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Screening of Newborns for Sickle Cell Disease by Automated High Performance Liquid Chromatography

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Abstract:

Sickle cell disease is a congenital genetic disorder with significant global public health issue with numerous Complications. Newborn screening helps to diagnose the disease even before the development of sign and symptoms which usually don't occur before 4 months of age. This would enable early detection and therefore early management that can improve both morbidity and mortality.

Objectives:

1. To screen for sickle cell disease and sickle cell trait in full term newborns delivered in J.J Hospital over a period of two months.
2. To find the birth prevalence of sickle cell disease and sickle cell trait among those full term newborns screened.

Settings and Design: This is a prospective, randomised, single centric, non-interventional, open labelled study.

Methods and Material: Umbilical cord blood (100 samples) of the newborn was taken. Automated HPLC (high performance liquid chromatography - BIORAD VARIANT™) was performed.

Results: We found that, birth prevalence of sickle cell trait was two out of hundred births ie; 2% among the total population and 8% among backward and tribal communities of our society.

Conclusions: 8%(present study)sickle cell trait prevalence among backward and tribal communities should be taken into consideration for recommending a universal neonatal screening programme in high prevalent areas of India to identify babies with sickle cell disease and commence comprehensive care.

Keywords: Screening, Sickle cell disease, high performance liquid chromatography, HPLC

Introduction:

Sickle cell disease is a congenital genetic disorder with significant global public health issue with numerous Complications. Newborn screening helps to diagnose the disease even before the development of sign and symptoms which usually don't occur before 4 months of age.[1] Sickle cell disease is an inherited condition caused by abnormal haemoglobin i.e. sickle haemoglobin (HbS)[2]due to a point mutation in beta chain of haemoglobin with a substitution of valine for glutamic acid at position 6. The complications of this disorder are numerous. Most are due to

ischaemia and infections resulting in lung tissue damage (acute chest syndrome), pain episodes (arms, legs, chest and abdomen), stroke and priapism (painful prolonged erection). It also causes damage to most organs including the spleen, kidneys and liver. Stroke is the most serious complication of sickle cell disease [3].

The main aim of this study was to find the birth prevalence of sickle cell anaemia and sickle cell trait among the full-term newborns which helps to diagnose the disease even before the development of sign and symptoms.This would enable early detection and therefore early management that can improve both morbidity and mortality.

Objectives:

1. To screen for sickle cell disease and sickle cell trait in full term newborns delivered in J.J Hospital over a period of two months.

2. To find the birth prevalence of sickle cell disease and sickle cell trait among those full term newborns screened.

Material and Methods:

Study design – This is a prospective, randomised, single centric, non-interventional, open labelled study.

Site of sample collection – Labour room and operation theatre , department of obstetrics and gynaecology, J.J hospital ,Mumbai.

Site of sample study – Biochemistry laboratory, department of Biochemistry, J.J hospital, Mumbai.

Inclusion criteria-

- Normal and abnormal full term newborn babies.
- Newborns with a family history of sickle cell disease.
- Newborns without a family history of sickle cell disease.

Exclusion criteria-

- Premature babies. Premature infants may have very low levels of adult haemoglobin at birth, which may be less than the limit of detection.
- Newborns with age > 1 day.

Materials–

- EDTA bulbs
- Umbilical cord blood sample.
- Sickle cell short program recorder pack, contains
  (whole blood primer, wash solution – deionised water, elution buffer 1, elution buffer 2, analytical cartridges, retention time marker set of retention time marker 1 (FAES) and of retention time marker 2 (FADC) contains lyophilised human red blood cell hemolysates with preservatives.
- Procedure –

Sample collection-

Umbilical cord sample, about 2 ml was collected from the labour room or the operation theatre from the department of obstetrics and gynaecology, of JJ hospital immediately after the delivery of the baby, in EDTA bulbs, from a convenience sample of 100 normal or abnormal full term newborns born by vaginal delivery, with or without oxytocin augmentation or by elective cessarean delivery.

Sample storage-

The sample was stored in refrigerator at 2-8^0 c till further assay.

Sample preparation –

A dilution adjustment of the sample may be required due to variation in sample collection, transport and storage. 5 micro lit of umbilical cord sample is taken in a vial each time with the help of a pipette. A 0.5 micro lit deionised water is added to each sample vial which is allowed to stand for 30 min at room temperature. Each sample vial is mixed by inversion. The sample vial is placed into the sample tray.

