Bangladesh J Obstet Gynaecol, 2024; Vol. 39(2): 81-90

# Red Blood Cell Indices as a Primary Tool for Carrier Screening of Thalassemia & E Disease in Pregnancy

TABASSUM PARVEEN[1], MAHFUJA ASMA [2], NAHREEN AKHTAR[3] , NIGAR SULTANA[4]
SELIMA KAWSAR [5], LUBNA NAZNIN[6]

**Abstract**

***Backgrounds:*** *â thalassemia major and Hemoglobin (Hb) E-β thalassemia appears as a significant threat for the health status of a country including Bangladesh. Pregnancy is a state when many women come for antenatal care and complete blood count (CBC) is done as a routine test. The component of this test the red blood cell (RBC) indices include mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). These* measure the size, shape, and physical characteristics of the RBC and can be utilized *as a first round screening tool for carrier screening of defective Hb and then subjected to diagnostic testing i.e. Hb-electrophoresis which is a costly and not readily available test in all health facilities. The cutoff value at which the RBC indices are most sensitive and specific for the subsequent diagnostic test was the main objective of this study.*

***Method:*** *This cross sectional study was conducted from January 2023 to July 2023 among pregnant women attended for antenatal care between 10-18 weeks of gestation in the Out Patient Department (OPD) of Fetomaternal Medicine Department of Bangabandhu Sheikh Mujib Medical University. After getting the complete blood count (CBC) report by convenient sampling 112 pregnant women were selected whose MCV value was < 80fl and were subjected to Hb-Electrophoresis to detect defective Hb i.e. â thalassemia and E trait.*

***Result:*** *Cut off value of MCV for detection of defective Hb was 75.1 fl with the sensitivity 81.8% and specificity 64.3%. For MCH best cut off point was 23.5pg with the sensitivity 72.7%% and specificity 62.9%.*

***Conclusion:*** *During pregnancy RBC indices showing MCV ≤75 fl and/or MCH ≤23.5pg should be advised for Hb Electrophoresis for detection of defective Hb in the form of thalassemia trait, E trait or E disease.*

***Key words:*** *Hb E β Thalassemia, E trait, Thalassemia minor*

**Introduction:**

Inherited disorders of abnormal or defective hemoglobin synthesis is one of the commonest single gene disorders and commonly fall into two groups; the structural haemoglobin (Hb) variants and the Thalassemia. Among the thalassemia's, β-thalassemia is quantitative defect in production of β-globin chains of Hb due to mutation on β-globin (HBB) gene resulting in

1. Professor & Chairman; Department of Fetomaternal Medicine, Bangabandhu Sheikh Mujib Medical University (BSMMU).
2. Assistant Professor, Department of Obs & Gynae , Sir Salimullah Medical College & Mitford Hospital Hospital.
3. Professor & Treasurer, Department of Fetomaternal Medicine, Bangabandhu Sheikh Mujib Medical University (BSMMU).
4. Assistant Professor , Department of Obstetrics & Gynaecology , BSMMU. nigarrumy1@gmail.com
5. Senior Consultant, Dept. of Obstetrics & Gynae, BSMMU, Dhaka.
6. Classified Specialist in pathology, Armed Forces Medical Institute (AFMI), Bangladesh.

**Address of Correspondence:** Prof. Tabassum Parveen, Room no – 809, 7th floor, C block, Bangabandhu Sheikh Mujib Medical University (BSMMU). Email: drtparveent@gmail.com

ORCID ID : https://orcid.org/0009-0003-0115-7616

insufficient ($\beta^{+}$–$\beta^{++}$) or absent ($\beta^{0}$) synthesis of β-globin chain. β-thalassaemia trait is the carrier state with one defective or missing beta gene. When there is co-inheritance of a β-thalassaemia trait from both parent, the offspring has 25% chance of becoming thalassemia major. On the other hand structural Hb variants occurs due to substitution of single amino acid of either α or β chain of Hb.[1] Among 700 structural hemoglobin variants, Hemoglobin E disease (HbE) is extremely common and occurs at high frequencies throughout Asian countries including Bangladesh. In HbE, at position 26 of beta chain of Hb there is a change in the amino acid sequence, from glutamic acid to lysine.[2] When there is co-inheritance of β-thalassaemia trait from one parent and the HbE trait from the other parent there is 25% chance to have Hb E/β-thalassaemia in the offspring which often produce features similar to β-thalassaemia major. Worldwide, Hb-E/β-thalassaemia represent approximately 50 per cent of those affected with severe beta thalassaemia.[3]

