Original Research Paper

Volume - 12 | Issue - 10 | October - 2022 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Pathology

STUDY OF EFFECTIVENESS OF SOLUBILITY TEST FOR SICKLE CELL DISEASE IN FEMALES IN REPRODUCTIVE AGE GROUP AS SCREENING TOOL AND HEMATOLOGICAL CHANGES DURING ANTENATAL VISITS – INSTITUTIONAL ANALYSIS.

Bela Sharda	Assistant Professor, Department of Pathology, Sri Aurobindo Medical College and Hospital, Indore, MP.
Gaurav Pawar*	Associate Professor, Department of Pathology, Sri Aurobindo Medical College and Hospital, Indore, MP. *Corresponding Author
Chandradevi Korant	Assistant Professor, Department of Pathology, Zydus Medical College and Hospital, Dahod, GJ.
Kirtan Ratanpara	Assistant Professor, Department of Pathology, Zydus Medical College and Hospital, Dahod, GJ.

ABSTRACT Sickle cells Disease is a result from inheritance of sickle cell gene that codes for Beta- globin chain. This change leads to single base A →T in the sixth codon of Beta globin gene so that there is substitution of thymine for adenine, this in turn causes substitution of valine for glutamic acid at position 6 of β polypeptide chain. **Context:** The purpose of the Retrospective study is to identify unknown identities in Tribal Zone having Sickle cell Disease as well to trace such females in Reproductive age groups. **Aims:** Utility of Solubility test in early detection and identifying the cases with Sickle disease and reliability of the Methodology. **Setting And Design:** Study conducted on females with different trimesters, Total 853 patients from Antenatal clinic, period of three months from October to December 2021. **Methods And Material:** We collected the data from Central laboratory and performed Complete blood count and Solubility Test on Females within reproductive age group. **Statistical Analysis Used:** We carried out a logistic regression analysis with variable Sickle and explanatory and predictive analysis variables HB, Age, Platelet and total count. **Result-** 0.12% of patient was missing (1/853) with Negative 740/853 (87%) and Positive cases 112//853(13%), mean Hb (9.72± 1.59) P < 0.001 with Welch double test. Age in sickle positive cases had Mean (24.1± 3.45) with P= 0.58, Odds ratio 0.989 and P= 0.68. Mean Age and Hb display a Nonlinear Relationship with p= 0.23. Age and Platelet count have shown a linear Correlation with p=0.021, Age and total count have also showed significant correlation with P=< 0.001. **Conclusion-** In this analysis we found that using a Method having fast accessibility and having good time approval test result like solubility gives a preliminary outlook towards SCD, though the test is having few limitations in differentiating Sickle cell trait and disease.

KEYWORDS : Sickle disease, Tribes, Hemoglobinopathies, Solubility

INTRODUCTION –

Sickle cell anemia has been an integral part of the Hematological disorder and its prevalence among the tribal population of India and as well indigenous community residing in the interiors of states. Sickle cell disorder is the second most common hemoglobin disorder next to thalassemia in India.[1] Majority of the population is living in states Gujarat, Maharashtra and Orissa, Jharkhand and Chhattisgarh and Madhya Pradesh. We here have covered institutional data and the visits from different groups of communities belonging to different sects and religions and followers of different deities. In Gujarat, the Dhodia, Dubla, Gamit, and Naika tribes have a high prevalence of HbS (13-31 %)10. More recently very extensive population surveys have been done by the Indian Red Cross Society, Gujarat State Branch where 1,68,498 tribals from 22 districts were screened and the overall prevalence of sickle cell carriers was 11.37 per cent. Some tribal groups in south Gujarat like Chaudry, Gamit, Rohit, Vasava and Kukana have shown both a high prevalence of HbS (6.3 to 22.7%) as well as β-thalassaemia trait (6.3 to 13.6 %). These tribal groups would have the likelihood of co-inheriting both these genes.[2] Tribal accounts 15 % of the total population of Gujarat and distributed in various districts of the state such as Sabarkantha, Banaskantha, Panchmahal, Vadodara, Narmada, Bharuch, Surat, Valsad, Dang and Div-Daman.[4] Combating with these inherited disorder prevention and screening is they will be backbone. Various Screening test are available for diagnosing sickle cell disorders. The impact of the disease and burden is mostly not covered at state and national levels, National health Mission, Ministry of Health and family welfare has implicated and structured an outline with the prevention and early diagnosis as the primary motive to detect Hemoglobinopathies, 2016 with focus on tracing and improving the lifestyle and prevent the disease modality and control the adversities faced by the population.[7]

