ORIGINAL RESEARCH PAPER

Volume-6 | Issue-12 | December-2017 | ISSN No 2277 - 8179 | IF : 4.176 | IC Value : 93.98

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

AN ASSESSMENT OF BETA THALASSEMIA TRAIT AND IRON DEFICIENCY ANEMIA AMONG INFANTS AND CHILDREN: A SECONDARY DATA ANALYSIS

Medicine

Dr. Chandrahas Prasad Associate Professor, Department of Laboratory Medicine Rajendra Institute of Medical Sciences, Ranchi

Dr. Shashi Bhushan Singh* Associate Professor Cum Statistician, Department of Preventive and Social Medicine, Rajendra Institute of Medical Sciences, Ranchi *Corresponding Author

ABSTRACT

Objective of the study to assess the Beta Thalassemia Trait and Iron Deficiency Anemia by using automated haematoanalyzer and HPLC- Variant II.

Material and methods: A total 107 cases of infants and children aged (1 month to 18 years) of both sexes who presented with anemia and their peripheral blood film revealed microcytic and hypochromic red cell picture was included in this study. A study was done in the year 2013. A clinical history was taken regarding the progressive / persisting pallor symptoms. Basic hematological parameters were performed using an automated analyzer. Mentzer index (MCV/red blood cell count) was used to differentiate thalassemia trait from iron deficiency. Detailed picture of hemoglobin was obtained by High Performance Liquid Chromatography.

Results: It was observed that out of 107 cases 13 (12.1%) cases were found to be with β thalassemia trait, while 21 (19.6%) patients were with iron deficiency anemia. However the MCV was slightly lower in traits than the iron deficient subjects. In a few selected cases Mentzer count ratio was counter checked which confirmed the patients with iron deficiency or thalassemia trait. 13 (Thirteen) cases with higher mean Hb concentration, higher mean red blood cell count and low mean red cell indices were selected and run chromatographically. All the cases showed a high HbA2 with a mean value 5.4%. On the other hand the range and mean values of HbF and of HbA was in normal limit.

Conclusion: Percentage of children with beta thalassemia trait was less as compared to iron deficient children. However, thalassemia sustains affected children with blood transfusions. It is important to screen for beta thalassemia trait in the inhabitants to stop the propagation of the gene.

KEYWORDS

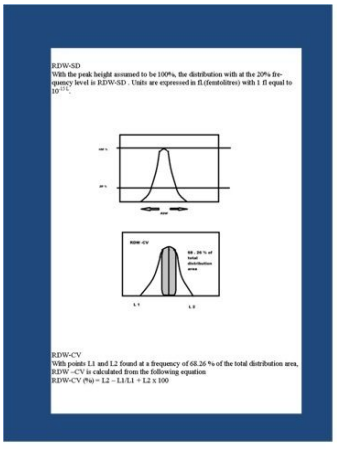
Automated Hematoanalyzer, Beta thalassemia trait, iron deficiency anemia and red blood cell indices.

INTRODUCTION

The most commonly encountered disorders with mild microcytic anemia are iron deficiency anemia (IDA) and β-thalassemia trait (BTT). 1,2 It is important to distinguish between IDA and BTT to avoid unnecessary iron therapy and the development of hemosiderosis. Red blood cell counts red blood cell distribution (RDW) and other discriminant functions calculated by blood cell analyzers have been helpful in distinguishing between the two disorders. We were especially interested in comparing the effectiveness of the RDW-SD, a recently introduced function, and the RDW-CV, currently used in most clinical laboratories. The RBC count and E & F Index were found to be very useful functions while the RDW-CV was less effective. 22,23 However, the study revealed that the RDW-SD was the most useful discriminant function for distinguishing IDA from BTT. 3

Figure 1

Figure 1: RDW-CV and RDW-CV (Sysmex 2000 i - Operating Manual)



(Figure 1) demonstrates how RDW-CV and RDW-SD were calculated (CV = coefficient of variance. SD = one standard deviation). A Sysmex XT 2000i, Five -part differential analyzer was used to obtain data.