â thalassemia major and HbE-β thalassemia appears as a significant threat for the health status of a country and is a public health concern. Southeast Asia, are considered as part of the 'world thalassaemia belt'. Being part of this Bangladesh appears as a highly prevalent zone for thalassemia.[4] Study shown the number of patients suffering from â thalassemia major and HbE-β-thalassemia are approximately 60,000–70,000 in Bangladesh[5]. In addition 6–12% of the population (about 10–19 million people) in Bangladesh are carriers of a gene either β or E trait that can lead to thalassemia[6]. With the birth rate of 21.6/1000, it could be estimated that nearly 2500 thalassemia major/ Hb-E β-thalassemia cases are added every year in Bangladesh.[5]

To face this challenge the only way is the effective screening to find out a person at carrier state or trait and then adopting primary and secondary prevention of this condition. Considering the burden of the disease on the family as well as society, thalassemia prevention program is the need of the hour in Bangladesh. To prevent and control births of the new cases, an accurate identification of couple who both carry the genes and capable of transmitting it to their offspring is needed. American College of Obstetricians and Gynecologists (ACOG) recommends screening for β thalassemia in couples of Southeast Asian population.[7] World health organization (WHO) recommends to incorporate the policy of screening to identify carriers of hemoglobinopathy in basic health services.[8] The organization also recommends to assess the risk of producing severely affected progeny, followed by genetic counseling and give the provision of options to avoid such births, ideally prior to conception.[9] Many countries get success in reducing the prevalence of thalassemia by effective screening program. In Turkey voluntary screening and nondirective counselling showed reduction of the birth rate of thalassaemia major from 1:250 live births to 1:4,000 .[10]

Two possible methodological approaches for carrier screening are commonly practiced. First one is a primary screening procedure by RBC indices followed by a secondary screening by Hb Electrophoresis in subjects with reduced MCV and/or MCH. Second one is complete screening at the outset by Hb Electrophoresis in all subjects.

In a country like Bangladesh with constraints of availability of Hb Electrophoresis in all level of health facilities, the first one is more feasible i.e. RBC indices as it is a common test and cost effective test of prenatal care. To make it a feasible approach it is logical to know the cut off values of RBC indices to be subjected for Hb Electrphoresis in this population. In this study it was the main objectives of the study.

Red blood cell (RBC) indices as component of complete blood count (CBC) namely mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) along with Hb level have been shown a promising screening tool in this aspect. RBC indices along with other parameters, complete blood count is done by an automated without any extra effort and cost. This machine is available in many government and private sector even in remote corners of the country. With the general availability of electronic cell counters, red cell indices are now automatically measured in all blood count determinations.

Among the indices MCV defines the size of the red blood cells and is expressed as femtoliters ($10^{"15}$; fl) or as cubic microns (ìm$^3$). The normal values for MCV are considered 87 ± 7 fl. MCH quantifies the amount of hemoglobin per red blood cell. The normal values

for MCH are 29 ± 2 picograms (pg) per cell. MCHC indicates the amount of hemoglobin per unit volume. It is expressed as g/dl of red blood cells or as a percentage value. The normal values for MCHC are 34 ± 2 g/dl. Studies shown that MCV less than 80 fL and/or MCH value of less than 27 pg i.e. when they are at the lower limit of their normal range, can be used as cutoff levels for further screening of thalassemia trait or E-trait by Hb Electrophoresis.[11,12.] If RBC indices are found suggestive then patient can be referred for Hb Electrophoresis for confirmation of the carrier state. This is also recommended in the National guidelines on thalassemia management for physician in Bangladesh.[13]

But so far known no studies were done in context of Bangladesh to explore the cut off value of the RBC indices in pregnancy which is most sensitive and specific for further screening by Hb Electrophoresis to detect defective Hb. This small study was intended to explore the sensitivity and specificity of different RBC indices as a first round screening tool for detection of thalassemia trait and E trait in our population.

**Materials and Methods:**

The study was carried out among women attending for regular antenatal care (ANC) at their first booking visit between 10-18 weeks of gestation in the Out Patient Department (OPD) of Fetomaternal Medicine Department of Bangabandhu Sheikh Mujib Medical University (BSMMU) after taking verbal consent.Patient having any known hematological disorders or serious illness were excluded from the study. Those who received blood transfusion for any reason in the past 1 month were excluded from the study.It was a cross sectional study done from January 2023 to July 2023. Complete blood count was done as a routine antenatal test by collecting blood sample in EDTA tube in the department of Hematology of Bangabandhu Sheikh Mujib Medical University (BSMMU). Hemoglobin and red blood cell indices (MCH, MCV, and MCHC) were measured using automated blood cell counter (Sysmex KX-21) on the same day in the Haematology department of the institute. By convenient sampling 112 pregnant women whose MCV value was < 80fl were selected as samples. After taking verbal consents, samples were subjected for Hb Electrophoresis and results were noted in a predesigned data sheet. β-thalassemia trait and E trait were diagnosed as per protocol of the national guideline.[13] β-thalassemia trait was diagnosed when Hb Electrophoresis report showed Hb A 90-95%, Hb A2 level ≥3.5% &/or Hb F level 1-5%. Hb E trait was diagnosed when Hb E level >15%, Hb A2 level 2-7% and Hb F level <1%, and rest were HbA. Hb E disease was diagnosed HbA 0%, Hb F<5%, Rest is Hb E+ HbA2. All abnormal Hb was designated as 'abnormal or defective Hb' in this study.

