ORIGINAL RESEARCH PAPER

Volume-9 | Issue-4 | April-2020 | PRINT ISSN No. 2277 - 8179 | DOI : 10.36106/ijsr

INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

DIAGNOSTIC ROLE OF CBC IN β -THALASSEMIA

Pathology

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ABSTRACT

Beta-thalassemia is most common inherited red cell disorder worldwide and single gene disorders in India . Effective population screening of β thalassemia trait can dramatically decrease the incidence of birth of a beta-thalassemia major child. Through genetic counseling birth rate of β-thalassemia major can be reduced by 90%.Aim of the study was to determine the prevalence of beta-thalassemia in patients with microcytic hypochromic anemia and to assess the suitability of using CBC, PBF, NESTROFT and confirmation by HPLC. A total of 666 cases from January 2016 to December 2018of microcytic hypochromic anaemia submitted for HPLC in the Department of Pathology, JawaharLal Nehru Medical College, Ajmer.Out of 666 microcytic hypochromic cases, 12 cases were diagnosed as -thalassemia major (BTM), 106 cases beta-thalassemia trait (BTT) and rest were of non - thalassemia micocytic hypochromic(NBT) anemia.. Majority of beta-thalassemia (37.28%) were seen in the paediatric age group of 0-15 years. BTM was more common in males than females.Majority of the cases were Hindus(66.94%) followed by Muslims(16.94%) and Sindhi population (14.40 %). Mean RBC count ,Hb g/dl, and Hct were significantly higher and MCV , MCH and RDW were significantly lower in BTT . Mentzers index and Green and Kings index were highly reliable with high specificity and sensitivity along with NESTROFT(Naked Eye Single-Tube Red Cell Osmotic Fragility Test).A set of screening tests like NESTROFT, Mentzers index and Green and Kings index along with routine hemogram data (RBCcount ,Hb,MCV,MCH,RDW) in microcytic hypochromic cases can effectively discriminate between BTT and Non BTT, and diagnosis of BTT can be reliably done by HPLC and HbA2 quantitation by elution with HbA2>3.5%.

KEYWORDS

β–Thalassemia, Microcytic hypochromic anemia, MCV, MCH.

INTRODUCTION:

Thalassemias are group of autosomal recessive disorders where there is an inhibition of the production of alpha or beta globin chains of haemoglobin resulting in varying levels of anaemia.[1,2]

It is estimated that 1.5% of world population are carrier of β-beta-thalassemia with an estimated 60,000 new carrier born annually.[3] -beta-thalassemia is one of the most common single gene disorders in India with an overall prevalence of 3-4%. A WHO update on beta-thalassemia in India indicate a similar overall carrier frequency of 3-4%, which with the current national population would translate to between 35.6 and 47.5 million carriers of the disorder nation-wide.[4]

Complete blood count provided by a routine automated blood counter and the red blood cell indices derived from them are major contributors for extensive and appropriate detection of beta- thalassemia trait.[5,6]

Effective population screening of β thalassemia trait (βTT) can dramatically decrease the incidence of birth of a thalassemia major child. Through genetic counseling birth rate of β-thalassemia major can be reduced by as much as 90%.[6,7]

MATERIALS AND METHODS :

The present study was done over a period of 3 years, from January 2016 to December 2018. Total 666 cases were studied that includes all cases of microcytic hypochromic anemia patients submitted for HPLC presenting in the Department of Pathology, JawaharLal Nehru Medical College and Associated Group of Hospital, Ajmer.

Procedure :3ml of venous blood samples were collected aseptically by standard phlebotomy technique by trained phlebotomist from each subject into Di-potassium ethylene diamine tetra-acetic acid (K2 EDTA) anticoagulant containing vial. The sample were be well mixed (though not shaken) and aspirated into automated haematology analyzer within 4 hours of collection and provides a print out with Complete blood count, RBC indices, and RDW.[8]

The blood samples of patients were analysed for complete haemogram on haematology analysers and peripheral blood smear study was carried out in selected cases. The patient of suspected β-thalessemia cases were investigated. CBC and RBC indices and HPLC were done in all cases. NESTROF test was also done in some cases.[8]

