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MODIFICATION OF SICKLING TEST FOR MASS SCREENING OF SICKLE CELL DISEASE USING CAPILLARY BLOOD IN ANTI COAGULANT AND REDUCING SUBSTANCE PRE COATED CAPILLARY GLASS TUBES

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ABSTRACT Mass screening for sickle cell disease is the need of the hour. A screening test should be cost effective, easy to perform, less cumbersome, should have a high sensitivity and T.O.T. of the test (Turn Over Time, meaning time taken for a single cycle of test which excludes time taken for sample collection) should be short.

Sickling test is a well established and time tested test. But preparation of fresh 2% solution of sodium metabisulphite and slide preparation for sickling by covering the edges of the coverslip using wax is cumbersome. Solubility test is not reliable. Agarose gel electrophoresis is an art that needs a skilled technician, costly instrumentation and consumables, TOT is long, and cannot differentiate between Hb S,C,D disease in alkaline pH. Cellulose acetate electrophoresis is similar to Agarose gel electrophoresis but much more expensive than Agarose gel. HPLC is a very expensive setup, available only at tertiary level and therefore not suitable as a screening test in Indian scenario. Our modified sickling test is easy to carry out, cost effective, less cumbersome, no need of a freshly prepared solution of sodium metabisulphite, and most importantly, it uses capillary blood thereby doing away with the need for venepuncture.

KEYWORDS : MASS SCREENING, MODIFIED SICKLING TEST, CAPILLARY BLOOD,ANTI COAGULANT AND REDUCING SUBSTANCE COATED CAPILLARY GLASS TUBE , ELECTROPHORESIS.

INTRODUCTION

SICKLING TEST - The principle of sickling test is based on microscopic observation of sickling of red blood cells when exposed to low oxygen tension. Equal volume of EDTA blood is mixed with 2% sodium metabisulphite. A drop of blood and reducing substance mixture is taken in a glass slide, covered with a cover slip and the four sides of the cover slip is sealed with wax. The slide is seen under high power microscope after 2 hrs, 4 hrs and 24 hrs.

In our modified sickling test, we used pre-coated capillary glass tubes; coated with a reducing substance and an anticoagulant. We used a solution of 2 g sodium metabisulphite and0.2 g EDTA in 100 ml of distilled water. We rinsed the capillary tubes with the above solution and dried the capillary tubes over night at 50°C in an incubator. These tubes were later kept in air tight container. Each bunch contained less than 25 capillary tubes. The air tight container with the capillary tubes was sealed with adhesive tape.

The capillary tube pack was opened when sufficient number of suspected patients were collected for screening. Taking all aseptic and antiseptic measures, by finger prick, capillary blood was allowed to half fill the pre- coated capillary glass tube. Capillary blood was mixed well by tilting the capillary tube a few times, and then a drop of this blood mixture in the capillary glass tube was poured in a clean glass slide, covered with a glass cover slip, all the four sides of the coverslip was sealed with ordinary nail varnish and viewed under high power microscope after 2 hrs.

MATERIAL AND METHODS-

The study was conducted in RIMS, Ranchi. OPD Paediatric patients who presented with only anaemia were included. Sample size was 394. Since the study was conducted to establish the efficacy and reliability of a modified sickling test using pre-coated capillary tubes with capillary blood, general population sampling was not done .

RESULTS-

Out of 394 patients ,Hb electrophoresis detected 372 HbAA, 19 HbAS and 3 HbSS. Sensitivity of modified sickling using capillary blood and pre-coated capillary tube test was found to be 83.33%, which is similar to normal sickling test preparation using anticoagulated venous blood and freshly prepared reducing substance solution.

TABLE-

VARIABLE	MODIFIED SICKLING TEST	Hb ELECTROPHORESIS
TRUE POSITIVE	20	22
FALSE POSITIVE	2	0
FALSE NEGATIVE	4	0
TRUE NEGATIVE	368	372
TOTAL	394	394
SENSITIVITY	83.33%	100%
SPECIFICITY	0.54%	0%
PREDICTIVE VALUE OF POSITIVE TEST	90.90%	100%
PREDICTIVE VALUE OF NEGATIVE TEST	98.92%	100%
PERCENTAGE OF FALSE NEGATIVE	16.66%	0%
PERCENTAGE OF FALSE POSITIVE	0.54%	0%

DISCUSSION-

Despite the availability of an array of tests for Sickle cell disease, Sickling test remains a good screening test. While all the methods can detect homozygous SS state but can't detect heterozygous AS state.

Modified sickling test is better than the normal sickling test because of the following reasons-

1. It does not need venepunctre, so it is more acceptable to the patient.
2. In rural setup, sample of capillary blood can be easily collected where skilled lab technicians are not available.
3. There is no need of a freshly prepared chemical solution, so being less cumbersome, it is better for the doctors.
4. It is a very simple and easy test to carry out.
5. It has a short T.O.T. of 2 hrs which saves time of the treating doctor, pathologist and the patient.
6. It is cost effective.

CONCLUSION-

Sickling test is the most reliable, cheapest, easiest to perform and has a high sensitivity and specificity. Modified sickling test using capillary blood in reducing substance and anticoagulant prefilled capillary glass tube was found to be the best one for the mass screening of Sickle cell disease.