journal homepage: https://jablm.acclmp.com/

Journal of Applied Biochemistry & Laboratory Medicine (2023) 04 (2):1-6

Original article

Utility of MCH, MCV, RDW, and HbF for differentiating between the HbE trait patients and normal subjects

Biswas Teresa[1]*, Thakuria Ghanashyam[2]
[1]Laboratory Director & Consultant Biochemist, Bioscan Diagnostics, Guwahati, Assam.
[2] Consultant Pathologist, Bioscan Diagnostics, Guwahati, Assam

Keywords:
Thalassemia E, thalassemia E trait, HbF, MCH, MCV, PCV, RDW.

ABSTRACT

HbE is becoming one of the most common thalassemia among the population of the Indian subcontinent. However, screening or provisional diagnosis of its trait stage is very difficult using routine hematological tests due to significant overlap with the normal parameters and different combinations of thalassemia.

Objectives:
The objectives of the present study were to find out the utility of routine hematological tests and fetal hemoglobin (HbF) to differentiate between the HbE trait patients and the normal subjects.

Materials and Methods:
All routine blood parameters like hemoglobin, MCV, PCV, MCH, MCHC, and RDW were measured using an automated 5-part cell counter from suspected hemoglobinopathies during one-year period. Hemoglobin variants and HbF were measured using HPLC. Mean values of the data from the HbE trait population and normal persons were compared using an independent t-test. Cut-off values of data showing significant differences were obtained using ROC curve analysis. Association between the study parameters of the case group was done using Pearson's bivariate correlation study.

Results:
RDW and HbF were significantly higher in the trait group with significantly lower values for MCH and MCV. The association of RDW with the MCH and MCV was significantly negative. The ROC curve analysis showed cut-off values of 13.8, 0.85, 75.0, and 24.9 for RDW, HbF, MCV, and MCH respectively. Analysis of all ROC curves revealed that RDW showed a better cut-off value than the other parameters.

Conclusion:
RDW along with the other routine hematological parameters like MCV, MCH, and FHb can be used as good screening parameters for differentiating the HbE trait patients at a very initial stage. Furthermore, their cut-off values may be useful to segregate the HbE trait persons from the normal population at an early stage.

INTRODUCTION

HbE is caused by a point mutation at codon 26 of the beta chain that replaces glutamic acid with lysine. This results in the introduction of a cryptic splicing site in the beta-globin mRNA producing a premature termination and lowered synthesis of the globin chain in the affected person. The prevalence of HbE has been reported to be substantially high in some countries of Asia including India.[1, 2]. Furthermore, the variable combination of HbE with different categories of alpha and beta thalassemia makes the clinical picture further complicated, particularly in the context of a high prevalence of beta-thalassemia in these regions. The clinical scenario may vary from completely asymptomatic to transfusion-dependent cases. In many countries, E-beta thalassemia has replacbeta-thalassemia as the commonest type of thalassemia.

*Correspondence:
Dr. Teresa Biswas, Laboratory Director & Consultant Biochemist, Bioscan Diagnostics, Guwahati, Assam
Email : teresabiswasguha@gmail.com

Due to immigration changes and demographic reasons, the prevalence of E-beta thalassemia is predicted to be substantially high shortly in several South Asian countries including India.[3] During this immigration phase and demographic changes, several patients with HbE traits are found who are most difficult to differentiate from normal persons as they are mostly asymptomatic and show minimal difference in routine blood profile except a mild microcytosis that may be often confused with iron deficiency. Homozygotes for EE may show mild anemia with microcytosis. However, their diagnosis of an almost asymptomatic HbE trait is crucial in the context of transmission of E-beta thalassemia to the next generation. Although most of the routine hematological tests fail to differentiate between the trait and normal persons, efforts are there to find out parameters that can play vital roles in distinguishing between the HbE trait and normal persons using routine blood tests and fetal hemoglobin (HbF). HbF is the commonest parameter that is found to be increased in the E-beta thalassemia trait, but its increase is reported to be closely associated with some polymorphic variation of the genes linked to beta-thalassemia.[4, 5] Similar variabilities in HbF have been found in patients suffering from E-alpha thalassemia.[6, 7] However, very few studies were found that described a significant difference between the routine blood parameters that could differentiate between E thalassemia trait patients and the normal population. Till now, although some of the routine blood indices like MCV, MCH, red cell distribution width (RDW), and HbF are higher in the HbE trait persons, studies have underscored the fact that a suitable cut-off value could not be established for these factors that could distinguish the HbE trait persons with significant high sensitivity and specificity [8].

