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Research Article

DIAGNOSTIC PREDICTIBILITY OF COMPLETE BLOOD COUNT (CBC) IN IDENTIFYING THALASSEMIA TRAIT IN PREGNANT FEMALES

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ABSTRACT

Background- Thalassemia is among the most common genetic disorders worldwide. They are a group of autosomal disorders where there is an inhibition of the production of α or β globin chains of hemoglobin resulting in varying level of anemia.

Objectives- 1) To study the diagnostic predictibilty of CBC and various indices in identifying β-thalassaemia trait (β-TT) in pregnant females. 2) For prevention of β-thalassaemia major (β-TM) by genetic counseling.

Material and Methods - 400 patients having microcytic hypochromic anemia were analysed for Hb, RBC, MCV, MCH, MCHC, TLC, platelet count; and various indices including Mentzer index, England and Fraser index, RBC count and Green and King formula. The blood samples from patients with possibility of β-TT were used for HbA_2 estimation by high performance liquid chromatography (HPLC) for confirmation.

Results - Out of 400 cases of microcytosis, 40 were confirmed to have β-TT and 2 cases of sickle cell trait by HPLC. In our study the incidence of β-TT was 10% in the pregnant females. Out of 40 positive females only 25 females turned up with their husbands. Two husbands were turned out positive for β-TT.

Discussion and Conclusion- Present study demonstrates that set of cost effective screening tests like Mentzer index, England & Fraser index, RBC count and Green & Kind index with routine hemogram data (RBC and Hb) in microcytic cases can effectively discriminate between β-TT and Non β-TT. Diagnosis of β-TT can be reliably done by HPLC and HbA_2 quantitation by elution with $HbA_2 \geq 3.8\%$.

INTRODUCTION

Thalassaemia are a group of autosomal disorders where there is an inhibition of the production of α or β globin chains of hemoglobin resulting in varying level of anemia[1]. Sindhis, Gujratis, Bengalis, Punjabis and Muslims account for most of cases. Prevalence of β-TT varies from 1.0-14.9% in various region of India[2]. The disease has tremendous variation ranging from silent, asymptomatic carriers (β-TT) to transfusion dependent patients (Cooley's Anemia)[3].

Identification of β-TT is essential for the prevention of a birth of a β-TM by genetic counselling and it can be reduced to 90%.[4] .The present study is undertaken to detect β thalassemia trait in pregnant women attending the antenatal clinic on the basis of red blood cell indices and its confirmation by high performance liquid chromatography thereby finding the sensitivity, specificity and validity of the various red blood cell indices. Husbands of women who were confirmed to be carrier on HPLC analysis were screened to identify the "high-risk couples". Risk factor for offspring were calculated and informed to the couples. Counselling and prenatal diagnosis

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was offered to the couple when both husband and wife were thalassemic trait positive.

MATERIAL AND METHOD

A total 400 patients having microcytic hypochromic anemia with signed informed consent and details regarding the socioeconomic demographic characteristics, the obstetric history and family history were investigated from January 2012 to December 2013. For determination of RBC indices blood was collected in potassium EDTA vacutainer (4 ml of whole blood in K2-EDTA vaccutainer). The blood samples were analysed for complete hemogram on five part differential cell counter (Sysmex XT-4000i) and following parameters were analysed in CBC- Hb, RBC, MCV, MCH, MCHC, TLC and platelet count. IDA and β-TT are recognized as the most important cause of hypochromasia and microcytosis. High RBC count and low MCV is a characteristic of β-TT. TLC and platelet count were undertaken to rule out any ongoing infection or thromboembolic disorder. Four indices were calculated which is based on the hematological parameters derived from complete blood count reports.

The indices used were

Mentzer Index[5]

The Mentzer index is a commonly used calculation to help identify patients who may β-TT or iron deficiency anemia.
Mentzer formula = (MCV) / (red blood cell count)

Interpretation

Iron deficiency is indicated by a value >13
β-thalassemia minor is indicated by a value <13

England Fraser Index[6]

The England-Fraser index is intended to help in distinguishing between β-TT and other causes of microcytosis.

