International Journal of Life Sciences, Biotechnology and Pharma Research Vol. 14, No. 2, February 2025 Online ISSN: 2250-3137
Print ISSN: 2977-0122

DOI: 10.69605/ijlbpr_14.2.2025.69

ORIGINAL RESEARCH

Diagnostic Predictability Of Complete Blood Count Using Various Red Cell Parameters In Detection Ofβ-Thalassemia Trait- A Tertiary Health Care Based Study.

[1]Dr. Bharti Parashar, [2]Dr. Stuti Kumari, [3]Dr. Shagufta Tahir Mufti

[1]Associate Professor, Department of Pathology, Career Institute of Medical Sciences, Lucknow .
[2]Assistant Professor, Department of Pathology, Career Institute of Medical Sciences, Lucknow .
[3]Associate Professor, Department of Pathology, Career Institute of Medical Sciences, Lucknow .

Corresponding author :
Dr. Bharti Parashar
[1]Associate Professor, Department of Pathology, Career Institute of Medical Sciences, Lucknow

Received: 12 January, 2025 Accepted: 10 February, 2025 Published: 14 February, 2025

Abstract

Background: -Thalassemias are a heterogeneous group of autosomal disorders caused by inherited mutations that decrease the synthesis of α and β globin chains of hemoglobin causing varying degree of anemia. They are among the most common genetic disorders worldwide with a prevalence of thalassemia carriers being 1.5-7%. Symptoms vary from silent, asymptomatic carriers to transfusion dependent anemia.

Aim of the study: - To evaluate the diagnostic usefulness of complete blood count using various red cell parameters in detection of thalassemia trait in microcytic hypochromic anemia cases.

Material and methods: - All samples received in central laboratory for CBC were analyzed for Hb, RBC count, MCV, MCH, MCHC and RDW**.** A total of 550 cases were selected based on criterion of Hb < 12 g /dl , MCV < 80 fl and RDW(CV)- < 18%. All the samples were sent for HbA2 estimation by HPLC.

Results: -Out of 550 microcytic hypochromic cases, 397 were diagnosed with microcytic hypochromic anemia with normal HPLC pattern, whereas 153 cases were diagnosed with β thalassemia trait with HPLC A2 value more than 3.8.
In our study, the incidence of beta thalassemia trait came out to be approx. 27.8 % in microcytic hypochromic cases.

Conclusion:- CBC is a very simple and cheap technique with high predictive value in screening of beta thalassemia trait in microcytic hypochromic anemia. It can be used for mass screening where Thalassemia prevalence is high.

Introduction

Thalassemias are a heterogeneous group of autosomal disorders with agenetically determined reduction in the synthesis of normal hemoglobin which is caused by inhibition of α or β globin chain production resulting invarying level of anemia [1].

These are commonest genetic disorders of hemoglobin [2]. According to World Health Organization (WHO)[3] reports,7% of worldpopulation is carrier for hemoglobin disorders. There is a prevalence of1.0-14.9% of β thalassemia trait (β-TT) in various parts of India [4].The clinical spectrum of these inherited disorders varies from asymptomatic conditions tocarriers, and to serious diseases like thalassemia major and hydrops foetalis.β-thalassemiais caused by deficient synthesis of β chains. Diagnosis of β- TT is often challenging due to its overlapping and similarclinical presentations with iron deficiency anemia. There are many similaritiesin both conditions even on peripheral smear and blood counts, such as reducedHb, MCV, MCH, microcytosis and hypochromasia. Clinical features do not helpmuch in differentiation of these conditions [5]. A correct diagnosis in patients with microcytic anemia is very important as iron supplementation is required in iron deficiency anemia patients while avoiding unnecessary iron therapy inthalassemia trait [6].Early and timely diagnosis and identification of β- TT is very essential as ithelps in a) marriage and genetic counselling, so that birth of a babywith β thalassemia major and other serious disorders can be prevented. b) forproper awareness among heterozygous carriers. c) to ensure appropriate maternal care and help facilitate diagnosis in newborns. The severity of this diseasealong with high treatment cost and complications and high prevalence incertain regions of India justifies the role of its screening. One of the mosteffective ways is to screen

DOI: 10.69605/ijlbpr_14.2.2025.69

these populations by means of red cell indices whichis also cost effective and can be done at mass level.The present study is done to evaluate the diagnostic predictability of complete blood count (CBC) using various red cell parameters in detection of β thalassemia trait by analysing the CBC on the basis of red cell indices in microcytichypochromic cases and its confirmation by HPLC. thereby finding thesensitivity, specificity and validity of the various red blood cell indices.

