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RESEARCH ARTICLE

NESTROFT AS A SCREENING TEST FOR BETA THALASSEMIA

Dr.Himaxi Rathod, Dr.Nandita Mehta, Dr.Priyanka Gohel

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Corresponding Author

Dr.Himaxi Rathod

Abstract

NESTROFT (Naked Eye Single Tube Red Cell Osmotic Fragility Test) is simple, inexpensive and very sensitive test for screening of beta thalassemia, a form of congenital hemolytic anemia. , It is used to assess osmotic fragility of red cells at a single concentration of buffered saline (0.36% in single tube) visually without a spectrophotometer. Positive NESTROFT indicates decreased red cell osmotic fragility and increased resistance to osmotic lysis. Here we studied 100 random cases and positive cases were later confirmed by HbA2 electrophoresis.

Introduction

Thalassemia, a form of congenital hemolytic anemia, is characterized by microcytic hypochromic anemia.It is also accompanied by ineffective hematopoiesis, spleno-hepatomegaly,etc. It has many subtypes. The majority of cases found in India are of the β-thalassemia subtypes.Although the differential diagnosis of homozygous β-thalassemia is relatively easy on the basis of medical interviews and physical findings, the heterozygous β-thalassemia may be difficult to diagnose solely on the basis of manifestations. Thalassemia minor does not always require treatment; however, its diagnosis must be made for medical counseling regarding potential homozygous birth resulting from marriage of a couple of heterozygous parents. In the present study, we investigated β-thalassemia cases based on using NESTROFT (Naked Eye Single Tube Red Cell Osmotic Fragility Test) as a screening test to count sensitivity of NESTROFT in country like India[1,6] .

MATERIAL AND METHOD:

The samples assayed were obtained from 100 random patients at the hematology department, civil hospital, Ahmedabad. Total of 100 random subjects were screened. Samples were collected in EDTA vacutte, as ananticoagulant. NESTROFT was performed in all the cases as described below.

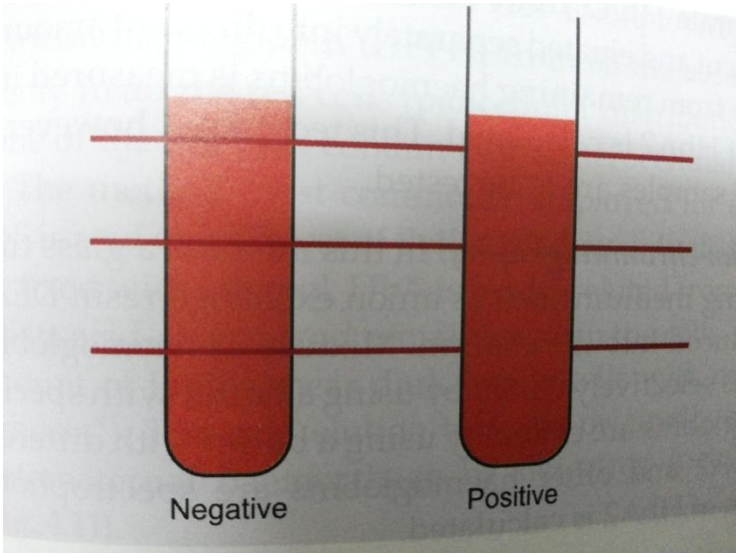
As its name implies, NESTROFT is used to assess osmotic fragility of red cells at a single concentration of buffered saline (0.36% in single tube) visually without a spectrophotometer. A stock solution of 10% buffered saline (pH 7.4) is prepared by taking NaCI 90g, Na_2HPO_4 13.655 g and NaH_2PO_4, $2H_20$ 2.4 g, and dissolving them in 1 litre of distilled water. From this, a litre of 1 buffered saline is prepared by 1:10 dilution with distilled water. 0.36% buffered saline is prepared by diluting 36 mL of 1 % buffered saline with 64 mL distilled water to make 100 mL. 2mL of 0.36% buffered saline is taken in one tube (10 cm x 1 cm diameter) and 2 mL distilled water is taken in another. A drop of blood is added to each of the tubes, which are left undisturbed for half an hour at room temperature. After half an hour the contents of both the tubes are shaken and the tubes held against a white paper on which a thin black line is drawn.

