Original Article

PRE-MARITAL SCREENING TESTS OF β-THALASSEMIA TRAIT IN DAKSHINA KANNADA POPULATION OF KARNATAKA

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ABSTRACT

Objective: β-Thalassemia is one of the familiar single gene disorders which passes from parents to offspring. The prevalence of β-thalassemia trait varies from 1-14% in different regions of India. Every year almost 9000 β-thalassemic major children are being born in the Indian sub-continent. In the present study, the prevalence of β-thalassemia trait was checked and some screening tests were performed to detect it among the Dakshina Kannada population of Karnataka.

Methods: A total of 800 youngsters were selected for the study, males being above 21 y and females above 18 y. Two ml of blood was drawn and collected in K2 EDTA bottles and complete hemogram was immediately checked. Samples which have Mean Corpuscular Volume (MCV)<80 fico litres(fl) were selected for the study. Five discriminant functions were calculated. NESTROFT (Naked Eye Single Tube Red Cell Osmotic Fragility Test) was performed in all the samples. The samples which show positive for NESTROFT and at least 2 discriminant functions were further checked for HbA2 level using cellulose acetate electrophoresis to confirm the β-thalassemia trait. A comparison was made with the normal samples which have MCV > 80fl.

Results: Prevalence of β-thalassemia trait was found to be 5.125 % in this population. The obtained values were analyzed using unpaired Student's't' test using GraphPad prism (Version-3.0). Samples of β-thalassemia trait have significant changes in the white blood corpuscles (WBC p=0.1266), red blood corpuscles (RBC p=0.0130), hemoglobin (Hb p<0.0001), hematocrit (HCT p<0.0001), MCV(p<0.0001), mean corpuscular hemoglobin (MCH p<0.0001), mean corpuscular hemoglobin concentration (MCHC p<0.0001), platelets (PLT p=0.0005), HbA2(p<0.0001) compared to normal controls.

Conclusion: The present study shows that the people with β-thalassemia trait have a significant variation in complete hemogram compared to normal; NESTROFT and discriminant functions can be used for the screening of β-thalassemia trait in the population.

Keywords: β-Thalassemia trait, Complete hemogram, Discriminant functions, NESTROFT, Cellulose acetate electrophoresis

INTRODUCTION

β-Thalassemia is one of the familiar single gene disorders which pass from parents to offspring, which is caused due to impaired production of β hemoglobin chains [1]. As per the information β-thalassemia is one of the common genetic disorders in the world [2]. Various studies have found that the prevalence of β-thalassemia trait varies from 4-15% in different regions of India. Every year almost 9000 β-thalassemic major children are being born in the Indian subcontinent and the carrier frequency of β-thalassemia varies from 3 to 20% [3]. Prevalence is found to be more in northern, western and eastern parts of India. Compared to all the states of India, Gujarat has the highest frequency of β-thalassemia trait (10.0 to 15.0%) which is followed by Calcutta (10.2%), Punjab (6.5%), Delhi (5.5%), Tamil Nadu (4.0%), Bengal (3.5%), Mumbai (2.6%), Maharashtra (1.9%) and Kerala (0.6%) [4]. India which is known for various cultural backgrounds with different ethnicities has an elevated inherited disorder rate in certain communities. In population screening it has been revealed that some of the communities have a risk of β-thalassemia and the carrier status prevalence is as high as 17% [5]. Based on the population distribution [6-13], a higher frequency of β-thalassemia is found in the people of Bhanishali (15.0%), Lohana (13.6%), Sindh (8.0%), Assam (5.0%), Saraswelt North West (4.4%), Saraswelt West (3.5%), Bangalen (3.7%) and Ahom (1.0%), Panjabis and Jains (4-7%). β-Thalassemia has been classified into three types-minor, intermedia and major. β-Thalassemia minor is also called as β-thalassemia trait(BTT) or carrier state and the affected person carries one normal and one mutated thalassemia β globin chain. There are 25% chances of developing a homozygous β-thalassemic major child for a β-thalassemia carrier couple [14]. Thalassemia is cost effective. To get an ideal treatment for one thalassemic child it costs around Rs.1,25,000/annum [15]. There is a lack of awareness about this disease among the people. There are many different screening techniques available to screen β–thalassemia.

