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An Inexpensive and Easy Protocol for Detection of Beta-Thalassemia Carrier

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ABSTRACT

Thalassaemia, a hereditary haemolytic anaemia, is a disease of utmost medical importance. It occurs due to synthesis of one or more globin polypeptide chains in reduced rate. The carriers of these defective genes show no clinical manifestation, and thus considered medically fit apparently. But when both the parents are carriers of thalassaemia, the siblings show the thalassaemia disease. Thus, screening of thalassaemia carrier is medically very important before marriage. But in a country like India where many peoples live in a low socio-economic condition, this carrier detection is still now not widely accepted. It is mainly due to the high cost of the screening procedure using HPLC. The method used in this system requires simple bench centrifuge, glass goods and biochemical reagent solutions, and thus can be done in any health care centre even in remote areas. The reagent solution used in this study can be stored at 4^oC for 1 year. The turbidity test can be visualized easily by any semi-trained person. So, sophisticated trained personnel are also not required here. For a Gold Standard test using HPLC requires at least Rs. 650/-, but this biochemical method only requires Rs. 5/- per test, maintaining equal accuracy, sensitivity, and specificity. Thus, this simple, easy-to-do, low-cost biochemical method can be implemented for mass-screening of beta-thalassaemia carrier detection.

Keywords: Beta-thalassaemia, detection, biochemical method, low-cost, sensitivity,specificity.

INTRODUCTION

Thalassaemia, a hereditary haemolytic anaemia, is a disease of utmost medical importance. It occurs due to synthesis of one or more globin polypeptide chains in reduced rate. The carriers of these defective genes show no clinical manifestation, and thus considered medically fit apparently (Shinar *et al.,* 1989; Weatherall and Clegg, 2001a). But when both the parents are carriers of thalassaemia, the siblings show the thalassaemia disease. Thus, screening of thalassaemia carrier is medically very important before marriage. But in a country like India where many peoples live in a low socio-economic condition, this carrier detection is still now not widely accepted. It is mainly due to the high cost of the screening procedure using HPLC. So, for mass-scale acceptability of the thalassaemia carrier detection, a simple and low-cost procedure should be implemented, so that each and every person can be ready to do so. In the

present study, a simple biochemical method has been employed for thalassaemia carrier detectionin very low-cost maintaining the quality equal to HPLC test. The method used in this study is very easy and needs minimum equipments. Further, this handy protocol can be done by semi-trained personnel and thus can be implemented in remote areas.
Thalassemia is among of the foremost prevalent single-gene diseases in which healthy hemoglobin synthesis is disrupted. This autoimmune disease creates a disequilibrium in the globin chain synthesis by reducing the formation of either the α-globin or β- globin molecules which make up human hemoglobin HbA(α2β2) (Marengo-Rowe, 2007).

This condition is most frequent in the Mediterranean, the Middle East, tropical Africa, the Indian subcontinent, and Central Asia, and it is one of the most universal human hereditary illnesses. Approximately 10% of the globe's thalassemia transmitters live in India (Farashi and Harteveld, 2018).

Thalassemia Major (TM) and Thalassemia Intermedia (TI) are the most common forms of the illness in India. Standard lifetime blood donations and iron neutralization are frequently used to treat diseases. These thalassemia abnormalities are produced by both family members inheriting aberrant thalassemia genes, or one parent inheriting defective beta-Thalassemia gene as well as the other parent inheriting faulty mutant hemoglobin allele. Some other hemoglobin condition which necessitates lifetime care and leads to newborn and adolescent illness and death is sickle cell disease (Higgs, 2009).

One of the most prevalent inherited genetic disorders is Thalassemia, which is caused by mutations in the gene mutations (Weatherall and Clegg, 2001b.). Because the Thalassemia transmitter form is medically undetectable, diagnosing it is difficult. Because the process requires sophisticated equipment’s expert labor, and time, the present gold benchmark technology is unlikely to be adopted for actual pathogen identification.

Due to their muted genotype such bearers may evade detection in regular populationscreening procedures. Yet, because homozygosity for extremely moderate and undetectable genotypes, or complex heterozygosity for moderate and especially extreme thalassemia phenotypes, generally manifests in diminished versions of thalassemia intermedia, this would not have a significant impact (Piel and Weatherall, 2014).

According to NHM guidelines, another hemoglobin condition, Sickle Cell Disease (SCD), necessitates lifetime treatment and leads to newborn and adolescent death worldwide. SCD is triggered by the inheriting of two defective HbS alleles, one in each parent, or one HbS mutation and the another HbE or β thalassemia allele. Sickle Cell Disease, also known as Sickle Cell Anemia (SCA), and infections caused by the sickle cell genotype in combination with different hemoglobinopathy like Hb C, E, or β thalassemia are all examples of sickle cell abnormalities (Old*et al.,* 2016).

People that have only one of these mutations are referred to as "providers" since they never have any symptoms yet inherit the faulty genome and pass it on to the future generation (Thein, 2017). Donors can only be identified by specific blood testing, not through medical examination When both the woman and the man are transmitters, their offspring may receive the faulty mutation from both sources and develop major thalassemia or Sickle Cell syndrome, or they may be healthy without the aberrant allele or providers like their parents (Gambari, 2012).