England-Fraser index = {(MCV) – [(5 (hemoglobin)) + (RBC) + K)]}
K = 3.4 if the hematocrit is corrected for plasma trapping (usual situation) or 8.4 if it is not.
Interpretation:
Iron deficiency: > 0 (positive)
β-thalassaemia minor: < 0 (negative).

RBC Count

RBC count itself is used as an index
Interpretation:
B-thalassaemia minor: RBC count >5 $\times 10^6/mm^3$
Iron deficiency anemia: RBC count <5$\times 10^6$ /mm3

Green And King Formula[7]

The Green and King formula is used to help identify the cause of microcytosis.
Formula = $[(MCV)^2 \times (RDW) / [(Hb) \times 100]$
Interepretataion:
Iron deficiency >72
β-thalassaemia minor <72

After application of these indices on 400 microytic patirnts, 61 patients with possibility of β-TT were selected. 4 ml venous blood in EDTA vial which is drawn for CBC from all these patients, the remaining venous blood was used for HbA_2 estimation by high performance liquid chromatography (HPLC) for confirmation. The system used for HPLC is BIO-RADIANT VARIANT Beta Thalassemia short program. A level of more than 3.8% by HPLC is taken as diagnostic of heterozygous β-TT. The levels between 3.4-3.8% should be regarded as borderline and the assay should be repeated both on the same sample and a fresh sample. The sensitivity and specificity of the various red blood cell indices used to screen β-TT were also calculated and the test best index according to Youdens index will be found.
Younden's index = (sensitivity) + (specificity) – 1
Interpretation:
minimum index: -1
maximum index: +1
A perfect test would have a Younden's index of +1.
Pregnant females who come out to be positive for thalassaemia trait by HPLC, further testing of their husbands is done to prevent the birth of a thalassaemic major child.

OBSERVATION AND RESULTS

Out of total 400 microcytic hypochromic cases studied, 40 were diagnosed with β-TT, 2 were diagnosed as sickle cell trait and remaining cases were diagnosed as microcytic hypochromic anemia (table–1).

Table 1 Frequency of Beta Thalassaemia Trait In Pregnant Females

Total no. of microcytic hypochromic cases	Total no. of cases undergone HPLC	β-thalassaemia trait (diagnosed by HPLC)	Normal HPLC pattern	Sickle cell trait (diagnosed by HPLC)
400	60	40(10%)	18	2

Out of total 400 microcytic hypochromic cases studied, 40 were diagnosed with β-thalassaemia trait, 2 were diagnosed as sickle cell trait and remaining cases were diagnosed as microcytic hypochromic anemia.

Majority of the cases belonged to Muslim community (17.5%) followed by Punjabis (15%), Baniya (12.5%)) and Sindhi (12.5%) (table-2).

Table 2 Ethnical Distribution of Females Diagnosed With β-Thalassaemia Trait

Ethnical group	β-thalassaemia trait	
Muslim	7	(17.5%)
Punjabi	6	(15%)
Baniya	5	(12.5%)
Sindhi	5	(12.5%)
Brahmin	3	(7.5%)
Soni	3	(7.5%)
Rajput	2	(5%)
Jat	2	(5%)
Jatav	2	(5%)
Meena	2	(5%)
Yadav	1	(2.5%)
Bairwa	1	(2.5%)
Meghwal	1	(2.5%)
Total	40	(100%)

Majority of the cases belonged to Muslim community (17.5%) followed by Punjabis (15%), Baniya (12.5%)) and Sindhi (12.5%).

RBC count is much higher and MCV and MCH is lower in β-TT group. There is no significant difference in Hb, MCHC and RDW between the two groups (table-3).