In the present study, an attempt was made to screen β-thalassemic trait among the youngsters in Dakshina Kannada district, in Karnataka and to bring awareness among them. Dakshina Kannada is one of the coastal districts in Karnataka which have western ghats [16, 17]. Dakshina Kannada is considered as the number one in literacy in Karnataka [18] but many of them do not know about β-thalassemia. In the prosperous families too, β-thalassemic children are being born.

Hence, well-educated youngsters were selected to screen for β-thalassemia prevalence in Dakshina Kannada and also enumerated various tests available to screen.

MATERIALS AND METHODS

Sample collection

A total of 800 youngsters were selected for the study. Informed consent was obtained from all the subjects. Ethical clearance was obtained from Yenepoya University Ethics Committee YUEC 38/05/02/2015.

Inclusion Criteria: Unmarried people, males being above 21 y and females above 18 y.

Exclusion Criteria: People who underwent recent blood transfusion.

Informed consent was obtained from all the people.

Methodology

Two ml of blood was collected in K2 EDTA bottle and complete hemogram was investigated immediately using Sysmex XP-100.

Samples which have MCV<80 fico litres were selected for the study. NESTROFT (Naked Eye Single Tube Red Cell Osmotic Fragility Test) was performed in all the samples using 0.36% buffered saline [19].

Five discriminant functions (DF) were calculated using the formula and cut off points [20].

1.	DF1= MCV-RBC-(5xHb)-3.4.	BTT<0	(England and Fraser)[21]
2.	DF2= MCVRBC.	BTT<13	(Mentzer ratio)
3.	DF3= MCH/RBC	BTT<3.8	(Srivastava ratio)
4.	DF4= (MCV)²x MCH x 0.01	BTT<1530	(Shine and Lal product)
5.	DF5= RBC Counts	BTT>5x10^{12}/l	(Klee)

The samples which show positive for NESTROFT and positive for at least 2 discriminant functions were further checked for HbA2 level using cellulose acetate haemoglobin electrophoresis [22] to confirm the β-thalassemia trait. If HbA2>3.5%, then it is considered as positive for β-thalassemia trait. Cellulose acetate strips and control (HbA and HbA2) were purchased from HELENA laboratory.

Statistical analysis

The obtained values were analyzed using unpaired Student's 't' test using GraphPad prism (Version-3.0) to find out the significant mean values of lab parameters. SPSS version 16 was used to find out the sensitivity, specificity, positive predictive value and negative predictive value. Microsoft excel was used to generate the graphs.

RESULTS AND DISCUSSION

Totally 800 samples were collected for this study and a complete hemogram was immediately checked. MCV<80fl was further screened for the β-thalassemia trait using NESTROFT. Totally 15% of the population have MCV<80fl (fig. 1).

NESTROFT is the simple low cost test which is used for population screening for β-thalassemia trait. Out of 120 samples, 72 samples were positive for NESTROFT. In the positive samples, the turbidity was observed and the line behind the test tube was not visible (fig. 3). In the negative samples, since there is no turbidity, the line was visible (fig. 2).
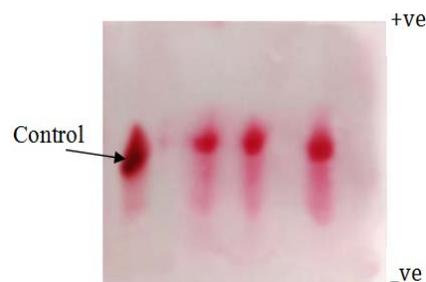
Samples which show positive for NESTROFT and for at least 2 discriminant functions were further checked for HbA2 level using cellulose acetate haemoglobin electrophoresis to confirm β-thalassemia trait. HbA2>3.5% is considered as β-thalassemia trait (fig. 4).