Iron deficiency anemia (IDA) and beta thalassemia trait are the most common causes of hypochromia and microcytosis[1]. Beta-thalassemia syndromes are a group of hereditary blood disorders characterized by reduced or absent beta globin chain synthesis, resulting in reduced Hb in red blood cells (RBC), decreased RBC production and anemia. Most thalassemias are inherited as recessive traits. [4] Beta thalassemia and IDA are the most common microcytic hypochromic anemia in India.[5,6] Thalassemia affects men and women equally and occurs in approximately 4.4 of every 10,000 live births.

.

Iron deficiency anemia is the most common microcytic hypochromic anemia world wide especially in third world including India .[7,8] Iron deficiency modulates the synthesis of Hb-A_2, resulting in reduced Hb-A_2 levels in patients with IDA.[9] The decreased Hb-A_2 levels in iron deficiency could be due to decreased transcription and or translation of the delta gene. Another possible explanation is competition between Hb-A beta chains and Hb-A_2 delta chains in binding the limited quantities of available iron to their haem groups, as the ratio of beta: delta chains in normal RBC is 49:1. [10,11]

Most persons with thalassemia trait are found incidentally when their complete blood count shows a mild microcytic anemia. Microcytic anemia can be caused by iron deficiency, thalassemia, or anemia of chronic disease. The mean corpuscular volume (MCV), red blood cell distribution width (RDW), and the patient's history can exclude some of these etiologies. The MCV is usually less than 75 fl with thalassemia and rarely less than 80 fl in iron deficiency until the hematocrit is less than 30 percent. For children, the Mentzer index (MCV/red blood cell count) can help distinguish between iron deficiency and thalassemia**.** In iron deficiency, the ratio is usually greater than 13, whereas thalassemia yields values less than 13. A ratio of 13 would be considered uncertain. [12] Diagnosis of BTT was based on levels of HbA2 greater than 3.5%. [13] Reduction of HbA2 because of coincident iron deficiency did not preclude detection of BTT[12].

Quantitative Hb analysis by HPLC identifies the amount and type of Hb present. The Hb pattern in beta-thalassemia varies according to beta-thalassemia type. In beta^{0}thalassemia homozygote, HbA is absent and HbF constitutes the 92-95% of the total Hb. In beta$^+$ thalassemia homozygotes and beta$^+$/beta^0 genetic compounds HbA levels are between 10 and 30% and HbF between 70-90%. HbA2 is variable in beta thalassemia homozygotes and it is enhanced in beta thalassemia minor. [4]

OBJECTIVE

To asses the Beta Thalassemia Trait and Iron Deficiency Anemia by using automated haematoanalyzer and HPLC- Variant.

MATERIALAND METHODS

107 (Hundred and seven) cases of infants and children aged (1 month to 18 years) of both sexes who presented with anemia and their peripheral blood film revealed microcytic and hypochromic red cell picture was included in the study. A clinical history was taken regarding the progressive pallor symptoms. For complete blood count 3.0 ml of venous blood was taken in a vacutainer tube containing EDTA as an anticoagulant. Basic hematological parameters were performed using an automated analyzer **(Sysmex XT 2000i)**. A total of 107 cases, in the year 2013 for Hb variant analysis were studied for various hemoglobinopathies and variants. 107 blood samples in the year 2013, were run in 5 parts SYSMAX hemato- analyzer before performing HPLC to obtain the Hemoglobin values and indices in the Department of Laboratory Medicine, Rajendra Institute of Medical Sciences, RIMS, Ranchi, Jharkhand. In order to account the abnormalities in CBC, normal reference ranges for hemoglobin, red cell indices, and reticulocyte count as per Sysmex XT 2000 parameters. The reticulocytes were stained and their number was counted in 1000 erythrocytes and the result was reported as a reticulocyte count percent.

