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Newborn Screening of Sickle Cell Anemia by Lateral Flow Chromatography Immunoassay Test

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ABSTRACT

INTRODUCTION
Sickle Cell Anemia is a rare inherited haematological disorder in which RBCs become sickle-shaped in the abnormal haemoglobin S variant's presence.1 This makes severe sickle-shaped blood difficulty passing through small blood vessels, obstructing the normal blood flow, damaging tissues, and finally leading to Sickle Cell Disorder complications.2 The red blood cells contain haemoglobin S live only about 16 days compared to 120 days for normal RBCs.3 Sickle Cell conditions exist in many types, with the most common being Sickle Cell Trait (HbAS), Sickle Cell Disease (HbSS), Sickle-Hb C Disease (HbSC), and Sickle-Hb C Trait (HbAC).

MATERIALS AND METHOD:
Aim of Study: To screen all Newborn babies born in the hospital at N. K. P. Salve institute of medical sciences & research centre and LataMangeshkar Hospital, Nagpur for Sickle cell anemia by Lateral flow chromatographic immunoassay test.
Prospective Study. All New-born babies born in the hospital at NKPSIMS to be screened by Lateral flow chromatographic immunoassay test- Scan test kit, Heel prick blood-5microlitre, Buffer solution. Blood collected by heel prick in capillary tube mixed with buffer solution available with the scan test kit and five drops are added to slide, Results interpreted as per markings on the slide after 5 mins. A number of the sample (n) Female Baby 103, and Male Baby 135. Study Duration 7 months (March 2020 to September 2020),

RESULT AND CONCLUSION:
The incidence of sickle cell anemia in new-borns by lateral chromatographic immunoassay method by the quick and cheap way in a country like India with minimal resources is the need of an hour, and this newer method will be beneficial to detect and diagnose cases of sickle cell anaemia in new-borns and thereby reduce mortality and morbidity in new-borns due to sickle cell disease.

Keywords:
Sickle Cell Anemia, Lateral Flow Chromatographic immunoassay test, scan test kit, new-born babies

Introduction

Sickle Cell Disease is a haematological disorder that causes red blood cells to become sickle-shaped because of the abnormal haemoglobin S variant.1

These rigid sickle-shaped blood cells have difficulty passing in small blood vessels, resulting in obstruction of the normal blood flow and damage to the body's tissues and eventually leading to various Sickle Cell Disorder complications.2 The red blood cells contain mainly Hb-S live only

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about 16 days compared to 120 days for normal RBCs.3

There are types of Sickle Cell conditions which with the most common, is Sickle Cell Trait (HbAS), Sickle Cell Disease (HbSS), Sickle-Hb C Disease (HbSC), and Sickle-Hb C Trait (HVAC). The diagnosis (preferably as a newborn) of Sickle Cell anaemia becomes necessary at an early stage to initiate early treatment such as penicillin prophylaxis, vaccination against pneumococcus bacteria, folic acid supplementation, pain management, blood transfusions, and hydroxyurea if needed .3,4

Sickle cell trait is milder condition and, harmful complications are possible when there are extreme environmental conditions like high atmospheric pressure, at higher altitudes, decreased oxygen levels, athletic activities, or severe dehydration).5

Caution should be taken in sickle cell disease carriers and situations for individuals' genetic counselling and family planning.

The Sickle test is a lateral flow chromatographic qualitative immunoassay that helps in the rapid diagnosis of sickle cell disorders of haemoglobins A, S, and C using fingerstick. It involves a clinical professional such as a doctor, physician assistant, nurse, clinical or medical assistant, or laboratory technician.

MATERIAL AND METHODS

Aim: To screen all Newborn babies born in the hospital at **N. K. P. Salve institute of medical sciences & research centre and LataMangeshkar Hospital, Nagpur** for Sickle cell anemia by Lateral flow chromatographic qualitative immunoassay test.

Objectives:
1. To study the incidence of sickle cell anemia in the newborn in a tertiary care hospital.
2. Identify the magnitude of sickle cell anemia in newborns in a N. K. P. Salve institute of medical sciences & research centre and LataMangeshkar Hospital, Nagpur.

The present study was conducted at N. K. P. Salve institute of medical sciences & research centre and LataMangeshkar Hospital, Nagpur, in the Department of Paediatrics at a tertiary care center from March 2020 to September 2020.

