Research Article

A study of thalassemia screening of 1000 medical students and comparison of various screening methods

Om Vrajlal Bodarya, Hardik Makwana, Nayana Lakum, Atul Shrivastav, Jayesh Joshi, Ashok Agnihotri

C.U. Shah Medical College, Surendrangar, Gujarat, India.
Correspondence to: Om Vrajlal Bodarya, E-mail: ovbodarya@gmail.com

Received May 28, 2015. September 09, 2015

Abstract

Background: β-thalassemias are widespread throughout many countries including India. India has 3.5 crores thalassemic carriers with about 10,000 thalassemic birth every year in India. In Gujarat, there are about 6000 thalassemic children. The prevalence of β-thalassemia trait (BTT) is not uncommon in India and its incidence is rising in certain states including Gujarat.

Objective: (1) To evaluate the prevalence of BTT among medical students above 18 years in C.U. Shah medical college; (2) To compare specificity of NESTROFT (Naked Eye Single Tube Red Cell Osmotic Fragility Test) with Hb-Electrophoresis lmethod for screening of BTT; (3) To compare specificity of Mentzer's index with Hb-Electrophoresis method for screening of BTT; (4) To create awareness among medicos about BTT.

Materials and Methods: Blood (2 mL) was collected from 1000 medical students; complete blood count (CBC), peripheral smear examination, and NESTROFT were done on all samples. HbA2 levels were measured by fully automated cellulose acetate electrophoresis GENIOS-INTERLAB on suspected samples for BTT on basis of CBC, peripheral smear findings, and NESTROFT. HbA2 levels >3.5 were taken as gold standard for diagnosing BTT.

Results: In this study, we found the incidence of BTT being 4.1% (41/1000), that of iron deficiency anemia being 7.3% (73/1000) and that of megaloblastic anemia being 5.4% (54/1000). Three students were found to be sickle cell trait and one to be Hb D. These four cases were confirmed by HPLC at higher center.

Conclusion: Prevalence of BTT is high in India and aggressive control measures should be taken to prevent it. Government and NGOs should concentrate attention to educate MEDICOS and public at large. This will decrease financial burden on families of thalassemia major patients and society at large.

KEY WORDS: Medical students, thalassemia, screening methods

Introduction

Thalassemia (from Greek, thalassa, haima, blood; British spelling, "thalassaemia") is an inherited autosomal recessive blood disease. It is a quantitative problem in globin synthesis.[1] Every year, 10,000 children with β-thalassemia major are born in India, which constitutes 10% of the total number in the world.[2] Inherited hemoglobin disorders are an important cause of morbidity and mortality. The curative treatment such as bone marrow transplantation is costly and so prevention is the cost effective strategy, which includes population screening, genetic counseling, and prenatal diagnosis.[3] The highest frequency of β-thalassemia is reported in Gujarat (10–15%) followed by Tamil Nadu (8.5%), Punjab (6.5%), Maharashtra, and northern India;[4–6] and certain communities in India, such as Sindhis and Punjabis from northern India; Bhanushalis, Kutchis, and Lohanas from Gujarat; Mahars, Neobuddhists, Kolis, and Agris from Maharashtra; and Gowdas and Lingayats from Karnataka have a higher carrier rate.[7] India spends nearly 1000 crores per annum in the treatment of thalassemia patients.[8] Only 3.6% had thalassemia minor[7]

Access this article online	
Website: http://www.ijmsph.com	Quick Response Code:
DOI: 10.5455/ijmsph.2016.2805201585	

and it was found that 4% of Ahmedabad (major city of Gujarat) population (as per 2010 estimate) is carrying thalassemia trait and it is on the rise. There are 1000 children who are on constant blood supply to stay alive.[9] So, it is important to screen medical college student as they consist of major and important part of community and with that comparison of various methods of screening is also important.

Materials and Methods

- Study period was 2009–2014. Total 1000 blood samples were collected and processed.
- From each medical student of CUSMC, Surendranagar, Gujarat, India, 2 mL blood was collected; complete blood count (CBC) that included Hb, RBC count, PCV, MCV, MCH, MCHC, and RDW by fully automated cell counter, peripheral smear examination, and NESTROFT (Naked Eye Single Tube Red Cell Osmotic Fragility Test) was done on all samples.
- HbA2 levels were measured by fully automated cellulose acetate electrophoresis GENIOS-INTERLAB on suspected samples for BTT on basis of CBC, peripheral smear findings, and NESTROFT.
- HbA2 levels >3.5 were taken as gold standard for diagnosing BTT.
- Prior permission to perform this study was taken from our institutional ethical committee.

Results

In this study we found the incidence of BTT being 4.1% (41/1000), that of iron deficiency anemia being 7.3% (73/1000) and that of megaloblastic anemia being 5.4% (54/1000). Three students were found to have sickle cell trait and one to be Hb D. These four cases were confirmed by HPLC at higher center.

Table 2 shows 23 (2.3%) true positive cases and 20 (2.0%) false positive cases by NESTROFT. Similarly 18 cases (1.8%) are false negative and 59 cases (5.9%) are true negative by NESTROFT. The sensitivity and specificity of NESTROFT are 56% and 75%, respectively.