A few samples were also counter checked by applying the Mentzer index (MCV/red blood cell count) which was used to differentiate thalassemia trait from iron deficiency. These patients presented with mild anaemia (mean Hb level of 10.4gm/dl) and all showed microcytic – hypochromic blood picture with low mean corpuscular volume RDWSD < 46 fl, RDW CV < 16 % [(MCV) of <80fl]. For the suspected cases of beta thalassemia trait who had raised RBC count or family history of thalassemia, a detailed picture of hemoglobin was obtained by cation Exchange, Liquid High Performance Liquid Chromatography (HPLC) on the Variant II (Bio Rad).

RESULTS

Laboratory records of 107 patients of anemia with suspected hemoglobinopathies were analyzed and 63 (67.40% %) patients showed different abnormal hemoglobin variants (table 2). Out of total 107 cases, 71 (75.97 %) were male and 36 (24.03 %) were female. Criteria for suspecting hemoglobinopathy in these cases included: results of screening tests eg., various discriminant functions (so obtained on hematology cell counters, findings obtained from the peripheral smear examination, family history and relevant clinical signs and symptoms suggestive of hemoglobinopathy. 44 (47.08%) cases which were labeled as "No-Hemoglobinopathies" also included cases of Iron Deficiency anemia & Malaria amongst the other ones having other causes of anemia. The age range of patients was from 1 month to 18 years.

Of these 13 (21.1 %) patients were diagnosed to have beta-heterozygous thalassemia (Table 2) based on the high level of HbA2 (>3.9%). These patients presented with mild anaemia (mean Hb level of 10.4gm/dl) and all showed microcytic – hypochromic blood picture with low mean corpuscular volume RDWSD < 46 fl , RDW CV < 16 % [(MCV) of <80fl].

5 (4.6%) of 107 cases were diagnosed as beta – homozygous thalassemia (Table 2). All these patients had increased HbF values (> 40 %). Clinically they presented with severe pallor requiring regular blood transfusion and had moderate to marked hepatosplenomegaly. 9 (8.4%) cases of d B Thal Trait [[Hb A2 (4 – 7%) & (7 – 9%)] were diagnosed & 7 (6.5%) of HPFH Trait (Hb F 15 -40 %) along with variable degree of anemia showing anisopoikilocytosis and microcytic hypochromic (MCHC) blood picture. Hb F levels were raised with a variable reduction in Hb A. and these patients were not dependent on blood transfusion.

(Table - 1)

Spectrum of Thalassemia & Sickle Cell Diseases recently observed in Department of Laboratory Medicine , RIMS, Ranchi

Type	Total	Percent
B Thal Trait	13	12.1 %
d B Thal Trait	9	8.4 %
Sickle Homo	12	11.2 %
Sickle Thal	7	6.5 %
HPFH Trait	7	6.5 %
Sickle Trait	10	9.3 %
B Thal Major	5	4.6 %
IDA	21	19.6 %
MP	18	16.8 %
Others	5	4.6 %
Total	107	

IDA (Iron def. Anemia)

All patients were investigated to find the frequency of β thalassemia trait and iron deficiency. Complete blood count was done with particular emphasis on hemoglobin, red blood cell count and red blood cell indices as mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). HbA2 was used to differentiate the patients. i.e. *whether they have beta thalassemia trait or iron deficiency.*

Thirteen cases with higher mean Hb concentration, higher mean red blood cell count and low mean red cell indices were selected and run chromatographically using High Performance Liquid chromatography. All the cases showed a high HbA_2 with a mean value 5.4%. On the other hand the range and mean values of HbF and of HbA was in normal limit (Table 3 & 4).

Thalassemia trait: Cell counter report shows low hemoglobin average of 9.8 g/d1 with a higher red cell count of 5.3 million, with low MCV and MCH, highly suggestive of thalassemia trait. HbA, estimation is advised. HPLC Chromatogram (Bio-Rad Variant Thal - program) shows HbF of 1.1%, HbA is 86% and HbA2 of 5.9% (> 4%). Diagnosed as thalassemia trait. [15]

(Table -2) Distribution of cases by blood count & others in this study.

