

Detection of carriers for β -thalassemia: A case study of Jammu (J&K)

VIJAY BHARAT*, SUBASH GUPTA and RACHNA GUPTA

P.G. Department of Zoology, University of Jammu, Jammu- 180 006 (India).

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ABSTRACT

The present work embodies screening of about 450 individuals for β -thalassemia carrier detection through NESTROFT and CBC. Of 450 individuals, 125 individuals were found to be NESTROFT positive and 325 were found negative. The sensitivity, specificity, positive predictive and negative predictive values were found to be 100%, 98.5%, 96% and 100% respectively. The efficiency of the NESTROFT was found to be 98.8%. Of the 125 NESTROFT positive individuals, 50 individuals were subjected to CBC analysis and it was found that the hematological parameters like Hb level, red cell count, PCV/HCT, MCV, MCH and MCHC levels varied from 8.1-14.4 g/dl with a mean of 10.63 ± 0.21 g/dl, $2.57-9.80 \times 10^{12}$ /litre with a mean of $4.49 \pm 1.13 \times 10^{12}$ /litre, 25.4-39.0% with a mean of $33.5 \pm 3.49\%$, 53.3-101 fl with a mean of 72.64 ± 1.34 fl, 16.5-31.3 pg with a mean of 22.7 ± 0.49 pg and 29.4-33.4 g/dl with a mean value of 30.8 ± 0.86 g/dl respectively.

Key words: β -thalassemia, NESTROFT, CBC and carrier detection.

INTRODUCTION

β -Thalassemia is one of the commonest single gene disorders resulting from mutations in and around the α -globin gene located as a cluster on the short arm of chromosome number 11 i.e.11p15 (Lin *et al.*, 1985) causing severe anemia. In this disorder, there is relative excess of insoluble β -globin chains that precipitates within the red cells causing ineffective erythropoiesis and severe anemia (Weatherall and Clegg, 1981; Susanne *et al.*, 2000).

In the Jammu region of the J&K state, no high risk community for β -thalassemia is known as there is no previous record of screening for β -thalassemia carrier detection. The present study is an account of the carrier screening for β -thalassemia trait in Jammu region of the J&K state. The aim of the study was to provide genetic counselling to the carriers thus detected and their

relatives that would help in preventing the transmission of the β -thalassemia trait. A number of authors have already studied this aspect viz., Weatherall and Clegg, 1981; Kattamis *et al.*, 1981; Varawalla *et al.*, 1991; Balgir, 1999; Balgir, 2000; Susanne *et al.*, 2000; Singh and Gupta, 2008 and Wiwanitkit. (2009).

MATERIAL AND METHODS

During the present study, screening for the carrier detection was carried out by NESTROFT (Naked Eye Single Tube Red cell Osmotic Fragility Test) and Complete Blood Count (CBC).

A total of 450 individuals were screened and the screening included two categories of people:
Individuals belonging to the families having the history of β -thalassemia i.e. the families where at least one β -thalassemic child was born, and,
Randomly selected individuals.

NESTROFT

NESTROFT was performed using 0.36% buffered saline solution following the method of Singh and Gupta (2008) with least modifications. Based on the observations, sensitivity, specificity, positive and negative predictive values and efficiency of the test were calculated by following the formulae given below (Singh and Gupta, 2008):

Sensitivity	$100 \times TP / (TP+FN)$
Specificity	$100 \times TN / (TN+FP)$
Positive predictive value	$100 \times TP / (TP+FP)$
Negative predictive value	$100 \times TN / (TN+FN)$
Efficiency of test	$100 \times (TN+TP) / (TP+FP+TN+FN)$

CBC (Complete Blood Count)

Of the 125 NESTROFT positive samples, only 50 samples were put to CBC analysis because of latter being expensive and cumbersome. CBC was carried out by using the ERMA PC 604 Cell counter within 2-4 hours after collection of the blood samples.

RESULTS**NESTROFT**

Out of the 450 individuals screened through NESTROFT, 125 individuals were found positive with respect to NESTROFT while the remaining 325 individuals were found negative. Further, of these 125 NESTROFT positive individuals, 61 were females and 64 were males. The nature and number of the individuals with respect to NESTROFT is shown in the table 1.