Table 3 shows 28 (2.8%) true positive cases and 12 (1.2%) false positive cases by Mentzer's index. Similarly 13 cases (1.3%) of false negative and 67 cases (6.7%) of true negative are observed by Mentzer's index. The sensitivity and specificity of Mentzer's index is 68% and 84%, respectively.

Discussion

The disease was first reported among children in Mediterranean countries. The other names of thalassemia are Mediterranean anemia and Cooley's anemia (after Thomas Cooley in 1925).[1]

India has a population of 1.21 billion according to the Census in 2011. There are 4693 endogamous communities that includes 427 tribal groups. Although β-thalassemia and other haemoglobinopathies are seen in all the states, the prevalence is quite variable.[10] The frequency of β-thalassemia trait (βTT) has variously been reported from <1% to 17% and an average of 3.3%.[11] Birth rates of homozygous β-thalassemia in different parts of the world have reduced considerably. Some smaller countries have reported no newborns with the disease. This has been achieved by control programs involving screening population surveys for heterozygous β-thalassemia, antenatal diagnosis along with increasing awareness in the medical profession, and in the population by large-scale education and counseling. Control programs in Sardinia have substantially reduced the birth of homozygous thalassemics from 1:250 to as low as 1:4000 births.[11]

The distribution of β-thalassemia is not uniform in the Indian subcontinent. Though certain communities are identified to have high prevalence, it has been detected in almost every Indian population. The prevalence of BTT varies from 1% to 17% in different populations of India.[12–17]

Result of NESTROFT is compared with result of other studies. In our study, sensitivity of NESTROFT is 56% and specificity is 75%. Chakrabarti et al.[18] showed sensitivity of 100% and specificity of 85.47%. Mehta et al.[19] showed sensitivity of 100% and specificity of 87.7%. So, we found low sensitivity and specificity of NESTROFT. It is not reliable test for screening of BTT.

In our study, Mentzer's index had specificity of 84% whereas Aysel et al.[20] showed specificity of 82.3%. In our study, sensitivity of England and Fraser index is 58% and specificity of 84% whereas Aysel et al.[20] showed sensitivity of 66.2% and specificity of 85.3%.

Mentzer's index and England & Fraser had more sensitivity and specificity than NESTROFT. Cases suspected by these indices are 120 and of which 41 cases are of BTT.

As we have seen the incidence of BTT is high among Medicos and they are being unaware of their BTT status, the screening for BTT should be carried out on a large scale throughout India.

The tests such as MCV, MCH, NESTROFT, Mentzer's index, England & Fraser index have low sensitivity and specificity, as seen in the results, and should not be used for screening BTT as there are greater chances of false positive and false negative.

In Cyprus, 1 in 7 individuals carries the β-thalassemia gene, which translates into 1 in 49 marriages between carriers and 1 in 158 newborns expected to have β-thalassemia major. As a result, preventive measures established and enforced by public health authorities have been very effective in decreasing the incidence among their populations.

In Cyprus, students in all the schools and universities are tested for the disease.

Thus, we in India should also carry out BTT screening on large scale and people should be educated about the disease. Screening can be done in schools, colleges, industries, antenatal clinics, etc. This will help in decreasing incidence of thalassemia major.

Table 1: Laboratory values in blood samples of students with or without BTT

Parameter	All students (μ = 1000)	Classic BTT (n = 41)
Hb (g/dL)	13.33 ± 1.72 (7.10–17.70)	10.80 ± 0.82 (8.3–12.2)
RBC Count (×10^{12}/L)	5.02 ± 0.75 (1.77–7.05)	5.62 ± 0.73 (3.65–7.05)
MCV (fL)	85.41 ± 10.86 (53.80–114.2)	66.71 ± 5.62 (53.8–77.7)
MCH (pg)	25.38 ± 4.32 (15.6–32.58)	22.39 ± 4.27 (15.8–33.2)
RDW (%)	16.49 ± 3.99 (5.20–34.6)	19.37 ± 9.77 (14–34.6)
HbA2 (%)	3.98 ± 3.90 (1.5–35.5)	5.10 ± 1.57 (3.6–9)

BTT, β-thalassemia trait; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume; RDW, red cell distribution width.

Table 2:

NESTROFT	Electrophoresis	
	Positive	Negative
Positive	23 (true positive)	20 (false positive)
Negative	18 (false negative)	59 (true negative)

Table 3:

Mentzer's index	Electrophoresis	
	Positive	Negative
Positive	28 (true positive)	12 (false positive)
Negative	13 (false negative)	67 (true negative)

Table 4: Comparison of false positive and negative result obtained by NESTROFT and Mentzer's index

	False positive (%)	False negative (%)
Nestroft	2.0	1.8
Mentzer's index	1.2	1.3

Table 5: Sensitivity and specificity of various parameters and formulas

Parameter	Cutoff	Sensitivity (%)	Specificity (%)
1)RBC count (x10^{12} /L)	>5	79.31	75.86
2) MCV (fL)	<76	87.5	61.29
3) MCH (pg)	<27	82.75	41.1
4) RDW (%)	>13.6	42.58	32.9
5) Mentzer's index	<13	68	84
6) England and Fraser index	<0	58	84
7) NESTROFT	–	56	75

MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume; RDW, red cell distribution width; NESTROFT, Naked eye single tube red cell osmotic fragility test.

