



Comparison of various hematological indices in patients of β Thalassemia trait of Jharkhand ,Ranchi, India:A study conducted on 50 patients over 3 years duration

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ABSTRACT

The two most frequent types of microcytic anemia are beta thalassemia trait (β -TT) and iron deficiency anemia (IDA). We retrospectively evaluated the reliability of various indices for differential diagnosis of microcytosis and β -TT in the same patient groups.

Methods. A total of 50 carefully selected patients with no age restriction were evaluated. We calculated 7 discrimination indices in all patients with hemoglobin (Hb) values of 5.1 to 11.6 g/dL. The patient groups were evaluated according to red blood cell (RBC) count; red blood distribution width index, Mentzer, Shine and Lal, England and Fraser, Srivastava, Green and King and Ricerca indices

Results: Most sensitive index was Shine and Lal, followed by Mentzer index.

KEYWORDS : Hematological indices, β Thalassemia trait, Iron deficiency anemia.

Introduction

The two most common causes of Microcytic anemia are iron deficiency anemia and β -thalassemia. Microcytic anemia in a case of thalassemia results from impaired globin chain synthesis and decreased hemoglobin (Hb) synthesis, resulting in microcytosis and hypochromia¹. Individuals with the beta thalassemia trait (β -TT) are usually asymptomatic and may be unaware of their carrier status unless diagnosed by testing. β -TT is the most common type of hemoglobinopathy transmitted by heredity. It is estimated that about 50% of the world's population with β -TT are in Southeast Asia².

A definitive differential diagnosis between β -TT and IDA is based on the result of HbA₂ electrophoresis, serum iron levels, and a ferritin calculation³. But all these investigations are quite expensive, especially in developing country like ours. Electronic cell counters have been used to determine red cell indices as a first indicator of β -TT. The purpose of using indices to discriminate anemia is to detect subjects who have a high probability of requiring appropriate follow-up and to reduce unnecessary investigative costs. Since 1970, a number of complete blood count indices have been proposed as simple and inexpensive tools to determine whether a blood sample is more suggestive of β -TT or IDA^{4,12}. The need of a sensitive index for screening of the patients has led to the commencement of this study. Here, we have compared the ability of 7 different Indices in patients of β thalassemia trait and calculated their sensitivity for the disease.

Materials and Methods:

A total of 50 patients with β thalassemia trait were included in the study. Patients were otherwise medically fit and no clinical symptoms of acute or chronic inflammation or infectious diseases was present in them. 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. Red blood cell (RBC) count and red blood

cell distribution width (RDW) were assessed on a Sysmex XT 2000i Hematology Analyzer. HbA₂ was detected by high-performance liquid chromatography (Bio-Rad Variant II, California, USA) which confirmed the cases to be of β thalassemia trait. 7 indices were taken in consideration and sensitivity was calculated for each of them to determine which index has highest sensitivity in the subpopulation of Jharkhand Different RBC indices and mathematical formulas used to differentiate between β -TT and IDA is shown in table 1. Complete hemogram of 50 patients and Indices value are shown in table 2.

Hematological index	Formula	Cut off value for β -TT
Mentzer index (MI) (1973)	MCV/RBC	<13
Srivastava (1973)	MCH/RBC	<3.8
England & Fraser(E and F) (1973)	$MCV - (5 \times Hb) - RBC - 3.4$	<0
Shine and Lal (S and L) (1977)	$MCV \times MCV \times MCH/100$	<1530
Green & King (G and K) (1989)	$MCV \times MCV \times RDW/Hb \times 100$	<72
Ricerca (1987)	RDW/RBC	<3.3
RDWI (1987)	$MCV \times RDW/RBC$	<220

Table1- Different RBC indices and mathematical formulas used to differentiate between β -TT and IDA

Results and Analysis:

Data were analyzed with computerized Medcalc software version 9.6.4.0. The differential values for each discrimination index were applied as defined in the original published reports: red blood distribution width index (RDWI), Mentzer index⁴, the Shine and Lal index⁵, the England and Fraser index⁶, the Srivastava⁷, the Green and King index⁸, the Ricerca et al. index⁹.

The following results were obtained-Range of hemoglobin values lie between 5.1 and 11.6gm%.