The research question of the present study was whether there could be any blood parameters that could be found to differentiate between the E thalassemia trait and normal persons using routine hematological tests. Furthermore, our aim was also to ascertain any suitable cut-off point for those parameters that were found to be significantly higher in the trait persons. Based on this, the present study was undertaken to find out whether any parameters generally included in routine laboratory tests can be used to differentiate between the E thalassemia trait and normal persons in a statistically significant way.

MATERIALS AND METHODS:

The present study was conducted from the data availed from a tertiary care laboratory in Guwahati, Assam, that performed routine and specialized analysis of different hemoglobinopathies with an established quality control program.

Selection of study population:

The study population for the present study was the persons attending the laboratory for screening and confirmatory tests for different diagnosed and suspected hemoglobinopathies. Data from patients of all age groups and both sexes were selected for one year i.e. 2022-23. Data from the patients who received a blood transfusion within the last four months or suffered from any other chronic disorders, malignancies, or any endocrinological disorders were excluded. Data from the patients who were pregnant were also excluded.

Procedural methods:

All data included in the present study were generated from validated and calibrated procedures. The hematological data were generated from 5 5-part cell counters and HPLC data of hemoglobin fractions were obtained from the Bio-Rad D10 HPLC analyzer.

Data analysis:

Data collected for the study period as mentioned above were analysed for their distribution pattern using the Smirnov Kolmogorov test. Their mean values were ascertained using the independent t-test after ascertaining their normal distribution pattern. Study parameters that showed a significant difference between the HbE trait and the normal subjects were further analyzed to obtain a suitable cut-off value with the highest possible sensitivity and specificity using the receiver and operator characteristic (ROC) curve. We used the SPSS 21 software package (IBM) for performing all statistical analysis. Association among the study parameters was ascertained by Pearson's bivariate correlation study. A 'p' value of .05 or less was significant for statistical interpretation at a confidence level of 95 percent.

RESULTS:

For the above-mentioned study period, we availed data for 53 persons diagnosed with the HbE trait (test population) and 175 data from normal persons (control subjects). The Smirnov Kolmogorov test for assessing the normal distribution of data was carried out first the result of which (not shown in the table) suggested that all data were distributed following an approximate normal pattern ($p>.05$). Hence, we selected the parametric test procedure for comparison of means of the study variables.

The independent t-test (Table 1) showed that the MCV and MCH values in the test population were found to be significantly lower in the HbE trait patients ($p<.001$) while the RDW and HbF values in the trait group were significantly higher ($p<.001$). Although the hemoglobin level, PCV, and MCHC were found to be lower in the trait group the difference was not significant statistically ($p > .05$). The ROC curve for these parameters showed significant differences between the HbE trait and normal groups i.e the RDW and HbF showed that their cut off values were 13.8 and 0.85 percent with an area under the curve (AUC) of 0.806 and 0.696 respectively (Table 2, Fig 1). The 13.8 cut-off value of RDW has a sensitivity and false positivity (1-specificity) of 0.94 and 0.37 The parameters e.g. MCV and MCH showed a cut-off value of 75.0 and 24.9 and the AUC as 0.773 and 0.729 respectively (Table 2, Fig 2). The correlation study revealed a significant negative correlation of the RDW with MCV and MCH in the trait group ($P < .001$, Table 3, Fig 3).