Mentzer index showed the highest sensitivity of 90% and the lowest specificity of 94.7%. England & Fraser index had lowest sensitivity of 37.5% but high specificity of 98.3%, while

RBC count had sensitivity of 80% and specificity of 95%. The Green & King index showed a sensitivity of 72.5% but a high specificity of 99.4% (table-4).

Table 3 Comparasion of Parameters between B-TT and Microcytic, Hypochromic Group

Parameter	β-TT	MC,HC	P value	Significance
RBC ($\times 10^6$/Cumm)	5.46	4.36	<0.05	Significant
Hb (gm/dl)	10.21	9.62	>0.05	Non significant
MCV (fl)	60.86	73.17	<0.001	Very significant
MCH (pg)	18.93	21.80	<0.01	Significant
MCHC (gms/dl)	31.09	29.73	>0.05	Non significant
RDW (%)	18.5	19.6	>0.05	Non significant

The above table shows the comparasion of red cell parameters between β-thalassaemia trait and microcytic, hypochromic group. RBC count is much higher and MCV and MCH is lower in β-thalassaemia trait group. There is no significant difference in Hb, MCHC and RDW between the two groups.

Table 4 Comparison of The Results Obtained Using The Four Indices To Detect β-TT

Index	Sensitivity	Specificity	PPV	NPV	Younden Index
Mentzer	90	94.7	65.4	98.8	**84.7**
E & F	37.5	98.3	71.4	93.4	**35.8**
RBC Count	80	95	66.6	97.7	**77**
G & K	72.5	99.4	93	97	**71.9**

*gold standard test- HPLC

The above table shows the comparasion of four red cell indices used for screening of beta-thalassaemia trait. Mentzer index showed the highest sensitivity of 90% and the lowest specificity of 94.7%. England & Fraser index had lowest sensitivity of 37.5% but high specificity of 98.3%, while RBC count had sensitivity of 80% and specificity of 95%. The Green & King index showed a sensitivity of 72.5% but a high specificity of 99.4%.

Mean HbA_2 of β-TT was 5.51±0.56 & that of Microcytic, Hypochromic group was 2.45±0.41. This difference was significant statistically (p>0.05); (table-5).

Table 5 Comparasion of mean value of HbA_2 between β-TT & Microcytic, Hypochromic Group

	GROUP	N	Mean	Std. deviation	P Value	Significance
HbA_2	β-TT	40	5.51	0.56	<0.05	**Significant**
	MC, HC	20	2.45	0.41		

The above table compares shows the comparison between the HbA_2 of β-TT and MC, HC group. Mean HbA_2 of β-TT was 5.51±0.56 & that of MC, HC group was 2.45±0.41. This difference was significant statistically (p>0.05).

DISCUSSION

Out of the 400 cases of microcytosis, only 60 cases were suspected to have β-TT based on the indices. Out of these 60 cases, 40 were confirmed to have β-TT, 2 were diagnosed as sickle cell trait by HPLC. Remaining 18 out of 60 cases were reported to have normal hemoglobin pattern. The remaining 358 cases had been referred to as Microcytic, Hypochromic group. Further testing of 25 females who turned up with their husbands was done and two are positive for thalassaemic trait.

The distribu tion of β-thalassaemia gene is not uniform in the Indian sub-continent. In our study the incidence of β-TT was 10% in the pregnant females.

Kulkarni *et al* (2013) reported in their study the prevalence of the β-TT among 210 pregnant women in Bangalore is 8.5% (n=18). Mendiratta *et al* (2015) reported 7.9% (n=79) in 100 pregnant females. Balgair *et al* (2006) reported the incidence of β-thalassaemia is 9.1% (n=94) in 480 females which is comparable to our study[8,9].

Dipanshu *et al* (2016) in their study on 1083 pregnant femlaes in West Bengal concluded the prevalence of thalassemia trait is 4.61% (n=50). Baxi *et al* (2013) reported 2.78% (n=28) incidence of β-TT in 1006 pregnant females in urban population of Indore, MP and Panda *et al* (2009) in their study in Southern Orissa showed the prevalence of β-TT is 1.5% (n=1) in 62 pregnant females. which is in contrast to our studies[10,11].