METHODS AND MATERIAL-

We here have documented a total number of females in Ante Natal clinic registered at outpatient department were 853 with Positive Total number of cases documented in this descriptive analysis are 853 at the ante natal clinic ,the test performed were in ANC profile includes CBC , Sickle cell screening by Solubility test (Dithionate Qualitative Solubility test) ,the hematological changes and impact of Sickle disease on Parameters was studied which include Age , HB level, Total count and platelet, further a predictive analysis, with as the outcome variable SICKLE and as the predictor variables Age, HB, Platelet and Total count. Statistical analysis Methods - Predictive analysis relied on logistic regression to obtain a probability for everyone to belong to the group SICKLE = POS. We then drew a ROC curve to present the different decision thresholds. Solubility tests this test is easy to perform and inexpensive. It suffers from a false-negative result when utilized for newborns, due to the presence of a high amount of hemoglobin F and when the HbS is less than 10% of the total hemoglobin. Furthermore, false-negative results are observed in patients with coinheritance of α-thalassemia trait and severe anemia. In contrast, false positives are observed in patients with high serum viscosity, erythrocytosis, highly marked leukocytosis and in some cases of anemia. Moreover, the sickle solubility tests cannot differentiate between sickle cells trait (SCT) and SCD, and they are insensitive to the detection of hemoglobin AS (HbAS). These disadvantages make them difficult to use in screening programs.[3]

RESULTS -

We selected the candidate predictor variables from the set of collected variables in such a way that there were less than 20% of patients with missing data or variables with less than 5% missing values. The predictor variables Age, HB, Platelet and Total count were defined a priori based on data from the literature. The candidate predictor variables were included in a Least Absolute Shrinkage and Selection Operation (LASSO) penalized regression model. The penalty coefficient (lambda) was chosen to provide an estimation error lower than one standard deviation of the minimum error obtained by 10-fold cross-validation, while being as parsimonious as possible.

We carried out a logistic regression analysis with variable Sickle and explanatory and predictive analysis variables HB, Age, Platelet, and total count. Model Performance was calculated from bootstrap resampling. Welch two sample test was performed for univariable analysis, total 0.12% of patient was missing (1/853) with Negative 740/853 (87%) and Positive cases 112//853(13%). Out of 853 visits from a period of three months from October to December 2021, Age ranging from 18 to 45 years, Sickle positive 112/852, having mean Hb (9.72± 1.59) P < 0.001 with Welch double test. Age in sickle positive cases had Mean (24.1± 3.45) with P=0.58, (Table 1,2,3) Odds ratio

0.989 and P= 0.68. Mean Age and Hb display a Nonlinear Relationship with P= 0.23.(Table 4). Age and Platelet count have shown a linear Correlation with p=0.021, Age and total count have also showed significant correlation with P=< 0.001. A p-value<0.05 was considered significant. In total, 157,473 deliveries occurred at MNH during the study period, of which 149 were SCD (incidence of 95 SCD per 100,000 deliveries). The incidence of SCD had increased from 76 per 100,000 deliveries in the 1999–2002 period to over 100 per 100, 000 deliveries in recent years. The mean maternal age at delivery was lower in SCD (24.0±5.5 years) than in non-SCD deliveries (26.2±6.0 years), p<0.001. Compared with non-SCD (2.9±0.7 Kg), SCD deliveries had less mean birthweight (2.6±0.6 Kg), p<0.001. SCD were more likely than non-SCD to deliver low APGAR score at 5 minutes (34.5% Vs 15.0%, OR=3.0, 95%CI: 2.1–4.2), stillbirths (25.7% Vs 7.5%, OR=4.0, 95%CI: 2.8–5.8). There was excessive risk of maternal deaths in SCD compared to non-SCD (11.4% Vs 0.4%, OR=29, 95%CI: 17.3–48.1). The leading cause of deaths in SCD was infections in wholly 82% in contrast to only 32% in non-SCD. In conclusion SCD in pregnancy is an emerging problem at MNH with increased adverse fetal outcomes and excessive maternal mortality mainly due to infections.[3] This study in Tanzania Tertiary center and our predictive Logistic regression has same result regarding the health outcome of females with positive Sickle trait.[4] A highly significant association of anemia was found with the mother's age group ($\chi 2$ =28.38, p0.05) and dietary habits ($\chi 2$ =2.37, p>0.05) were not significantly associated with anemia. All the women with multiple pregnancies were found to be anemic, though it was only moderate in nature. Pregnancy in sickle cell disease is at very high risk. Many reports have documented a substantial maternal risk of morbidity and mortality and high perinatal adverse consequences. Women with SCD have an increased risk of pre-eclampsia and maternal death, stillbirths, preterm deliveries, and small-for-gestational-age newborns. The prevalence of sickle-cell anemia is extremely common in the tribal belt of Southern and Central parts of India which includes tribal in the states of Gujarat, Madhya Pradesh, Maharashtra, Chhattisgarh, Orissa, Tamil Nadu, and Kerala[5] This coincides with our study relative to Age and Hemoglobin level. Sensitivity of solubility test is 50% and specificity 10.54%[6] Out of 23420 large cases 4555 patients screened were positive on Solubility test, this test had specificity 91.66% and 100 % sensitivity with predictive value of positive test 80% and negative test 100%. the sensitivity and specificity of solubility test were 100% for samples collected during the year 2008. The predictive value of positive and negative test was 100%. For samples collected during 2009, the sensitivity of the solubility test was 100%, whereas 2012 the specificity was 87.93%.(Table 5,6) The predictive value of positive and negative solubility test was 80% and 100%, respectively. In 2010, the sensitivity of the solubility test was 100%, whereas the specificity was 86.18%.[7]