INTERPRETATION OF RESULTS:

The line is clearly visible through the contents of the tube containing distilled water due to complete lysis. If the line is visible through the contents of the tube with buffered saline, the test is negative, whereas if the line is not visible, the test is positive[1,6]. The tubes are then left undisturbed for a few hours. At the end of this period, contents of the

tube with distilled water remain uniformly pink with no sediment at the bottom. In the case of a negative test, the tube containing buffered saline also presents a similar picture. With a positive test, the tube shows a sediment of the red cells at the bottom and the top part of the saline is colourless. This is an additional confirmatory evidence of a test earlier interpreted as positive.

A positive NESTROFT indicates that all red cells in the tested sample have not undergone lysis in 0.36% buffered saline. These unlysed red cells result in the hazy appearance of the contents of the tube and render the line on the paper indistinct. These red cells also sediment as a button at the bottom. of the tube when it is left undisturbed for some time. Thus a positive NESTROFT indicates decreased red cell osmotic fragility and increased resistance to osmotic lysis.HbA2 was performed in all NESTROFT positive subjects to compute sensitivity of the NESTROFT.



INTERPRETATION OF RESULT

RESULTS:

A total of 100 random patients at the hematology department, civil hospital, Ahmedabad tested. Out of 100 random subjects taken NESTROFT was performed in all. Results of test performed is tabulated as below:

TEST NAME	TOTAL POSITIVE CASES	TOTAL NEGATIVE CASES
NESTROFT	32	68

HbA2 was performed in all 32 NESTROFT positive cases, which shows following results[7]:

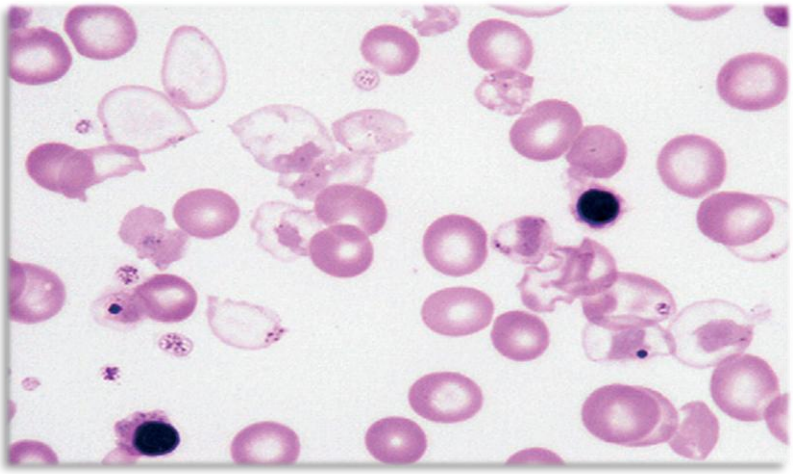
TEST NAME	TOTAL NESTROFT POSITIVE CASES	CASES VALUED HbA2 >3.2 (True positive)	CASES VALUED HbA2<3.2 (False positive)
HbA2 ESTIMATION	32	29	3

This shows the sensitivity of the NESTROFT is 90.62%

DISCUSSION:

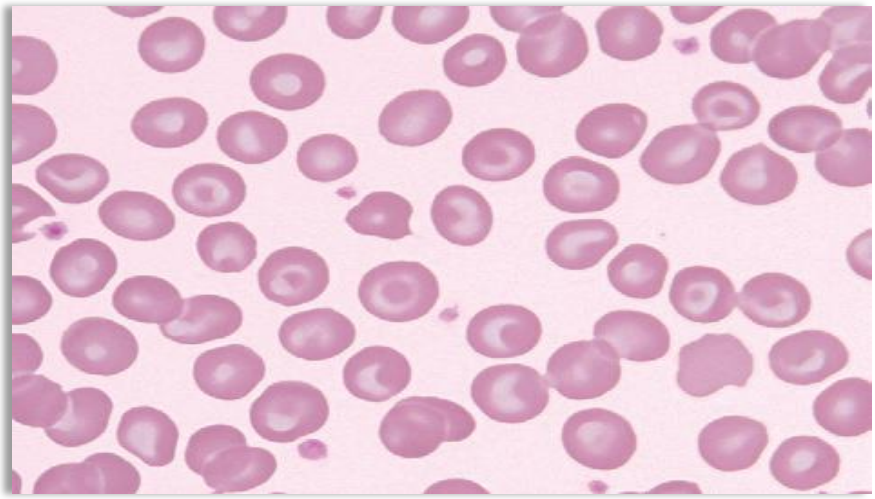
The thalassaemia syndromes are a heterogeneous group of inherited conditions characterised by defects in the synthesis of one or more of the globin chains that form the haemoglobin tetramer. The clinical syndromes associated with thalassaemia arise from the combined consequences of inadequate haemoglobin production and of unbalanced accumulation of one type of globin chain. The former causes anaemia with hypochromia and microcytosis; the latter leads to ineffective erythropoiesis and haemolysis. Clinical manifestations range from completely asymptomatic microcytosis to profound anaemia that is incompatible with life and can cause death in utero. This clinical heterogeneity arises as a result of the variable severity of the primary genetic defect in haemoglobin synthesis and the coinheritance of modulating factors, such as the capacity to synthesize increased amounts of Hb F. Identification of beta-thalassemia ideally requires globin chain synthesis or gene mapping which is possible only in research laboratories. But practically, measurement of elevated levels of HbA2 is sufficient enough to diagnose the beta thalassemia. Measurement of HbA2 is an expensive procedure. That is why simple and inexpensive single screening methods are sought. Proposed tests are RBC-indices and discriminant functions as generated by automated cell counters, and NESTROFT.