Table 1: Showing the number of True Positive, False Negative, False Positive and True Negative individuals

Nature of Individuals with respect to NESTROFT	Number of Individuals
True Positive (TP)	120
False Negative (FN)	0
False Positive (FP)	5
True Negative (TN)	325

Table 2: Showing the Sensitivity, Specificity, Positive predictive value, Negative predictive value and the Efficiency of NESTROFT

Parameters	Formulae	Values Found
Sensitivity	$100 \times TP / (TP+FN)$	100 %
Specificity	$100 \times TN / (TN+FP)$	98.5 %
Positive predictive value	$100 \times TP / (TP+FP)$	96 %
Negative predictive value	$100 \times TN / (TN+FN)$	100 %
Efficiency of test	$100 \times (TN+TP) / (TP+FP+TN+FN)$	98.8 %

Table 3: Ranges and Means of different hematological parameters of β -thalassemia carriers

S. No.	Hematological Parameters	Range	Mean	Standard Deviation
1.	Hb (g/dl)	8.1-14.4	10.63	0.21
2.	Red cell count ($\times 10^{12}$ /litre)	2.57-9.80	4.49	1.13
3.	PCV/HCT (%)	25.4-39.0	33.5	3.49
4.	MCV (fl)	53.3-101	72.64	1.34
5.	MCH (pg)	16.5-31.3	22.7	0.49
6.	MCHC (g/dl)	29.4-33.4	30.8	0.86

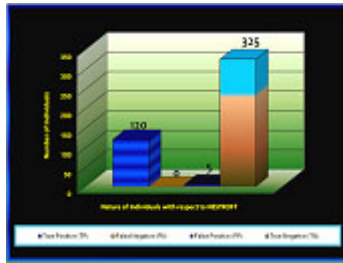


Fig. 1:

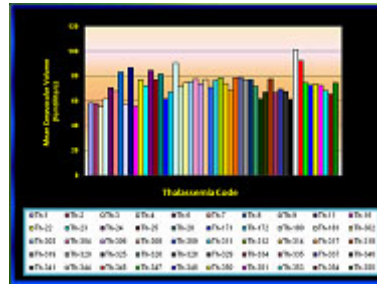


Fig. 3(c):

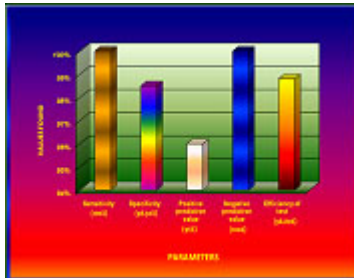


Fig. 2(a):

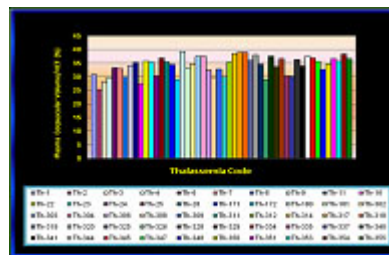


Fig. 3(d):

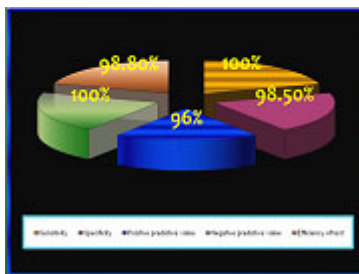


Fig. 2(b):

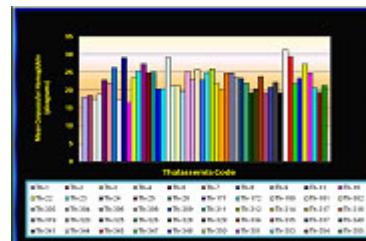


Fig. 3(e):

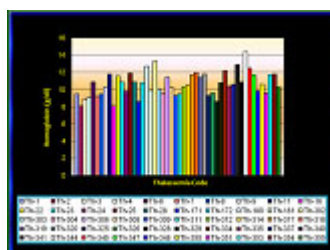


Fig. 3(a):

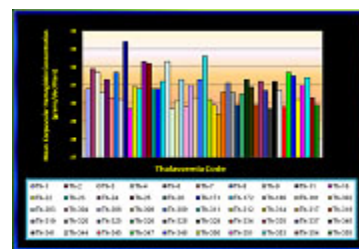


Fig. 3(f):