S.No	RBC	Hb	PCV	MCV	MCH	MCHC	RDW-CV	PLT	MI	SHRI.	E & F	S & L	G & K	RICERCA	RDWI
1	6.45	10.3	31.2	76	16	33	30.8	162	11.78	2.48	1	57.60	172.72	4.78	362.91
2	5.52	10.2	33	63.8	18.5	29	22	122	11.56	3.35	7.28	40.52	87.79	3.99	254.28
3	6.11	10.9	35	55.5	17.8	32.2	23	275	9.08	2.91	-5.11	30.62	65.00	3.76	208.92
4	6.2	10.9	33.7	58.4	17.6	30.1	22	469	9.42	2.84	-2.3	33.93	68.84	3.55	207.23
5	5.6	10	39	69.5	17.9	25.7	15.9	345	12.41	3.20	13.9	48.12	76.80	2.84	197.33
6	6.16	10.2	43.9	71.3	19.8	27.8	17.9	186	11.57	3.21	14.14	50.64	89.21	2.91	207.19
7	6.46	11.5	3.87	59.9	18.3	30.5	17.2	234	9.27	2.83	-4.06	35.70	53.66	2.66	159.49
8	5.69	10.8	35	61.5	19	30.9	17.4	314	10.81	3.34	1.81	37.63	60.94	3.06	188.07
9	6.54	11.5	45.7	69.9	23.4	33.5	15.8	180	10.69	3.58	5.86	48.63	67.13	2.42	168.87
10	3.39	5.6	20.7	61.1	16.5	27.1	20.9	239	18.02	4.87	29.71	37.17	139.33	6.17	376.69
11	6.39	10.4	40.9	64	19.7	30.8	18.4	93	10.02	3.08	5.61	40.76	72.47	2.88	184.29
12	1.9	5.4	20.3	20.7	28.4	26.6	17.4	97	10.88	14.95	-8.23	3.99	13.77	9.16	189.32
13	6.42	10.6	40.9	63.7	19.9	31.3	18	241	9.92	3.10	4.28	40.38	68.90	2.80	178.60
14	3.29	6.3	21.8	66.3	19.1	28.9	27.5	137	20.15	5.81	31.51	43.77	191.88	8.36	554.18
15	5.42	11.1	34.9	64.4	20.5	31.8	14.9	225	11.88	3.78	3.48	41.27	55.67	2.75	177.04
16	2.77	6.3	20.1	72.6	22.7	31.3	18.2	68	26.21	8.19	38.33	52.48	152.27	6.57	477.01
17	2.6	7.2	23.6	90.8	27.7	30.5	21.1	261	34.92	10.65	52.2	82.17	241.61	8.12	736.88
18	3.08	5.6	20.8	67.5	18.2	26.9	16.1	42	21.92	5.91	36.42	45.38	130.99	5.23	352.84
19	5.03	11.2	35.6	70.8	22.3	31.5	16.7	162	14.08	4.43	9.77	49.90	74.74	3.32	235.06
20	4.49	11.4	35.6	79.3	25.4	32	12.8	492	17.66	5.66	17.81	62.63	70.61	2.85	226.07
21	5.83	11.3	37.8	64.8	21.1	32.5	19.4	132	11.11	3.62	2.47	41.78	72.09	3.33	215.63
22	4.2	7.4	25.1	59.8	17.6	29.5	21.6	116	14.24	4.19	18.6	35.58	104.38	5.14	307.54
23	6.23	11.6	42.9	68.9	20.2	29.4	18.3	122	11.06	3.24	4.67	47.27	74.89	2.94	202.39
24	5.68	10.4	40.5	71.3	21.8	30.6	16.8	75	12.55	3.84	13.62	50.62	82.12	2.96	210.89
25	8.36	10.9	58.1	69.5	20.2	29.1	20.5	231	8.31	2.42	6.64	48.10	90.84	2.45	170.42
26	3.89	10	37	95.1	30.8	32.4	16.8	286	24.45	7.92	41.21	90.13	151.94	4.32	410.71
27	6.38	11.6	45.6	71.5	22.9	32	20.1	298	11.21	3.59	7.12	50.89	88.58	3.15	225.26
28	5.32	10.3	34.8	65.4	19.4	29.6	16.9	129	12.29	3.65	8.58	42.58	70.18	3.18	207.76
29	5.31	9	27.5	51.8	16.9	32.7	18.2	383	9.76	3.18	1.49	26.66	54.26	3.43	177.54
30	5.58	11.5	38.9	69.7	22.4	32.1	14.7	58	12.49	4.01	6.62	48.36	62.10	2.63	183.62
31	4.97	10.7	33.9	68.2	21.5	31.6	15	145	13.72	4.33	9.73	46.30	65.20	3.02	205.84
32	6.72	9.3	41.6	61.6	19.8	32.1	19.6	394	9.17	2.95	8.38	37.75	79.97	2.92	179.67
33	3.89	8.4	27.1	69.7	21.6	31	17.9	366	17.92	5.55	23.81	48.36	103.52	4.60	320.73
34	5.13	11.5	40	78.9	22.8	28.8	17.2	141	15.38	4.44	16.27	62.02	93.11	3.35	264.54
35	2.17	5.1	20	77.4	23.5	30.4	17.8	130	35.67	10.83	49.73	59.67	209.09	8.20	634.89
36	6.42	10.8	40.9	63.7	19.9	31.3	18	241	9.92	3.10	3.28	40.38	67.63	2.80	178.60
37	3.9	5.4	20.3	65.6	28.4	26.6	16	97	16.82	7.28	34.7	42.75	127.51	4.10	269.13
38	5.9	10.6	34.2	58	98	31	16.1	46	9.83	16.61	-0.9	32.66	51.09	2.73	158.27
39	6.39	10.6	40.9	64	19.7	30.8	25	93	10.02	3.08	4.61	40.76	96.60	3.91	250.39
40	3.39	5.6	20.7	61.1	16.5	27.1	27	239	18.02	4.87	29.71	37.17	179.99	7.96	486.64
41	5.92	9	31.7	70.9	20.5	28.6	18.7	241	11.98	3.46	19.98	50.06	104.45	3.16	223.96
42	5.18	11.1	31.7	77.4	21.4	27.7	17.2	242	14.94	4.13	16.72	59.69	92.83	3.32	257.00
43	4.35	10.9	13.2	84.1	25.1	29.8	16.2	362	19.33	5.77	25.25	70.48	105.12	3.72	313.20
44	5.53	11.5	38	68.9	20.8	30.2	18.1	255	12.46	3.76	5.87	47.26	74.72	3.27	225.51
45	5.86	9.3	40	72.4	21.8	29	19.3	115	12.35	3.72	20.04	52.20	108.78	3.29	238.45
46	5	11.6	26.4	68	21	32.1	15.1	262	13.60	4.20	5	46.03	60.19	3.02	205.36
47	7.11	10.9		61.3	19.5	31.9	19.1	364	8.62	2.74	-0.31	37.38	65.85	2.69	164.67
48	4.79	9	29.3	61.2	18.8	30.7	16.4	235	12.78	3.92	11.41	37.27	68.25	3.42	209.54
49	6.02	10.8	34.4	57.1	17.9	31.4	19.5	1.5	9.49	2.97	-2.92	32.43	58.87	3.24	184.96
50	5.19	10.9	34.3	66.1	21	31.8	16.3	1.7	12.74	4.05	6.41	43.48	65.34	3.14	207.60