Table 1: Discrimination limits (cut-off points) for BTT and non-BTT

	Discriminant Functions	BTT	Non –BTT
England and Fraser[9]	DF1=MCV-RBC-(5XHb)-3.4	<0	>0
Mentzer ratio[10]	DF2=MCV/RBC	<13	>13
Srivastava ratio[11]	DF3=MCH/RBC	<3.8	>3.8
Shine and Lal product[12]	DF4 =(MCV)2 /MCHX0.01	<1530	>1530
Klee[13]	DF5=RBC Counts	>5X1012/L	<5X1012/L
Green and King[14]	DF6= [(MCV)2x(RDW)/ [(Hb)x 100]	<72	>72

RESULTS :

Out of total 666 microcytic hypochromic cases studied, 12 cases diagnosed with -thalassemia major (BTM), 106 cases diagnosed with beta-thalessemia trait (BTT), 548 cases diagnosed as non beta-thalassemia microcytic hypochromic anemia (NBT).

In this study, 44 cases (37.28%) of beta-thalassemia were seen in the paediatric age group of 0 -15 years followed by 38 cases (32.2%) were in the age group of 16 – 45 years, 23 cases (19.5%) were in age group of 31 -45 years and minimum number of cases 13(11.02%) were seen in the >46 years of age and mean age was 22.8years. -thalassemia was more common in males 66 cases (56 %) than in females 56 cases(44%).

In our study, the maximum cases of beta-thalassemia were found commonly in Hindu community 79 cases(66.94%),followed by Muslim 20 cases (16.94%),Sindhi 17 cases (14.40%) and 2 cases (1.69%) in Jain community.

In our study comparision between BTT,BTM and NBT with CBC parameter (Mean Hb, TRBC, Hct, MCV, MCH, MCHC, RDW) was done. Theirdifference was significant statistically.MeanHb A2 of BTM was 4.37±1.56& Mean HbF of BTM was 91.75±6.47. Mean HbA2 of BTT was 5.5±1.29 &Mean Hb- F of BTT was 1.82±1.58 and Mean HbA2of NBT was 2.6±3.7 and MeanHb-F of NBT was 1.66±5.02.[Table 2]

Table 2:Group wise Hemogram, HPLC Study Data

Parameters/risk factor (mean ±SD)	BT.MINOR	BT MAJOR	NBT

WBC (cells/mm3)	7.92±7.3	8.9±4.9	8.13±5.13
RBC(x 1012cells/ L)	5.55±.61	3.89±.66	4.2±.82
Hb (gms /dl)	10.02±1.67	7.08±1.21	8.74±2.01
HCT (%)	33.66±4.51	23.88±2.36	29.17±6.03
MCV(fl)	60.12±5.99	62.25±6.92	70.2±6.99
MCH (pg)	18.03±4.86	18.6±4.39	20.4±2.5
MCHC (gms/dl)	29.6±4.89	29.69±4.86	29±2.21
RDW (%)	16.5±2.12	14.64±2.12	20.1±4.48
HbA2% Mean±SD	5.5±1.29	4.37±1.56	2.6±3.7
HbF % Mean±SD	5.5±1.29	91.75±6.45	1.66±5.02

The most sensitive DF was found to be DF4 with sensitivity of 98.11% however the specificity was lowest (3.5%). DF1, DF2 , DF3, DF6 and NESTROFT showed sensitivity of 72.64%, 91.5%, 82.35% , 90.56% and 90%, specificity were 89.59 %, 84.48 % , 91.78 % , 89.96 % and 92.22 % respectively. The discriminant functions DF1, DF2 , DF3, DF6 and NESTROFT showed higher accuracy (86.85%, 85.62%. 76.58% , 90.06% and 91.33 %) despite comparatively low positive predictive values [Table 3]

Table 3: Diagnostic values of Discriminant Functions with respect to HPLC

Discriminant Functions	Diagnostic values in relation to HPLC						
	Sensitivity	Specificity	FN	FP	PPV	NPV	Accuracy
DF1(<0.0) MCV-RBC- (5 x Hb)-3.4	72.64	89.59	27.36	10.41	57.46	94.42	86.85
DF2(<13.0) MCV/RBC	91.5	84.48	8.5	15.52	53.29	98.09	85.62
DF3(<3.80) MCH/RBC	86.79	91.78	13.21	8.22	67.15	97.29	90.97
DF4(<1530.0) 0.01 x MCH x (MCV)2	98.11	3.5	1.89	96.5	16.42	90.47	18.80
DF5(>5.0) RBC COUNTS	81.13	85.58	18.87	14.42	52.12	95.51	84.86
DF6(<72)	90.56	89.96	9.44	10.04	63.57	98.01	90.06
NESTROFT	90	92.22	10	7.78	88.52	93.25	91.33

DISCUSSION:

A total of 666 cases of suspected -thalassemia , 106 cases positive for - Thalassemia minor, 12 cases positive for beta-thalassemia major, 548 were non thalassemic microcytic hypochromic (NBT).