Conclusion

Prevalence of BTT is high in India and aggressive control measures should be taken to prevent it. Alone NESTROFT and other indices should not be used for screening BTT. Electrophoresis or chromatography (HPLC) must be done as and when required. Government and NGOs should focus attention to educate MEDICOS (including Ayuverda, Homeopath, etc.) and public at large. This will decrease financial burden on families of thalassemia major patients and society at large.

References

1. *The Thalassemia Saga*. Available at: http://www.expresshealthcare.in/200708/knowledge02.shtml (last accessed on April 2, 2015).
2. Varawalla NY, Old JM, Sarkar R,Venkatesan R, Weatherall DJ. The spectrum of beta thalassemia mutations on the Indian subcontinent: the basis for prenatal diagnosis. Brit J Hematol 1991;78(2):242–7.
3. Patel AP, Naik MR, Shah NM, Sharma NP, Parmar PH. Prevalence of common hemoglobinopathies in Gujarat: an analysis of a large population screening program. Nat J Commun Med 2012;3(1):112–6.
4. Balgir RS. The burden of hemoglobinopathies in India and the challenges ahead. Curr Sci 2000;79:1536–47.
5. Balgir RS. The genetic burden of hemoglobinopathies with special reference to community health in India and the challenges ahead. Indian J Hematol Blood Transfus 2002;20:2–7.
6. S Gujarat turning hub of thalassemia cases may 2009. *The Times of India*. Available at: http://timesofindia.indiatimes.com/city/surat/S-Gujarat-turning-hub-of-thalassemia-cases/articleshow/4500667.cms#ixzz1CvBrbywZ
7. Talsania S, Talsania N, Nayak H. A cross sectional study of thalassemia in Ahmedabad city, Gujarat. (Hospital based). Healthline 2011;2(1):48–51.
8. India spends Rs 1 cr on thalassemia. *Business Standard*. Available at: http://www.business-standard.com/india/news/india-spends-rs-1000-crthalassemia/300186/ (last accessed on April 2, 2015).
9. Available at: http://www.prathama.org/thalassemia-eradication-in-ahmedabad-city.php. (last accessed on April 2, 2015).
10. Mohanty D, Colah RB, Gorakshakar AC, Patel RZ, Master DC. Prevalence of β-thalassemia and other haemoglobinopathies in six cities in India: a multicentre study. J Commun Genet 2013;4:33–42.
11. Madan N, Sharma S, Sood S K, Colah R, Bhatia H M. Frequency of β-thalassemia trait and other hemoglobinopathies in northern and western India. Indian J Hum Genet 2010;16:16–25.
12. Swarup-Mitra S. In: *Medical Genetics in India*, Verma IC (Ed.). Pondicherry: Auroma enterprises, 1978. pp. 199–213.
13. Sharma RS, Parekh JG, Shah KM. Hemoglobinopathies in western India. J Assoc Physicians India 1963;11:969–73.
14. Flatz G, Chakravartti MR, Das BM, Delbrück H. Genetic survey in the population of Assam. I. ABO blood groups, glucose-6-phosphate dehydrogenase and haemoglobin types. Hum Hered 1972;22(4):323–0.
15. Sukumaran PK, Master HR. The distribution of abnormal haemoglobin in the Indian population. Proceedings of the First Conference of the Indian Society for Human Genetics, Mumbai, 1974. pp. 91–4.
16. Misra RC, Ram B, Mohapatra BC, Das SN, Misra SC. High prevalence & heterogenicity of thalassemias in Orissa. Indian J Med Res 1991;94:391–4.
17. Dash S. Beta thalssemia trait in the Punjab (North India). Br J Haematol 1985;61(1):185–6.
18. Chakrabarti I, Sinha SK, Ghosh N, Goswami BK. Beta thalassemia carrier detection by NESTROFT: an answer in rural scenario. Iranian J Pathol 2012;7(1):19–26.
19. Mehta BC, Gandhi S, Mehta JB. Naked eye single tube red cell osmotic fragility test for beta thalassemia population survey. Ind J Haemat 1988;6:187–90.
20. Vehapoglu A, Ozgurhan G, Demir AD, Uzuner S, Nursoy MA, Turkmen S, et al. Hematological indices for differential diagnosis of beta thalassemia trait and iron deficiency. Anemia. 2014;2014 Article ID 576738, 7 pages. Available at: http://dx.doi.org/10.1155/2014/576738

How to cite this article: Bodarya OV, Makwana H, Lakum N, Shrivastav A, Joshi J, Agnihotri A. A study of thalassemia screening of 1000 medical students and comparison of various screening methods. Int J Med Sci Public Health 2016;5:272-275

Source of Support: Nil, **Conflict of Interest:** None declared.