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Original Research Article

Application of Various RBC Indices to Differentiate Iron Deficiency Anemia and Beta Thalassemia Trait Among Antenatal Women in A Tertiary Care Hospital

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Abstract

Introduction: The most frequent causes of microcytic anemia among antenatal women in India are iron deficiency anemia (IDA) and beta thalassemia trait(βTT). Hence it is important to differentiate between both the conditions to avoid unnecessary iron therapy in βTT patients. This study was done to screen antenatal women with β thalassemia trait using formulas derived from various Red cell indices.

Aims And Objectives: This study aims to screen all microcytic (MCV<80fl) hypochromic (MCH<27pg) anemia for β thalassemia trait, to differentiate IDA from βTT and confirm the screened cases by HPLC. Also attempt has been made to assess sensitivity and specificity of various indices to identify βTT.

Materials And Methods: A cross sectional comparative study was done in 200 antenatal women for a period of 2 years from October 2019 to September 2021 in department of clinical pathology at Tertiary care hospital. Complete blood counts and various red cell indices were evaluated. There are 16 cases of hemoglobinopathies out of which 9 are β thalassemia trait and 184 cases are iron deficiency anemia. Four indices were calculated i.e., the Mentzer index (MI), Srivastava index (SV), Shine and Lal index (SL) and Red cell Distribution Width (RDW) index. These indices were calculated in patients having microcytosis (MCV<80fl) and hypochromia (MCH<27pg) and, all the cases are further subjected to HPLC for confirmation.

Results: In the present study the index with highest sensitivity for diagnosing β thalassemia trait was Shine and Lal index (100%) and Mentzer index showed highest specificity with 99.5%. Positive predictive value (PPV) was highest for Mentzer index and Negative predictive value (NPV) was highest for Shine and Lal index. Youden's index was highest for RDW. In the present study the percentage of correctly identified patients was highest for Mentzer index.

Conclusion: It is vital to establish a screening model based on HbA_2 levels and red cell indices for the differentiation of β TT from IDA in microcytic hypochromic anemia cases. According to the results of the present study MI and RDW index are considered as the most

useful and reliable indices for screening β thalassemia trait, though HPLC confirmation is mandatory.

Keywords: RBC indices, Antenatal women, β thalassemia, iron deficiency anemia, HPLC.

Introduction

Globally, anemia is a public health problem affecting both developing and developed countries with major consequences for human health as well as social and economic development. It is the result of wide variety of causes but the most significant contributor to the onset of microcytic anemia in antenatal women are iron deficiency anemia (IDA) and β thalassemia trait[1] in India.

β Thalassemia is an autosomal recessive hematological disorder where β globin chain production is defective. β Thalassemia is further categorized into two subtypes β Thalassemia major and beta thalassemia minor/ trait (βTT).[2] 10% of world thalassemia patients are born in India every year with average carrier rate being 3.3%[3]. In India prevalence of βTT varies from 1 to 14.9% in various regions4. According to Lokeshwar M.R, et al study 20 million β thalassemia trait cases were estimated in India, and around 8000-10,000 children are born every year with β thalassemia major[5]. Prenatal screening for β thalassemia trait in antenatal women and genetic counselling plays a crucial role in decreasing number of thalassemia major cases in the newborns[6].

In India Iron deficiency anemia has been reported in 65-75% of pregnant females[7]. IDA occurs in pregnant women either due to failure to meet an increased physiological need of iron or as a late manifestation of prolonged negative iron balance. According to WHO standards, Hemoglobin value <11 gms is considered as anemia in pregnant women, thus it is necessary to screen these women.[8] The clinical and hematological picture of βTT and IDA is very similar, so it is very important to differentiate between both of them to avoid fatal impact on quality of life and unnecessary iron therapy in β thalassemia trait patients[9].

βTT presents with microcytic hypochromic blood picture similar to that of iron deficiency anemia. We can differentiate between these two based on HbA2 levels by Hb electrophoresis, HPLC, serum levels of iron, ferritin and total iron binding capacity. These investigations are quite expensive causing an economic burden. Hence using red cell indices derived from various parameters of automated hematology analyzers like Mentzer's index, Shine and Lal index, Srivastava index, RDW index, are calculated for screening of β thalassemia trait. All these cases are further subjected to HPLC for confirmation as it is the gold standard tool for diagnosis of various Hemoglobinopathies.