The demographic and clinical details were noted as part of routine antenatal care and recorded on the antenatal card and data collection sheet made for the purpose of the study.

**Statistical analysis :**

Statistical analysis was performed with SPSS v.20.0 Distribution of study subjects based on Hb Electrophoresis was expressed as frequency and percentage. Data of the different study parameters for normal & abnormal Hb states were expressed as, Mean±2SD. Comparison between the normal & defective Hb states were done by unpaired t-test and one way ANOVA. p value ≤0.05 was considered statistically significant. Plotting of ROC curve was carried out for different variables to demonstrate the cut off value; sensitivity & specificity.

**Results:**

Among 112 samples who had MCV value < 80fl, 65.2% were found to have normal Hb. Among the rest, 18.8% had E trait and 13.4% had beta thalassemia trait and 2.7% had Hb-E disease (Table I).

**Table-I**

*Distribution of Study subjects according to report of Hb Electrophoresis*

| Variants of Hb | Frequency | Percentage |
|---|---|---|
| Normal | 73 | 65.2 |
| Hb E trait | 21 | 18.8 |
| B thalassemia trait | 15 | 13.4 |
| Hb E Disease | 03 | 2.6 |
| Total | 112 | 100.0 |

Among 112 samples, 65.2% were found normal. Among the rest, 18.8% had E trait and 13.4% had beta thalassemia trait and 2.7% had Hb-E disease.

**Table-II**

*Mean value of the study parameters in normal & defective Hb states*

| Traits | Mean ± SD | | | |
|---|---|---|---|---|
| | Normal | Hb-E trait | Thalassemia trait | Hb-E disease |
| Age | 27.37 ± 4.50 | 28.71 ± 3.99 | 25.3 ± 3.81 | 28.3 ± 1.53 |
| Hb% (gm/dL) | 10.39 ± 1.38 | 10.19 ± 1.43 | 9.74 ± 0.62 | 8.57 ± 0.51 |
| MCV (fl) | 76.83 ± 3.68 | 70.11 ± 4.89 | 64.19 ± 6.07 | 60.97 ± 5.92 |
| MCH (pg) | 24.92 ± 2.51 | 22.80± 1.94 | 20.13 ± 2.43 | 20.73 ± 2.15 |
| MCHC(gm/dL) | 32.28 ± 1.79 | 32.55 ± 1.65 | 31.56 ± 1.41 | 34.00 ± 0.40 |

Other than Hb E disease , in carrier state Hb level , MCV & MCH were observed lowest in thalassemia trait group. MCV was found too low in thalassemia trait ( 64.19 ±6.07 fl). MCHC was found almost normal in all cases of defective Hb states.

**Table-III**

*Comparison of study parameters in normal and defective Hb states*

| Study Parameters | Mean ± SD | | p value |
|---|---|---|---|
| | Normal (n=73) | E-Trait, Thalassemia trait and E disease (Defective Hb) (n= 39) | |
| Age | 27.37 ± 4.50 | 27.38 ± 4.07 | 0.986 |
| Hb%(gm/dL) | 10.39 ± 1.38 | 9.89 ± 1.20 | 0.51 |
| MCV(fL) | 76.83 ± 5.68 | 67.13 ± 6.27 | 0.00 |
| MCH(pg) | 24.92 ± 2.51 | 21.61 ± 2.47 | 0.00 |
| MCHC(gm/L) | 32.28 ± 1.79 | 32.28 ± 1.63 | 0.991 |

Among the RBC indices MCV & MCH were found significantly less in defective Hb states when compared to normal

Other than Hb-E disease, in carrier state lowest Hb level, MCV, MCH were observed in thalassemia trait group. MCV was found too low in thalassemia trait (64.19 ± 6.07 fl). (Table II). MCHC was found almost normal in all the cases of defective Hb states.

Among the RBC indices MCV & MCH were found significantly less in defective Hb states when compared to normal. (Table III)

By constructing the ROC curve, for the variable haemoglobin (Hb), 10.65gm was found the best cut off point for detection of Thalassemia Trait or E Trait/ disease with sensitivity 77.3% and specificity 52.9% (Fig. 1).