The sensitivity was found to be 94.8% and specificity of study was 87.8 %. Positive predictive value of this test is 97.73% and negative predictive value of this test is 94.40 %, NHM Guidelines for Prevention and Control of Hemoglobinopathies, Ministry of Health & Family Welfare Government of India[8]

Descriptive Analysis

Quantitative Variables Table 1

	Mean (sd)	Median [Q25-75]	Min	Max	n
Age	24.3 (4.00)	24.0 [21.0; 26.0]	18.0	45.0	852
HB	10.2 (1.73)	10.4 [9.20; 11.4]	2.80	18.4	853
Platelet	2.83 (0.763)	2.73 [2.31; 3.28]	0.340	6.72	852
Total count	9720 (2396)	9500 [8100; 11200]	1200	21700	853

Qualitative Variables- Table 2

		n (%)
SICKLE	NEG	740 (87%)
	POS	112 (13%)

Univariable Predictive Analyses- Table 3

	SICKLE NEG (n = 740)	SICKLE POS (n = 112)	n	p	test
Age, mean	24.3 (±4.08)	24.1 (±3.45)	852	0.58	Welch
HB, mean	10.3 (±1.73)	9.72 (±1.59)	852	**<0.001**	Welch

Logistic Regression Model - Table 4

	Odds-Ratio	p	Coefficients
Intercept	0.371 [0.0507; 2.75]	0.33	-1.08
Age	0.989 [0.936; 1.04]	0.68	-0.0101
HB	0.831 [0.742; 0.932]	**<0.01**	-0.165
Platelet	1.29 [1.00; 1.66]	**0.047**	0.229
Total count	1.00 [1.000; 1.00]	0.26	0

Univariable Predictive Analyses Table No. 5

	SICKLE NEG (n = 740)	SICKLE POS (n = 112)	n	p	test
Age, mean	24.3 (±4.08)	24.1 (±3.45)	852	0.58	Welch
HB, mean	10.3 (±1.73)	9.72 (±1.59)	852	<0.001	Welch
Platelet, mean	2.80 (±0.746)	2.99 (±0.855)	852	0.031	Welch
Total count, mean	9671 (±2384)	10047 (±2466)	852	0.13	Welch

Multivariable Analysis

With a 5% risk, by adjusting for Age, Platelet and Total count, there is a statistically significant relationship between SICKLE and HB. SICKLE is also significantly linked to Platelet.

- HB favours SICKLE = NEG (p = **<0.01**): when HB increases by 1 unit(s) (for instance from 10 to 11), odds of SICKLE = NEG is multiplied on average by 1.20.
- Platelet favours SICKLE = POS (p = **0.047**): when Platelet increases by 0.1 unit(s) (for instance from 2.8 to 2.9), odds of SICKLE = NEG is multiplied on average by 0.975.

Table No. 6

	Odds-Ratio	p
HB	1.20 [1.07; 1.35]	<0.01
Age	1.01 [0.960; 1.07]	0.68
Platelet (+0.1)	0.975 [0.951; 1.000]	0.047
Total count (+1000)	0.952 [0.875; 1.04]	0.26

Sensitivity / Specificity Tables - 7

Age

Threshold	Sensitivity	Specificity
0	100% [97% - 100%]	0% [0% - 0.5%]
9	100% [97% - 100%]	0% [0% - 0.5%]
18	100% [97% - 100%]	1.8% [0.94% - 3%]
27	17% [11% - 25%]	83% [80% - 85%]
36	0.89% [0.023% - 4.9%]	98% [97% - 99%]
45	0% [0% - 3.2%]	100% [100% - 100%]