Material and methods

The present prospective study was undertaken for 2 years from January 2021 toJanuary 2023 in the central laboratory, Career institute of medical sciences, Lucknow among patients attending OPD and IPD. The data is composed of CBC and HPLC reports. The sample wascollected in 2 ml of whole blood in POTASSIUM(K3)-EDTA vacutainers. In CBC, Hb value andRBC indices are included for analysis and in HPLC report, Hb A2 value was analysed. The complete blood count was done using a 5-part automateddifferential cell counter (Mindray/ BC - 5150 Auto hematology analyzer) and HPLC was done using BIO-RAD D-10 hemoglobin testing system as perinstructions of machine manufacturer. All the cases presenting at the central laboratory (both out-patient and in-patient) for complete blood count (CBC)were analysed and screened for Hb, RBC count, MCV, MCH, MCHC and RDW along with peripheral smear examination. All patients with CBC showing following inclusion criteria were selected and the same EDTA blood was sent for HPLC for Hb A2 estimation and confirmation.

Inclusion criteria include-
1. Hb </= 12 g / dl
2. MCV </= 80 fl
3. RDW </=18 %

A total of 550 cases having microcytic hypochromic anaemia were selected.All the samples with HbA2 value ≥ 3.8 % were termed as BTT (Beta ThalassemiaTrait) and those with HbA2 value less than 3.8% were termed asnon- BTT subjects. Samples with HPLC reports of other hemoglobinopathieswere excluded from the study.The data was analysed using SPSS 21(IBM).

Observation and Result

Out of 550 microcytic hypochromic cases, 397 were diagnosed with microcytic hypochromic anemia with normal HPLC pattern, whereas 153 caseswere diagnosed with β thalassemia trait with HPLC A2 value more than 3.8 %.Among 550 cases, 196 (35.6%) were females and 354 (64.4%) were males.Out of 550 cases, 265 (48.2%) patients were of ≤18 years of age, 270 (49.1%) were adults and only 15(2.7%) patients were elderly.On analysis, we found mean RBC count much higher in BTT than microcytic hypochromic group with p value being </= 0.05 making observation significant. Whereas MCV, MCH and MCHC were lower in BTT group than MCHC group. There is no significant difference in Hb and RDW between these two groups.We found, out of 153 cases of BTT, 118(77.1%) were males and 35 (22.9 %) were females.Mean HbA2 of Non BTT group was 2.52±0.55 whereas that of BTT was 4.78±0.73.This Difference Was Statistically Significant With P- Value< 0.01.On analysis of RBC count, we found in BTT group, 97 cases (63.4%) having RBC count >/= 5 million whereas in microcytic group only 36.3% cases having RBC count &>/= 5 million. with p value <0.01, making the observation clinically significant.

Table 1:- Frequency of β thalassemia trait in our study

Total no. of microcytic hypochromic anemia studied	Total no. ofβ thalassemia trait diagnosed by HPLC	Normal HPLC Pattern cases
550	153 (27.8%)	397 (72.2%)

Table 2 :- Gender wise distribution of study subjects

	Frequency	Percent
Female	196	35.6 %
Male	354	64.4 %
Total	550	100

Table 3: Age group wise distribution of study subjects

Age Group (Years)	Frequency	Percent
≤18	265	48.2 %
18-60	270	49.1 %
>60	15	2.7 %
Total	550	100.0

DOI: 10.69605/ijlbpr_14.2.2025.69

Table 4- Comparison of parameters between BTT and Non- BTT group

Parameters	BTT	Non- BTT	P VALUE	SIGNIFICANCE
RBC (X 10^6/cu mm)	5.48 ± 0.85	4.29 ± 0.72	<0.05	SIGNIFICANT
Hb (gm/dl)	9.93 ± 2.35	9.82 ± 2.70	0.668	Not significant
MCV (fl)	65.04 ± 12.15	73.95 ± 14.73	<0.01	significant
MCH (pg)	20.02 ± 4.20	24.14 ± 5.37	<0.01	significant
MCHC (gm/dl)	30.76 ± 2.09	31.73 ± 1.88	<0.01	significant
RDW (%)	16.82 ± 4.19	17.12 ± 4.91	0.5	Not significant