Table 2-Complete hemogram and indices value of 50 patients.

Sensitivity is calculated as-
$$\frac{\text{True positive}}{\text{True positive} + \text{False negative}} \times 100$$

Sensitivity obtained for different indices were- Mentzer index-64%, Shine and Lal index-90%, England and Fraser index -14%, Srivastava index-52%, the Green and King index-40%, the Ricerca index-52% and Red blood distribution width index (RDWI)-56%. While England and Fraser index showed least sensitivity, Shine and Lal proved to be most sensitive (90%), followed by Mentzer Index (64%).

Discussion

β-TT and IDA are among the most common types of microcytic anemia. Distinguishing β-TT from IDA has important clinical implications because each disease has an entirely different cause, prognosis, and treatment. Thalassemia is endemic in Jharkhand. Up to now, many investigators have used different hematological indices to distinguish β-TT from IDA using only a complete blood count. This process helps to select appropriate individuals for a more detailed examination; however, no study has found 100% specificity or sensitivity for any of these RBC indices. RBC count has been considered a valuable index¹³, but RBC alone is not a reliable tool for distinguishing β-TT from IDA. Different studies have shown different reliable results. In a 2010 study, Ferrara et al. demonstrated that RDWI had the highest sensitivity (78.9%)¹⁴. In 2007, Ntaios et al. reported that the Green and King index was the most reliable index, as it had the highest sensitivity (75.06%)¹⁵. Our study here shows that Shine and Lal

is the most sensitive index. Mentzer index can also be used, as it is very simple to calculate with moderate degree of sensitivity.