HB

Threshold	Sensitivity	Specificity
0	100% [100% - 100%]	0% [0% - 3.2%]
4	100% [99% - 100%]	0% [0% - 3.2%]
7	96% [94% - 97%]	8.9% [4.4% - 16%]
11	37% [34% - 41%]	79% [70% - 86%]
15	0.14% [0.0034% - 0.75%]	100% [97% - 100%]
18	0.14% [0.0034% - 0.75%]	100% [97% - 100%]

Platelet

Threshold	Sensitivity	Specificity
0.0	100% [97% - 100%]	0% [0% - 0.5%]
1.3	98% [94% - 100%]	1.1% [0.47% - 2.1%]
2.7	57% [47% - 66%]	49% [45% - 53%]
4.0	11% [5.7% - 18%]	95% [93% - 96%]
5.4	1.8% [0.22% - 6.3%]	99% [98% - 100%]
6.7	0.89% [0.023% - 4.9%]	100% [100% - 100%]

Total Count

Threshold	Sensitivity	Specificity
0	100% [97% - 100%]	0% [0% - 0.5%]
4000	98% [94% - 100%]	0.81% [0.3% - 1.8%]

DISCUSSION-

SCD being a national subject of discussion since the implication of the project under the National Rural and urban health program 2006. Total tribal population residing in India with different blood lineage and origin and living in different vicinities in 29 states of India. Our Institutional Study shows a linear correlation between Age, Hb Platelet and Total count with significant relation between them. Solubility test has proven to be a gold Standard Screening tool to give a predictive analysis of HbS cases.

Screening being an important standard at community based was implicated by majority of researchers. Delivering health care to tribal populations is a challenge and a village based model has been described in Bardoli in Gujarat where an outreach program is being undertaken with the help of a mobile clinical unit and a local villager has also been given basic health care training to regularly visit and monitor sickle cell

disease patients and send those with significant complications to the hospital coordinating the program Most of the early studies on epidemiology of sickle hemoglobin in different parts of the country used the sickling or the solubility test and in many reports this was followed by Hb electrophoresis to determine the phenotypes.[9-13]

Conflict Of Interest:-
The authors have no conflict of interest to declare.

Acknowledgments:-
The authors thank to Technicians and the collection team for sample processing and validation.

Financing:- There is no external financing during the project.

REFERENCES-

1) Agarwal MB, Mehta BC. Sickle Syndrome – A study of 44 cases from Bombay. Indian Pediatrics.
2) Patel AG, Shah AP, Sorathiya SM, Gupte SC. Hemoglobinopathies in South Gujarat population and incidence of anemia in them. Indian J Hum Genet 2012; 18: 294-8.
3) Micromachines 2021, 12, 519. https://doi.org/10.3390/mi12050519
4) Sickle Cell Disease in Pregnancy: Trend and Pregnancy Outcomes at a Tertiary Hospital in Tanzania; Projestine S. Muganyizi ,Hussein Kidanto; https://doi.org/10.1371/journal.pone.0056541
5) Kuntal Devesh Patel et al. Prevalence of Sickle Cell Anemia in Pregnancy: A Prospective Study in Tertiary Health Center. International Journal of Science and Healthcare Research Vol.2; Issue: 3; July-Sept. 2017
6) Dr Satyabrata Patra, MD et al JMSCR Volume 05 Issue 01 January 2017 Page 16056
7) Vasikar et al 2012, Efficacy of Solubility test in Diagnosis of Sickle cell Disorders Journal of Research in Medical Education and ethics Vol.2, No3, November 2012, pp-214-216
8) NHM Guidelines for Prevention and Control of Hemoglobinopathies, Ministry of Health & Family Welfare Government of India
9) Study of effectiveness of NESTROFT ANS Solubility test as a screening test for the detection of haemoglobin disorders at Nanded Region, IJHSR, Vol.4; Issue 9; September 2014
10) Raman V, et al. BMJ Global Health 2021;6:e004322. doi:10.1136/bmjgh-2020-004322
11) Colah RB, Mehta P, Mukherjee MB. Newborn screening for sickle cell disease: Indian experience. Int J Neonatal Screen 2018; 4:31.
12) Colah R, Surve R, Nadkarni A, et al. Prenatal diagnosis of sickle syndromes in India: dilemmas in counselling. Prenat Diagn 2005; 25:345–9.
13) Nimgaonkar V, Krishnamurti L, Prabhakar H, et al. Comprehensive integrated care for patients with sickle cell disease in a remote Aboriginal tribal population in southern India. Pediatr Blood Cancer 2014; 61:702–5.