L are among the most reliable haematological parameters of all the indices for screening of βTT. Mussarrat Ali et al and Ghafouri et al reported M.I. as best index with sensitivity and specificity of 89and 81% and 90.9 & 80.3% respectively [22].

The Y.I was used to assess various RBC indices and YI was highest in the following order: M.I>S&L>S.I>E&F with highest for M.I.E hsani too found M.I as a good discriminative tool [23]. This was in contrast to studies by Suad et al [24], Al Fadehli [25] and Demir et al [19] who showed that the E&F index had the highest Y.I and S&L index was found ineffective.

M.I also had the best ARUC (0.879) in the present study just as in that of Shen et al(0.819).

Summing up the results of screening parameters at our cut offs with the original standard values given by Mentzer et al, Srivastava et al, Shine and Lal et al and England and Frazer et al study (Table 3), we found that sensitivity and PPV were found to be marginally better at our calculated ROC cut offs for MI, S.I and S&L. It showed marked increase in specificity and NPV for the new cut off of E&F.

Table 3. Comparison of RBC indices at our own and standard cut off values

RBC Indices	Cut Off	Se (%)	Sp (%)	PPV (%)	NPV (%)	YI
Mentzer Index	<13	90	71.49	46.9	96.3	0.61
	≤12.45	85.41	81.75	56.6	95.3	0.67
Srivastava Index	<3.8	90.27	57.32	37.1	95.5	0.48
	≤3.3	78.38	78.58	50.5	92.9	0.57
Shine and Lal Index	<1530	100	1.81	22.1	100	0.02
	≤595	77.84	87.63	63.7	93.4	0.65
England and Frazer	<0	43.78	93.36	64.8	85.6	0.37
	≤-1.39	40.00	95.63	71.8	85.1	0.81

Table 4. Comparison of RBC Indices in Diagnosis of βTT in our study with other studies

	RBC INDEX	Accepted Cut Off	Cut Off in Study	Se (%)	Sp (%)	PPV (%)	NPV (%)	YI	ARUC
Our Study	M.I	<13	≤ 12.45	85.41	81.75	56.6	95.3	0.671	0.879
Ebrahim Mir et al (2014)			<13	72.00	82.00	68.00	67.00	0.54	0.819
Sahli et al (2013)			<12.5	77.00	100.00	88.00	82.00	0.77	0.954
Our Study	S&L	<1530	≤ 595	77.84	87.63	63.7	93.4	0.654	0.872
Ebrahim Mir et al (2014)			<1004	64.00	44.00	57.00	75.00	0.08	0.709
Sahli et al (2013)			<1083	86.00	88.00	68.00	96.00	0.74	0.951
Our Study	S.I	<3.8	≤ 3.3	78.38	78.58	50.5	92.9	0.569	0.830
Ebrahim Mir et al (2014)			<4.1	64.00	44.00	57.00	74.00	.08	0.696
Sahli et al (2013)			<3.7	88.00	100	100.00	88.00	0.88	0.928
Our Study	E&F	<0	≤ -1.39	40.00	95.63	71.8	85.1	0.523	0.815
Ebrahim Mir et al (2014)			<6.5	67.00	90.00	94.00	53.00	0.57	0.907
Sahli et al (2013)			<5.3	44.00	96.00	100.00	55.00	0.40	0.944

A comparison of our indices and cut off values are comparable to those reported by Kotwal et al [8] and Ebrahim et al who used individual ROC cut off for their studies too in Table 4.

5. Conclusion

From this study, we thus conclude that although no screening test can diagnose thalassemia minor with 100% sensitivity or specificity, MCV among the hematological parameters, most efficiently discriminates βTT from other microcytic, hypochromic anemia.

Similarly, no RBC index is good enough to diagnose thalassemia trait but Mentzer Index is best and most easily calculated among the broadly available formulae that can be used reliably in screening.

We can also see that although the cut offs of the indices available are good for screening purposes, it is more useful to take out one's own most suitable values as the prevalence of nutritional anemia and demographics varies from region to region and no standard value can be used to ably apply in the screening of the cohort.

Thus we conclude that since prevention is the most effective way in controlling β-thalassemia, an appropriate, reliable and cost effective way to screen the carriers can be done through a careful examination of their haemogram parameters and RBC indices so calculated.

Abbreviations

βTT – Beta thalassemia trait; **Hb** – Haemoglobin; **MCV** – Mean corpuscular volume; **MCH** – Mean corpuscular haemoglobin; **TRBC** – Total Red Blood cell count; **ROC** – Receiver operating characteristic; **IDA** – Iron deficiency anaemia; **HPLC** – High performance liquid chromatography; **MI** – Mentzer Index; **SI** – Srivastava index; **S&L** – Shine and Lal Index; **E&F** – England and Frazer index; **Se** – Sensitivity; **Sp** – Specificity; **PPV** – Positive predictive value; **NPV** – Negative predictive value; **YI** – Youden Index; **ARUC** – Area under ROC curve

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