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Original Article

Utility of basic hematological indices in early detection of hemoglobinopathies in paediatric population of southern India

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ABSTRACT

Background: Cases diagnosed as microcytic hypochromic in paediatric age group will be subjected to basic hematological indices and further confirmed in cases of high suspicion through hemoglobin electrophoresis and High-Performance Liquid Chromatography. This study aimed to identify simple indices and cost-effective methods to detect undiagnosed cases of thalassemia syndromes being treated for nutritional deficiency anemias.

Materials and Methods: The study included 100 pediatric patients, from a period of January 2019 to June 2020. Case selection was based on children whose hemoglobin levels were below the cutoff value according to age. They were further subjected to Sehgals and Mentzers indices and suggestive cases were subjected to gel electrophoresis and High-Performance Liquid Chromatography.

Results: 43% of the cases showing abnormality on screening hematological indices were further subcategorized on gel electrophoresis and confirmed on High-Performance Liquid Chromatography. 27% of the cases were diagnosed to be β Thalassemia Trait, which constituted the most frequent type of hemoglobinopathy detected. There were 8% of the cases diagnosed to be as Sickle Cell disease which included major and minor forms. 5% of the study cases were diagnosed as β Thalassemia major. 3% of the cases were diagnosed as HbE disease, HbE trait and 8β Thalassemia.

Conclusions: Hematological indices can be used effectively for early detection of suspected cases of hemoglobinopathies.

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INTRODUCTION

Hemoglobinopathies are the most common single gene disorders which can be quantitative (Thalassemia Syndrome) or qualitative (variant HbS) and are a major burden to humanity particularly in South East Asian countries like India. The World Health Organisation (WHO) figures estimate a total of 5% of the world population as a carrier of Hemoglobin disorders, with the cumulative gene frequency of hemoglobinopathies in India estimated to be 4.2% with a population of over 1.2 billion and over 12,000 infants born each year with a clinically significant hemoglobinopathy.[1,2]

The Indian Council of Medical Research (ICMR) estimates an overall incidence of β-thalassemia trait to be 2.78%, exhibiting geographical variations.[3] HbS prevalence is seen in Orissa, HbE in West Bengal, and HbD among the Sikhs and Punjabis, HbE is highest in northeast states of India constituting 16.2% to 47.3%, and is one of the highest in the world.[3] India spends nearly 1000 crore per annum in the treatment of Thalassemia patients.[4] The clinical manifestations of these Hemoglobinopathies vary from lifelong transfusion-dependent anemia with multi-organ system involvement and severely reduced life expectancy to asymptomatic carriers. Identification and management of these disorders are important epidemiologically and aid in alleviating the psychological and economic burden associated with their longevity.[5]

The majority of the urban health centers in India use conventional methods for screening and diagnosis, which include clinical and family history, laboratory investigations like complete blood counts, red cell indices, sickling test, estimation of HbF, and HbA2 through Hemoglobin Electrophoresis and HPLC.[6] In developing countries predominant subset of pathologists and physicians, particularly untrained residents who are posted in rural areas with minimal diagnostic methods are unable to pick up the cases based on blood count parameters alone. These basic thalassemia indices help raise suspicion and patients can be referred to higher medical centers at urban cities for further testing and genetic counseling.

MATERIALS AND METHODS

After obtaining the Institutional ethical committee clearance, a study was conducted for a duration of 18 months (January 2019 to June 2020) with a sample size of 100 pediatric patients (from birth to 18 years).

This study included children (newborn to 18 years) attending the outpatient and inpatient department of pediatrics in various hospitals in southern India. Venous blood samples collected in EDTA vials and received in the department of hematology were run on an automated analyzer SYSMEX XT-2000i. The SYSMEX XT-2000i is based on the principle that Sulfolyser is added to hemolyse the red blood cells and the hemoglobin is converted to SLS-Hb (sodium lauryl sulphate-Hb). The concentration of SLS-Hb is measured as light absorbance and calculated by comparison with the absorbance of the diluent measured before the sample was added. The following parameters were recorded: Hematocrit, RBC count, WBC count, Platelet count, RBC indices -mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and red cell distribution width (RDW). After obtaining complete blood count (CBC), a peripheral smear was prepared using Leishman stain.