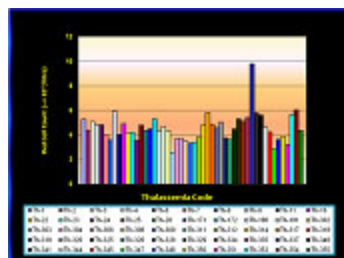


Fig. 3(b):

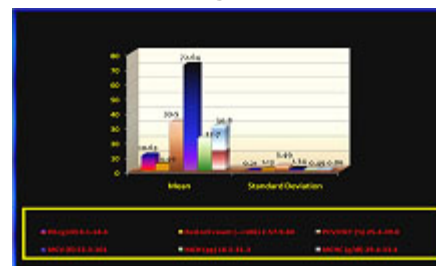


Fig. 4:

Table 4: Usefulness of NESTROFT in the detection of β -thalassemia carriers: a Comparative analysis of data with earlier studies

Parameter	Present Study	Kattamis <i>et al.</i> (1981)	Mehta <i>et al.</i> (1988)	Gorakshaker <i>et al.</i> (1990)	Raghavan <i>et al.</i> (1991)	Thomas <i>et al.</i> (1996)	Manglani <i>et al.</i> (1997)	Mareshwari <i>et al.</i> (1999)	Sirichotiyakul <i>et al.</i> (2004)	Singh (2006)
Sensitivity	100%	98.4%	95.0%	98-100%	95.5%	98.4%	94.4%	91.0%	97.6%	97.7%
Specificity	98.5%	91.0%	82.1%	82-84%	87.0%	66.6%	64.2%	95.0%	72.9%	83.3%
Positive Predictive Value	96%	91.3%	73.1%	50.0%	70.5%	81.5%	97.6%	55.0%	33.6%	95.5%
Negative Predictive Value	100%	98.35	97.0%	99-100%	98.3%	96.5%	35.3%	99.0%	99.5%	90.9%

The graphical representation of the number of True Positive, False Negative, False Positive and True Negative individuals in Figure 1.

In detecting the heterozygous β -thalassemia cases, the sensitivity of NESTROFT was 100%, specificity 98.5% and the positive and negative predictive values were 96% and 100% respectively. The efficiency of the test was 100%. (Table 2 and Figure 2a & 2b).

The values found for the various parameters of NESTROFT can also be shown in a graphical representation in figure 2a and 2b.

CBC

The values of different hematological parameters like Hemoglobin, Red Cell Count, Mean Corpuscular Volume (MCV), Plasma Corpuscular Volume (PCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) of 50 samples in which CBC was done are depicted in the form of graphs shown in the figures 3a,3b,3c,3d,3e and 3f respectively.

In the present study, most of the carriers were diagnosed on the basis of their hematology with special emphasis on complete blood count (CBC). The Hb level in carriers varied from 8.1-14.4 g/dl with a mean of 10.63 ± 0.21 g/dl. The red cell count was relatively high and ranged from $2.57-9.80 \times 10^{12}$ /litre with a mean value of $4.49 \pm 1.13 \times 10^{12}$ /litre. The MCV and MCH were markedly reduced and ranged from 53.3-101 fl and 16.5-31.3 pg with mean values of 72.64 ± 1.34 fl and $16.5-31.3 \pm 0.49$ pg respectively.

Ranges and mean values of different hematological parameters of the β -thalassemia carriers are shown in the table 3 and graphically in the figure 4.

DISCUSSION

β -Thalassemia is the major genetic health problem in the world (Khattak *et al.*, 2006). Carrier frequency of β -thalassemia is the highest in the countries like Italy (0.5-19%), Sardinia (11-19%) and Cyprus (15-17%) (Weatherall and Clegg, 2001b). Worldwide carrier frequency of β -thalassemia is 1.4-

Table 5: Hematological values in β -thalassemia carriers in different populations of the world