In this study, the most common incidence among the beta-thalassemia was of - thalassemia trait (BTT) 106 cases (15.8%), and beta-thalassemia major 12 cases (1.8%).These results are comaparable to Uddim MM et al[15] and Bhalodia JN et al[16]

Majority of patients were diagnosed as BTT in the reproductive age group and BTM were diagnosed in only early pediatric age group, this study included age ranging from 4 months to 84 yrs. This study results were similar to the study done by Srivastav et al[17].

The occurrence of cases were most common in males in our study which was similar to Srivastav A et al[17] and comparable to that of Uddim MM et al[15].This may be due to the prevalent socio- culture factor in our society, that more male patients seek medical attention.

Hindu community was having higher incidence of beta-thalassemia followed by Muslim and Sindhi community which result comparable to Sinha S et al[18].In a diverse country like India, the frequency of BT has been found to vary in various religions and sub castes.[19]The present study 67 % of patients were Hindus. We could not correlate the frequency with various sub castes due to non- availability of records.

In this study, in - thalassemia minor (BTT) the mean±SD of Hb was 10.02±1.67, RBC 5.5±1.61, Hct 33.66 ± 4.51, MCV 60.12 ± 5.99, MCH 18.03± 4.86, MCHC 29.6±4.89, RDW 16.5 ± 2.12 and .-thalassemia major (BTM) the mean±SD of Hb was 7.08 ±1.21, RBC 3.89 ±0.66, Hct 23.88 ±2.36 , MCV 62.25 ± 5.92, MCH 18.6 ± 4.39, MCHC 29.69 ±4.86, RDW 14.64 ± 2.12 . These findings are comparable to Philip J et al[20]

In this study, the mean ±SD result of HbA2% and HbF% in - Thalassemia minor(BTT) were similar to that of Philip et al [20], and comparable with Baruah MK et al[21], but findings were different form Uddin MM et al[15].

In our study, the mean ±SD result of HbA2% in beta-thalassemia major(BTM) was 4.37±1.56 andmean±SD result of HbF% in -beta-thalassemia major was 91.75±6.47 ,which similar to that of Philip J et al119 and comparable with Baruah MK et al [21] and Uddim MM et al[15] but findings were different form Shrivastav A et al[17].

Target cells and fine basophilic stippling are encountered more frequently in BTM.[22] Peripheral blood film examination usually reveals microcytosis (with mild anisocytosis), target cells and fine basophilic stippling than with IDA given the same level of anemia.[22] England and Fraser suggested that the presence of significant target cells and basophilic stippling in RBC's in microcytic cases strongly suggest BTT.[12]

In the present study sensitivity of DF1, DF2, DF3, DF4, DF5, and DF6 were found to be 72.6%, 91.5%, 86.9%, 98.11%, 81.13% and 90.21% respectively comparable to the study done by ElaheBordber et al23 andBhusan R et al24.Hence DF2, DF3 and DF6 were found to be a good combination to screen BTT to the best of our knowledge. .

NESTROFT were sensitivity is slightly lower (90%), the specificity is higher (97.34%) in the present study This were comparable to Manjula et al[25] and Chakraboty et al[26] present study we conclude as Susanna Thomas et al that NESTROFT can be used as a mass-screening test which is cost effective, simple and rapid when combined with DF's.

CONCLUSION:

A set of screening tests like NESTROFT, Mentzers index and Green and Kings index along with routine hemogram data (RBCcount ,Hb,MCV,MCH,RDW) in microcytic hypochromic cases can effectively discriminate between BTT and Non BTT, and diagnosis of BTT can be reliably done by HPLC and HbA2 quantitation by elution with HbA2>3.5%.

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