DISCUSSION:

In the present study, we found that the values of RDW and HbF were significantly higher in the trait group in comparison to the normal population (Table 1). The mean values of RDW and HbF were 15.6 and 1.1 respectively in the trait which are significantly higher than the corresponding values in the control group. In one of our previous studies, we showed that these values were 16.51 and 4.76 respectively in the HbE homozygous patients.[9] The data in our present study thus demonstrate that these values are relatively lower in the HbE trait subjects, albeit significantly higher than in the normal population.

The RDW is a measure of RBC anisocytosis that has been used to differentiate between iron deficiency anemia and different types of thalassemia traits and a range of cut-off values from 13.4 to 21.0%.[10-16] With this wide range of cut-off values of RDW obtained from multiple studies, in the present study we found a cut-off value of RDW to be 15.6 in the Assamese population which corroborates well with the cut-off values of this index throughout the world. So, we propose that with a significantly higher value in the HbE trait persons and a valid cut-off value obtained through a valid ROC analysis, RDW may be used as a valid and strong marker for differentiating between the HbE trait persons and the normal subjects.

HbF has been one of the most important predictors of morbidity in patients suffering from HbE, but its level depends upon several co-existent hemoglobin variants as well as different genetic susceptibilities like co-existent β thalassemia and Xmn I(+) polymorphism.[17-19] Furthermore, co-inheritance of α thalassemia in its different forms may also affect the levels of HbF in HbE trait patients. In our present study, significantly raised HbF

values in the trait population (Table 1) prompted us to check for a suitable cut-off value that could differentiate between the trait and healthy normal persons. However, the cut-off value found here (0.85) was not so robust as the sensitivity and specificity were not very high (0.52 and 0.13 respectively) with a medium-range AUC of 0.696. From these results, we suggest that the HbF parameter should not be used as a single parameter to differentiate between the HbE trait and normal persons, rather it is more useful in conjunction with the RDW values which have more robust AUC, sensitivity, and specificity as indicated by the corresponding ROC analysis (Fig 1, Table 2).

On the other hand, the two common parameters of the routine blood tests, MCV and MCH showed better results in the ROC analysis with AUC of 0.773 and 0.729 respectively with a good sensitivity and specificity (Fig 2, Table 2). Moreover, these two parameters showed a significant difference between the case and control groups with lower values in the HbE trait with P <.001 (Table 1). In our study, no significant difference was observed in the MCHC values between the two groups (p = .80). Our study results correlate with some previous studies where similar outcomes were observed for the values of MCH, MCV, and MCHC.[20] In the bivariate correlation analysis (Table 3, Fig 3), the RDW values were found to exhibit a significant negative association with MCH and MCV values in the HbE trait patients (P < .001). This reciprocal relationship of RDW with MCV and MCH in the HbE trait patients further validated the fact that these routine blood parameters could be used as dependable markers for differentiating HbE trait patients from the normal population through a wide range of values.

In conclusion, the present study provides some important guidelines for differentiating HbE trait patients from normal subjects using routine hematological assays and HbF measurements. The RDW was found to be the best indicator for this differentiating task with a cut-off value of 13.8. Other parameters like HbF, MCV, and MCH were also relevant and valid for differentiating HbE traits and normal subjects. We propose that these laboratory parameters may be used as successful routine screening tests for HbE traits at even small laboratories before pursuing the more specific and definitive tests like HPLC and DNA testing that are available only at high-end laboratories.

REFERENCES:

1.Williams TN, Weatherall DJ. World distribution, population genetics, and health burden of the hemoglobinopathies. Cold Spring Harb Perspect Med. 2012;2(9):a011692Available from: https://www.ncbi.nlm.nih.gov/pubmed/22951448.

2.Mondal SK, Mandal S. Prevalence of thalassemia and hemoglobinopathy in eastern India: A 10-year high-performance liquid chromatography study of 119,336 cases. Asian J Transfus Sci. 2016;10(1):105-10Available from: https://www.ncbi.nlm.nih.gov/pubmed/27011683.

3.Weatherall DJ, Clegg JB. Inherited hemoglobin disorders: an increasing global health problem. Bull World Health Organ. 2001;79(8):704-12Available from: https://www.ncbi.nlm.nih.gov/pubmed/11545326.