Table 5- Gender group wise comparison of study subjects according to presence of thalassemia

	Normal		TT	
	Frequency	Percent	Frequency	Percent
Female	161	40.6	35	22.9
Male	236	59.4	118	77.1
Chi square value	15.04			
p value	<0.01*			

*Statistical

Table 6- Comparison between HbA2 of BTT and Non BTT cases.

	group	number	mean	Std deviation	p- value	Significance
HbA2	B TT	153	4.78	0.73	<0.01	SIGNIFICANT
	Non BTT	397	2.52	0.55		

Table 7- RBC grading wise comparison of study subjects according to presence of thalassemia. (count in millions)

Diagnosis	<5		>5	
	Frequency	Percent	Frequency	Percent
Normal	253	63.7	144	36.3
TT	56	36.6	97	63.4
Chi square value	33.01			
p value	<0.01*			

*Statistically significant

Table 8- Comparison of results obtained using the criteria to detect thalassemia trait

	Sensitivity	Specificity	PVV	NPV		Younden Index
RBC	81.88	91.25	68.73	95.4		73.13

Discussion

Thalassemia is a common genetic disorder which is widely prevalent in some parts of our country,which not only causes health issue to the affected individual but also causes huge socioeconomicburden on family and society. The incidence of B thalassemia varies in different parts of Indian sub-continent.The present study provides a systematic approach of screening of b thalassemia trait by differentiating it from othermicrocytic hypochromic conditions especially iron deficiency anaemia.In our study, a total of 550 cases were studied based on above mentioned criterion to predict how manycases of microcytic hypochromic anaemia turn out to be thalassemia trait and how can we predict in a better way just on blood count. So, out of 550 microcytic hypochromic cases, 27.8% were found to be BTT.Decreased MCV with normal RDW and increased RBC count in microcytic hypochromic cases was foundto be very reliable, convenient and cost-effective approach for screening of beta thalassemia at mass level,as in our study we found approx. 27.8 % cases of TT in 550 microcytic hypochromic cases.In our study, 196 (35.6%) were females and 354 (64.4%) were males. Amongthese, 265 (48.2%) patients were of ≤18 years of age, 270(49.1%) were adultsand only 15(2.7%) patients were elderly.We found total RBC count (x 106/cu.mm) of 5.48 ± 0.85 in BTT and 4.29±0.72 in microcytichypochromic group which was statistically significant with p-value </=0.05. A study done byAdlekha et al (2013) [10], found mean RBC count of 5.43 ± 0.97 in BTT cases and that of 4.16 ± 0.82 in IDAcases. Bhairwa ET AL (2019) [11]found mean RBC count of 5.4 ± 0.56 in BTT cases and that of 4.36 ± 0.56 in IDAcases. Our findings are also in line with observations by studies of Beshlawy et al [12]. This is in line with asimilar observation laid by the studies of Bencaiova et al.,[13]. Ehsani et al [14]. and rahim et al [15]. In our study, there was no significant different in the Hb value in both groupswith P value 0.668 because we included all microcytic cases with Hb </= 12gm/dl in our study. The mean Hb (g/dl) in BTT group was 9.93 ± 2.35 and thatof 9.82 ± 2.70 in microcytic hypochromic

DOI: 10.69605/ijlbpr_14.2.2025.69

group. In our present study, we found significantly lower MCV (fl) value of 65.04 ±12.15 in BTT as compared to MCV of 73.95 ± 14.73 in microcytic hypochromicgroup. Ehsani et al (2009) [14] found MCV of 62.02 ± 4.57 in BTT and that of70.04± 7.94 in IDA group. Vehapoglu et al. (2014) [16] also have similarobservation.MCH and MCHC are also statistically significant in our study with p value </=0.01. MCH and MCHC value found in BTT patients are 20.02 ± 4.20 and 30.76 ±2.09 respectively, whereas those of non- BTT patients are 24.14 ± 5.37 and31.73 ± 1.88. Our findings are in line with Vehapoglu et.al (2014) [16] and Ehsani etal (2009) [14].In our study, RDW- CV (%) is analysed in both cases and found no significantdifference was found between the two groups. In BTT, it was 16.82 ± 4.19 andthat of 17.12 ± 4.91 in microcytic hypochromic group.In this study, we closely analysed RBC count, and we observed that out of 153BTT patients, 97 (63.4%) had RBC count (x 106/cu.mm)>/= 5 million, while in non BTTgroup only 36.3% had RBC count>/= 5 million. This is statistically significant with pvalue<0.01. According to our study, RBC count had 81.88% sensitivity and 91.25 %specificity. It has PPV 68.73 and NPV - 95.4. with Youden index of 73. Youden index is defined as sensitivity+specificity-100[11].Bhairwa et al [11] reported 80% and 95% as sensitivity and specificity of RBC countrespectively with PPV 66.6% and NPV 97.7% with Younden index 77.Rahim et al (2008) [15] reported in their study sensitivity and specificity of 94%and 84% while PPV and NPV of 91% and 87% with Youden index of 78. Ntioset al (2007) [17] and ehsani et al [14] had similar results.In our study, the mean Hb A2 value in BTT group is 4.78 ± 0.73 and while thosein non BTT group is 2.52 ± 0.55. Our findings are statistically significant with pvalue < 0.01. In a study done by Bhairwa et al,[11] they reported mean Hb A2 in BTTgroup as 5.51 and those of 2.45 in non BTT group, which is comparable to ourstudy.

