

## EFFECTIVENESS OF OSMOTIC FRAGILITY SCREENING WITH VARYING SALINE CONCENTRATION IN DETECTING $\beta$ -THALASSEMIA TRAIT

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### • ABSTRACT

**Background:** Thalassemia is a major health problem in Iran. The prevalence of thalassemia minor is high and screening program for detecting  $\beta$ -thalassemia trait has been established in southern parts of Iran since 1992.

**Objectives:** To determine the efficacy of naked-eye single-tube red cell osmotic fragility test (OFT) as a screening test for  $\beta$ -thalassemia trait.

**Methods:** Three different concentrations (0.36%, 0.37% and 0.38%) of buffered saline solutions were used. OFT were applied to three groups of subjects: 50 normal individuals, 50 subjects with genetically proven  $\beta$ -thalassemia trait, and 15 patients with proved iron-deficiency anemia.

**Results:** The results demonstrated that 0.37% saline was the best solution for OFT. It could detect 98% of heterozygous  $\beta$ -thalassemia patients compared to 96% and 84% detection rate obtained with respective 0.36% and 0.38% saline. Specificity of OFT with 0.37% saline was 96% whereas that of 0.36% and 0.38% saline were 84% and 94%, respectively. The OFT with 0.37% saline was also positive in 5 (33.3%) patients with iron-deficiency anemia.

**Conclusion:** OFT done with 0.37% buffered saline solution provides more accurate results. Since the test is inexpensive and practical it might be considered as the single screening test to be used in areas with limited laboratory facilities and economic resources.

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**Key Words:** • Hemoglobinopathy • thalassemia • screening test for thalassemia • osmotic fragility

### Introduction

The thalassemias are a group of hereditary blood disorders that occur with considerable frequency in ethnic groups tracing their origins to countries that border the Mediterranean sea, the Middle East and Southeast Asia.<sup>1,2</sup> The condition results from genetic defects causing deficient synthesis of hemoglobin polypeptide chains.

In its homozygous state, thalassemia genes cause severe and often lethal disease. In heterozygous state, however, the trait is worldwide in distribution. In certain countries, however, the high prevalence of the abnormal gene causes serious public health problems.<sup>3</sup>

Ideally, a screening test for thalassemia should be reliable and specific yet not expensive or time-consuming; Most of the available methods fall short of these requirements: Measurement of HbA<sub>2</sub> is expensive, and time-consuming; the determination of erythrocyte indices requires equipment not available in many routine laboratories and demonstration of characteristic changes in the proportions of HbA<sub>2</sub> and F or unbalanced polypeptide chain synthesis are somewhat complicated and expensive.

This study was undertaken to determine the efficacy of simple and inexpensive methods of naked-eye single-tube red-cell-osmotic-fragility test (OFT) as a screening test for detecting heterozygous thalassemia.

### Materials and Methods

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Three buffered saline solutions with concentrations of, 0.36%, 0.37% and 0.38%, were prepared by diluting a 10% stock solution of sodium chloride (90 g), disodium hydrogen phosphate (13.65 g) and sodium dihydrogen phosphate (2.43 g) in 1000 ml of distilled water (pH 7.4).

Twenty  $\mu$ l of whole blood collected in ethylenediaminetetraacetic acid (EDTA) was pipetted into four glass test tubes (100 mm x 10 mm) which contained 4 ml of 0.36%, 0.37%, and 0.38% saline solution. Tubes were shaken well and allowed to stand at room temperature. After thirty-min. contents of the tubes were again shaken and the tubes were held immediately against a piece of paper. Words were clearly visible and could be easily read, through the tube containing distilled water (control). If the words were similarly visible through the tube containing buffered saline, the test was considered negative whereas if words were not clearly visible, the test was considered positive.

The test was performed in three groups: group I; 50 normal male with normal hematological parameters, group II; 50 fathers of patients with proven thalassemia major, and group III; 15 males with proved iron-deficiency anemia (serum iron < 50  $\mu$ g/dl, and total iron binding capacity of > 400 mg/dl).

Blood samples of each individual case were drawn and subjected to a battery of tests including OFT, complete hemogram, hemoglobin electrophoresis, HbA<sub>2</sub> estimation, and iron studies (serum iron, total iron binding capacity, serum transferrin and percentage saturation of iron binding capacity). HbA<sub>2</sub> >3.5% was treated as the gold standard for diagnosis of thalassemia trait.

## Results

The results are summarized in [Table 1](#). With the 0.36%, 0.37% and 0.38 saline, 42, 48 and 47 of the normals gave negative results corresponding to a specificity of 84%, 96% and 94%, respectively. The differences in false positive error rates among three different dilutions were not statistically significant, however, the difference between two solution concentrations (0.36% and 0.37%) was statistically significant ( $p < 0.05$ ).

The rates of false negative results among those with thalassemia minor patients were 4% and 16% for 0.36% and 0.38% saline, respectively. By contrast, 98% of those with thalassemia trait were detected with the 0.37% saline buffered solution, which is quite satisfactory for a screening test.

With 0.37% saline solution, the positive predictive value (PPV) for the test (96%) is higher than those for other dilutions; 85.7% for 0.36% solution and 93.3% for 0.38% saline, respectively. Negative predictive value (NPV) of test for 0.37% saline (97.9%) is also higher than that of 0.36% saline (95%) and that of 0.38% solution (85.4%). With 0.37% saline buffered solution, accuracy of the test was 97%, indicating that only 3% (error rate of the test) of all subjects screened are misclassified. The error rates for 0.36% and 0.38% saline solutions were 10% and 11%, respectively.

OFT with 0.37% saline in 15 subjects with iron-deficiency anemia was positive in 5 (33%) ([Table 1](#)).

## Discussion

Any program for prevention of Cooley's anemia requires, as a preliminary step, the reliable identification of young people with thalassemia. Screening for thalassemia trait is extremely difficult. This is mainly because of the heterogeneity of thalassemias and the absence of a single test to uncover all thalassemia variants.<sup>5</sup> In spite of these difficulties, many attempts have been made to establish a screening test capable of detecting all thalassemia variants. The proposed tests are red cell osmotic

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fragility test, determination of mean cell volume, and estimation of HbA<sub>2</sub> level. Apart from OFT, the two other tests need well-equipped laboratories and technical experience. OFT has additional advantages; it is simple, inexpensive, and easy to apply.

This study showed that the concentration of 0.37% was more efficient in detecting heterozygous thalassemia patients than the two other saline strengths (i.e., 0.36% and 0.38%).

Although screening with OFT using 0.37% saline was successful in detecting 98% of subjects with thalassemia trait, it was also positive in 33.3% of subjects with severe iron-deficiency anemia, and 4% of normal subjects.

It is well known that there are limitation to all screening tests proposed for detection of heterozygous thalassemia. However, all screening tests give valuable information by excluding normal subjects and thus restricting further investigation for the precise diagnosis to the small proportion of suspects subjects. From our results, OFT with 0.37% buffered saline seems to be the most valuable and reliable test to be used as a single test in areas with limited laboratory facilities and economic resources.

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