In the present study β-TT was distributed among various ethnic groups as Muslim (17.5%, 7cases), Punjabi (15%, 6 cases), Baniya & Sindhi together (25%, 10 cases). Balgair *et al* (2006) in their study which includes n=185 (18.2%) β-TT concluded that β-TT was prevalent among General castes followed by SC/STs which are comparable to our results[9]. Dipanshu *et al* in their study of 50 thalassemia carrier females concluded that β-TT is more prevalent in Muslims (20%, 10 cases), followed by Marwari (16%, 8 cases) and Punjabi (16%, 8 cases) which is comparable to our study[12].

In present study shows a highly significant lower MCV (in fl) of 60.24±3.77 and MCH (in pg) of 18.93±1.39 as compared to MCV of 73.17±5.43 and MCH of 22.80±2.91 in microcytic, hypochromic group. Vehapoglu *et al* (2014) observed the mean MCV & MCH of 61.11±3.49 & 18.8±1.37 respectively in the β-TT group and MCV & MCH of 67.49±7.14 & 21.33±3.09 respectively in the microcytic, hypochromic group comparable to our study[14]. Ehsani *et al* (2009) found an average MCV of 62.02±4.57, MCH of 19.669±1.53 in β-TT group and MCV of 70.04±7.94, MCH of 21.30 in the IDA group[13]. [

The result of our study matched with the above studies. MCV & MCH are a key diagnostic factor in categorizing patients with β-TT and those without it.

There was not much variation between the hemoglobin values as our study inclusion criteria is microcytic, hypochromic cases and between the mean corpuscular hemoglobin concentration of β-TT group and microcytic hypochromic group (p>0.05, non significant).

RDW-CV is studied in our study instead of RDW-SD as this parameter is influenced by MCV value, so gives better differentiation between β-TT and Iron deficiency anemia. There was no significant difference in the red cell distribution width (RDW-CV) between the two groups. It was 18.5±2.01 in the β-TT group and 19.6±3.51 in the microcytic, hypochromic group.

Rahim *et al* (2008) in his study of 94 cases of β-TT found the mean value of RDW-CV to be 15.12±2.37 which was contrast to our study. RDW is normal in patients with β-TT and increased in iron deficiency anemia. This may be explained by coexisting iron deficiency anemia in some patients with β-TT[14].

Total red blood cell count ($x10^6$/Cumm) in the β-thalassaemia group in our study was 5.46±0.56 while it was 4.36±0.56 in the microcytic, hypochromic group which was statistically significant. Ehsani *et al* (2009) found a mean TRBC of 5.89±0.59 in the β-TT group & 4.41±0.55 in the IDA group[13].

Rahim *et al* (2008) noted a mean TRBC of 5.94±0.7 in 323 cases of β-TT[14]. All the studies are comparable to our study.

Sensitivity (Sn) & specificity (Sp) of the Mentzer index were 90% & 94.7% respectively, while the positive predictive value (PPV) & negative predictive value (NPV) were 65.4% & 98.8%. Overall, the validity of the index according to youndens index was +0.84. Vehapoglu *et al* (2014) studied 290 patients of β-TT and found Sn & Sp of 98.7% & 82.3%. PPV, NPV& YI of 86.3%, 98.2% & 81[16]. Ehsani *et al* (2009) reported a Sn & Sp of 95.5% & 94.6% respectively for mentzer index with overall validity by Younden's index of +0.91[13]. Natios *et al* (2007) reported a Sn & Sp of 59.7% & 99% respectively with YI of +0.58[15]. Hence Mentzer index has a high validity in our study. The results of our study are comparable to other studies.

