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BETA THALASSEMIA SCREENING BY NASTROF: A RELIABLE METHOD.

KEY WORDS:

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ABSTRACT

Background: Thalassemia is the commonest inherited hemoglobinopathy. Beta Thalassemia Trait is asymptomatic. Beta Thalassemia Major presents with severe anemia and requires lifelong blood transfusion, so screening and prenatal counselling should bedone to affected parents.
Methods: It was a study on 67 subjects with microcytic hypochromic anemia who underwent Naked Eye Single Tube Red Cell Osmotic Fragility Test (NESTROFT), High Performance Liquid Chromatography (HPLC).
Results: In our study the sensitivity and specificity of NESTROFT was 100% and 84.2% respectively.
Conclusions: For low resource countries similar to India, screening for beta thalassemia by NESTROFT is a cheaper and more reliable method with a high sensitivity and specificity and can be performed easily.

Introduction

The Thalassemia syndromes are the most common genetic disorder . In these disorders, there is decreased synthesis of either the alpha-globin chains (alpha thalassemia) or the beta-globin chains (beta thalassemia) of haemoglobin. Worldwide, frequency of thalassemia trait is about 3%[1]. Certain communities, such as Sindhis, Kutchis, Lohanas, Bhanusalis, Punjabis, Mahars, Agris, Gaud, Saraswats, Gowdas etc. in India have a higher frequency[2].

Haemoglobin electrophoresis (HPLC) is done for thalassemia trait which shows elevated HbA2 levels which are confirmatory. Some other haematological investigations available are helpful in diagnosing beta thalassemia trait. However, these investigations are either expensive or time-consuming or cumbersome and often require sophisticated equipment. Hence, we require such a test which can be an effective tools for population screening.

For screening purposes, a test should be inexpensive, does not require sophisticated equipment and can be applied on the population. Kattamis et al first described a modified osmotic fragility test "NESTROFT" (Naked Eye Single Tube Red Cell Osmotic Fragility Test)[3]. The present study was undertaken, to evaluate the validity of NESTROFT as a screening test for the diagnosis of beta thalassemia trait

Material and methods

The study comprised of 55 patients presenting with various complaints associated with anemia. Haematological investigations such as haemogram and peripheral blood film (PBF) were done to confirm anemia.

12 cases were that of the families of thalassamia were also included. NESTROFT and HbA2 levels were done in all the cases.

Normally, red cells put in saline solution begin to lyse at a saline concentration of 0.4-0.5% and lysis is complete at 0.32%. However, in beta thalssemia trait, due to alteration in osmotic resistance of the affected RBC's due to volume/surface area ratio changes,[4] lysis begins at a saline concentration between 0.4-0.35% and it may not be completed even at 0.1% solution. NESTROFT is done at a saline concentration of 0.36%.

Material

0.36% buffered saline (BS) prepared by diluting 36ml of 1% buffered saline with 64ml of distilled water (DW) to make 100 ml (Test Reagent).

Procedure

Two test tubes labelled as BS (2ml) and DW (2ml) were taken and a drop of blood was added to each of the tubes, which were then left undisturbed for half an hour at room temperature. Following this, contents of both tubes were gently shaken and held against a white paper on which a thin black line was drawn. The line was clearly visible through DW tube and if it was the same in BS tube; it was considered negative, otherwise test result was interpreted as positive.

The tubes were left undisturbed for 3 hours. At the end of 3 hours, the DW tube was seen to be homogeneously pink with no sediments. In the BS tube the negative test showed similar findings as DW tube where as in a positive case, a clear supernatant and a sediment at bottom was observed[5].

HbA2 estimation was done by electrophoresis. The observations collected from the NESTROFT test and the Hb electrophoresis was recorded. By means of statistical analysis, an attempt was made to validate a correlation between NESTROFT positive samples and HbA2 levels in those cases

Results

NESTROFT was performed on all selected cases. 10 cases out of 55 gave positive results with NESTROFT while 45 cases tested negative. 9 out of 12 cases tested positive with NESTROFT, while 3 gave a negative result.

HbA2 levels were also estimated in all cases. Out of 10 NESTROFT positive cases in group I, only 1 had HbA2 levels more than 3.5%. The remaining 9 cases were false positive since HbA2 levels were less than 3.5%. In group II, all the 9 cases showing positive NESTROFT had HbA2 levels more than 3.5%. In this group, no false positive case was identified. None of the NESTROFT negative cases showed HbA2 levels more than 3.5%. So, all these cases were true negative.

From the data thus obtained; sensitivity, specificity, positive and negative predictive values of NESTROFT were obtained. The test showed a sensitivity of 100%, specificity of 84.2%, a positive predictive value of 52% and a negative predictive value of 100%

	NASTROF +	NASTROF -
HBA2>3.5	10	0
HBA2<3.5	9	48

Discussion

Correct identification of beta thalassemia trait is important, as the management of a patient with beta thalassemia major expensive. Also, as the red cell morphology in beta thalassemia trait is microcytic hypochromic; these patients are often misdiagnosed, as those suffering from iron deficiency anaemia and given unnecessary iron medication.

In this setting, a reliable screening test becomes very much important for screening purposes. Many authors in India have found NESTROFT as a suitable test to identify carriers of beta thalassemia trait, especially in rural settings[6].

Conclusion

From the above data, it is clear that NESTROFT is a highly sensitive and reasonable specific test for detection of beta thalassemia trait. The test is economical, as the cost of performing a single test is less than a rupee, quick, is easy to perform. A high negative predictive value can reasonably rule out beta thalassemia trait cases. So, it should be adopted as a screening test for beta thalassemia trait.

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