The study group consisted of 238 newborn babies delivered by regular vaginal or caesarean section in the institute's obstetric department.

They were enrolled in the study after obtaining written informed consent from parents and Institutional Ethics Committee.

Study duration: 7 months (March 2020 to September 2020)

Study centre: Postnatal care unit of tertiary care institute.

Sample size: 238 Sample size was calculated using Open Epi version 3 software calculators for unmatched case-control study design, as mentioned below. However, due to study tenure

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limitation, only 238 neonates could be accessed for statistical evaluation.

Study design: This is a prospective observational study conducted in the postnatal ward from March 2020 to September 2020. All healthy and stable newborn babies born in the hospital were enrolled in this study.

INCLUSION CRITERIA:

1. All newborn babies born in a hospital with postnatal age of fewer than 28 days were admitted.
2. All non-sick newborn babies in hospital

EXCLUSION CRITERIA

1. All babies age more than 28 days.
2. Babies are sick and shifted to Nicu for intensive care.
3. Out born babies

Selection of cases
The babies born in the hospital by either normal vaginal delivery or caesarian section and are healthy and hemodynamically stable admitted in the postnatal ward for postnatal care in the department of paediatrics are selected for newborn screening test by lateral flow chromatographic immunoassay test.

Clinical Assessment
All newborn babies delivered in the hospital were enrolled in the study. The demographic data like name, sex, age at admission, registration number, Gestational age, birth weight, a sickle test of mother and father, maternal haemoglobin were recorded.

Data collection and Statistical analysis
Data was collected in structured data collection forms. All findings and observations were coded and entered in MS Excel master sheet. The distribution of neonates included demographic parameters like age and gender in terms of numbers and percentage. The birth weight of neonates and gestational age of mothers, the incidence of sickle cell anemia (AS pattern or SS pattern) were expressed in terms of mean, standard deviation, median and range. The distribution of neonates according to various findings on neonates was also summarized in numbers and percentage.

This study's statistical significance was calculated using Pearson's Chi-square test for categorical variables in this study. The distribution of various non-parametric data was obtained in numbers and percentage. All the analyses were performed using SPSS ver20.0 (IBM Corp), Minitab 16, and the statistical significance was tested at a 5% level ($p < 0.05$).

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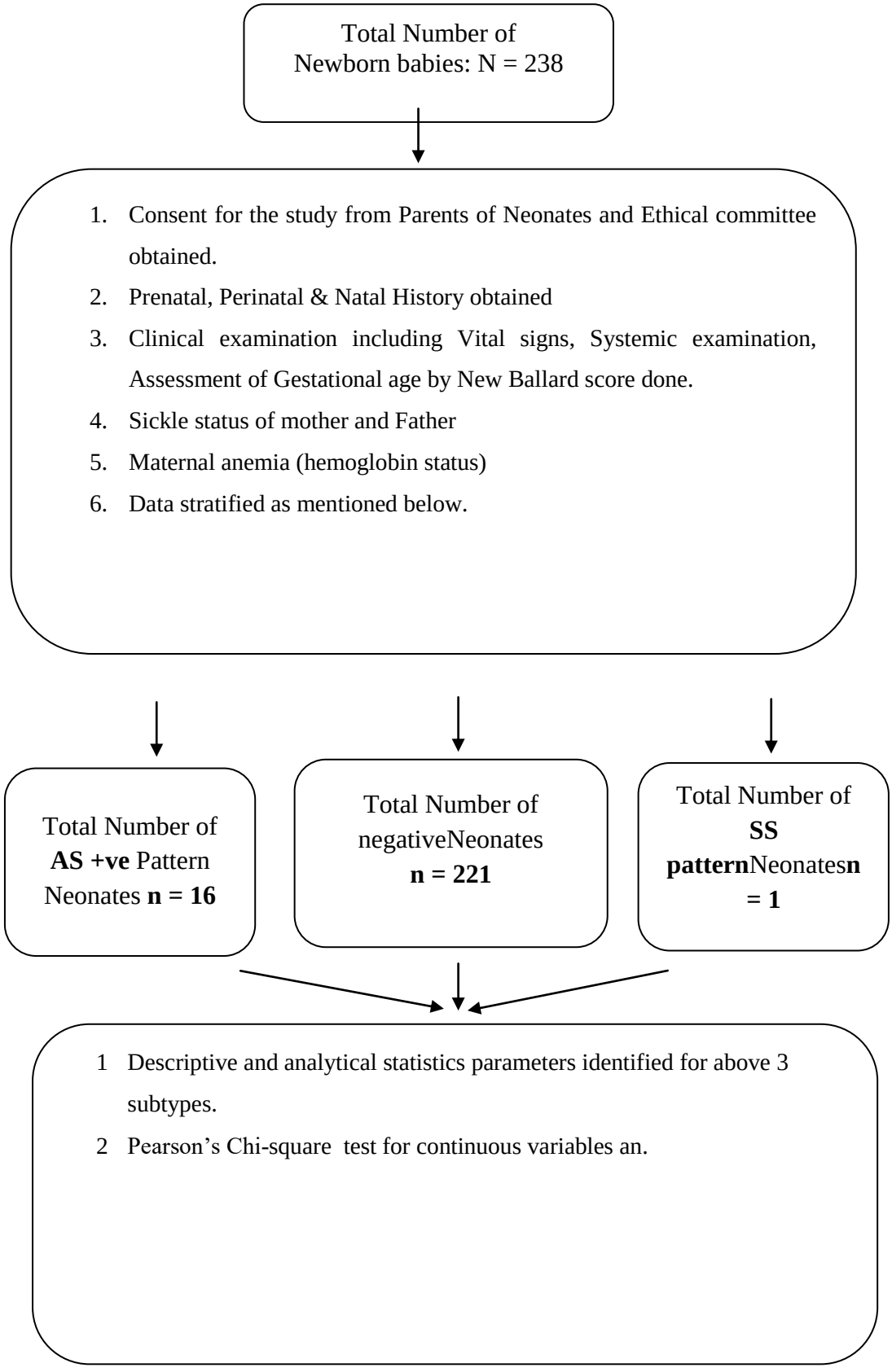
Figure: 1. Study Profile: Patient Flow Chart



Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 1, 2021, Pages. 401 - 411
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OBSERVATIONS AND RESULTS

The study conducted at tertiary care hospital in postnatal wards had revealed the following observations.

Table 1: Descriptive statistics for mother's and baby's characteristics (n=238)

Characteristics		Mean	SD	Median	Minimum	Maximum	n (%)
Day of life		2.79	1.97	2.00	1.00	18.00	
Sex	Female						103 (43.28)
	Male						135 (56.72)
Mother: Gestation (weeks)		38.18	1.74	38.29	30.00	41.00	
Baby birth weight (Kg)		2.72	0.44	2.80	1.10	4.10	
Mother: Hb (%)		11.32	1.28	11.20	7.10	15.70	

Table 1 gives the descriptive statistics for mother's and baby's characteristics according to scale of measurement. The babies included in the study had mean live days of 2.79 (SD: 1.97). The proportion of male babies (56.72%) was marginally higher than that of female babies (43.28%). The mean gestational age of mothers was 38.18 weeks (SD: 1.74 weeks) and ranged between 30 – 41 weeks. The mean weight at birth was 2.72 kg (SD: 0.44 kg) and ranged between 1.10 – 4.10 kg. The mean haemoglobin level of mothers was 11.32% (SD: 1.28%), which ranged between 7.10% – 15.70%

Table 2: Sickle cell test outcomes in father, mother and new born (n=238)

Sickle cell test		**n (%)**
Father	Negative	238 (100)
Mother	AS	6 (2.52)
	Negative	232 (97.48)
Baby	AS	16 (6.72)
	SS	1 (0.42)
	Negative	221 (92.86)

Table 2 gives the sickle cell outcome in father, mother and baby categories. Out of 238 studied cases, all fathers (100%) were negative. As regards mothers, 6 (2.52%) were of AS type, while remaining 232 (97.48%) were negative. Among babies, 16 (6.72%) were of AS type, 1 (0.42%) was of SS type and 221 (92.86%) were negative.

Thus, the prevalence of AS type babies was 6.72%, while SS type was 0.42%. The overall prevalence of sickle cell anaemia in new born babies was 7.14%.

