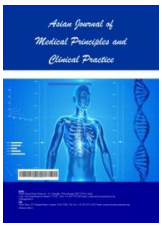

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Evaluation of One Tube Osmotic Fragility as a Screening Test for Beta Thalassaemia Trait

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Authors' contributions

Authors AP and MT wrote the protocol, managed the literature survey, carried out the data collection, performed data analysis and wrote the first draft of the manuscript. Author CK designed the study, corrected the protocol and literature survey, supervised and guided methodology and data analysis and corrected and modified the final manuscript up to this version. Authors RW and JN supervised and guided the handling of screening population at the National Thalassaemia Centre. All the authors read and approved the final manuscript.

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ABSTRACT

Background: Thalassaemia is highly prevalent in Sri Lanka. The highest number of patients are seen in the North Western province, especially in the Kurunegala district. Screening of children and adults to detect beta thalassaemia carriers/trait using automated full blood counts and high performance liquid chromatography (HPLC) are only limited to few centers in Sri Lanka. In this context, the value of one tube osmotic fragility test (OFT) as a screening test is immense. Our study tried to determine the sensitivity and specificity of OFT in beta thalassaemia trait (BTT) in Sri Lanka.

Materials and Methods: This was a cross sectional study on randomly selected 700 subjects, carried out at National Thalassaemia Center (NTC), Kurunegala. Participants were categorized into four groups based on red cell indices, HPLC and serum ferritin assay as normal group, BTT group, iron deficiency anaemia (IDA) group and other haemoglobinopathies. OFT was performed on all cases.

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Results: Out of 700 subjects 396 subjects (56.6%) were females and 304(43.4%) were males.
OFT gave definitely positive or equivocal results in 194 of 201 patients with BTT and 96.52% of sensitivity was observed. The test was false positive in 2 out of 268 (0.75%) normal subjects and 99.25% of specificity was observed. There were 3.48% (7/201) false negative results with a negative predictive value (NPV) of 97.44% and the positive predictive value (PPV) of the test was 98.97%.
Conclusion: One tube osmotic fragility test is a sensitive, cost effective, rapid and reliable primary screening test for detection of BTT in a population with financial restrictions.

Keywords: Beta thalassaemia trait; iron deficiency anaemia; one tube osmotic fragility test; haemoglobinopathies.

1. INTRODUCTION

Thalassaemia is a form of inherited haemolytic anaemia. Thalassaemia syndromes are identified as the most common single gene disorder worldwide; about 3% of the world population (150 million) carries the β thalassaemia genes [1].

Homozygous beta thalassaemia carries a huge burden to the health system of a country owing to the patient's life long need for monthly blood transfusions and treatment with iron chelating agents [2].

If there is no concomitant decrease in the number of new thalassaemia major births, there will be a cumulative increase in number requiring treatment [3]. Screening programmes for detection of beta thalassaemia trait (BTT) allow couples at risk to avoid having a thalassaemia major child [4]. The most reliable methods for diagnosis of thalassaemia trait include HbA_2 estimation by high performance liquid chromatography (HPLC), globin chain synthesis ratio and DNA studies for specific mutations. However, these methods are expensive and not applicable for mass screening programmes [5].

Many studies have been done to evaluate the utility of decreased osmotic fragility of the hypochromic, microcytic red cells seen in thalassaemia [6]. These thalassaemic red cells are resistant to lysis when placed in hypotonic solutions due to their abnormal osmotic resistance. This can be identified by using a modified single-tube osmotic fragility test (OFT) which is a screening test for thalassaemia that has been proposed for the use in laboratories in developing countries [7].

For OFT, different studies have recommended the use of 0.32%, 0.34%, 0.36% and 0.4% buffered saline solutions [8,9,10,11]; but most of the studies have used 0.36% solution and have shown satisfactory results with high sensitivity rates. However, specificity was affected by iron deficiency cases which gave false positive results and this becomes a problem in areas where iron deficiency is prevalent.

In addition to iron deficiency anaemia (IDA), haemoglobin (Hb) E heterozygosity also leads to a significant proportion of positive tests [8,11], but detection of Hb E heterozygosity has an added advantage rather than a weakness of the test since the detection of Hb E is also relevant to antenatal counseling for disorders of globin chain synthesis [7].

Sickle cell trait results in positive tests in 13-40% of instances, depending on the degree of hypotonicity of the saline solutions [8,12], but the results in haemoglobin C trait have not been reported [7].