When there is red cell picture of microcytic hypochromic anemia
Discriminant functions in distinguishing Beta Thalassemia Trait and Iron Deficiency Anemia

Points	BTT	IDA
Morphology	Microcytic hypochromic may be with inclusion bodies	Microcytic hypochromic may be with ring cells
RDWSD	<46 fl (N 38 to 58)	>46
RDW CV	< 16 % (N 11 to 19)	>16
Hb	Minimal decrease(around 5 million)	May be markedly decrease(<5 million)
Nestroft test	Positive	Negative
Hb A2	>3.5 % up to (5 – 9 %)	
HbF	>2% up to (5 to 15%)	
Hepatomegaly	May be present	May not be present
Spleenomegaly	May be present	May not be present

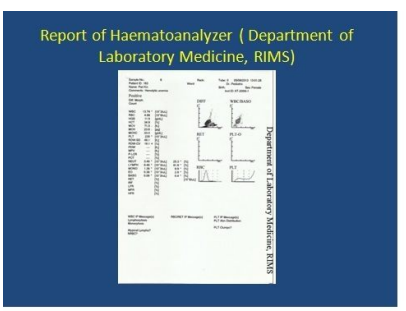
(Table 3) Levels and ranges of different forms of hemoglobin using the technique of chromatography.

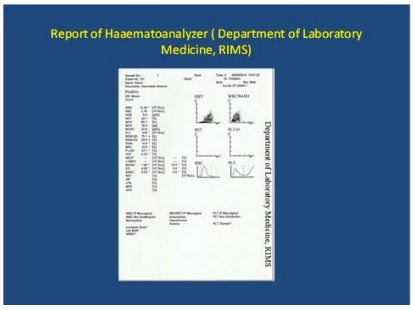
	Range
Hb F%	0.2-9.0
Hb A_2%	4.1-6.8
Hb A %	89-95

DISCUSSION

Highly significant decrease of level of Hb, RBC count was observed in patients with thalassemia trait when compared with iron deficient patients. RBC indices showed that both the values of MCV and MCH were decreased in thalassemic patients as compared to patients with iron deficiency but significant difference was only observed in case of MCV. Mean value of MCHC was increased in patients with thalassemia trait. *RDW & Mentzer count was used to distinguish between iron deficiency and thalassemia.12 It is observed that in iron deficiency, the ratio is greater than 13 i.e. 20.5, whereas thalassemia yields values less than 13 i.e.10.1.* Our study is in accord to number of studies who observed that in both iron deficiency and beta thalassemia the level of MCV is low i.e. <70fl in children. Mentzer index for children was >13 in iron deficiency and <13 in beta thalassemia.

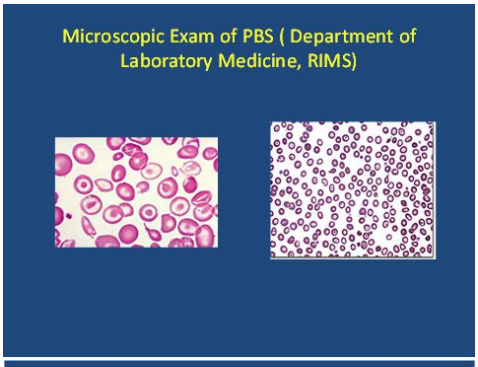
(Fig. 2)

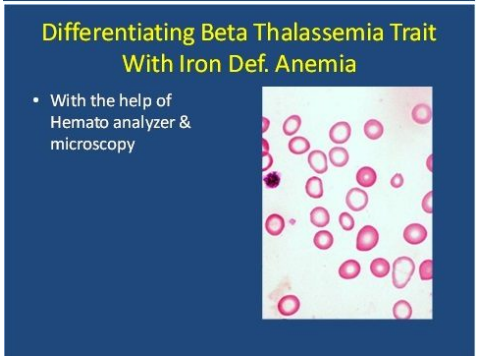




However a study contradictory to our study which reported that none of RBC indices or formulas appears reliable to discriminate between betaTT and ID subjects.17 On the other hand a group of workers reported that in typical beta-thalassemia carriers there is low MCV, low MCH and reduced MCHC2.