BLOOD SMEAR IMAGE OF THALASSEMIA POSITIVE PATIENT:



Homozygous β-thalassemia:

A few cells contain hardly any haemoglobin (Hb), and the Hb is often precipitated at the membrane. Bizarre target cells, Howell-Jolly bodies, and poorly hemoglobinized nucleated red blood cells are seen[1,6].



β-Thalassemia trait

Hypochromic, microcytic red blood cells with many target cells. Anemia is generally mild[8]

β –THALASSEMIA TRAIT: This is caused by the β^0-thalassemia gene with absent, or the β^+thalassemia gene with reduced, β-globin chain synthesis. There are usually no symptoms or abnormal physical signs. The only clinical presentation might be a refractory anemia of pregnancy[1,6]

DIAGNOSIS: Diagnosis rests on the detection of an increased haemoglobin A2 percentage. The increased haemoglobin A2 percentage is due to an absolute rather than merely a relative increase in d chain synthesis .The use of a one-tube visual osmotic fragility test reduces the number of samples that have to be referred to a central laboratory for definitive diagnosis.

UTILITY OF NESTROFT :

Red cell osmotic fragility is decreased in BTT, resulting in a positive NESTROFT. NESTROFT can be a useful test to screen the general population for BTT, and has been used for this purpose in India and in other countries where BTT is common.it is also positive in other conditions like iron deficiency anemia, liver disease[3] and hemoglobinopathies. It is necessary to identify individuals with β-thalassemia because:

- **Premarital screening and genetic counselling:**
  (identification of of heterozygous individuals as heterozygous individuals should not marry another heterozygote for the same gene due to risk of having affected children, 25% children are affected**.** In genetic counselling- Various options such as prenatal diagnosis followed by termination of pregnancy and alternative couples are at risk, in which both of the partners are genetic carriers, are explained various options like termination of pregnancy, artificial insemination or adoption. **)**

- **Prevention of beta thalassemia,**

- **Population screening for beta thalassemia trait,**

- **Antenatal screening** (performed between 6-10 weeks of gestation, to prevent birth of child with BTM[2,4] by globin chain synthesis studies or foetal DNA),
- **Avoidance of unnecessary iron therapy,**

- **Rational approach to hypochromic microcytic anemia.**

Need for a screening test for Beta-thalassemia:

The diagnostic test for BTT is estimation of hemoglobin A2 by electrophoresis or column chromatography. Both these techniques are expensive and time consuming, and need expensive equipment and laboratory expertise. Because of these limitations, these tests can only be carried out on a limited number of samples and only in some laboratories. Cost effectiveness can be improved if definitive tests were to be performed only on samples with high

chances of yielding positive results. Universal use of definitive tests for population surveys or in antenatal clinics or in hematology clinics would not be acceptable due to the cost and effort involved. The availability of an initial screening test which, while being inexpensive and easy to perform even under field conditions, can identify positive samples with great sensitivity would be technically and financially attractive.

NESTROFT does not require any specialized equipment, expertise or rigid conditions, and can be used in field conditions[1,].

CONCLUSION:

NESTROFT is a very sensitive screening method.It is also inexpensive and simple and proves to be an ideal cost effective population screening method for beta thalassemia[1,6], in comparison with PS examination, red cell indices, osmotic fragility, and free red cell porphyrins, as they are expensive, time consuming and require sophisticated equipments.

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