The samples were subjected to two discrimination indices, Sehgal (MCV X MCV/RBC count) and Mentzer (MCV/RBC), and values <972 and <13 were taken as cut off for thalassemia and hemoglobinopathies respectively. EDTA blood samples were procured simultaneously for Hemoglobin electrophoresis, HELENA SAS-MX EPS 600 Platinum. Under each run of the machine, 9 tests can be performed each time with one control sample. SAS-MX Acid/alkaline Hb gel is a qualitative system for the identification of hemoglobin bands. Using a control material containing Hb A, S and A2, the same band patterns were seen within a single gel and between different gels. No bands were missing and no additional bands were observed between applications. It has a sensitivity of 0.08 g/dL per band, determined as the lowest concentration of hemoglobin which was evident as a discrete band on the completed gel. It shows a linearity of 4 g/dL per band, based upon the maximum concentration of hemoglobin per band which allows separated bands to be satisfactorily resolved from each other without protein overloading

Interpretation of Results for Acid/Alkaline Hb gel electrophoresis:

Qualitative evaluation is where the possible identity of the hemoglobin types present in the samples can be determined by visual evaluation of the completed gel. The Hemo controls provide a marker for band identification. Figure 1.01 Shows commonly encountered hemoglobin types. Quantitative evaluation is the relative percent of each hemoglobin type on the gel which can be determined by densitometry of the completed gel at 595nm.

The patients' EDTA samples were also subjected to HPLC using the BIORAD Variant D-10 for further confirmation. Biorad Variant D-10 Hemoglobin testing works on the principle of HPLC (High Performance Liquid Chromatography).

In the D10 HPLC, the samples are automatically diluted, injected into the analytical flow path, and applied to the analytical cartridge. The D-10 delivers a programmed buffer gradient of increasing ionic strength to the cartridge, where the hemoglobins are separated based on their ionic interactions with the cartridge material. The separated hemoglobin fraction is measured by photometer.

The D10 software performs reduction of raw data collected from each analysis. The raw data are integrated by the Clinical Data Management software (Bio-Rad Laboratories), and a chromatogram/sample report is generated. The integrated peaks are assigned to manufacturer-defined windows derived from the retention time, i.e., the time in minutes from sample injection to the maximum point of the elution peak, of normal hemoglobin fractions and common variants (fig. 1.02). If a peak elutes at a retention time not predefined, it is labeled as an unknown.

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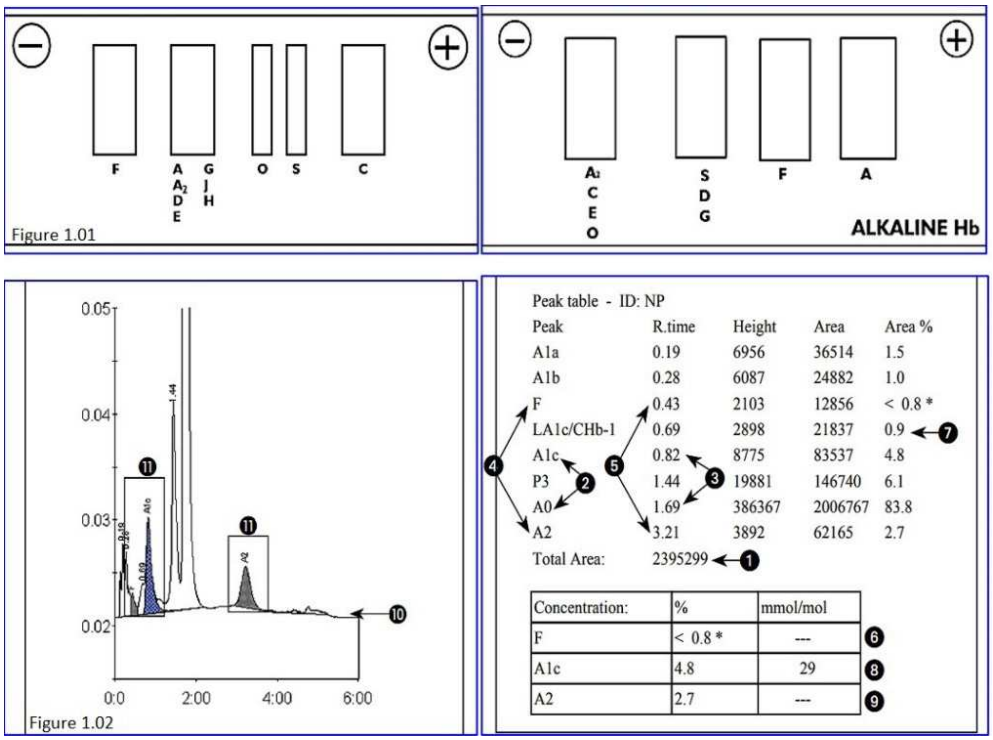