Conclusion

The present study found a significant differences in CBC red cell indiceswith respect to MCV values, RBC count and MCH value between betathalassemia trait and iron deficiency anaemia patients. CBC can be usedfor mass screening of beta thalassemia trait in all regions where itsprevalence is high as it is easy, inexpensive and with appreciablesensitivity and specificity. HPLC is a very simple and accuratetechnique along with CBC for the confirmation of b thalassemia trait. Byearly diagnosis, the patient or couple can be counselled accordingly andfurther adverse outcome can be minimized.

References

1. Wintrobe MM, Richard G, Boggs DR, Bithell TC, Foerster J, Athens WJ et al. Clinical hematology. USA: Philadelphia; 1981.p1886-7.
2. Weatherall DJ, Clegg JB, Thalassemia-a global public health problem. Nat Med 1996; 2:847-9.
3. WHO Management of Hemoglobin Disorders. Report of Joint WHO-TIF Meeting on Management of
4. Hemoglobin Disorders. Nicosia, Cyprus,16-18 November 2007. World Health Organisation2008:1-2.
5. Available from: http://www.who.int/genomics/WHO-TIF genetics.
6. Madan N, Meera S, Satendra S, Rusia U, Kela K. Red cells Indices and Discriminant functions in the detection of Beta – Thalassemia Trait in apopulationwith High prevalence of Iron Deficiency Anemia.
7. Indian J PatholMicrobiol 1999; 42(1):55-61.
8. Rahim F, Saki NAge specific cutoff in discriminating iron deficiency anemia from beta thalassemiatraits. IJBC 2010; 2:197.
9. Hoffmann et al.: Meta-analysis of discriminant indices for microcytic anemia
10. Adlekha S, Chadha T, Jaiswal RM,Singla A. Screening of b -thalassemia trait by means of red cellindices and derived formulae. Med J DY Patil Univ 2013;6 71-4.
11. Shilpa Bhairwa et al, Diagnostic predictability of complete blood count (CBC) in identifying thalassemiatrait in pregnant females. International journal of recent scientific research vol, issue, 01(A), pp 30234-30238, january, 2019.
12. El-Beshlawy A, Kaddah N, Moustafa A, Mouktar G, Youssry I. Screening for β thalassaemia carriers in Egypt:Significance of the osmotic fragility test. East Mediterr Health J 2007; 13:780-6.
13. Bencaiova G, Burkhardt T, Kraft A, Zimmerman R. Screening for β-thalassaemia trait in anaemic pregnantwomen. GynecolObstet Invest 2006; 62:20-7.
14. Ehsani MA, Shahghoi E, RahiminejadMS,Seighali F, Rashidi A. A new index for discrimination between irondeficiencyanemia and beta thalassemia minor: results in 284 patients.Pak J Bio Sci 2009; 12(5): 473-5.
15. Rahim F, Ahadi R. Thalassemia and hemoglobin disorders in the Khuzston Province of Iran. J clin Diagn res 2008;3:820.
16. Vehapoglu A, et al, Hematological indices for differential diagnosis of Beta thalassemia trait and iron deficiencyanemia. 10 Apr 2014, 2014:576738 pubmed.
17. Ntaios G, Chatzinikolaou A, Saouli Z, et al: Discrimination indices as screening tests for βthalassemic trait. AnnHematol 2007; 86: 487 – 491