Both Percentage of reticulocyte and serum iron levels were increased in patients with thalassemia trait as compared to in patients with iron deficiency **.** Many observed that anemia of iron deficiency and chronic disease is suggested with low iron levels and decreased total iron-binding capacity. 18 However a study **also** stated that estimation of serum iron and iron-binding capacity is rarely needed .**4, 16**





Chromatographic results of 13 cases with higher mean Hb concentration, higher mean red blood cell count and low mean red cell indices showed that all cases have high HbA_2 with a mean value of 5.4%. On the other hand the range and mean values of HbF and of HbA was in normal limit. A study also used High Performance Liquid Chromatography for quantification of HbA2 and found increased mean values of Hb A2. Their Study stated the usefulness of technique i.e. it is simple in terms of sample preparation, having superior resolution, and accuracy, combined with complete automation of the method. [19] However a study stated that interpreting results of high performance liquid chromatograms with borderline HbA2 values is often problematic. [20]

(Table -4)
Interpretation of HPLC results in this study at a glance: -

Hb F (when A 2 is normal)	**When Hb F is more than 40 %**
• 0 - 2%. - Normal • 5 - 15%. - d B - Thal trait • (RBC raised) • 15 - 40% - H PFH trait (CBC normal)	B Thal homozygous or B Thal major

Hb A2	**Hb S Trait**
• 0 - 1 %. = New born • 2- 3.5 % = normal • 3.5 - 4 % = borderline case 5 - 9 % = B Thal trait	• A2 - less than 5% • Hb F - less than • CBC – normal
Sickle - Thal	**Hb S – homozygous**
• If Hb S more than 50% and A2- more than 5% • F - more than 2% • CBC – low	• If Hb S more than 50% and A2- less than 5% • F - more than 2 % • CBC – normal

Our study is in accord with a number of studies which observed that patients with beta-thalassemia trait usually have elevated levels of hemoglobin A2.[15, 4] A study reported that imbalances of globin chains cause hemolysis and impair erythropoiesis. [21]

History of recent blood transfusion must be sought along with correct age so as to aid in an accurate diagnosis. Conditions with borderline Hb A2 need careful interpretation. Iron deficiency may lead to low Hb A2 and hence may mask a thalassemia trait where as B12/folate deficiency may lead to slightly raised Hb A2 leading to a false diagnosis of a trait.

CONCLUSION

The percentage of children with beta thalassemia trait was less as compared to iron deficient children. However thalassemia affected sustain children with blood transfusions. It is important to screen for beta thalassemia minor in the population to stop the propagation of the gene. The high prevalence beta thalassemia trait needs proper screening at different levels for the prevention of thalassemia. The high rate of consanguinity in our society is also responsible for an increased rate of thalassemia. Population screening programs need to be initiated in schools and in prenatal clinics.

Genetic counseling of the parents and family members of thalassemic patients is extremely important for the awareness of the ignorant.

Automated cell counter (Haematoanalyzer) & HPLC provide a rapid technically reliable method for screening of Thalassemia Trait & Iron Def. Anemia. Further studies should be conducted for detecting its role in screening microcytic anemias or asymptomatic anemias. We believe our findings are meaningful and should stimulate the evaluation of RDW –SD in clinical & research setting.

Authors acknowledge the support of faculty. Authors are giving thanks to **Mr. Jawed Akhtar, senior lab tech.** for rendering technical help and lab work support during the course of this study,which is gratefully acknowledged.