Materials and Method

The present study is a cross sectional comparative study conducted in 200 antenatal women for the period of 2 yrs. from October 2019 to September 2021 who attended the obstetrics department at Tertiary care hospital. An informed and written consent from subjects was taken before enrolling them. Blood samples from these women were collected and evaluated in clinical pathology department.

In the present study Antenatal women of age group of 18–40 years with

microcytosis (MCV<80fl) and hypochromia (MCH -27pg) and with informed valid consent were included in the study. The Pregnant women with anemia due to chronic diseases, who are on iron therapy and received blood transfusion in last 3 months, were excluded.

Blood samples from antenatal women satisfying all the inclusion criteria were run on Sysmex 800 XI Cell Counter and complete blood counts were obtained which includes Hemoglobin %, PCV or hematocrit %, Red blood cell count in millions/mm^3 & RDW. Samples with normal MCV, MCH are not tested further and samples with microcytosis (low MCV i.e., <80fl) and hypochromia (low MCH i.e. <27pg) are further assessed and differentiated using Mentzer's index, Shine and Lal index, RDW index and Srivastava index using the above CBC parameters. In Thalassemia suspicious cases proper clinical history of the patient is taken.

The following indices are calculated for screening in this study, MENTZER INDEX=MCV/RBC count (<13 is βTT,>13 means IDA), RDW INDEX-=MCV X RDW/RBC (<220 –βTT,>220 is IDA), SHINE and LAL INDEX=MCV^2X MCH/100 (<1530 is βTT and >1530 is IDA) and SRI VASTAVA INDEX= MCH/RBC count (<3.8 is βTT and >3.8 is IDA). (Table-1)

Table 1: Various RBC indices formulas and cut-off values to differentiate between β thalassemia trait and IDA

Index	Iron Deficiency Anemia	B Thalassemia Trait
1.Mentzer Index	>13	<13
2.Rdw Index	> 220	< 220
3.Shine & Lal Index	>1530	<1530
4.Srivastava	>3.8	<3.8

With valid informed and written consent from the patient are taken, 5ml of venous blood is collected under aseptic conditions in EDTA tube and are further subjected to HbA2 estimation using HPLC for confirmation. HbA2 level >3.5% are diagnosed as β thalassemia trait patients. Serum Iron, TIBC, and serum ferritin is done to confirm Iron deficiency anemia. Cases with reduced serum iron, ferritin and increased TIBC are labelled as iron deficiency anemia.

Results

In the present study out of total 200 cases there are 16 cases of hemoglobinopathies out of which 9 cases are β thalassemia trait and 184 cases are iron deficiency anemia. These 184 cases showed normal HPLC pattern. In the present study other than β thalassemia trait (9 cases), other hemoglobinopathies like β thalassemia major (3 cases), Sickle beta thalassemia (1 case), Sickle cell Anemia (1 case) and Sickle cell trait (2 cases) were observed. (Table-2). The most common hemoglobinopathy in our study in antenatal women is β thalassemia trait. Pallor was the most common clinical sign observed in the patients.

Table 2: Percentage of total correctly identified patients

	βTT	IDA	Total correctly identified patients	
			Count	%
SL				
Positive	9	167	26/193	13%
Negative	0	17		
MI				
Positive	5	1	188/193	97%
Negative	4	183		
SV				
Positive	4	1	187/193	97%
Negative	5	183		
RDW				
Positive	8	6	186/193	96%
Negative	1	178		

Shine and Lal index (100%) and RDW index (96.9%) showed highest sensitivities respectively in identifying β thalassemia trait patients. Highest specificities were denoted by Mentzer Index (99.5%) followed by Sri Vastava index (99%). The highest PPV was demonstrated by Mentzer Index (83.3%) and least PPV by Shine and Lal Index (5%). Shine and Lal index showed highest NPV with100% followed by RDW index with 99.5%. The Youden's Index was highest for RDW index (85.8%) and least for Shine and Lal index (9.9%). Mentzer Index correctly identified maximum number of patients with 97% (188 cases) followed by Srivastava index (97%) and RDW index with 96% and least for S&L index (13%) (Table-3)