For the variable MCV, the best cut off point was found 75.1fl with sensitivity 81.8% and specificity 64.3% (Fig. 2).

For the variable MCH, the cut off point was 23.5 pg. showing sensitivity 72.7%% and specificity 62.9% (Fig. 3).

For the variable MCHC, the cut off point was 32.1gm/dl and the sensitivity for detection of defective Hb was 54.5% and specificity 61.4% (Fig. 4)

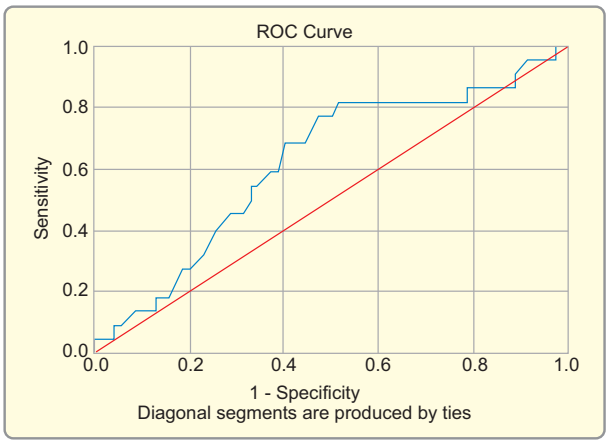


**Figure 1:** *Plotting of ROC curve for the variable Hb level for detection of Trait*

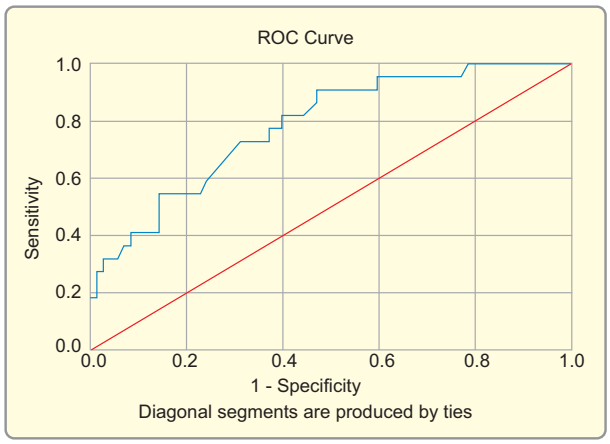


**Figure 2:** *Plotting of ROC curve for the variable MCV for detection of Trait*

| Test Result Variable(s): Hb | | | | |
|---|---|---|---|---|
| Area Under the Curve | | | | |
| AUC | Std. Error[a] | p-value | Asymptotic 95% Confidence Interval | |
| | | | Lower Bound | Upper Bound |
| .622 | .069 | .085 | .488 | .756 |

Cut off point= 10.65; Sensitivity = 77.3%; Specificity = 52.9%
For the variable haemoglobin (Hb), 10.65gm cut off point for detection of Thalassemia Trait or E Trait/disease was 77.3% and specificity 52.9%

| Area Under the Curve | | | | |
|---|---|---|---|---|
| Test Result Variable(s): MCV | | | | |
| Area | Std. Error[a] | Asymptotic Sig.[b] | Asymptotic 95% Confidence Interval | |
| | | | Lower Bound | Upper Bound |
| .814 | .050 | .000 | .716 | .912 |

Cutoff point= 23.5; Sensitivity = 72.7%; Specificity = 62.9%
For the variable MCH, at a cut off point 23.5 the sensitivity for detection of Trait was 72.7%% and specificity 62.9%

| Area Under the Curve | | | | |
|---|---|---|---|---|
| Test Result Variable(s): MCH | | | | |
| Area | Std. Error[a] | Asymptotic Sig.[b] | Asymptotic 95% Confidence Interval | |
| | | | Lower Bound | Upper Bound |
| .782 | .054 | .000 | .677 | .888 |

Cutoff point= 23.5; Sensitivity = 72.7%; Specificity = 62.9%
For the variable MCH, at a cut off point 23.5 the sensitivity for detection of Trait was 72.7%% and specificity 62.9%