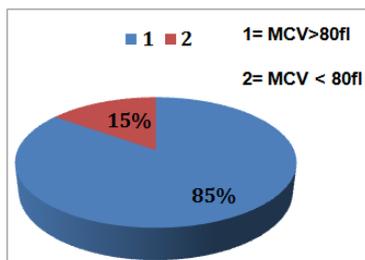**Fig. 4: Cellulose acetate electrophoresis**

Fig. 1: Prevalence of MCV<80fl

Fig. 2: Sample negative for NESTROFT

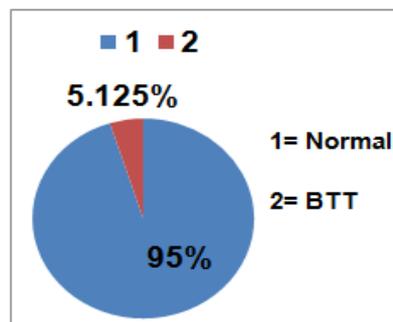**Fig. 3: Sample positive for NESTROFT**

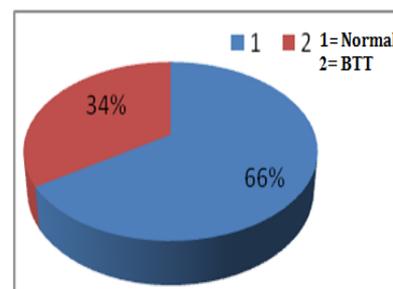**Fig. 5: Prevalence of β-thalassemia trait**

Fig. 6: Prevalence of β-thalassemia trait in MCV<80fl

The prevalence of β-thalassemia trait was found to be 5.125% in this population (fig. 5). Thirty-four percent of the population is affected with β-thalassemia trait whose MCV value is less than 80fl (fig. 6).

Table 1: Comparison of complete hemogram between β-thalassemia trait and healthy control blood samples

S. No.	Parameters (mean±SD)	β-thalassemia trait (n=41)	Healthy controls (n=41)	p value
1.	WBC(x10³/µl)	7.883±0.2918	8.605±0.3655	0.1266
2.	RBC(x10⁶/µl)	5.081±0.1067	4.767±0.06265	0.0130
3.	Hb (g/dL)	10.67±0.3238	13.34±0.2319	p<0.0001
4.	HCT (%)	35.97±0.7252	41.05±0.5727	p<0.0001
5.	MCV (fl)	70.70±0.8396	85.85±0.5937	p<0.0001
6.	MCH (pg)	20.91±0.4188	27.88±0.3061	p<0.0001
7.	MCHC (g/dL)	29.51±0.3354	32.44±0.1976	p<0.0001
8.	Platelet(x10³/µl)	379.1±14.22	315.1±10.59	0.0005

HCT = Hematocrit; MCV= Mean corpuscular volume; MCH= Mean corpuscular hemoglobin; MCHC= Mean corpuscular hemoglobin concentration.

Complete hemogram of the blood samples was compared between β-thalassemia trait (BTT) and healthy controls (table1). WBC value was slightly high in control (8.605±0.3655) when compared to BTT (7.883±0.2918); RBC level was high in BTT (5.081±0.1067) compared to controls (4.767±0.06265); Hb, HCT, MCV, MCHC were significantly low in BTT when compared to the controls and the platelets were significantly high in BTT (379.1±14.22) compared to the controls (315.1±10.59).

Table 2: Comparison of discriminant functions between β-thalassemia trait and healthy control blood samples

Parameters (mean±SD)	β-thalassemia trait (n=41)	Healthy controls (n=41)	p value
DF1	8.907±1.579	11.04±1.121	0.2746
DF2	14.20±0.3845	18.08±0.2908	p<0.0001
DF3	4.801±0.5224	5.854±0.09788	0.0512
DF4	1061±42.92	2069±52.05	p<0.0001
DF5	5.081±0.1067	4.767±0.06265	0.0130

DF2 and DF4 were significantly low in BTT when compared to controls while DF1 and DF3 were slightly lower in BTT than the healthy controls. DF5 is slightly elevated in BTT when compared to controls (table 2).