Principle–

This is based on the principle of cation exchange high performance liquid chromatography (HPLC - BIORAD VARIANT ™). All steps are automated. Diluted specimens are maintained at 12 +/- 2^0 c in the automatic sampler chamber. Each specimen is sequentially injected into the analysis stream and then separated by the analytical cartridge. Two dual piston pumps and a pre-programmed gradient control the elution buffer mixture flow through the analytical cartridge. The ionic strength of the elution buffer mixture is increased by raising the percentage of elution buffer 2. As the ionic strength of the mixture increases, more strongly retained haemoglobin elute from the analytical cartridge. A dual wavelength filter photometer (415 and 690 nm) monitors the elution from the cartridge. As the haemoglobin elute from the cartridge and pass through the photometer flow cell, changes in the absorbance at 415 nm are detected . The secondary filter at 690 nm corrects the baseline for changes caused by the buffer gradient. Changes in absorbance are monitored versus time, producing a chromatogram (graph of absorbance versus time). Each haemoglobin has a characteristic retention time. Retention time is measured from the time of sample injection to the maximum point of each peak. Identification of unknown haemoglobin is accomplished through the comparison of the unknown haemoglobin's retention time with the retention time of known haemoglobin, analyzed on the same system. A built in integrator performs reduction of the raw data collected from each analysis. At the end of each sample analysis, a copy of the chromatogram and report data is automatically printed.

Results:

The screening was completed over a period of two months of the hundred samples collected, results were obtained as chromatograms.

TABLE- 1: Table shows observations of Hb screening patterns.

Hb screening pattern	No of samples
FS	0
FAS	2
FA	98
Total	100

The above table shows observations of Hb patterns obtained on screening the hundred samples, as 0 samples gave FS pattern, 2 samples gave FAS and 98 samples gave FA pattern.

Our study population consisted of 14% OBC, 9% SC and 1% ST, a total of 25%. Hence, the expected carrier rate was 2.5% and sufferer rate was 0.125%. thus, the result obtained in our study (2%) is supported. 2/25 i.e.; 8% of backward class newborn was found positive for the trait.

The 2% newborns found trait positive must have inherited the gene from either of their respective parents, who could be carriers or sufferers, since it is an autosomal recessive defect. Since the parents were asymptomatic for the disease, there is very little possibility of them being sufferers. If either of their parents was a carrier then the newborn had a 25% chance of inheriting the trait. If both their parents were carriers they had a 50 % chance of inheriting the trait. If one parent was a sufferer and the other carrier then the newborns had a 50 % chance of inheriting the trait. If one parent was sufferer and the other normal then the newborns had a 100 % chance of inheriting the trait. Thus, there are four possibilities.

The possibilities imply that the siblings of the carriers have a possibility of being normal or sufferers or carriers, making a parental counselling essential.

On interpretation of the results, the birth prevalence for sickle cell trait was found to be 2%. 98% of the samples were negative for sickle cell trait or anaemia. There were no samples found to be sickle cell anaemia positive. This result is implying that further study requires to be performed on the birth prevalence of the disease on a larger sample size.

POSITIVE CHROMATOGRAMS:

Fig 1- RESULT-1

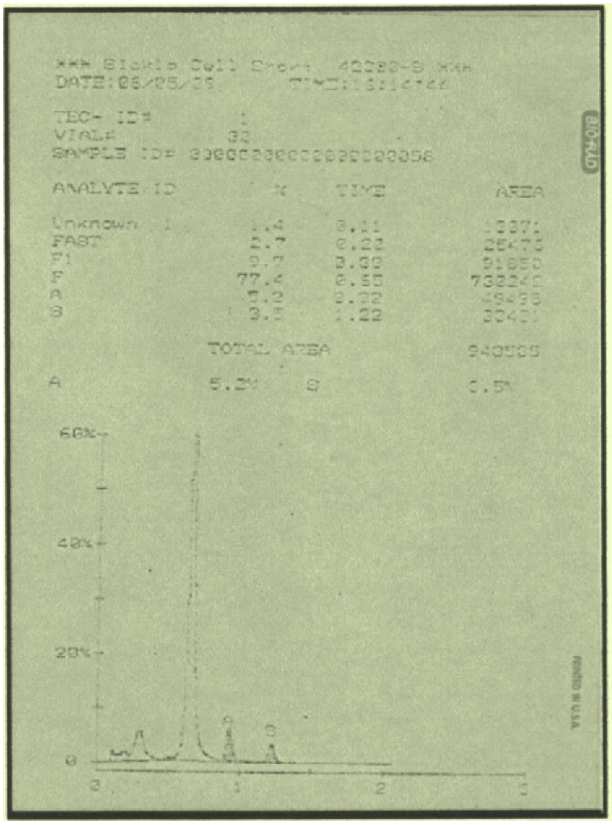
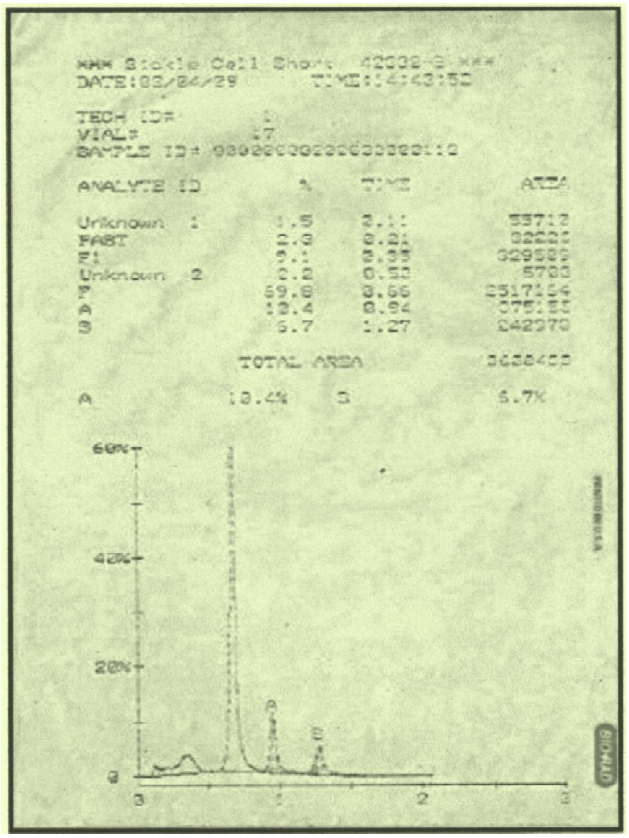