Table 3: Sensitivity, Specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV) and Youden's index to differentiate between β thalassemia trait and IDA

	Sensitivity	Specificity	PPV	NPV	Accuracy	LR+	LR-	Youden's index
SL	100	9.9	5	100	9.5	1.1	0.00	9.9
MI	55.6	99.5	83.3	97.9	97.5	111.2	0.45	55.1
SV	44.4	99	66.7	97.4	96.5	44.4	0.56	43.4
RDWI	88.9	96.9	57.1	99.5	96.5	28.7	0.11	85.8

Discussion

The present study was done in Department of pathology in a tertiary care hospital from October 2019 to October 2021. In this study attempt has been done to apply different RBC indices and the different formulas derived from them in order to differentiate between IDA and βTT.

Nutritional Iron deficiency is the most common deficiency disorder in this world. The two most important causes of microcytic anemia are IDA and β-thalassemia trait in India. Thus, in Antenatal women it is important to differentiate between both iron deficiency anemia (IDA) and β thalassemia trait so as to avoid unnecessary iron therapy. Among the spectrum of β-thalassemia syndrome forms, βTT is the most common form encountered in India and is responsible for causing anemia other than IDA. βTT is due to presence of β thalassemia gene mutation on one chromosome and a normal beta globin gene on another chromosome [10] Usually these patients are asymptomatic except in periods of stress like pregnancy. In βTT carriers

during pregnancy with preexisted hypochromic microcytic anemia the plasma volume expansion may cause pronounced anemia, which make them symptomatic.

βTT patients present with a hematological picture similar to IDA like reduced hemoglobin, microcytic hypochromic anemia picture [11]. Hence it is very challenging to identify and differentiate βTT from IDA appropriately as the course of treatment completely differs.

CBC of βTT and IDA shows low hemoglobin, decreased MCV (<75fl), decreased MCH (<25pg). RBC count is elevated in βTT (> 5 million/mm3) whereas reduced count is seen in IDA. In the present study mean Hb in βTT group is 10.28 gm/dl, RBC is 5.14 million/ cu.mm., mean MCV is 65.98 fl and MCH 20.17pg which is closely similar to the study done by Bhushan R et al[12]. where mean Hb is 10.88 gm/dl, RBC count is 5.62 million/cu mm, the mean MCV value is 67.02 fl and MCH is 20.96 pg (Table -5)

Table 5: Relevant hematological parameters in β thalassemia trait in various studies in comparison to present study

Study	Hb(g/dl)	RBC (million/cu mm)	MCV (fl)	MCH (Pg)
Shrivastav et al [2013][13]	10.4	5.38	62.1	19.4
Mondal[14] et al [2014]	9.7	3.8	70.4	21
Tripathi [15] et al [2015]	6.82	4.75	59.9	16.81
Reema Bhushan et al [2018][12]	10.88	5.62	67.02	20.96
Present Study	10.28	5.14	65.98	20.17

In IDA group in present study the mean hematological parameters are, Hb 8.43 gm/dl, RBC 3.79 million/ cu.mm., mean MCV 71.59 fl and MCH 22.18pg which is similar to the study done by Bhushan R et al[12], where mean Hb is 8.8 gm/dl, RBC count is 3.7million/cu mm, the mean MCV value is 73.83 fl and MCH is 24.48pg

In current study the percentage of correctly identified patients is highest for MI (97%) followed by SV [13] index (97%), and RDW index (96%) and the least being S&L index (13%). According to the results of the present study the two accurate indices which could detect most of the cases of β thalassemia trait were MI index and SV index.

According to Demir et al[16] (2009) the results, the percentage of correctly identified patients is highest for Shine and Lal index (92%) followed by Srivastava index (88.43%) and the least being Mentzer index (88%). In their study, in patients younger than 10 years RBC count and Shine and Lal index showed highest diagnostic accuracy in differentiating beta thalassemia trait from Iron deficiency anemia. In the study conducted by Ehsani et al [17](2009) the percentage of correctly identified patients is highest and most valid index is Mentzer index [18](94.71%) followed by Srivastava index (86.97%), . Aysel V et al [19](2014) study results showed nutritional iron deficiency is the most common deficiency disorder in this world the percentage of correctly identified patients is highest for Mentzer index (91%) followed by Srivastava[13] index (79.3%) and least being Shine and Lal [20](57.9%).