4.Gibbons R, Higgs DR, Olivieri NF, Wood WG. The β and δβ thalassaemias in association with structural hemoglobin variants. In: Weatherall DJ, Clegg JB, eds. The Thalassaemia Syndromes (Fourth ed). Oxford, United Kingdom: Blackwell Science; 2001:393-449.

5.Fisher CA, Premawardhena A, de Silva S, Perera G, Rajapaksa S, Olivieri NA, et al. The molecular basis for the thalassaemias in Sri Lanka. Br J Haematol. 2003;121(4):662-71Available from: https://www.ncbi.nlm.nih.gov/pubmed/12752111.

6.Sripichai O, Fucharoen S. Genetic polymorphisms and implications for human diseases. J Med Assoc Thai. 2007;90(2):394-8Available from: https://www.ncbi.nlm.nih.gov/pubmed/17375650.

7.Sripichai O, Whitacre J, Munkongdee T, Kumkhaek C, Makarasara W, Winichagoon P, et al. Genetic analysis of candidate modifier polymorphisms in Hb E-beta 0-thalassemia patients. Ann N Y Acad Sci. 2005;1054:433-8Available from: https://www.ncbi.nlm.nih.gov/pubmed/16339693.

8.Tatu T. Laboratory Diagnosis of β-Thalassemia and HbE [Internet]. Beta Thalassemia. IntechOpen; 2020. Available from: http://dx.doi.org/10.5772/intechopen.90317.

9.Biswas T, Thakuria G. Establishment of diagnostic cut-off values of MCV, Fetal hemoglobin and Red Cell Width Distribution for HbE disease and HbE trait. Journal of Applied Biochemistry & Laboratory Medicine 2023; 04 (1):5-9.
Access the link for this article https://jablm.acclmp.com/2022/04/01_5-9.pdf.

10.Plengsuree S, Punyamung M, Yanola J, Nanta S, Jaiping K, Maneewong K, et al. Red Cell Indices and Formulas Used in Differentiation of Beta-Thalassemia Trait from Iron Deficiency in Thai Adults. Hemoglobin. 2015;39(4):235-9Available from: https://www.ncbi.nlm.nih.gov/pubmed/26076394.

11.Bessman JD, Gilmer PR, Jr., Gardner FH. Improved classification of anemias by MCV and RDW. Am J Clin Pathol. 1983;80(3):322-6Available from: https://www.ncbi.nlm.nih.gov/pubmed/6881096.

12.AlFadhli SM, Al-Awadhi AM, AlKhaldi D. Validity assessment of nine discriminant functions used for the differentiation between iron deficiency anemia and thalassemia minor. J Trop Pediatr. 2007;53(2):93-7Available from: https://www.ncbi.nlm.nih.gov/pubmed/17166936.

13.Ntaios G, Chatzinikolaou A, Saouli Z, Girtovitis F, Tsapanidou M, Kaiafa G, et al. Discrimination indices as screening tests for beta-thalassemic trait. Ann Hematol. 2007;86(7):487-91Available from: https://www.ncbi.nlm.nih.gov/pubmed/17476506.

14.Aulakh R, Sohi I, Singh T, Kakkar N. Red cell distribution width (RDW) in the diagnosis of iron deficiency with microcytic hypochromic anemia. Indian J Pediatr. 2009;76(3):265-8Available from: https://www.ncbi.nlm.nih.gov/pubmed/19205647.

15.Hoffmann JJ, Urrechaga E, Aguirre U. Discriminant indices for distinguishing thalassemia and iron deficiency in patients with microcytic anemia: a meta-analysis. Clin Chem Lab Med. 2015;53(12):1883-94Available from: https://www.ncbi.nlm.nih.gov/pubmed/26536581.

16.Zaghloul A, Al-Bukhari TA, Bajuaifer N, Shalaby M, Al-Pakistani HA, Halawani SH, et al. Introduction of new formulas and evaluation of the previous red blood cell indices and formulas in the differentiation between beta thalassemia trait and iron deficiency anemia in the Makkah region. Hematology. 2016;21(6):351-8Available from: https://www.ncbi.nlm.nih.gov/pubmed/26907523.