Table 3: Cross-tab showing association of sickle cell outcome between mother and baby

Sickle cell status		Baby		
		AS	SS	Negative
Mother	AS	2	1	3
	SS	0	0	0
	Negative	14	0	218

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 1, 2021, Pages. 401 - 411
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P-value < 0.0001

The cross-tab shows the association of sickle cell outcome between mother and baby. Out of 6 mothers with AS type, 2 (33.3%) babies were AS type and 1 (16.6%) had SS type. However, out of 232 negative mothers, 14 (6.03%) had AS type babies, while 218 (93.97%) had normal babies. The association between the two attributes was studied using Pearson's Chi-square test, which resulted into a p-value < 0.0001, suggesting a significant association. In other words, the proportion of abnormal outcome in babies born to abnormal mothers was significantly higher than abnormal babies born to normal mothers.

Figure 1: Bar chart showing association of sickle cell status between mothers and babies

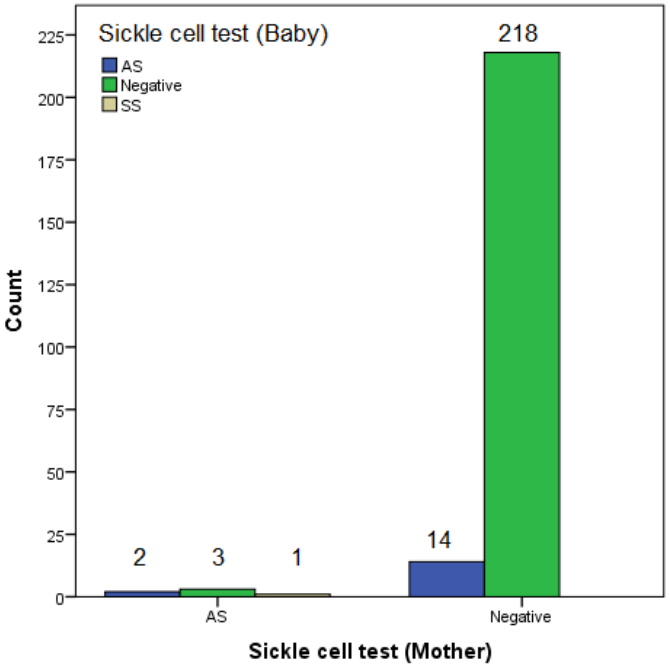

Table 4: Cross-tab showing association of baby's sex with sickle cell outcome

Sickle cell status		Baby		
		AS (n=16)	SS (n=1)	Negative (n=221)
Sex	Male	8	0	95
	Female	8	1	126

P-value: 0.587

The cross-tab shows the association between baby's sex and the sickle cell outcome. The proportion abnormal outcome in males was 7.77%, while that in females was 6.67%. The difference in the proportion was statistically insignificant with a p-value of 0.587 using Pearson's Chi-square test. In other words, there was no evidence of association between sex and sickle cell outcome in babies.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 1, 2021, Pages. 401 - 411
Submitted20 October2020;Revised20 November 2020; Accepted 14 December 2020.

Figure 2: Bar chart showing association of sickle cell status between baby's sex and sickle cell status