Figure: *1.01,1.02 shows common encountered Hemoglobin types on acid and alkali gel electrophoresis, And Chromatogram exhibiting normal Retention windows (BIORAD VARIANT D10)*

Statistical Package for Social Sciences [SPSS] for Windows, Version 22.0. was used to perform statistical analyses. Descriptive statistics (categorical data in terms of number & percentage whereas in Mean, SD & SEM for continuous data) and inferential-Statistics were employed. Independent Student t test was used to compare the mean values of study parameters between Hemoglobinopathy and Iron deficiency Anemia. And similarly, comparison of mean values of different types of Hemoglobin estimated by Electrophoresis and HPLC Report and also between Sickle cell disease & trait was done using the same test.

Mann Whitney U test was used to compare the Mean values of different types of Hemoglobin between β Thalassemia disease and trait. Sensitivity & Specificity Analysis was done to estimate the accuracy between different indices and procedures for detecting the actual Patients with Hemoglobinopathies. The level of significance [P-Value] was set at P<0.05.

RESULTS

Out of the 100 pediatric cases, the most common age group were in the 11-18 years, followed by 1-5 years. There was a slight female preponderance. Generalized weakness was the most common presenting symptom. 32% of the children had positive parent history. 12 children had organomegaly and history of blood transfusion was present in 13 children. (Table 1.0)

Table 1: Distribution of Demographic and Medical History characteristics among the study patients.

Variables	Categories	n	%
Age group	<1 yr	6	6%
	1 - 5 yrs	42	42%
	6 - 10 yrs	7	7%
	11 - 18 yrs	45	45%
Sex	Males	49	49%
	Females	51	51%
Past history	Dyspnea	5	5%
	Gen. Weakness	52	52%
	Hematuria & Abd.Pain	7	7%
	Loss of Apetite	2	2%
	Icterus	1	1%
	No Rel. History	33	33%
Parent history	Present	32	32%
	Nil	68	68%
Clinical findings	Mild Hepatosplenomegaly	5	5%
	Hepatosplenomegaly	7	7%
	NAD	88	88%
H/O blood transfusion	Yes	13	13%
	No	87	87%

Peripheral smears stained with Leishman's and visualized on 100x of Olympus CX21i showed predominantly microcytic hypochromic blood picture (92 cases). 8 cases showed drepanocytes. The mean parameters MCV, RBC and Hb values of Iron Deficiency Anemia and Hemoglobinopathies were calculated and were compared using independent student t test for mean difference. There was statistical significance in parameters MCV and RBC count with a p value of <0.001 and <0.001 respectively. (Table 2.0)

Table 2: Comparison of mean values of study parameters b/w Hemoglobinopathy and ID Anemia conditions using Independent Student t-test .