S. No.	Population	No.	RBC ($\times 10^{12}$)	MCH (pg)	MCV (fl)	Reference
1	Jammu region	50	2.57-9.80 (4.49 \pm 1.13)	16.5-31.3 (22.7 \pm 0.49)	53.3-101 (72.64 \pm 1.34)	Present study
2	Jammu region	87	4.23-6.37 (5.52 \pm 0.45)	16.8-29.6 (21.14 \pm 2.97)	53.4-81.9 (64.4 \pm 7.12)	Singh (2006)
3	Greek	85	5.1-7.8 (6.3 \pm 0.4)	18.0-29.0 (22.0 \pm 0.4)	-	Malamos <i>et al.</i> (1962)
4	Thai and Chinese	168	4.20-7.87 (5.95 \pm 0.78)	16-34 (20.0 \pm 3.0)	49.0-106.0 (67.0 \pm 9.0)	Pootrakul <i>et al.</i> (1973)
5	British	32	4.60-6.60 (5.60 \pm 0.60)	18.6-25.6 (21.5 \pm 1.3)	63.1-77.1 (70.5 \pm 4.2)	Knox-Macaulay <i>et al.</i> (1973)
6	Italian	82	3.70-6.70 (5.40 \pm 0.69)	15.0-31.0 (23.5 \pm 1.02)	50.0-98.0 (76.29 \pm 1.03)	Mazza <i>et al.</i> (1976)
7	Turkish	64	3.90-6.70 (5.20 \pm 0.70)	18.0-25.0 (22.0 \pm 2.0)	66.0-82.0 (74.0 \pm 5.0)	Dincol <i>et al.</i> (1979)
8	Sardinian	43	5.0-7.0 (6.1 \pm 0.50)	14.0-25.0 (22.0 \pm 2.0)	59.0-85.0 (66.0 \pm 4.0)	Galanello <i>et al.</i> (1979)

7.96%. It is probably the commonest inherited hemoglobin disorder in India, with an uneven distribution amongst the different endogenous populations (Sukumaran, 1975; Varawalla *et al.*, 1991a, b; Venkatesan *et al.*, 1992; Verma, 1994; Verma *et al.*, 1997; Balgir, 1999; Balgir, 2000).

The test is very sensitive and specific. A comparison of the present findings with those of the earlier reports has been shown in the table is shown in the table 4.

The sensitivity of NESTROFT in the present study was found to be 100% which is comparable with sensitivity reported by Gorakshaker *et al.* (1990) and to some extent with the reports of Thomas *et al.* (1996). The specificity in the present study has been found to be 98.5%. Although the specificity as reported by majority of the workers is less than the one found in the present study, yet, the value of specificity as recorded in the present study (98.5 %) is more or less comparable with the findings of Maheshwari *et al.* (1999) who reported the value of specificity to be 95.0%.

Similarly, the positive predictive value in the present study has been found to be 96% and it is quite high quite high but the positive predictive value is comparable with the studies made by Kattamis *et al.* (1981), Manglani *et al.* (1997) and Singh (2006). The negative predictive value of the test in the detection of β -thalassemia carriers in the present study has been found to be 100%. The same is well comparable with the studies made by Kattamis *et al.* (1981), Mehta *et al.* (1988), Gorakshaker *et al.* (1990), Maheshwari *et al.* (1999) and Sirichotiyakul *et al.* (2004) respectively. Thus, a comparison of different parameters has largely been found to be more or less similar to the earlier reports. This suggests that these parameters have their practical significance in the screening of β -thalassemia trait.

The red cell indices have been found as a valuable diagnostic tool for the detection of β -thalassemia carriers and have also been used in the present study. In the carriers detected during the present study, there was a relatively high red cell count, however, the red cells were microcytic and hypochromic having markedly reduced MCV and MCH with averages of 72.64 fl and 22.7 pg respectively.

Carriers for β -thalassemia trait, in general have reduced hemoglobin, reduced MCV (≤ 80 fl) and reduced MCH (< 27 pg) (Karimi and Rasekhi, 2002; Leung *et al.*, 2005; Sirichotiyakul *et al.* 2005; Al-Suliman, 2006). They have relatively high red cell count ($>4.5 \times 10^{12}$ /liter) (Gomber *et al.*, 1997), but with pronounced microcytosis and hypochromia. Reticulocyte count is slightly increased around twice the normal value.

A comparison of the data obtained on red blood indices during the present work with other workers is given in the table-5.

The findings of reduced MCV and MCH values in majority of the cases has been used as a basis of population screening for $\hat{\alpha}$ -thalassemia trait (Pearson *et al.*, 1973 & 1974; Klee, 1980). In the present study also, similar approach was used for the diagnosis of carriers and this approach worked well in the screening programme.

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