Thalassaemia is highly prevalent in Sri Lanka. Highest number of patients are seen in the North Western province, especially in the Kurunegala district in comparison to the other provinces in the country. Annually about 60 -70 new patients get registered at National Thalassaemia Centre (NTC) [13].

Screening of children and adults using automated full blood counts and HPLC are only limited to few centers in Sri Lanka. So, the diagnostic process is not easy in under resourced laboratories in a developing country like ours.

In this context, the value of one tube osmotic fragility test as a screening test is immense. It is an easy, cheap and simple test which can be carried out in the field. This study tried to determine the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of one tube osmotic fragility test using 0.36% buffered saline for screening of beta thalassaemia trait in Sri Lanka.

2. MATERIALS AND METHODS

This was a cross sectional study carried out in the haematology laboratory of National Thalassaemia Centre at Kurunegala. Randomly selected 700 individuals undergoing screening for beta thalassaemia trait at NTC were included in the study.

As full blood count (FBC), HPLC and serum ferritin consist of part of the investigations in the diagnostic work up in a patient with suspected beta thalassaemia trait at NTC, we did not take any additional samples for the study. The study population was categorized according to routine investigations done during the screening process.

The blood sample used in the screening was a 2 mL venous sample collected to a K_2EDTA container from each individual and the OFT was done in the same sample.

Patients with suspected thalassaemia or other haemoglobinopathies who were not confirmed by HPLC were excluded from the study. Alpha and beta thalassaemia major patients and suspected iron deficiency anaemia patients who were not confirmed by serum ferritin level were also excluded from the study.

A full blood count (FBC) was performed on all blood samples using automated haematology analyzer Mindray BC-3000, within 2 hours of blood collection and participants were categorized into 2 groups based on red cell indices.

Group 1 comprised individuals with normal red cell indices. Group 2 comprised individuals with microcytosis (MCV <80fL) and hypochromasia (MCH<27pg) and peripheral smear suggestive of beta thalassaemia trait (BTT) or iron deficiency anaemia (IDA).

Then, the participants in group 2(study group) were further categorized into three (03) groups according to differential diagnosis of IDA, BTT and other haemaglobinopathies (Hb E trait and Hb S trait) based on serum ferritin done on Immulite 1000 hormone analyzer and HPLC done on BIO-RAD analyzer.

Group 2a (BTT)

Patients having beta thalassaemia trait which was confirmed by HPLC (>3.5% HbA2).

Group 2b (IDA)

Patients with iron deficiency anaemia with Hb <10 g/dL and low serum ferritin according to the age group.

Group 2c

Other individuals with abnormal Hb variants (Hb S trait and Hb E trait) who were confirmed by HPLC 0.36% OFT was performed on all samples within 4 hours of collection without knowledge of these categories while waiting for HPLC or ferritin reports.

2.1 One Tube Osmotic Fragility Test Procedure

A volume of 2 mL, 0.36% buffered solution was taken into a khan tube and labeled as test and 2 mL of distilled water in another khan tube was labeled as control. Then, 20 µL of well mixed EDTA venous blood was added to each of the tubes and was mixed gently. Tubes were left undisturbed for half an hour at room temperature [14].

After 30 minutes, both tubes were mixed gently by inverting 5 times and the tube was held 1.5 cm in front of a white card with two narrow black lines and was read for turbidity.

If the lines were clearly visible indicating complete lysis, the test was read as negative. If turbidity caused lines to be blurred, test was read as positive. Equivocal results were those in which there was a very fine cloudiness in the test tube and the edges of the lines slightly blurred. The control sample must be fully lysed; if not the result of test was considered invalid [15,16,17]. Only one control was performed with the batch of tests per day.

2.2 Principle of OFT

Hypochromic microcytic red cells of beta thalassaemia trait are resistant to lysis in 0.36% buffered saline, due to their decreased red cell osmotic fragility and increased resistance to osmotic lysis. Therefore, turbidity will remain in the tube indicating a positive OFT. In normal subjects, cells will lyse and a clear solution makes the lines visible indicating a negative OFT.

Finally, the sensitivity, specificity, positive and negative predictive values and efficiency of the

test in patients with BTT were calculated as validity statistics by the following formulae and 2x2 table.