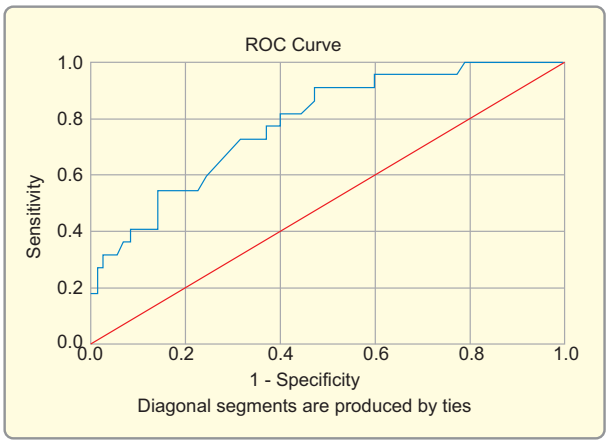


**Figure 3:** *ROC curve for the variable MCH for detection of Trait*

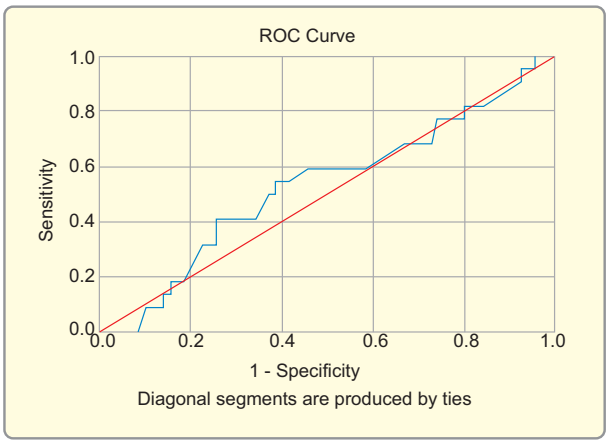


**Figure 4:** *ROC curve for the variable MCHC for detection of Trait*

| Area Under the Curve | | | | |
|---|---|---|---|---|
| Test Result Variable(s): MCHC | | | | |
| Area | Std. Error[a] | Asymptotic Sig.[b] | Asymptotic 95% Confidence Interval | |
| | | | Lower Bound | Upper Bound |
| .530 | .072 | .674 | .389 | .671 |

Cut off point= 32.1 with Sensitivity 54.5% & Specificity 61.4%
For the variable MCHC, at a cut off point 32.1 the sensitivity for detection of Trait was 54.5% and specificity 61.4%

**Discussion:**

In this study the distribution of defective Hb in the form of Hb E trait,β thalassemia trait and Hb E disease were 18.8%, 13.4% and 2.6% respectively i.e. in total 34.9% (Table 1), which were surprisingly very high. This may be due to the fact that samples of this study were those having MCV <80fl and almost all had Hb level <11gm/dl among which chance of defective Hb are high. In addition in a study high prevalence of Hb E & thalassemia trait were noted among anemic women in rural Bangladesh which was 28%.[14] In another study among apheretic donors those who had Hb 12.5gm/dl but MCV 80fl, hemoglobinopathy without concomitant iron deficiency was found in 36% cases which was very close to this study.[15] Findings of the present study and other studies showed high prevalence of defective Hb in cases of anemia and alerts proper assessment to find out the cause of anemia before starting treatment blindly. This findings also points to a fact that the actual prevalence of defective Hb in the form of thalassemia trait, E trait, even E disease in our population specially among pregnant population who had low MCV might be much higher than those considered as 6–12% in general population[16] and warranted further large studies in this field.

In this study in β Thalassemia trait mean Hb level, MCV and MCH were 9.74 ± 0.62 gm/dl, 64.19 ± 6.07 fl, 20.13 ± 2.43pg respectively (Table 2) which were close to the findings of the study of Wickramaratne et al where the mean Hb concentration, MCV and MCH in BTT (â thalassemia trait )group were 10.45 ± 1.6 g/dL, 62.1 ± 5.4 fl, and 19.7 ± 1.7 pg, respectively.[17] This study showed that the Hb level was almost comparable in normal and defective Hb group ( 10.39 ± 1.38 Vs. 9.89 ± 1.20 gm/dl). But MCV level remains too low and almost always <70fl (64.19 ± 6.07) in beta thalassemia trait. MCH level was also found remarkably low in thalassemia trait, <22pg in almost all cases. In a retrospective analysis, CBC and Hemoglobin (Hb) electrophoresis of 378 patients in Baghdad university, MCV was found 65.3, MCH 19.4 among BTT which were very close to the present study.[18] In another study in Iraq on utility of Red Blood Cell Indices in the diagnosis of â-Thalassemia minor,

MCV and MCH were found to be highly sensitive as screening tests with the mean MCV 61.97±5.20 fL ($p < 0.01$) which was also very close to this study. [19] In this study MCV level was also low in Hb E trait when compared to normal (70.11 ± 4.89 Vs. 76.83 ± 5.68) and almost always <75 fl but it was not that much low which was observed (<70 fl) in beta trait. E trait also cause smaller-than-normal red blood cells i.e. microcytic RBC which can lead to low MCV. Findings of this study was close to the findings of Anjali Sharma et al of India where mean MCV was 74.7fl in E trait.[20] E trait is very common in Bangladesh and its combination with beta trait can result in HbE-β thalassemia in the offspring and may require regular blood transfusion lifelong . So if E trait is detected in pregnancy, it immediately mandates carrier screening of the partner.