Parameters	Condition	N	Mean	SD	S.E.M	Mean Diff	t	P-Value
Hb-gm/dl	Hemoglobinopathy	43	8.81	1.27	0.19	-0.60	-2.470	0.02*
	ID Anaemia	57	9.41	1.15	0.15			
MCV	Hemoglobinopathy	43	56.04	2.64	0.40	-3.90	-7.734	<0.001*
	ID Anaemia	57	59.94	2.39	0.32			
RBC	Hemoglobuinopathy	43	5.13	0.19	0.03	0.38	10.290	<0.001*
	ID Anaemia	57	4.75	0.17	0.02			

The mean value of various hemoglobins obtained by hemoglobin electrophoresis and HPLC were compared using Independent Student t test, and the results showed insignificant mean difference. (Table 3.0)

Table 3: Comparison of mean values of types of Hemoglobins obtained from Electrophoresis and HPLC using Independent Student t test

Parameters	Method	N	Mean	SD	S.E.M	Mean Diff	t	P-Value
HbA	Electrophoresis	100	88.80	20.20	2.02	2.01	0.648	0.52
	HPLC Report	100	86.79	23.63	2.36			
HbA2	Electrophoresis	100	3.28	3.10	0.31	-0.23	-0.519	0.60
	HPLC Report	100	3.51	3.12	0.31			
HbF	Electrophoresis	100	5.97	18.53	1.85	2.12	0.930	0.35
	HPLC Report	100	3.85	13.23	1.32			
HbS	Electrophoresis	9	40.61	22.91	7.64	4.80	0.474	0.64
	HPLC Report	11	35.81	22.24	6.70			

In both the procedures, the most frequent hemoglobinopathy to be diagnosed was β Thalassemia Trait constituting to 27% of the cases. There were 2 cases of Sickle cell disease and 6 cases of Sickle cell Trait diagnosed by Gel Electrophoresis and which was confirmed by HPLC. There were 5 cases of β Thalassemia major diagnosed by both Gel Electrophoresis and HPLC. 2 cases showed elevation of HbA2 on gel electrophoresis which was confirmed as HbE disease by HPLC. 1 case showed combination disorder of 8β Thalassemia which was confirmed by Allele specific PCR technique. (Table 4.0)

Table 4: Frequency distribution of different diseases in study patients as diagnosed by different procedures.

Method	Diagnosis	n	%
Electrophoresis Diagnosis	Acid gel S/d band elevation	1	1%
	HbE Elevated	2	2%
	N/E Hemoglobinopathy	57	57%
	Sickle cell disease	2	2%
	Sickle cell trait	6	6%
	B-Thalassemia Major	5	5%
	B-Thalassemia trait	27	27%
HPLC Report	HbE Disease	1	1%
	HbE Elevated	1	1%
	N/E Hemoglobinopathy	57	57%
	P3 Window	1	1%
	Sickle cell Disease	2	2%
	Sickle cell Trait	6	6%
	B Thalassemia Major	5	5%
	B Thalassemia Trait	27	27%
Final Diagnosis	HbE Disease	1	1%
	HbE Trait	1	1%
	N/E Hemoglobinopathy	57	57%
	Delta-B Thalassemia	1	1%
	Sickle Cell Disease	2	2%
	Sickel cell Trait	6	6%
	B Thalassemia Major	5	5%
	B Thalassemia Trait	27	27%

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We performed Sensitivity and Specificity analysis for estimating the accuracy of Sehgal (Table 5) and Mentzer Index (Table 6) Vs HPLC Report in detecting the patients with Hemoglobinopathy, ROC is a plot of the true positive rate against the false positive rate for the different possible cut of points of a diagnostic test. (fig.2.0)