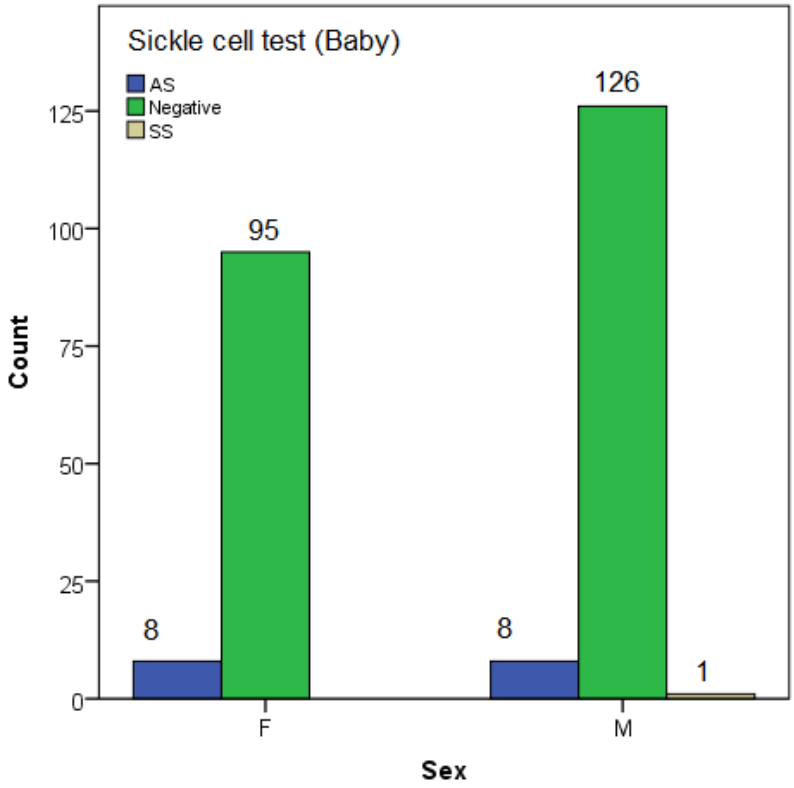

Table 5: Comparison of various parameters between sickle cell categories

Parameter	Sickle cell outcome (Baby)						P-value
	AS (n=16)		Negative (n=221)		SS* (n=1)		
	Mean	SD	Mean	SD	Mean	SD	
Baby birth weight (Kg)	2.65	0.41	2.73	0.45	2.98	-	0.493
Gestation (Weeks)	37.37	2.23	38.25	1.69	37.00	-	0.052
Haemoglobin (Mother)	10.63	1.37	11.37	1.26	10.60	-	**0.026**

*This group was ignored in analysis due to single observation; Bold p-value indicate statistical significance

Table 5 gives the comparison of maternal and baby's parameters across sickle cell outcome categories of baby. It is evident that the mean birth weight was insignificantly different between AS and negative categories, as indicated by a p-value of 0.493 using t-test for independent samples. The mean gestational age of mother also differed insignificantly between two groups, with a p-value of 0.052. However, the mean haemoglobin level of mothers in AS category (10.63%) was significantly lower than that of negative group (11.37%), with a p-value of 0.026.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 1, 2021, Pages. 401 - 411
Submitted20 October2020;Revised20 November 2020; Accepted 14 December 2020.

Figure 3: Bar chart showing mean levels of parameters in three sickle cell categories of babies