17.Fucharoen S, Ketvichit P, Pootrakul P, Siritanaratkul N, Piankijagum A, Wasi P. Clinical manifestation of beta-thalassemia/hemoglobin E disease. J Pediatr Hematol Oncol. 2000;22(6):552-7Available from: https://www.ncbi.nlm.nih.gov/pubmed/11132229.

18.Rees DC. Hemoglobin F and hemoglobin E/beta-thalassemia. J Pediatr Hematol Oncol. 2000;22(6):567-72Available from: https://www.ncbi.nlm.nih.gov/pubmed/11132232.

19.Rees DC, Porter JB, Clegg JB, Weatherall DJ. Why are hemoglobin F levels increased in HbE/beta thalassemia? Blood. 1999;94(9):3199-204Available from: https://www.ncbi.nlm.nih.gov/pubmed/10556208.

20.Vichinsky E. Hemoglobin e syndromes. Hematology Am Soc Hematol Educ Program. 2007:79-83Available from: https://www.ncbi.nlm.nih.gov/pubmed/18024613.

ACKNOWLEDGEMENT: NIL
CONFLICT OF INTEREST: Nil

Tables and Figures.

	Mean ± SD	**P value**
Hb (g/dl) Normal	10.4 ± 2.2	0.23
Hb (g/dl) trait	10.0 ± 1.8	
PCV (%) normal	31.0 ± 6.2	0.26
PCV (%) trait	30.0 ± 4.8	
MCV (fl) normal	79.2 ± 9.5	<.001
MCV (fl) trait	70.6 ± 6.5	
MCH (pg) Normal	26.2 ± 3.7	<.001
MCH (pg) trait	23.6 ± 3.2	
MCHC (g/dl) Normal	33.1 ±1.9	.803
MCHC (g/dl) trait	33.1 ± 1.7	
HbF (%) normal	0.85 ± 0.19	<.001
HbF (%) trait	1.1 ± 0.46	
RDW (%) normal	13.60 ± 1.77	<.001
RDW (%) trait	15.60 ± 2.32	

Table 1: Results of independent t-test for the study parameters between the case and control group
P value significant at P < .05 for 95% confidence interval

Table 2: Data for ROC analysis of the relevant study parameters

Parameters	**Sensitivity**	**False positivity (1-specificity)**	**Area under curve (AUC)**	**Cut off value**
RDW	0.94	0.37	0.806	13.8
MCV	0.70	0.26	0.773	75.0
MCH	0.70	0.37	0.729	24.9
HbF	0.52	0.13	0.696	0.85

P value significant at P < .05 for 95% confidence interval

Table 3: Results of bivariate correlation analysis among the study parameters of the case group.

Correlations					
		RDW	MCH	MCV	HbF
RDW	Pearson Correlation	1	-.697**	-.569**	.015
	Sig. (2-tailed)		.000	.000	.917
	N	53	53	53	53
MCH	Pearson Correlation	-.697**	1	.932**	-.066
	Sig. (2-tailed)	.000		.000	.638
	N	53	53	53	53
MCV	Pearson Correlation	-.569**	.932**	1	-.060
	Sig. (2-tailed)	.000	.000		.669
	N	53	53	53	53
HbF	Pearson Correlation	.015	-.066	-.060	1
	Sig. (2-tailed)	.917	.638	.669	
	N	53	53	53	53
**. Correlation is significant at the 0.01 level (2-tailed).					