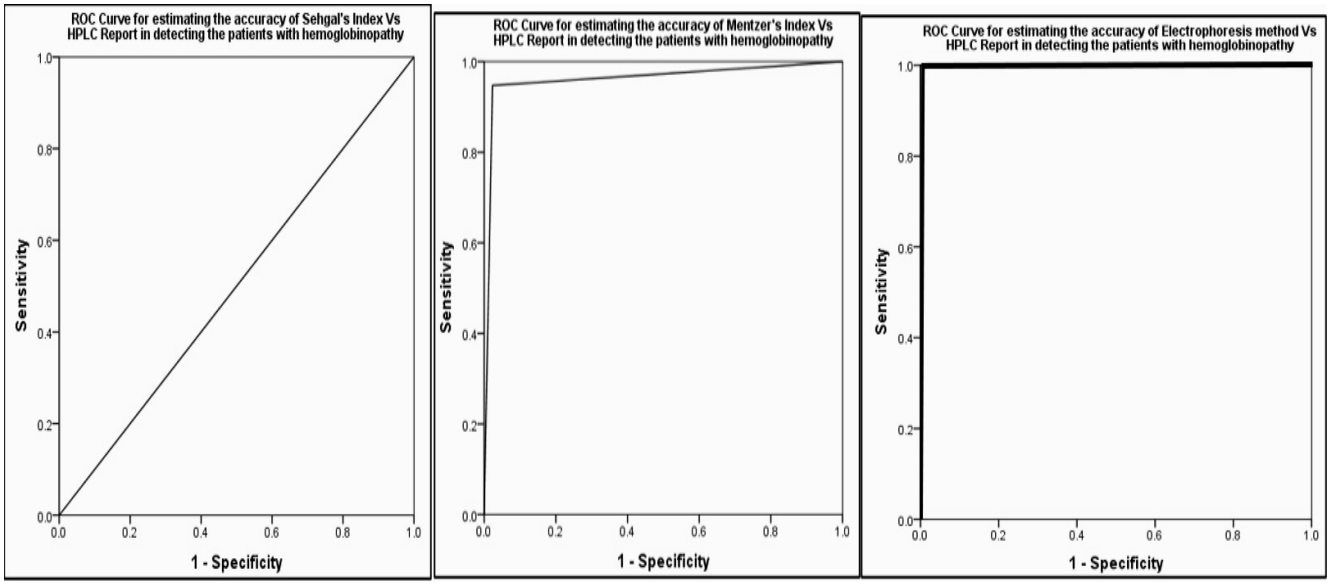

Figure 2.0: *ROC curves for estimating accuracy of Sehgal's, Mentzer and Electrophoresis.*

Accuracy is measured by the area under the ROC curve. An area of 1 represents a perfect test. AUC of Seghal index was 0.5 thus represents a worthless test. A rough guide for classifying the accuracy of a diagnostic test is .90 is excellent(A) and .80-90 is good(B). (Tables 5.0-6.0)

Table 5.0: Sensitivity and Specificity analysis for estimating the accuracy of Sehgal Index Vs HPLC Report in detecting the patients with hemoglobinopathy.

Sehgal's Index	HPLC Report		Total
	Hemoglobinopathy	ID Anaemia	
Hemoglobinopathy	43	57	100
ID Anaemia	0	0	0
Total	43	57	100

Contd.

Diagnostic Values of Sehgal Index	%	95% CI	
		Lower	Upper
Sensitivity	100.0%	91.8%	100.0%
Specificity	0.0%	0.0%	6.3%
PPV	43.0%	33.1%	53.3%
NPV	..	..	..
Accuracy	50.0%	39.0%	52.0%

Table 6: Sensitivity and Specificity analysis for estimating the accuracy of Mentzer Index Vs HPLC Report in detecting the patients with hemoglobinopathy.

Mentzer's Index	HPLC Report		Total
	Hemoglobinopathy	ID Anaemia	
Hemoglobinopathy	42	3	45
ID Anaemia	1	54	55
Total	43	57	100

Contd.

Diagnostic Values of Mentzer Index	%	95% CI	
		Lower	Upper
Sensitivity	97.7%	87.7%	99.9%
Specificity	94.7%	85.4%	98.9%
PPV	93.3%	82.3%	97.7%
NPV	98.2%	88.6%	99.7%
Accuracy	96.0%	91.0%	100.0%

Contd.