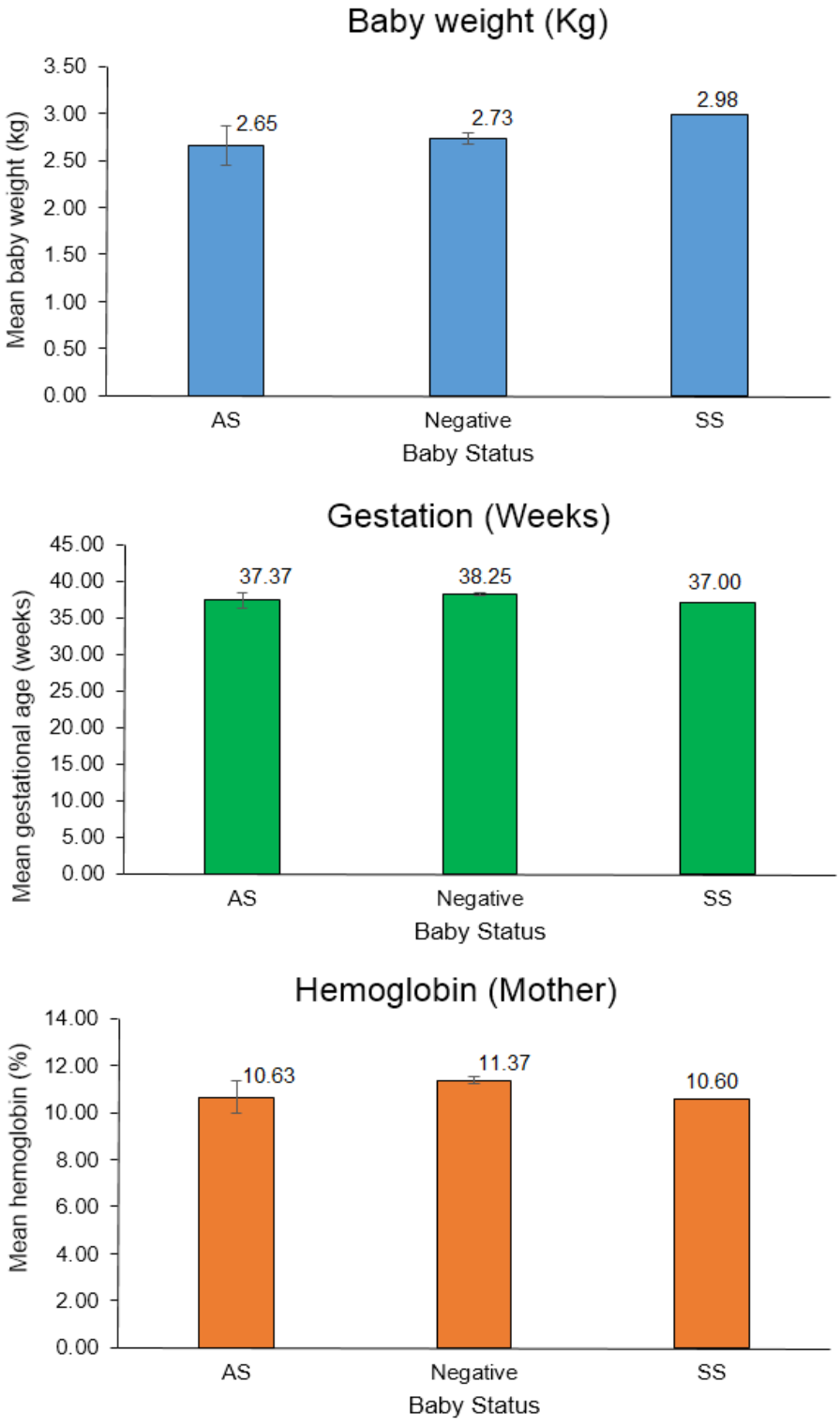

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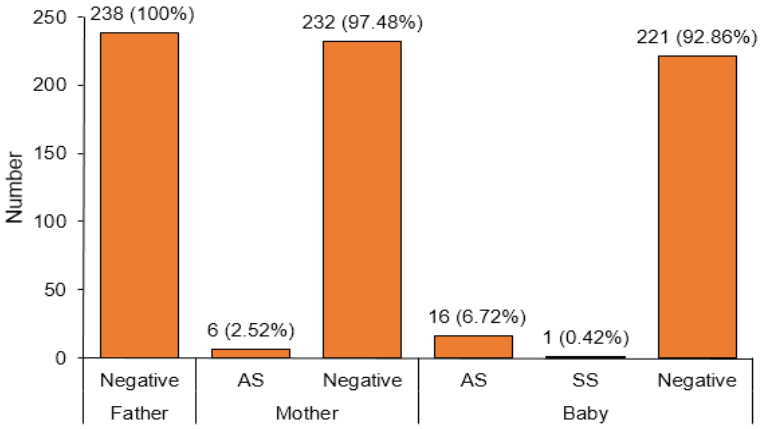
Figure 4: Bar chart showing number of cases according to sickle cell test outcome



DISCUSSION

The primary purpose of newborn screening for SCD is to early diagnose and reduce the morbidity and mortality associated with the disease. This study demonstrates that it is easy to adapt and use these methods in a developing country like India, mainly rural and mobile. Similar two studies from India have described newborn screening in single institutions.18, 19

In one study, 1158 babies were screened for sickle cell anaemia in Chhattisgarh (using the Bio-Rad Hemoglobin Variant Neonatal sickle cell short programme); 0.4% of babies had SCD, 5.26% had sickle cell trait, and 0.08% were double heterozygous for sickle-b-thalassaemia. 18

The study showed that it is feasible to use heel prick blood for diagnosis in remote areas. In India, which has more than 70% of the population living in rural areas, which has little access to infrastructure and sources, this was a targeted than universal screening approach, which considers more cost-effective screening newborns with a high prevalence HbS gene. The increase in newborn screening of conditions other than haemoglobinopathies and developing screening methods may have to consider universal screening in the future. A major drawback of this newborn screening was that they did not include the establishment of comprehensive care centres for the conditions detected and diagnosed, rather than providing Pneumococcal vaccination, regular clinical and work-up blood investigations, and folic acid supplementation to these babies. Penicillin prophylaxis did not begin as earlier studies in adults in this region had suggested that sickle cell anaemia patients belonging to these tribal populations have only mild infection. The current study will help inform policymakers on the future use of penicillin prophylaxis in newborns with SCD among these tribal groups. Data is very limited about the comprehensive care and complications or long term outcomes of newborns diagnosed with different types of haemoglobinopathies like sickle cell anaemia in India. The upcoming projects should include establishing comprehensive centres that provide comprehensive care and collect data on long-term outcomes of newborns diagnosed with these conditions, which can be screened easily.