As per National guide line, complementing illness care with preventive initiatives seems to be the most cost-effective method for decreasing the impact of hemoglobin abnormalities. Blood testing that are both affordable and effective can detect spouses who are at likelihood of bearing impacted babies. This assessment is notably useful prior wedding or childbirth, as it allows partners to talk about their family's safety. Following that, genomic consultation educates phenotype bearers about the dangers of passing the illness on to their offspring, the medication required if impacted by a hemoglobin deficiency, and the person's alternatives (Mettanand*et al.,* 2015; Traeger-Synodinos and Harteveld, 2014).

OBJECTIVES OF THE STUDY

1. The main objective is to develop a simple biochemical method has been employed for thalassaemia carrier detection in very low cost maintaining the quality equal to HPLC test.
2. Another is to promote a handy protocol can be done by semi-trained personnel and thus can be implemented in remote areas.

MATERIAL AND METHOD

For this study,126 known samples and 1478 unknown samples were taken from the persons attending to Mukherjee Clinical laboratory, Barasat and Barasat Cancer Hospital, Kolkata. Written informed consent were taken from all for this study, and permission was also taken from Ethical Committee and hospital review board. The persons having disease history, malaria, enteric fever, haematological disease within 3-6 months were excluded. The study was conducted with the age 14-45 years.

Protocol

2.5 ml of fresh bloods (not more than 24 hours of collection) with potassium EDTA were centrifuged at 6000 rpm for 5 minutes. For further study, the centrifuged blood was collected from the bottom level. If packed cell volume (PCV) more than 50%, re-centrifuged another 5 min. 25-50% PCV were used as stock solution for the study. Deionized water was taken as control. Known normal was considered as negative control.

A. Nacked eye single tube red cell osmotic fragility test (NESTROFT) for thalassemia syndromes:

2ml of 0.36% saline buffer of the stock solution was added with 20 µl centrifuged blood, mixed well, incubated at room temperature for 30 minutes. The degree of haemolysis was observed against a luminous background.

B. Basic turbidity test:

2 ml of buffered DCIP solution was added with 20 µl centrifuged blood, mixed well, incubated for 10 minutes at room temperature and then at 37^{o}C for 30 min in a waterbath. Turbidity test was performed immediately after decolourization against known negative control and assessed against luminous background. If the colour of the solution changes to yellow or brick red, enzyme deficiency to be suspected.

C. Urea di-thionite test:

10 ml of buffered sodium dithionite solution was added with 1.2gm urea (reagent grade). 2 ml of the above solution was added with 20 µl centrifuged blood and mixed well. The colour of the solution should be red or pink. Then incubated for 5 min at room temperature. The degree of turbidity was tested against negative and positive controls.

D. Haemoglobin S Solubility test:

2ml of buffered sodium dithionite solution was added with 20 µl centrifuged blood, mixed well (colour of the solution should be red or pink), incubated at for 5 min at room temperature, the degree of turbidity tested against negative and positive controls. Then centrifuged at 1200 rpm for 5 min for visualization of dark red ring.

RESULT AND DISCUSSION

The results were considered as 'suspected' depending on the relative turbidity and/or haemolysis in each step. When all the 'suspected' samples were confirmed with Gold Standard test, the result shows positive. It clearly confirmed that the method used in this study is accurate, sensitive and specific.

The 'suspected' samples of NESTROFT test indicates alpha, beta thalassaemia patients and carriers (alpha andbeta thalassaemia).

The 'suspected' samples of Basic turbidity test indicates HbE patients, carrier, unstable haemoglobins, RBC enzyme deficiencies (homozygous haemoglobin E disease, haemoglobin E carrier, G6PD).

The 'suspected' samples of Urea di-thionite test indicates none, only if cloudy in Haemoglobin S Solubility test and clear in Urea di-thionite test indicate Hb-S, and if turbidity persists suggested Non S – Sickling Haemoglobin.

The 'suspected' samples of Haemoglobin S Solubility test indicates Sickle cell patient, carrier (Sickle cell haemoglobin carrier and Sickle cell haemoglobin disease).

The 'suspected' samples of both NESTROFT test+ Basic turbidity test indicates E-Beta thalassaemia patient.

The 'suspected' samples of both Urea di-thionite test + Haemoglobin S Solubility test indicates HbC, HbD patient, carrier (haemoglobin D Punjab disease andhaemoglobin D Punjab carrier).

The sensitivity of all the test is 100%, thus this method can correctly screen the careers and diseases. The mean specificity is 95.21 % in known samples and 96.78% in unknown samples, thus it can truly predict true negatives. When more than one test were taken into account, it shows 100% specificity. The predictive accuracy is 96.25% for positive test in known samples and 92.56% in unknown samples. The predictive value of negative test is 100% in all the cases. The mean percentage of false positive is 1.65% in known samples and

1.36% in unknown samples. Further, the percentage of false negative is 0% indicating the error-free efficacy of the method.

The method used in this study requires simple bench centrifuge, glass goods and biochemical reagent solutions, and thus can be done in any health care centre even in remote areas. The reagent solution used in this study can be stored at 4^{o}C for 1 year. The turbidity test can be visualized easily by any semi-trained person. So, sophisticated trained personnel is also not required here. For a Gold Standard test using HPLC requires atleast Rs. 650/-, but this biochemical method only requires Rs. 5/- per test, maintaining equal accuracy, sensitivity and specificity. Thus, this simple, easy-to-do, low-cost biochemical method can be implemented for mass-screening of thalassaemia carrier detection.

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