Interestingly in this study 2.6% patients were found to have Hb E disease who were totally asymptomatic. In these cases both MCV and MCH were very low 60.97±5.92 fl and 20.73±2.15pg respectively i.e. lowest among all the cases but MCHC was quite normal (34.00 ± 0.40 gm/dl). MCHC was also not found to be raised in both E trait and BTT and even in E-disease (Table 2). MCHC indicates the average concentration of Hb per RBC. The study findings thus clearly indicates that when MCHC is normal and MCV & MCH are too low, some sorts of defective Hb either in the form of β trait, E trait or even E disease can be there and needs proper surveillance.

The value at which the normal range for a parameter changes to an abnormal range is called the cut-off value. The most widely used cut-off values of MCV and MCH for carrier screening of defective Hb are considered as 79 fl and 27 pg respectively. Values below are needed to make a precise diagnosis.[21] In Bangladesh the National Guidelines on Thalassemia Management for Physician emphasized that Hb electrophoresis should be done in any adult with or without anemia when RBC indices show MCV ≤80 fL or MCH ≤27 pg, to exclude the causes of microcytic anemia like BTT or iron deficiency anemia.[13] In Sri Lanka as well, the National Thalassemia screening program uses MCV below 80 fl and MCH below 27 pg as the cut off values to decide on performing confirmatory test to find out the carrier state of beta thalassemia.[12] The study of Wickramaratne et al of Srilanka showed that the application of these cut off values had 100% sensitivity and 27.2% specificity and assembled all true BTT individuals and additional 16 non-BTT individuals (n = 22) in to beta-thalassemia suspected group.[17] With this background keeping in mind in this study samples were taken who had MCV < 80fl. Amongst these samples the cut-off value of MCV as a screening test for BTT & E trait was derived by constructing the ROC curve. The area under curve for MCV was 0.814 (95% cf 0.716–0.912) (Fig. 1). In this study the most suitable cutoff value for MCV for detection of abnormal Hb was found 75.1 fl. At this cut-off, the sensitivity and specificity for detection of carrier state was 81.8% with specificity 64.3%. The study of Mayura Baliyan showed in their study the best cut off value for MCV 72fl and with this cutoff, the sensitivity and specificity were 63.67% and 68.31%, respectively. The positive predictive value was 9.7%, whereas the negative predictive value was 97.23%. [22] Next sensitive variable among RBC indices in the present study was MCH with cutoff point 23.5pg. Its sensitivity was found 72.7% and specificity 62.9%. In a study by Chatterjee et al in a population screening program in different cities of West Bengal, India on 18, 1666 cases showed that MCV values varied greatly in different conditions of hemoglobinopathies, whereas MCH provided a more stable measurement for BTT. They recommended that MCH value of <27.0 pg. is a suitable cut-off point for screening in their population. Participants with an MCH of <27.0 pg. should be investigated further to confirm or exclude a diagnosis of β-thalassemia trait.[23] In the study of Sahiratmadja et al of Indonesia recommended that the best cut off value for MCV was < 80fl or MCH was <27pg for the mass screening method for detection of BTC or E-trait in a limited capacity area.[24]

Hb level as a screening tool was not found promising in the present study. The best cut off point for detection of carrier state for Hb was 10.65 gm/dl which was almost close to the normal level. At this level it had sensitivity 77.3% but having poor specificity i.e. 52.9%. Interestingly in this study Hb level was found almost close with each other in cases of normal, E trait and BTT (Table 1). Similar observation was observed by Marwa and colleagues where Hb level were nearly equal for both groups i.e. who were normal and who had abnormal Hb.[19] So based on Hb level mass screening for thalassemia found not to be very much promising. The possible explanation for this is that β-Thalassaemia carriers (BTC) of either the $\beta^o$ or severe $\beta^+$ can lead to drop of haemoglobin levels to 2g/dl lower than normal, but vary widely. Moreover in Hb E

trait cases patient's Hb level may remain even normal. In a study on carrier screening of Thalassemia in Indonesia by Sahiratmadja et al, 79.4% of the subjects had low MCV and/or MCH with or without low Hb concentration.[24]

**Conclusion:**

This study showed that MCV<75fl and/or MCH<23.5 pg. are the best first round mass screening method for detection of abnormal Hb in the form of â-thalassemia carrier, E trait and even E disease during pregnancy

**Recommendation:**

To reduce thalassemia burden in Bangladesh, screening for thalassemia carrier state is urgently needed in pregnancy as early as possible preferably in first trimester. CBC should be recommended as a routine test of antenatal care in first prenatal visit instead of doing only Hb% for better understanding the need of further testing like Hb Electrophoresis. However, Hb Electrophoresis should be gradually installed regionally in various places wherever possible, as well as DNA analysis to confirm the mutation for an optimal carrier diagnosis.