P value significant at P < .05 for 95% confidence interval

Figure 1: ROC curve analysis to show the cut-off values RDW and HbF

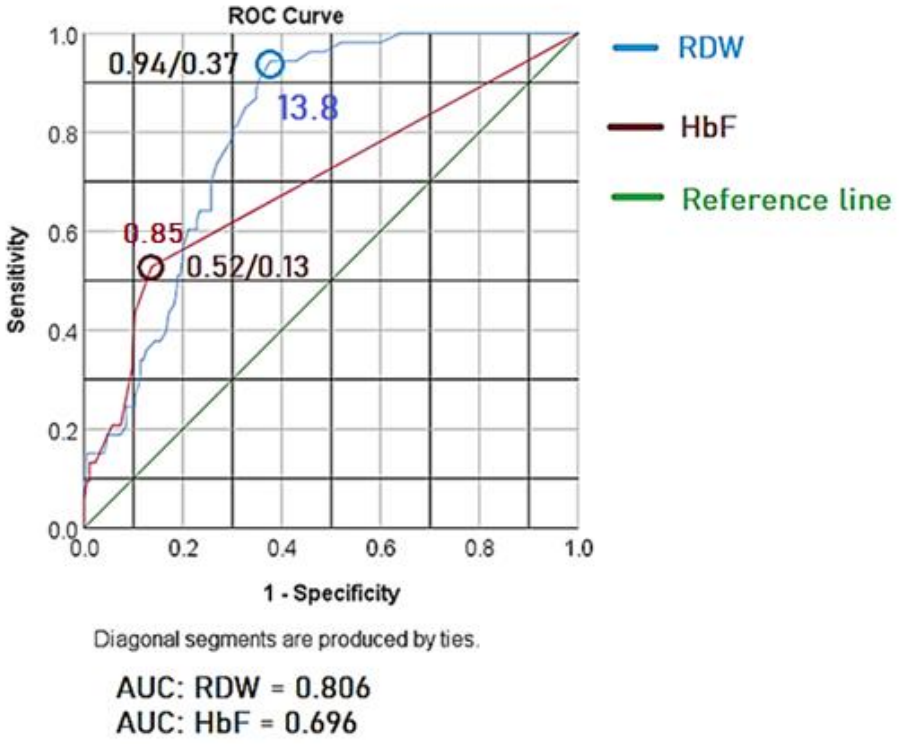

Figure 2: ROC curve analysis to show the cut-off values MCV and MCH

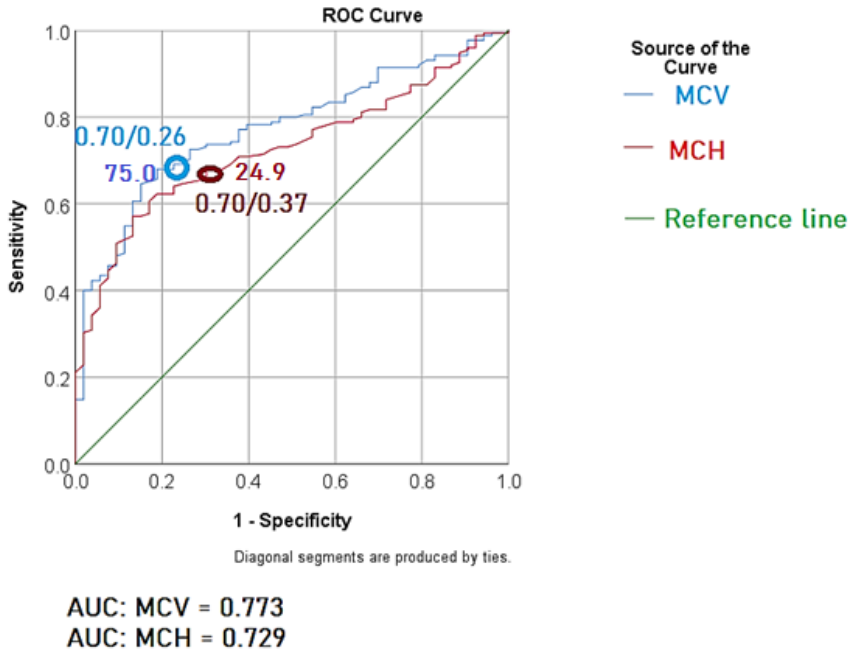

Figure 3: Results of bivariate correlation analysis among the study parameters of the case group.