ORIGINAL ARTICLE

Automated Measurement of Red Blood Cell Microcytosis and Hypochromia in Iron Deficiency and Beta Thalassemia Trait

RABIA NADEEM, NISAR AHMED, GHULAM RASOOL QURESHI

ABSTRACT

Aims: To diagnose two most common causes of microcytosis in children by red cell indices which are easy to obtain, low cost parameters and an early detection of anaemia of childhood.

Material and methods: 300 Infants and children aged (6 month to 18 years) of both sexes who presented with anaemia and their peripheral blood film revealed microcytic and hypochromic red cell picture were included in the study. Study duration was 12 months,from May 2005 to May 2006. A detailed clinical history was taken regarding the onset and duration of pallor symptoms and history of intake of haematinics was documented. 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. Serum iron was determined by autoanalyzer. Detailed picture of hemoglobin was obtained by Ion Exchange Liquid High Performance Liquid Chromatography.

Results: It was observed that 31 patients were found to be with β thalassemia while 269 patients were with iron deficiency anaemia. Mean Hb concentration, RBC count and serum iron level were significantly higher in traits than the iron deficient subjects ($P<0.001$). However the mean MCV was slightly lower in traits than the iron deficient subjects. Mentzer count ratio confirms the patients with iron deficiency or thalassemia trait. 31 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 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.

Conclusion: Percentage of children with beta thalassemia trait was less as compared to iron deficient children. However thalassemia centers sustain thousands of affected children with monthly blood transfusions. It is important to screen for beta thalassemia minor in the large number of population to stop the propagation of the gene.

Key words: Beta thalassemia trait, iron deficiency anemia and red blood cell indices.

INTRODUCTION

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 anaemia. Most thalassemias are inherited as recessive traits[2]. Beta thalassemia and IDA are the most common microcytic hypochromic anaemias in Pakistan. Thalassemia affects men and women equally and occurs in approximately 4.4 of every 10,000 live births[3,4].

Iron deficiency anaemia is the most common microcytic hypochromic anaemia world wide especially in third world including Pakistan[5,6]. Iron deficiency modulates the synthesis of $Hb\text{-}A_2$, resulting in reduced $Hb\text{-}A_2$ levels in patients with IDA[7]. The decreased $Hb\text{-}A_2$ 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 $Hb\text{-}A_2$ 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[8,9].

Most persons with thalassemia trait are found incidentally when their complete blood count shows a mild microcytic anaemia. Microcytic anaemia can be caused by iron deficiency, thalassemia, or anaemia 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

---

Department of Pathology, CMH Lahore Medical College, Lahore

Correspondence to Dr. Rabia Nadeem Assistant Professor Pathology, E-mail:dr.rabianadeem@hotmail.com

values less than 13. A ratio of 13 would be considered uncertain[10]. Diagnosis of BTT was based on levels of HbA2 greater than 3.5%[11]. 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[2].

AIMS & OBJECTIVES

A traditional approach followed by most general practitioners is a trial of iron treatment whenever anaemia and or microcytosis are encountered. However populations where thalassemias are common, like in Pakistan, this approach leads to unnecessary iron therapy , iron overload and failure to provide genetic diagnosis and counseling in subjects with BTT. Therefore it is important to diagnose these two most common causes of microcytosis in children by red cell indices which is easy to obtain, low cost parameters and an early detection of anaemias of childhood.

MATERIAL AND METHODS

Three hundred Infants and children aged (6 month to 18 years) of both sexes who presented with anaemia and their peripheral blood film revealed microcytic and hypochromic red cell picture were included in the study. A detailed clinical history was taken regarding the onset and duration of pallor symptoms and history of intake of haematinics was documented. Letter of consent was taken from attendant of patients. For complete blood count 3.0 ml of venous blood was taken in a tube containing EDTA as anticoagulant. Basic haematological parameters were performed using an automated analyzer (Sysmex SF 3000, Tokyo, Japan). In order to account the abnormalities in CBC, normal reference ranges for hemoglobin, red cell indices, reticulocyte count and serum iron were taken from Dacie and Lewis Practical Haematology. The reticulocytes were stained and their number was counted in 1000 erythrocytes and result was reported as reticulocyte count percent. Mentzer index (MCV/red blood cell count) was used to differentiate thalassemia trait from iron deficiency. Serum iron was determined by autoanalyzer (Hitachi 902) using a kit of Roche. 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 Ion Exchange Liquid High Performance Liquid Chromatography (HPLC) on the variant (Bio Rad).

Statistical Analysis: Data was analyzed by SPSS 14. Results were calculated and reported as mean, standard deviation and percentages. Student 't' test was used to compare the variables related to iron deficiency and beta thalassemia. P value <0.001 considered as highly significant difference.

RESULTS

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. It was observed that in 31 patients with β thalassemia Hb concentration in 61.3% cases was between 7-9 gm/dl and in 38.7% cases it was between 10-12 gm/dl. Iron deficiency anaemia was observed in 269 cases. In iron deficiency anaemia the Hb concentration in 5.9% cases was between 2-4 gm/dl,in 21.2% of cases was between 4-6gm/dl, in 36.8% of cases it was between 6-8 gm/dl and in 36.1% cases it was between 8.9-9.0 gm/dl. The mean Hb concentration was significantly higher in traits than the iron deficient subjects (P<0.001). Mean RBC count was also significantly higher in traits than the iron deficient subjects (P<0.001). However the mean MCV was slightly lower in traits than the iron deficient subjects. Mentzer count ratio confirmed the patients with iron deficiency or thalassemia trait..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 (Table 1).