**Limitations of the study:**

Iron profile to exclude iron deficiency anemia was not considered in our study is the limitation of this study.

**References :**


1. Weatherall D, Akinyanju O, Fucharoen S, et al. Inherited Disorders of Hemoglobin. In: Jamison DT, Breman JG, Measham AR, et al., editors. Disease Control Priorities in Developing Countries. 2nd edition. Washington (DC): The International Bank for Reconstruction and Development / the World Bank; 2006. Chapter 34. Co-published by Oxford University Press, New York. Available from: https://www.ncbi.nlm.nih.gov/books/NBK11727/
2. Young Kyung Lee, Hee-Jin Kim,[2] Kyunghoon Lee, Sang Hyuk Park, Sang Hoon Song, Moon-Woo Seong, Myungshin Kim, and Jin Yeong Han. Recent progress in laboratory diagnosis of thalassemia and hemoglobinopathy: a study by the Korean Red Blood Cell Disorder Working Party of the Korean Society of Hematology. Blood Res. 2019Mar;54(1):17–22.doi: 10.5045/br.2019.54.1.17. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6439293.
3. Olivieri NF, Pakbaz Z, Vichinsky E. Hb E/beta-thalassaemia: a common & clinically diverse disorder. Indian J Med Res. 2011;134(4):522-531. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3237252.
4. Hossain MS, Raheem E, Sultana TA, Ferdous S, Nahar N, Islam S, Arifuzzaman M, Razzaque MA, Alam R, Aziz S, Khatun H, Rahim A, Morshed M. Thalassemias in South Asia: clinical lessons learnt from Bangladesh. Orphanet J Rare Dis. 2017 May 18;12(1):93. doi: 10.1186/s13023-017-0643-z. PMID: 28521805; PMCID: PMC5437604. https://pubmed.ncbi.nlm.nih.gov/28521805.
5. Mandal PK, Maji SK, Dolai TK. Present scenario of hemoglobinopathies in West Bengal, India: An analysis of a large population. Int J Med Public Heal. 2014;4(4):496-99. https://www.cabidigitallibrary.org/doi/pdf/10.5555/20143400643.
6. Hossain MS, Hasan MM, Raheem E, Islam MS, Al Mosabbir A, Petrou M, et al. Lack of knowledge and misperceptions about thalassaemia among college students in Bangladesh: A cross-sectional baseline study. Orphanet J Rare Dis. 2020;15. doi: 10.1186/s13023-020-1323-y.https://ojrd.biomedcentral.com/articles/10.1186/s13023-020-1323-y
7. The American College of Obstetricians and Gynecologists. Carrier screening for genetic conditions. Committee Opinion No. 691.American College of Obstetricians and Gynecologists. Obstet Gynecol 2017;129:e41–55. https://www.acog.org/clinical/clinical-guidance/committee-opinion/articles/2017/03/carrier-screening-for-genetic-conditions
8. Modell B, Darlison M. Global epidemiology of haemoglobin disorders and derived service indicators. Bull World Health Organ. 2008; 86(6):480-487. https://pubmed.ncbi.nlm.nih.gov/18568278.
9. EuroGentest. **2017**. Recommendations for genetic counselling related to genetic testing.

Recomm. Doc., EuroGentest, Cent. Human Genet., Univ.Leuven, Belgium. http://www.eurogentest.org/fileadmin/templates/eugt/pdf/guidelines_of_GC56, 166. World Health Organization (WHO). **1998**. Proposed international guidelines on ethical issues in medical genetics and genetic services. Rep. WHO/HGN/GL/ETH/98.1,WHO, Geneva. http://www.who.int/genomics/publications/en/ethicalguidelines1998.pdf.

10. Cao A, Rosatelli MC, Galanello R. Control of beta-thalassaemia by carrier screening, genetic counselling and prenatal diagnosis: the Sardinian experience. Ciba Found Symp. 1996;197(137–51):151–155. https://pubmed.ncbi.nlm.nih.gov/8827372.