Table 3: HbA2 levels in BTT and control

Parameter (mean±SD)	β-thalassemia trait (n=41)	Healthy controls (n=41)	p value
HbA2 g/dL	4.951±0.1280	2.591±0.0799	p<0.0001

The HbA2 level above 3.5% up to 6.5% was considered as β-thalassemia trait and below 3.5% is considered as normal. When we screened the HbA2 using cellulose acetate haemoglobin electrophoresis, it was found that the mean value of HbA2 in BTT affected samples was significantly high when compared to controls (table 3).

Table 4: Association of complete hemogram withβ-thalassemia trait samples whose MCV<80fl

Parameters	BTT (n=41)	Non BT (Non β-thalassemia) (n=79)	Total (n=120)
WBC (>11000 x10³/µl)	4 (9.75%)	5 (6.32%)	9 (7.5%)
RBC (>5 x10⁶/µl)	20 (48.78%)	27 (34.17%)	47 (39.16%)
Hb (<10g/dL)	14 (34.14%)	18 (22.7%)	32 (26.66%)
HCT (<35 %)	17 (41.46%)	25 (31.64%)	42 (35%)
MCH(<26.5 pg)	40 (97.56%)	77 (97.46)	117 (97.5%)
MCHC (<31.8g/dL)	37 (90.24%)	70 (88.60%)	107 (89.16%)

BTT and non β thalassemia cases whose MCV<80fl were checked for risk factors of complete hemogram. In BTT, 4 (out of 41) and Non BT 5 (out of 79) totally 9 samples (out of 120) have shown WBC >11000 x10³/µl; 20 samples of BTT and 27 samples of Non BT and a total of 47 have RBC>5 x10 ⁶/µl; Hb<10 g/dL was found in 14 samples in BTT and 18 samples in non BT, totally 32 samples; in HCT<35 %, seventeen samples have shown positive in BTT cases and 25 samples showed positive in Non BT cases, thus entirely 42 cases showed positive; 40 BTT and 77 non BT and overall 117 samples had MCH<26.5 pg; MCHC<31.8 g/dL was initiated in 37 BTT and 70 Non BTT and a total of 107 samples (table 4). Discriminant functions in the population which have MCV<80fl was checked. Eight BTT and 17 Non BT, totally 25 subjects showed positive for DF1; positive for DF2 was present in 13 BTT and 24 Non BT subjects, totally 37; DF3<3.8 was seen in 11 cases in BTT and 5 cases in Non BT, a sum of 16 cases; 40 samples of BTT and 72 samples of Non BT, entirely 112 samples have DF4<1530; 37 samples of BTT and 70 samples of Non BT, a sum of 107 have DF5 >5 x10⁶/µl (table 5).

Table 5: Association of discriminant functions with β-thalassemia trait in samples whose MCV<80fl

Parameters	β-thalassemia trait (n=41)	Non β-thalassemia (n=79)	Total (n=120)
DF1(<0)	8 (19.5%)	17 (21.51%)	25 (20.83%)
DF2(<13)	13 (31.70%)	24 (30.37%)	37 (30.83%)
DF3(<3.8)	11 (26.82%)	5 (6.32%)	16 (13.33%)
DF4(<1530)	40 (97.56%)	72 (91.39%)	112 (93.33%)
DF5(>5x10⁶/µl)	37 (90.24%)	70 (88.60%)	107 (89.16%)

Among discriminant functions highest sensitivity was found in DF2, DF3, DF4 which showed 100% sensitivity followed by DF1 which showed 92.5% and DF5 with 65% sensitivity. DF5 have highest specificity of 100% followed by DF4 97.6%, DF1, DF2, DF3 have least specificity. False positive rate was found high in DF1 80.5%, DF3 73.2%, DF2 68.3%, DF4 had less and in DF5 false positive rate was absent. DF4 showed highest false negative rate followed by DF1 7.3% and in DF2, DF3, DF4 false negative rate was absent. NESTROFT showed 94.5% sensitivity, 97.1% specificity, 2.9% false positive rate, 5.4% false negative value (table 6).