Table 2 shows the range and mean values of reticulocyte count and serum iron in β thalassemia and iron deficiency. It was observed that the mean level of serum iron is also significantly higher in traits than the iron deficient subjects (P<0.001).

Thirty one 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).

Table 1: Distribution of cases by blood count

	Thalassemia trait		Iron deficiency	
Para-meters	**Range**	**Mean± SD**	**Range**	**Mean±SD**
Hb (gm/dl)	7.4-12.9	9.6± 1.4**	2.9-9.9	7.1±1.7
RBC count (x 10^{12} /L)	5.2-6.9	5.9± 0.4**	1.1-6.5	3.3±0.7
RBC Indices				
MCV (ft)	43.8-70.3	60.9± 5.9*	49.1-88.4	67.90±6.3
MCH (pg)	13.8-24.8	20.4± 2.5	12.6-30.5	22.30±3.5
MCHC (gm/L)	28.0-34.5	31.7± 1.9	21.2-36.2	28.7±2.5

**P<0.001 = Significant difference*
***P<0.001 = Highly significant difference*

Table 2: Distribution of cases by reticulocyte count and serum iron

	Thalassemia trait		Iron deficiency	
Parameters	**Range**	**Mean± SD**	**Range**	**Mean± SD**
Reticulocyte count (%)	0.2-2.2	1.2± 0.6	0.2-4.0	1.1± 0.6
Serum Iron (mg/dl)	97-165	124.9± 18.9**	12-165	51.33± 31.2

***P<0.001 = Highly significant difference*

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

	Range	**Mean ±SD**
Hb F%	0.2-9.0	1.1±0.8
Hb A_2%	4.1-6.8	5.4±0.9
Hb A %	89-95	92.5±1.7

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. Mentzer count was used to distinguish between iron deficiency and thalassemia. 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 6 month to 6 year of age. Mentzer index for children was >13 in iron deficiency and <13 in beta thalassemia[10,13].

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[14]. On the other hand a group of workers reported that in typical beta-thalassemia carriers there is low MCV, low MCH and reduced MCHC[2].
Both Percentage of reticulocyte and serum iron levels were increased in patients with thalassemia trait as compared to in patients with iron deficiency but significant difference (P<0.001) was only observed in case of serum iron. Our study is in accord with a study which observed that anaemia of iron deficiency and chronic disease is suggested with low iron levels and decreased total iron-binding capacity[15]. However a study stated that estimation of serum iron and iron-binding capacity are rarely needed[13].

Chromatographic results of 31 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[16]. However a study stated that interpreting results of high performance liquid chromatograms with borderline HbA2 values is often problematic[17].

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[14,2]. A study reported that imbalances of globin chains cause hemolysis and impair erythropoiesis[18].

CONCLUSION

Percentage of children with beta thalassemia trait was less as compared to iron deficient children. However thalassemia centers sustain thousands of affected children with monthly 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 programme need to be initiated in schools and in pre-natal clinics.

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

REFERENCES

1. Shen C, Jiang YM, Shi H, Liu JH, Zhou WJ, Dai QK, Yang H. Evaluation of indices in differentiation between iron deficiency anemia and beta-thalassemia trait for Chinese children. J Pediatr Hematol Oncol. 2010 Aug;32(6):e218-22.
2. Galanello R, Origa R. Beta-thalassemia. Orphanet J Rare Dis. 2010 May 21;5:11.
3. Ahmed S, Petrou M, Saleem M. Molecular genetics of beta-thalassaemia in Pakistan: a basis for prenatal diagnosis. Br J Haematol. 1996;94:476–482.
4. 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]
5. 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.
6. 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
7. 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.
8. Fritsch EF, Lawn RM, Maniatis T. Molecular cloning and characterization of the human beta-like globin gene cluster. Cell. 1980;19:959–972.
9. 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]
10. Mentzer WC Jr. Differentiation of iron deficiency from thalassaemia trait. Lancet. 1973;1(7808): 882.
11. Clarke G, Higgins TN. Laboratory investigations of hemoglobinopathies and Thalassemias; review and update. Clin Chem. 2000;46:1284–90.
12. Madan N, Sikka M, Sharma S, Rusia U. Frequency of coincident iron deficiency and beta-thalssemia trait. Clin Pathol. 1996;49:1021–2.
13. Marsh WL Jr, Bishop JW, Darcy TP. Evaluation of red cell volume distribution width (RDW). Hematol Pathol. 1987;1(2):117–123.
14. 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.
15. Van Vranken M. Evaluation of microcytosis. Am Fam Physician. 2010 Nov 1;82(9):1117-22.
16. Ou C-N, Rogerud CL. Diagnosis of hemoglobinopathies: electrophoresis vs. HPLC. Clinica Chemica Acta. 2001; 313:187–94.
17. 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]
18. Rund D, Rachmilewitz E. Beta-thalassemia. N Engl J Med. 2005;353(11):1135–1146.