11. Noor FA, Sultana N, Bhuyan GS, Islam MT, Hossain M, Sarker SK, Islam K, Khan WA, Rahman M, Qadri SK, Shekhar HU, Qadri F, Qadri SS, Mannoor K. Nationwide carrier detection and molecular characterization of â-thalassemia and hemoglobin E variants in Bangladeshi population. Orphanet J Rare Dis. 2020 Jan 15;15(1):15. doi: 10.1186/s13023-020-1294-z. PMID: 31941534; PMC6961315. https://europepmc.org/article/pmc/6961315

12. Perera DM, Gunasekara D, Wijekoon A, Petrou M. Thalassaemia control in developing countries—the Sri Lankan perspective. Ceylon Med J. 2000;45:12–6. https://pubmed.ncbi.nlm.nih.gov/11006614.

13. National Guidelines on Thalassaemia Management for Physician. Developed by Safe Blood Transfusion & Thalassaemia Management Hospital Services Management Directorate General of Health Services. Page 30. https://csc.dghs.gov.bd/assets/downloads/Thalassaemia% 20Management% 20Guideline_Print%20Version.pdf.

14. Merrill RD, Shamim AA, Ali H, Labrique AB, Schulze K, Christian P, West KP Jr. High prevalence of anemia with lack of iron deficiency among women in rural Bangladesh: a role for thalassemia and iron in groundwater. Asia Pac J Clin Nutr. 2012;21(3):416-24. PMID: 22705433. https://www.researchgate.net/publication/227170613.

15. Bryant BJ, Hopkins JA, Arceo SM, Leitman SF. Evaluation of low red blood cell mean corpuscular volume in an apheresis donor population. Transfusion. 2009 Sep;49(9):1971-6. doi: 10.1111/j.1537-2995.2009.02207.x. Epub 2009 May 11. PMID: 19453988; PMCID: PMC3421029. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3421029.

16. Khan, W.A.; Banu, B.; Amin, S.K.; Selimuzzaman, M.; Rahman, M.; Hossain, B.; Sarwardi, G.; Sadiya, S.; Iqbal, A.; Rahman, Y. Prevalence of beta thalassemia trait and Hb E trait in Bangladeshi school children and health burden of thalassemia in our population. Dhaka Shishu (Child.) Hosp. J. 2005, 21, 1–7. https://www.researchgate.net/publication/200176529.

17. Wickramaratne KAC, Wijewickrama DC. Screening for beta-thalassemia trait; applicability of red cell indices and parameters - A study in Sri Lanka. Int J Health Sci (Qassim). 2021 Jan-Feb;15(1):29-34. PMID: 33456440; PMCID: PMC7786441). https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7786441.

18. Laith S. Mahdi, Safa A. Faraj, Hasanein H. Ghali. Significance of Red Blood Cell Indices in Beta-Thalassaemia Trait. Mustansiriya Medical Journal 2015 December ;14(2) :27-30. https://www.iasj.net/iasj/article/108767.

19. Marwa Hasan Abbas.1, Zuhair M Almusawi.2, & Hosna Hasan Abbas.3. (2024). Utility of Red Blood Cell Indices in the Diagnosis of â-Thalassemia Minor. International Journal of Medical Science in Clinical Research and Review, 2024; 7(01):1–7. https://ijmscrr.in/index.php/ijmscrr/article/view/663).

20. Sharma A, Marwah S, Buxi G, Yadav R. Hemoglobin e syndromes: emerging diagnostic challenge in north India. Indian J Hematol Blood Transfus. 2013 Mar; 29(1):21-5. doi: 10.1007/s12288-011-0140-9. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3572259.

21. Old J, Harteveld CL, Traeger-Synodinos J, Petrou M, Angastiniotis M, Galanello R. Prevention of Thalassaemia and Other Haemoglobin Disorders, 2nd edn. Nicosia, Cyprus: Thalassaemia International Federation; 2012. https://www.ncbi.nlm.nih.gov/books/NBK190576.

22. Baliyan M, Kumar M, Nangia A, Parakh N. Can RBC Indices be Used as Screening Test for Beta-Thalassemia in Indian Antenatal Women? J Obstet Gynaecol India. 2019 Dec;69(6):495-500. doi: 10.1007/s13224-019-01220-8. Epub 2019 Mar 28. PMID: 31844363; PMCID: PMC6889085.

23. Chatterjee T, Chakravarty A , Chakravarty S (2015) Population Screening and Prevention Strategies for18,166cases. Hemoglobin 2015; 39:6, 384- 388, DOI: 10.3109/03630269.2015. 1068799). https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6889085.

24. Sahiratmadja E,Maskoen AM, Reniarti L, Prihatni D. Erythrocyte Indices MCV and/or MCH as First Round Screening Followed by Hb-analysis for β-thalassemia Carrier State. The Indonesian Biomedical Journal. Vol 14, No 3 (2022): 282-8. https://doi.org/10.18585/inabj.v14i3.1960.