According to Bhushan R et al[12] (2018), their study revealed the percentage of correctly identified patients is highest for Mentzer index (97%) followed by Srivastava index (96.1%), and RDW index (88.3%) and the least being Shine and Lal index (13%)[20].

Similar to our study, sensitivity values are highest for Shine and Lal index in studies done by Aysel V et al S&L (100%)[19], Bhushan R et al S&L (98.41%)[12], Sehgal et al [21]S&L (94.9%). According to Some other studies like Tripathi et al [15] and Ehsani et al[17] reported Mentzer index to have highest sensitivity.

In the present study, the specificities were highest for Mentzer index and Srivastava index with 99.5% and 99 % respectively, followed by RDW index with 96.9% and. Shine and Lal index showed the least specificity of 9.9% in the present study.

Specificity was also highest for MI in studies done by Bhushan R et al (99.66%)[12], Sehgal et al (87%)[21] and Aysel V et al [19](82.3%). But according to studies done by Ehsani et al Sri vastava index showed highest specificity value.

In the present study the Positive predictive value in differentiating β thalassemia trait from Iron deficiency anemia was highest for Mentzer index[18] with 83.3% followed by Srivastava index[13] with 66.7% and RDW index (57.1%) and least in Shine and Lal Index[20] with 5%. Also, in a study done by Aysel V et al (2014)[19] MI (86.3) % showed highest PPV.

In the present study, Negative predictive value was highest for shine and Lal index[20] with 100% followed by RDW index[12] with 99.5%, Mentzer index[18] (97.9%), and least Srivastava index[13] (97.4%). This was similar to the studies done by Aysel V et al[19] where NPV is 100% for S&L Index[20].

The descending order in regard to the ability of Youden's index to differentiate β thalassemia trait from iron deficiency anemia in the present study RDW Index (85.8%) >Mentzer Index (55.1%)> Srivastava index (43.4%)> Shine and Lal index (9.9%)

Similar to our study, Reema bhushan et al (2018)[12], in their study showed that Youden's index was highest for RDW index (63.1%), in screening of the β thalassemia trait cases from iron deficiency anemia. In a study done by Ehsani et al[17], Tripathi N et al[15], an Aysel V et al[19] Youden's Index was highest for Mentzer Index[18] with 90.1%, 67.1% and 81% respectively.

In the present study the percentage of correctly identified patients is highest for Mentzer Index (97%) followed by Srivastava index (97%), and RDW index (96%) and the least being Shine and Lal index (13%). This is similar to the results of studies done by Ehsani et al, Aysel V et al and Bhushan R et al where the percentage of correctly identified patients is highest for Mentzer Index followed by Srivastava Index.

According to the results of the present study considering the percentage of correctly identified individuals the best index to differentiate beta thalassemia trait is Mentzer index and considering the Youden's index the best differentiating index is RDW index.

Thus, hematological indices are a cost effective and easy way to screen β thalassemia trait patients in highly prevalent areas as HPLC is a costly investigation. Suspected and screened individuals are further confirmed using HPLC. Thus, using routine CBC parameters, we can identify carriers of β thalassemia trait and further prevent birth of β thalassemia major infants.

Conclusion

The best indices to differentiate β thalassemia trait from iron deficiency anemia in the present study is RDW Index followed by Mentzer Index. Taking into account the percentage of correctly identified patients as the criteria, Mentzer Index is the best index followed by Srivastava and RDW index. No particular index can accurately identify all the patients but utilizing combination of these indices can detect majority of β

thalassemia trait cases in real life situation. An ideal index should be easy to calculate and should have high sensitivity and specificity. Thus, simple CBC parameters can be used as cost effective and rapid test for initial screening of patients in high prevalent areas where gold standard investigations like HPLC are not available and technically it becomes difficult to perform on all the patients in a densely populated country like India.

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