Statistical formulae used in the study were;

Sensitivity% = TP/(TP+FN) x 100

Specificity% = TN/(TN+FP) x 100

PPV% = TP/(TP+FP) x 100

NPV% = TN/(TN+FN) x 100

Efficiency % = (TN+TP)/(TP+FP+TN+FN) x 100

TP –True Positive
NP – True Negative

Statistical package for the social sciences (SPSS) version 21 was used for data analysis.

3. RESULTS

Out of 700 subjects 396 subjects (56.6%) were females and 304(43.4%) were males. The female to male ratio was 1.3.

Out of 700 subjects 173 were below 20 years and 64 subjects were above 40 years. The highest number of participants were in the 21-40 years of age group (n= 463, 66.14%). Age distribution is shown in Table 1.

The distribution of the cohort depending on the diagnosis based on confirmatory investigations is shown in Table 2. There were 268 (38.3%) participants who had normal indices and beta thalassaemia trait was confirmed by HPLC in 201(28.7%) participants. Only two (02) participants had serum ferritin reports which confirmed IDA. Several cases with other haemoglobinopathies were diagnosed on HPLC, but suspected alpha thalassaemia trait was not confirmed by genetic studies.

Table 1. Age distribution of participants

Age group (years)	Frequency	Percent
< 20	173	24.72
21-40	463	66.14
>40	64	9.14
Total	700	100.0

OFT was performed on all participants and results are shown in Table 3. Out of 268 participants with normal indices OFT gave negative results in 266 (99.25%). There were two false positive cases in this normal group. Out of 201 beta thalassaemia patients, 194 (96.52%) showed a positive OFT. Out of 02 IDA cases one showed equivocal results. Other haemoglobinopathies showed variable results on OFT.

Table 4 is a 2x2 table showing the distribution of OFT results between normal and beta thalassaemia trait patients and using these data the sensitivity, specificity, positive predictive value, negative predictive value and efficiency of the test have been calculated.

A high sensitivity of 96.52% was noted together with high specificity, NPV, PPV and efficiency (Table 5).

4. DISCUSSION

An ideal screening test should be, easy to perform, less technically advanced, inexpensive, reliable, valid, less time consuming and should have on site result availability [18]. The findings the present study also agreed with the previous studies which suggested that the 0.36% OFT was a satisfactory screening test for BTT

Table 2. Final diagnosis of participants

Final diagnosis	Frequency	Percent%
Normal (MCV≥80,MCH≥27)	268	38.3
BTT	201	28.7
Iron deficiency anaemia (confirmed on Ferritin)	2	0.3
Hb E trait	9	1.3
Eβ thalassaemia	4	0 .6
HbS trait	1	0.1
δβ thalassamia trait	1	0.1
β thal major	3	0.4
No evidence of BTT but alpha thalassaemia trait possible	2	0.3
Suspected IDA (Ferritin not done for diagnosis)	102	14.6
Serum ferritin high	12	1.7
Serum ferritin normal(MCV< 80)	95	13.6
Total	700	100.0

Table 3. Screening test results of OFT

Final diagnosis	OFT			Total
	Equivocal	Negative	Positive	
Normal (MCV≥80,MCH≥27)	1	266	1	268
BTT	5	7	189	201
Iron deficiency anaemia	1	1	0	2
Hb E trait	2	4	3	9
Eβ thalassaemia	0	0	4	4
HbS trait	0	0	1	1
δβ thal trait	0	0	1	1
Beta thal major	0	1	2	3
No evidence of BTT, but alfa thal trait possible	1	1	0	2
Suspected IDA (Ferritin not done for diagnosis)	16	59	27	102
S.ferritin high	1	11	0	12
S.ferritin normal(MCV< 80)	17	65	13	95
Total	44	415	241	700

Table 4. Comparison of OFT results of BTT and normal group

OFT result	Diagnosis		Total
	BTT	Normal	
Positive	194 (TP)	02 (FP)	196
Negative	07(FN)	266(TN)	273
Total	201	268	469

Table 5. Sensitivity, specificity, positive and negative predictive values of 0.36% OFT in patients with BTT

Sensitivity	96.52%
Specificity	99.25%
PPV	98.97%
NPV	97.44%
Efficiency	98.08%

In this study OFT was able to pick up 194 out of 201 thalassaemia carriers and it gave only 7 false negative results. Thus, it gave a sensitivity of 96.52% which was comparable with sensitivities of 95%-100% reported in several previous studies [18,19,20]. As observed during the study, 266 normal subjects gave negative results with only 2 false positives and a specificity of 99.25% whereas previously reported specificities have varied from 34.1% [2] to 95.23% [17]. It was probably due to a variable proportion of individuals with iron deficiency or other haemoglobinopathies in the different populations investigated.