Fig 2- RESULT- 2



Discussion:

Sickle cell anaemia also called sickle cell cell disease, sickle cell disorder, haemoglobin SS disease, menisoscytosis or sicklemia, the defective haemoglobin (HbS) crystallizes readily at low oxygen tension. In consequence, erythrocytes from homozygotes change from the normal discoid shape to a sickle shape when the oxygen tension is low and these sickle cells become trapped in capillaries or damaged in transit. The inherited disorders of haemoglobin are the commonest monogenic disorders in India. The birth prevalence of sickle cell disease and trait in India is not known. If only one sickle cell gene has been inherited, it is called sickle cell trait. It is usually not regarded as a disease state because it has complications that are either uncommon or mild. Nevertheless, under unusual circumstances serious morbidity or mortality can result from complications related to polymerization of deoxy-haemoglobin S. Complications from sickle cell trait are important

because about three million people in the United States have this genotype, about 40 to 50 times the number with sickle cell disease [4].

The present study aims to find the birth prevalence of sickle cell anaemia and sickle cell trait among the full-term newborns which helps to diagnose the disease even before the development of sign and symptoms.

Several newborn screening studies have been carried out worldwide. E.V Adorno et al, Salvador, Bahia, Northeast Brazil determined haemoglobin profiles by HPLC in 581 of the 590 newborns 480(82.6%) had the normal profile FA, and 101 (17.4%) presented variant haemoglobins, of which 57 (9.8%) were heterozygous for HbS(FAS), and 38 (6.5%) were heterozygous for HbC (FAC). One (0.2%) baby was homozygous for HbS (FS) and five (0.9%) were double heterozygous for HbS and HbC (FSC)[5]. Grover et al carried out a newborn screening program in New York from January 1 to December 31, 1982. Of the 110,194 valid filter paper blood samples tested by cellulose acetate and citrate agar haemoglobin electrophoresis alternatively during the study period, 173 newborns were identified with sickle cell disease, (SS,SC,SpThal) and 4565 with trait haemoglobinopathies[6].

The population was randomized, representing an average neonatal population born in a tertiary health care centre in Mumbai (J.J Hospital). It consisted of 43% Hindus, 49% Muslims, 6% Buddhist, 1% Christian and 1% of unknown religion.

In a study performed by Kate et al, it was found that this disorder is mostly confined to economically and socially backward communities known as scheduled Caste (SC), Scheduled Tribe (ST) and other backward communities (OBC) groups of Maharashtra, expected carrier rate amongst them is 10% and sufferer rate is 0.5% [7]. It is rare in other communities.

During the last 54 years, several groups of investigators conducted hospital based or epidemiological surveys in various ethnic groups. Based on these surveys, prevalence of sickle gene is found to be 0-18% in north eastern India, 0-33.5% in western India, 22.5-44.4% in central India and 1-40% in southern India and the gene frequency of Hb-S varies between 0.03-0.41[8].

Prenatal screening had been performed only by Colah R. et al. they screened 85 at risk couples for antenatal diagnosis of sickle syndromes. Chorion villus sampling was done in the first trimester and DNA analysis using reverse dot blot hybridisation or restriction enzyme digestion in 65 cases. Cordocentesis was done in the second trimester and fetal blood analyses by automated HPLC in 20 cases who came late. 23.5% of fetus were affected (sickle cell anaemia- 18, sickle thalasemia- 2)[9].

Eastman et al performed automated HPLC to test dried blood spot specimens from newborns for haemoglobin F, A, S,C,E and D and presented the method and report on its performance determined during > 4 years of testing 2.5 x 10^6 newborns [10].

Conclusion:

2% birth prevalence for sickle cell trait is not high but 10% (8% in our study) prevalence among SC, ST, and OBC communities is high. It should be taken into consideration for recommending a universal neonatal screening programme in the rural and hilly areas of Maharashtra and selective implementation of screening among newborns in the cities.

The screening of newborns for sickle cell disease is highly significant in a country like India, where the incidence of sickle cell trait is high. It is highly essential to screen newborns for sickle cell disease for its proper management and to decrease the morbidity and mortality.

Further research is required on whether antenatal diagnosis is better than prenatal diagnosis

of SCD, since sickle cell anaemia has a relatively benign clinical course in some tribal groups in India.

Conflict of interest: No conflict of interest

Funding: No funding provided for this study.

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