REFERENCES

1. Wharton BA. Iron deficiency in children: detection and prevention. Br J Haematol 1999;106:270–80.
2. Lukens JN. The thalassemias and related disorders: an overview. In: Lee GR, et al., editors. Wintrobe's Clinical Hematology. 10th ed. Giza: Mass Publishing, 1999: 405-33.
3. Walters JG and RF Garrity RDW-SD and RDW-CV: Their relationship to RBC distribution curves and anisocytosis. Sysmex Journal International 1993; 3 (1); 40-45
4. Galanello R, Origa R. Beta-thalassemia. Orphanet J Rare Dis. 2010 May 21;5:11.
5. Ahmed S, Petrou M, Saleem M. Molecular genetics of beta-thalassemia in Pakistan: a basis for prenatal diagnosis. Br J Haematol. 1996;94:476–482.
6. Alsaeed AH. Prevalence of Hemoglobinopathy Disorders in Adult Patients Sent for Diagnosis of Anemia in Saudi Arabia. Genet Test Mol Biomarkers. 2011 Aug 23. [Epub ahead of print]
7. Sirdah M, Tarazi I, Al Najjar E, Al Haddad R. Evaluation of the diagnostic reliability of different RBC indices and formulas in the differentiation of the beta-thalassaemia minor from iron deficiency in Palestinian population. Int J Lab Hematol. 2008;30:324–330.
8. 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;53:93–97
9. Usman M, Moinuddin M and Ahmed SA. Role of iron deficiency anemia in the propagation of beta thalssemia gene. Korean J Hematol. 2011 March; 46 (1): 41–44.
10. Fritsch EF, Lawn RM, Maniatis T. Molecular cloning and characterization of the human beta-like globin gene cluster. Cell. 1980;19:959–972.
11. Proudfoot NJ, Shander MH, Manley JL, Gefter ML, Maniatis T. Structure and in vitro transcription of human globin genes. Science. 1980;209:1329–1336. [PubMed]
12. Mentzer WC Jr. Differentiation of iron deficiency from thalassaemia trait. Lancet. 1973;1(7808): 882.
13. Clarke G, Higgins TN. Laboratory investigations of hemoglobinopathies and Thalassemias; review and update. Clin Chem. 2000;46:1284–90.
14. Madan N, Sikka M, Sharma S, Rusia U. Frequency of coincident iron deficiency and beta-thalssemia trait. Clin Pathol. 1996;49:1021–2.
15. Dr. Tejindar Sigh, text book of Haematology,p.90(2nd edition-13).
16. Marsh WL Jr, Bishop JW, Darcy TP. Evaluation of red cell volume distribution width (RDW). Hematol Pathol. 1987;1(2):117–123.
17. Ferrara M, Capozzi L, Russo R, Bertocco F, Ferrara D. Reliability of red blood cell indices and formulas to discriminate between beta thalassemia trait and iron deficiency in children. Hematology. 2010 Apr;15(2):112-5.
18. Van Vranken M. Evaluation of microcytosis. Am Fam Physician. 2010 Nov 1;82(9):1117-

19. Ou C-N, Rogerud CL. Diagnosis of hemoglobinopathies: electrophoresis vs. HPLC. Clinic Chemica Acta. 2001; 313:187–94.
20. Rangan A, Sharma P, Dadu T, Saxena R, Verma IC, Bhargava M. β-Thalassemia mutations in subjects with borderline HbA(2) values: a pilot study in North India Clin Chem Lab Med. 2011 Sep 6. [Epub ahead of print]
21. Rund D, Rachmilewitz E. Beta-thalassemia. N Engl J Med. 2005;353 (11):1135–1146.
22. England JM and Fraser P. Discriminant between iron-deficiency and heterozygous thalassemia syndromes in differential diagnosis of microcytosis. Lancet 1979;1:145–8.
23. Junca J, Flores A, Roy C, et al. Red cell distribution width, free erythrocyte protoporphyrin, and England–Fraser index in the differential diagnosis of microcytosis due to iron deficiency or beta-thalassemia trait. A study of 200 cases of microcytic anemia. Hematol Pathol 1991;5:33–6.