AUC for Mentzer Index	Std. Error	P-Value	95% CI	
			Lower	Upper
0.96	0.02	<0.001*	0.91	1.00

The Sensitivity and Specificity analysis for estimating the accuracy of Electrophoresis Vs HPLC Report in detecting the patients with hemoglobinopathy were estimated. And area under curve were tabulated for electrophoresis when compared with HPLC. [Tables 7.00- 7.02]

Table 7.00: Sensitivity and Specificity analysis for estimating the accuracy of Electrophoresis Vs HPLC Report in detecting the patients with hemoglobinopathy.

Electrophoresis Method	HPLC Report		Total
	Hemoglobinopathy	ID Anaemia	
Hemoglobinopathy	43	0	43
ID Anaemia	0	57	57
Total	43	57	100

Table 7.01

Diagnostic Values of Electrophoresis Index	%	95% CI	
		Lower	Upper
Sensitivity	100.0%	91.8%	100.0%
Specificity	100.0%	93.7%	100.0%
PPV	100.0%	96.9%	100.0%
NPV	100.0%	97.4%	100.0%
Accuracy	96.0%	91.0%	100.0%

Table 7.02

AUC for Electrophoresis Index	Std. Error	P-Value	95% CI	
			Lower	Upper
1.00	0.00	<0.001*	1.00	1.00

The mean values of different types of Hemoglobin between Sickle cell disease and trait were compared using Independent Student t test [Table 8.0], along with Mean values of different types of Hemoglobin between β Thalassemia disease and trait which were also tabulated using Mann Whitney U test. [Table 9.0] All the results showed statistical significance.

Table 8: Comparison of Mean values of different types of Hemoglobin between Sickle cell disease and trait using Independent Student t test.

Hemoglobins	Diagnosis	N	Mean	SD	S.E.M	Mean Diff	t	P-Value
HbA	Sickle Cell Disease	2	2.25	0.35	0.25	-59.72	-14.085	<0.001*
	Sickle cell Trait	6	61.97	5.69	2.32			
HbA2	Sickle Cell Disease	2	2.20	1.13	0.80	-1.23	-2.574	0.04*
	Sickle cell Trait	6	3.43	0.45	0.19			
HbF	Sickle Cell Disease	2	17.50	3.54	2.50	16.00	12.184	<0.001*
	Sickle cell Trait	6	1.50	0.78	0.32			
HbS	Sickle Cell Disease	2	78.05	2.76	1.95	44.95	10.406	<0.001*
	Sickle cell Trait	6	33.10	5.66	2.31			

Table 9: Comparison of Mean values of different types of Hemoglobin between B Thalassemia disease and trait using Mann Whitney U test.

Hemoglobins	DIAGNOSIS	N	Mean	SD	S.E.M	Mean Diff	Z	P-Value
HbA	B Thalassemia Major	5	39.88	31.88	14.26	-53.25	-3.411	0.001*
	B Thalassemia Trait	27	93.13	3.96	0.76			
HbA2	B Thalassemia Major	5	7.62	12.07	5.40	3.03	-1.695	0.09
	B Thalassemia Trait	27	4.59	0.81	0.16			
HbF	B Thalassemia Major	5	52.50	34.03	15.22	50.72	-3.459	0.001*
	B Thalassemia Trait	27	1.78	2.88	0.55			

DISCUSSION

In our study, 45% of the children were in the age group of 11 to 18 years and 6% were in the age group of less than a year as most of hemoglobinopathies goes undetected with manifestation of significant clinical symptoms and signs beyond 1 year.[7]

Balgir et al. found a majority of the cases of hemoglobinopathy belong to the reproductive age group, that is 16 to 45 years, followed by the neonatal to childhood period (0-15 years).[8,9] Slight preponderance of female children was observed constituting 51% of the cases. Predominant studies reported male preponderance Niraj K M et al and Manan et al reported a marginal male predominance.[10,11] Yagnik and Balgir reported male preponderance of the study cases as well.[9,12] This disparity could be due to cultural practices exisiting amongst south Indian population particularly the tribes where gender roles plays a significant part in accessibility of healthcare.