Table 6: Diagnostic values of discriminant functions

Discriminant functions	Sensitivity	Specificity	False positive rate	False negative rate
DF1	92.5%	19.5%	80.5%	7.3%
DF2	100%	31.7%	68.3%	0%
DF3	100%	26.8%	73.2%	0%
DF4	100%	97.6%	2.4%	0%
DF5	65%	100%	0%	34. 9%
NESTROFT	94.6%	97.1%	2.9%	5.4%

Complete hemogram was compared with other studies, in which RBC value of our study was almost the same as other studies. Hb, MCHC values were little less and MCV, MCH values were slightly high in the present study when compared to other studies (table 7).

Table 7: Comparison of laboratory parameters among various studies in BTT

Study	Number of cases(n)	RBC (>5 x10⁶/μl)	Hb (<10g/dL)	MCV (fl)	MCH (pg)	MCHC (g/dL)
Gupta *et al.*, [23]	n=56	5.6±0.7	11.2±1.4	64.5±3.7	20±1.2	31.2±0.94
Mohamed *et al.*, [24]	n=382	5.45±0.71	11.3±1.45	64.81±4.72	20.75±1.64	-
Khin *et al.*, [25]	n=133	5.9±1.0	11.5±1.6	62.7±12.1	19.9±3.5	29.3±2.2
Madan *et al.*, [26]	n=337	5.56±0.76	11.6±1.6	64.7±4.8	20.6±3.6	-
Sujatha *et al.*, [20]	n=34	5.76±1.16	11.01±2.06	60.56±5.58	18.78±3.45	30.52±2.56
Present Study	n= 41	5.081±0.10	10.67±0.32	70.70±0.83	20.91±0.41	29.51±0.33
Present Study Control	n= 41	4.767±0.06	13.34±0.23	85.85±0.59	27.88±0.30	32.44±0.19

NESTROFT has been compared with the other studies. All the studies show greater sensitivity (above 90%) whereas 64.2% and 66.6% specificity was exhibited by the studies of Mangalni *et al.,* and Susanna *et al.*, respectively. In our study, we found 97.1% specificity (table 8).

Table 8: Comparison of NESTROFT among various studies in BTT

Study	Number of cases(n)	Sensitivity	Specificity
Maheshwari *et al.* [27]	n=1048	91%	95%
Mangalni *et al.* [28]	n=1695	94.4%	64.2%
Mehta *et al.* [29]	n=131	99.2%	75.8%
Susanna *et al.* [30]	n=137	98.7%	66.6%
Raghavan *et al.* [31]	-	95.5%	87.0%
Sujatha *et al.* [20]	n=222	90%	97.34%
Present study	n=41	94.6%	97.1%

CONCLUSION

Eight hundred unmarried youngsters were selected for the study and their complete hemogram was checked immediately in which MCV<80fl were selected and NESTROFT was done. Discriminant functions were calculated. The samples which showed positive for NESTROFT and the samples which showed positive for at least two discriminant functions were further checked for HbA2 using cellulose acetate haemoglobin electrophoresis to confirm β-thalassemia trait. Fifteen percent of the population have MCV<80fl. The pre-marital prevalence of β-thalassemia trait in Dakshina Kannada district was found to be 5.125%. NESTROFT showed 94.6% sensitivity, 97.1% specificity, 2.9% false positive rate, 5.4% false negative rate and DF4 which showed 100% sensitivity, 97.6% specificity, 2.4% false positive rate, 0% false negative rate. These are the best methods for population screening of β-thalassemia trait. NESTROFT, RBC >5 x10⁶/μl and lower values of Hb also seem to be the best combination to screen β-thalassemia trait. Mean HbA2 in β-thalassemia trait was found to be 4.95g/dL.

CONFLICT OF INTERESTS

Declared none

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