The primary step to tackle the early mortality of children with SCD is early diagnosis. This makes parents' education about how to look after their affected babies, identify important danger

signs, and prevent complications by vaccinations, antibiotics, and anti-malarial drugs. These measures can be easily afforded by many developing countries within the region 11,12, were successful outcome in decreasing the high early mortality that was previously seen in different parts of the world, and can be implemented in resource-limited settings 10.

Until to date, diagnostic facilities for SCD remain poor throughout many regions, which are all limited to private facilities and not to the majority who would be helpful.

There are different methods to diagnose SCD, of which the most common are haemoglobin electrophoresis, high-performance liquid chromatography (HPLC), isoelectric focusing and molecular approaches such as PCR 13. All these methods require a well-trained staff working with well-maintained equipment in good laboratory facilities, an adequate power supply r, and systems for the delivery and storage of reagents, the commercial costs of which alone can typically run 500-1000 RS/- per test. The laboratory-based approaches need a functional system to transport sample and return results. The pilot newborn screening studies in many resource-limited countries have used such approaches successfully 14, 15. The cost and logistic limitations mean that even when health workers suspect older children's condition, testing for SCD is rarely undertaken. In this context, the extensive availability of a rapid and reliable diagnostic test could change the outlook for children born with SCD in resource-limited settings. The newborn sickle screening test In research published in BMC Medicine, Kanter and colleagues show that a new point of care diagnostic device, called the Sickle scan test, can be used to accurately diagnose the most common forms of SCD (HbSS and HbSC) from a capillary sample of blood, in less than 5 minutes 16. This is an easy, rapid method that involves a sandwich format chromatographic immunoassay approach for the qualitative measurement of HbA, HbS and HbC, together with α-globin as a positive control method.

The test is similar to devices that are used for the rapid diagnosis of other diseases, like HIV and malaria, with which most practitioners in limited-resource settings will be familiar. This test has several advantages above traditional laboratory methods. This method being so rapid, the method could contribute to the immediate management of patients in whom clinicians suspect a diagnosis of SCD, which allows for the real-time communication of results and necessary referral to specialist services. Also, the test needs no electricity and should avoid the cost of sample transport and the feedback of results. The greatest results will be achieved if this test makes feasible the widespread testing of children as early in life as possible – preferably at birth (if delivered at a health facility) or at first contact with a health professional. The high and different concentration during the early life of red cell fetal haemoglobin (HbF) means that it cannot be assumed that the lateral flow chromatographic immunoassay test will be equally accurate during this period without further studies. Such studies are currently under still research, the results of which are suspected. Similarly, before its wide endorsement, this test's accuracy needs confirming in larger studies needs to be carried out in multiple communities under real-world scenarios.

SUMMARY AND CONCLUSION

Early detection and diagnosis of sickle cell anemia in newborn and proper follow-up can decrease mortality and morbidity due to sickle cell anemia . In this study, we prospectively enrolled 238 healthy newborns to study the incidence of sickle cell anemia in newborns in a tertiary care hospital.

Out of 238 new-borns, there were males, 135 (43.28%) were common than females 135 (56.72%). The mean birth weight was 2.72 kg with a range of 1.10 kg to 4.10 kg. The mean haemoglobin level of the mother was 11.32% (SD1.28%). The mean gestational age of mothers was 38.18weeks (SD 1.74Weeks) and ranged between 30-41 weeks. In our study, the incidence of sickle cell anemia (AS Pattern) was 16 (6.72%) out of 238 and SS Pattern 1 case (0.42)
.
This is a newer new-born screening test. SCD and its carrier status have a social stigma in many affected communities 17 and, as for other chronic conditions; unwanted consequences can be found if testing is conducted in the absence of adequate support.

The pre-and post-test counselling should be done by well-trained staff to identify such consequences, better parent education regarding SCD in affected communities, and improved advocacy to all affected parents. We need to enhance the widespread diagnosis of SCD in resource-limited settings; this test could make a valuable contribution and prove very useful for the same.

LIMITATION OF STUDY:

The test would not be possible when the sickle scan test kits are not available. The is has minimal charge of Rs. 250 and results are rapid of the spot. The awareness of the newer method test with quick results is not among the people. The sickle scan test would help identify sickle cell anemia in new-borns at birth, and the incidence and nature of the disease can be studied if the government supports and sponsors the tests kits.

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Conflict of Interest- The authors declare that they need no conflict of interest.

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