Generalized weakness was the main complaint constituting to 52% of the cases, followed by no relevant significant history constituting 33 % of cases, one patient had presented with icterus due to hemolysis. Hematuria and abdominal pain were seen in 7% of the children, and on further analysis they were found to have sickle cell disease and sickle cell trait who were on oxygen stress due to sulphonamides or high altitudes, correlating with study by Herklotz R et al.[13] 32% of the cases in the present study had evidence of genetic carrier history. 68% of the parent's genetic studies were unavailable.

Screening of individuals at increased risk of being carriers for thalassemias and hemoglobinopathies can help identify

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couples who have a 25% risk of having a pregnancy with a significant genetic disorder and for these cases prenatal diagnosis is possible.[14]

Ethnicity information and parental studies are extremely helpful in guiding the sequence of diagnostic tests for specific hemoglobinopathies. Parental testing to identify carriers for the purpose of defining an infant's diagnosis and providing genetic counseling typically includes a complete blood cell count and hemoglobin separation by IEF and/or HPLC. Carriers of β thalassemia mutations show a decreased mean corpuscular volume (MCV) and increased levels of Hb A2 and/or Hb F. Thus, accurate quantification of Hb F and Hb A2 is needed if the MCV is decreased.[15]

Mild and moderate hepatosplenomegaly was found in 5% and 7% of the children respectively. Thalassemia major children had moderate enlargement of the liver and spleen which constituted 7% of the cases and two cases of Sickle cell disease who presented as an early case had moderate enlargement of the liver and spleen. Sickle cell trait children had mild hepatosplenomegaly which was an incidental finding on ultrasound scan, correlating with Madan N et al and Shah S J et al.[12,16]

History of blood transfusion was present in 13% of the cases, in these cases quantification of HbF and HbA2 were inaccurate. After thorough history taking, repeat testing was performed after 3 months and a diagnosis of β thalassemia major and β thalassemia trait were made. The importance of elucidating this history lies in the fact that these abnormal fractions of hemoglobins cannot be quantified correctly on HPLC as well as gel electrophoresis and such children are advised to repeat testing after 3 months of receiving blood transfusion.[7,17]

Out of the 43 Hemoglobinopathies detected using above methods, Mean hemoglobin levels was 8.81g/dL. And out of 57 cases of Iron Deficiency Anemia, the Mean hemoglobin value was 9.41g/dL. The p-value calculated was 0.02 which was not statistically significant. Rathod et al. and Tripathi et al. showed a similar sensitivity of 95.56%, 22.35% and specificity of 91.89%, 22.32 % respectively in their study. The cut-off was sensitive in picking up the cases but not specific. [18,19,20]

Mean MCV obtained in Hemoglobinopathies was 56.04% and in IDA it was 59.94% with p-value of <0.001 which was significant, suggesting that MCV values are sensitive and specific in picking up cases of BTT. Rathod et al., Tripathi et al., Parthasarathy et al.'s study showed a sensitivity and specificity of 93.7%, 96.6%; 78.92%, 88.69%; 94.87%, 65.21% respectively. Our study showed a sensitivity of 95.65% and specificity of 94.67% with significant p-value of <0.001.[18, 19, 20]

The mean RBC count obtained in our study was 5.13 (million/microlitre) in Hemoglobinopathies and 4.75 (million/microlitre) in IDA with a sensitivity of 85.56%, and specificity of 92.32% and a p-value of <0.001 which was similar to study by Rathod et al., Tripathi et al., Parthasarathy V et al., which showed sensitivity and specificity of 95.5%, 90.5%; 78.92%, 88.69%; 76.92%, 81.37% respectively.

Evaluation of Thalassemic Indices:

Sehgal index:

Sehgal index cut of <972 was used to differentiate β-Thalassemia Trait (and other hemoglobinopathies) and IDA. Out of the 43 Hemoglobinopathies detected, the sensitivity was 100.0%, which means true positives were picked up easily. However, Sehgal's Index was not able to rule out True negatives hence we got a specificity of 0.0%. All the cases studied had a cut-off below the reference range of 972. The PPV was 43.0%. NPV was nil with an accuracy of 50.0%. [Table 10.0]

Mentzer Index:

Cut off used was 13. Less than 13 were considered as suggestive of Thalassemia syndrome.