Conclusion

In conclusion, the cell-count-based indices, particularly the Mentzer index, and Shine and Lal index are easily available and reliable methods for detecting β -TT. According to our results, the percentage of correctly diagnosed patients was the highest with the Shine and Lal index (90%) followed by the Mentzer index (64%), and they can be readily applied in patients in Jharkhand subpopulation.

References

1. D. A. Rathod, A. Kaur, V. Patel et al., "Usefulness of cell counter-based parameters and formulas in detection of β -thalassemia trait in areas of high prevalence," *American Journal of Clinical Pathology*, vol. 128, no. 4, pp. 585–589, 2007.
2. E. Urrechaga, L. Borque, and J. F. Escanero, "The role of automated measurement of RBC subpopulations in differential diagnosis of microcytic anemia and β -thalassemia screening," *American Journal of Clinical Pathology*, vol. 135, no. 3, pp. 374–379, 2011.
3. C. Thomas and L. Thomas, "Biochemical markers and hematologic indices in the diagnosis of functional iron deficiency," *Clinical Chemistry*, vol. 48, no. 7, pp. 1066–1076, 2002.
4. W. C. Mentzer Jr., "Differentiation of iron deficiency from thalassaemia trait," *The Lancet*, vol. 1, no. 7808, p. 882, 1973.
5. I. Shine and S. Lal, "A strategy to detect β thalassaemia minor," *The Lancet*, vol. 1, no. 8013, pp. 692–694, 1977.
6. J. M. England and P. M. Fraser, "Differentiation of iron deficiency from thalassaemia trait by routine blood-count," *The Lancet*, vol. 1, no. 7801, pp. 449–452, 1973.
7. P. C. Srivastava, "Differentiation of thalassemia minor from iron deficiency," *The Lancet*, vol. 2, pp. 154–155, 1973.
8. R. Green and R. King, "A new red cell discriminant incorporating volume dispersion for differentiating iron deficiency anemia from thalassemia minor," *Blood Cells*, vol. 15, no. 3, pp. 481–495, 1989.
9. B. M. Ricerca, S. Storti, G. d'Onofrio et al., "Differentiation of iron deficiency from thalassaemia trait: a new approach," *Haematologica*, vol. 72, no. 5, pp. 409–413, 1987.
10. M. Sirdah, I. Tarazi, E. Al Najjar, and R. Al Haddad, "Evaluation of the diagnostic reliability of different RBC indices and formulas in the differentiation of the β -thalassaemia minor from iron deficiency in Palestinian population," *International Journal of Laboratory Hematology*, vol. 30, no. 4, pp. 324–330, 2008.
11. M. A. Ehsani, E. Shahgholi, M. S. Rahiminejad, F. Seighali, and A. Rashidi, "A new index for discrimination between iron deficiency anemia and beta-thalassemia minor: results in 284 patients," *Pakistan Journal of Biological Sciences*, vol. 12, no. 5, pp. 473–475, 2009.
12. O. A. Telmissani, S. Khalil, and T. R. George, "Mean density of hemoglobin per liter of blood: a new hematologic parameter with an inherent discriminant function," *Laboratory Haematology*, vol. 5, pp. 149–152, 1999.
13. A. Demir, N. Yarali, T. Fisgin, F. Duru, and A. Kara, "Most reliable indices in differentiation between thalassemia trait and iron deficiency anemia," *Pediatrics International*, vol. 44, no. 6, pp. 612–616, 2002.
14. S. M. AlFadhli, A. M. Al-Awadhi, and D. AlKhalidi, "Validity assessment of nine discriminant functions used for the differentiation between Iron deficiency anemia and thalassemia minor," *Journal of Tropical Pediatrics*, vol. 53, no. 2, pp. 93–97, 2007.
15. G. Ntaios, A. Chatzinkolaou, Z. Saouli et al., "Discrimination indices as screening tests for β -thalassemic trait," *Annals of Hematology*, vol. 86, no. 7, pp. 487–491, 2007.