Sensitivity & Specificity of the E & F index were 37.5% & 98.3% respectively, while the PPV & NPV were 71.4% & 93.4% respectively. Overall the validity of the index according to Younden's index was 35.8. Mussarrat *et al* (2010) studied 312 patients of β-TT retrospectively and found Sn & Sp of 91% & 56%.PPV, NPV& YI of 74%, 82% & 47[16]. Ehsani *et al* (2009) reported a higher Sn & Sp of 69.5% & 99.2% respectively with overall validity by Youden's index of 68[13]. Lower validity of this index is obtained in our study as it gives misleading results in pregnancy so not applicable in pregnancy.

Present study reported Sn & Sp of TRBC were 80% & 95% respectively, while the PPV & NPV were 66.6% & 97.7% respectively with YI of 77. Ehsani *et al* (2010) reported a higher Sn & Sp of 98.1% & 86.2% while the overall validity by YI of 63. Natios *et al* (2007) reported a Sn & Sp of 64.34% & 99% respectively with YI of 63. Rahim *et al* (2008) reported a Sn & Sp of 94% & 84% while PPV & NPV of 91% & 87% with YI of 78[13-15]. The results of these studies are comparable to our results.

Present study reported Sn & Sp of the G& K index were 72.5% & 99.4% respectively, while PPV & NPV were 93% & 97% respectively. Overall the validity of the index according to the Younden's index was 71.9. Mussarat *et al* (2010) showed the Sn & Sp of 86% & 78%, while the PPV & NPV of 93% & 78% with YI of 64. Rahim *et al* (2008) showed the Sn, Sp, PPV, NPV & YI of 52%, 93%, 92%, 54% & 45[14,16].

According to the present study the Mentzer index had the best overall accuracy of 84.7 followed by RBC count YI of 77, Green & King index YI of 71.9 and the lowest accuracy of E&F Index of 35.8. Hence the Mentzer index is the best index to be used for screening patients of β-TT. Ehsani *et al* (2009) also reported Mentzer index (YI = 90) as the best discrimination index followed by RBC count (YI = 77) which is comparable to our study[13].

Mussarrat *et al* (2010) also reported Mentzer index (YI =70) as the best discrimination index followed by Green & King index (YI = 64) and the lowest YI of 35.8 of E&F I[16]. Rahim *et al* (2008) showed RBC count (YI = 78) as the best discrimination index in screening of β-TT followed by E & F I (YI of 76), MI (YI = 75) and G & K I (YI =45) which was in contrast to our study[14].

The diagnosis of β-TT relies on an accurate estimation of HbA_2 levels. A raised HbA_2 level (more than 3.8%) is the gold standard for the diagnosis of β-TT. HPLC was performed on 61 cases after screening 400 microcytic cases by four RBC indices in the present study.

CONCLUSION & SUMMARY

The frequency of β-TT was found to be 10% in 400 microcytosis cases and was predominantly seen among Muslims, General castes and SC/STs followed by Punjabis and Sindhis. 2 cases were diagnosed as sickle cell trait. Mean RBC count was 5.46±0.56 x 10^6 /Cumm in β-TT and 4.36±0.71 x 10^6 /Cumm in Microytic, hypochromic group. Hence an elevated RBC counts despite low Hb was found in β-TT. Mean MCV was 60.86±3.37 fl and MCH was 18.93±1.39 pg in β-TT as compared to 73.17±5.43fl and 21.80±2.91 pg in microcytic, hypochromic group. Hence low in β-TT.

Mentzer index was the best among the four discriminating indices used. Its validity was comparable to that obtained in other studies. It can be used for screening of β-TT.

HPLC is the most reliable and confirmatory investigation for diagnosing β-TT. HPLC were performed in all the suspected cases having Mentzer index<13, E & F I negative, RBC count >5x10^6 and G & K I <72 having the HbA2 >3.8 % were taken as cut off point. Hence we conclude that the differentiation of β-TT from MC, HC group has important clinical implication in hematology and medicine.

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