We had only two limited cases of IDA with serum ferritin values due to financial constrictions of getting the test done. One of them gave positive OFT whereas the other one was negative. Also, we had 102 cases of probable IDA based on FBC and blood picture findings and started on iron treatment, but serum ferritin was not done for diagnosis. Out of 102, 43 cases gave positive OFT and 59 gave negative OFT. Those patients were not included in the assessment of sensitivity and specificity. If those were included, it would have affected the specificity and it would be much worse as IDA is common in our population.

One of the most significant finding was a small number of false negative results (2.56%) with a negative predictive value (NPV) of 97.44%. That result was comparable with the studies of, Kattamis et al. [8], Chakrabarti et al. [21], Sumera et al. [22], Singh et al. [18], Rakholia et al. [19], Raghavan et al. [12] and Thomas et al [20]. This high NPV has an added advantage because the calculation of the NPV in the test almost rules out the possibility of BTT in the general population. This would reduce financial implications faced in performing other costly tests on the general population [18].

The positive predictive value (PPV) of the our study was quite high with 98.97% and

comparable to the studies of Kattamis et al [8], Singh et al. [18], Chow et al. [7] and Manglani et al. [23]. The PPV of the test has a significant value in a particular population with high prevalence of the disease [18].

OFT had been recommended in India [20,21,23,24], Greece [8] and England [7] to be used as a screening test for beta thalassaemia. But some studies done in Egypt [3] showed that OFT had a limited value in regions where iron deficiency was prevalent.

In the present study, 0.36% OFT led to both high sensitivity and specificity, which were desirable factors of a screening test.

The issue of cost was also very important in large scale screening, as the cost of one test of OFT was less than 01rupee when compared to the red cell indices by automated haematology analyzers which cost at least 150 rupees per test. Additionally, it is easy to perform (simple and short procedure), does not require sophisticated equipment or technical expertise and can be used as field studies due to its less time consuming and on site result availability (within 30 minutes result can be visually obtained). Therefore according to our findings OFT has fulfilled most of the requirements which are considered as ideal for a mass screening tool.

5. CONCLUSION

The present data of our study concluded that OFT is a simple test that neither requires any equipment nor any special expertise and can be performed in the field. On the other hand it is sensitive as well as specific enough that it can be used with reasonable reliability for the screening. of BTT and also other haemoglobinopathies as well.

6. LIMITATIONS

Due to the financial limitations we were able to get only 02 ferritin reports. So, we did not include those two cases with confirmed IDA in our calculations.

CONSENT AND ETHICAL APPROVAL

Ethical approval was obtained from the Ethics Review Committee, University of Sri Jayewardenepura. Informed consent was taken from the director, consultant haematologist and laboratory staff of NTC to carry out our study in their laboratory and utilize samples and reports of NTC.

Full blood count, HPLC, & serum ferritin were part of the investigations in a diagnostic work up in a patient with suspected beta thalassaemia trait. The OFT was carried out in the same EDTA sample obtained and no additional samples were taken for the study. The adults (>18 years) who were included in the study were explained about this additional test done in the same sample and verbal consent was taken. For patients below 18 years the verbal consent was taken from the guardian/parents.

When gathering data, personal identification was not used to ensure their privacy and confidentiality. Data was not accessible to any third party other than the research team.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Saxena A, Phadke SR. Feasibility of thalassaemia control by extended family screening in Indian context. Journal of health, population, and nutrition. 2002;20(1):31-5.
2. El-Beshlawy A, Kaddah N, Moustafa A, Mouktar G, Youssry I. Screening for beta-thalassaemia carriers in egypt: Significance of the osmotic fragility test. Eastern Mediterranean health journal = La revue de sante de la Mediterranee orientale = al-Majallah al-sihhiyah li-sharq al-mutawassit. 2007;13(4):780-6.
3. Perera DM, Gunasekara D, Wijekoon A, Petrou M. Thalassaemia control in developing countries-the Sri Lankan perspective. The Ceylon Medical Journal. 2000;45(1):12-6.
4. Leung TN, Lau TK, Chung T. Thalassaemia screening in pregnancy. Current opinion in obstetrics & gynecology. 2005;17(2):129-34.
5. Menon SR, Khatkhatay MI, Dandekar SP, Patel ZM. Development and validation of an ELISA for hemoglobin-A2: A novel method for β-thalassemia screening in developing countries. Journal of Immunoassay and Immunochemistry. 2004;25(2):135-46.
6. Sanchaisuriya K, Fucharoen S, Fucharoen G, Ratanasiri T, Sanchaisuriya P,

Changtrakul Y, et al. A reliable screening protocol for thalassemia and hemoglobinopathies in pregnancy an alternative approach to electronic blood cell counting. American journal of clinical pathology. 2005;123(1):113-8.
7. Fucharoen G, Sanchaisuriya K, Sae-ung N, Dangwibul S, Fucharoen S. A simplified screening strategy for thalassaemia and haemoglobin E in rural communities in south-east Asia. Bulletin of the World Health Organization. 2004;82(5):364-72.
8. Kattamis C, Efremov G, Pootrakul S. Effectiveness of one tube osmotic fragility screening in detecting beta-thalassaemia trait. Journal of Medical Genetics. 1981;18(4):266-70.
9. Cao A, Galanello R, Furbetta M, et al. Thalassaemia types and their incidence in Sardinia. J Med Genet. 1978;15:443–447.
10. Thool AA, Walde MS, Shrikhande AV, Talib VH. A simple screening test for the detection of beta thalassemia. Indian J Pathol Microbiol. 1998;41:423–426.
11. Panyasai S, SringamP, Fucharoen G, SanchaisuriyaK, Fucharoen S. A simplified screening test for a-thalassemia 1 (SEA type) using a combination of a modified osmotic fragility test and a direct PCR on whole blood cell lysates. Acta Haematol. 2002;108:74–78.
12. Raghavan K, Lokeshwar MR, Birewar N, Nigm V, Manglani MV, Raju NB. Evaluation of naked eye single tube osmotic fragility test in detecting beta-thalassemia trait. Indian Pediatr 1991;28:469–472.
13. National thalassaemia center, Kurunagela. Thalassaemia patients in Sri Lanka; 2008/2009. Available:http://thalassemia-srilanka.blogspot.com
14. Parikh U, Goswami H, Mehta R, Patel P, Gonsai R. Incidence of hemoglobinopathies in women attending antenatal clinics in their first trimester. NHL Journal of Medical Sciences. 2014;3(1).
15. Chow J, Phelan L, Bain BJ. Evaluation of single-tube osmotic fragility as a screening test for thalassemia. American journal of hematology. 2005;79(3):198-201.
16. Wild B, Bain BJ. Chapter 12 - Investigation of abnormal haemoglobins and thalassaemia. In: Bates SMLJB, editor. Dacie and Lewis practical haematology (tenth edition). Philadelphia: Churchill Livingstone. 2006;271-310.
17. Sarda H, Niveditha SR, Shivalingaiah N. Screening of β thalassemia trait among pregnant women with NESTROFT. Thalassemia Reports. 2015;5(1).
18. Singh S, Gupta S. Effectiveness of red cell osmotic fragility test with varying degrees of saline concentration in detecting beta-thalassaemia trait. Singapore medical journal. 2008;49(10):823-6.
19. Rakholia R, Chaturvedi P. Evaluation of nestroft as a screening test for beta thalassemia trait. JARBS. 2013;5(2):127-30.
20. Thomas S, Srivastava A, Jeyaseelan L, Dennison D, Chandy M. NESTROFT as a screening test for the detection of thalassaemia and common haemoglobinopathies—an evaluation against a high performance liquid chromato-graphic method. The Indian Journal of Medical Research. 1996;104:194-7.
21. Chakrabarti I, Kumar Sinha S, Ghosh N, Krishna Goswami B. Beta-Thalassemia Carrier Detection by NESTROFT: An answer in rural scenario? Iranian Journal of Pathology. 2012;7(1):19-26.
22. Sumera A, Ahmed S, Ali SA, Khanani R. Evaluation of Nestroft as a marker of differentiation between β-thalassemia trait and iron deficiency anemia. International Journal of Collaborative Research on Internal Medicine and Public Health. 2012;4:1560-6.
23. Manglani M, Lokeshwar MR, Vani VG, Bhatia N, Mhaskar V. 'NESTROFT'-an effective screening test for beta thalassemia trait. Indian pediatrics. 1997;34(8):702-7.
24. Himaxi R, Nanditha M, Priyanka G. Nestroft as a screening test for beta thalassemia. Journalijar. 2014;8:151-155.

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