Sensitivity obtained in our study was 97.7%, specificity was 94.7%, PPV 93.3%, NPV 98.2% and using the Mentzer's index one could diagnose a case with an accuracy of 96.0%. comparison with other studies yielded similar results. [Table 10.0]

Table 10.0: Comparison of Thalassemia Indices with other studies.

	RBC Index	Cut off	Cut off in study	Se(%)	Sp(%)	PPV(%)	NPV(%)	ARUC
Our study	MI	<13	<13	97.70%	94.70%	93.30%	98.20%	0.96
Tripathi N et al[20]	MI	<13	<12.45	85.41%	81.75%	56.60%	95.30%	0.879
Ebrahim et al[31]	MI	<13	<13	72.00%	82.00%	68.00%	67.00%	0.819
Sahli et al[33]	MI	<13	<12.5	77.00%	100.00%	88.00%	82.00%	0.954
Sehgal et al[32]	MI	<13	<12	58.97%	95.65%			0.77
Sehgal et al[32]	MI	<13	<13	76.90%	87.00%			0.82
Our study	Sehgal's	<972	<972	100.00%	0.00%	43.00%	-	0.5
Sehgal et al[32]	Sehgal's	<972	<972	89.74%	86.96%			0.8

Hemoglobin electrophoresis:

With Alkaline Agarose gel testing, some common hemoglobin variants co-migrate such as HbC, HbE, HbA2, HbO-Arab, and co-migration is seen with HbS, HbD, HbG.

To separate some hemoglobin variants that commonly co-migrate at alkaline conditions, the sample should be analyzed on gel at an acidic pH (6.0), in these conditions molecular charge will differ and migration patterns will change.

As a result, HbS can be differentiated from HbD, and HbC can be differentiated from HbE.

β thalassemia trait was the most frequent type of hemoglobinopathy diagnosed constituting 27%. The migration on bands was simple, and densitometric scans were able to quantify them accurately when compared to HPLC. 5 cases of β Thalassemia Major initially subjected to Electrophoresis on Alkaline pH showed a predominant migration band in the fetal position, which was later confirmed by Acid Gel Electrophoresis. The results were similar in HPLC.

Two cases of HbE were diagnosed as elevated levels of HbA2 in Electrophoresis as HbE co-migrates with HbA2, which was further confirmed by HPLC. Two cases of sickle cell disease were identified on Acid Gel Electrophoresis showing S band migration. Three cases of Sickle cell trait were diagnosed on Acid gel Electrophoresis. One case of significant HbA2 elevation on Gel Electrophoresis was confirmed as a combination of βδ Thalassemia by genetic studies.

Initial recommended manual method such as Alkaline gel electrophoresis can be used where cost is a limiting factor.[21] However the precision and accuracy of HbA2 measurements using densitometric scanning of electrophoretic gels is poor, especially when compared with HPLC techniques which showed a CV of 33.6% for densitometric scanning of electrophoretic gels at a HbA2 concentration of 2.41%. For column chromatography, the CV is 14.6% at a mean HbA2 concentration of 3.21%, and for HPLC, the CV is 4.3% at a mean HbA2 of 3.47%. However, most of the laboratories use combination of both Gel Electrophoresis and HPLC. [22]

High Performance Liquid Chromatography:

The results were similar to that of gel electrophoresis, however, two cases of HbE were detected by HPLC only. One case of δβ Thalassemia was diagnosed only in genetic studies, similar to Mandal et al.[2-,4] HbE results from a beta chain mutation (β26Glu→Lys) and tends to elute in A2 window on HPLC. It is the most common hemoglobin variant in southeast Asia and second most prevalent worldwide.[7]

HbE homozygotes have HbE values of usually 70-90%, and clinical features include anemia, with a blood picture showing microcytosis and moderate amount of codocytes. HbE heterozygotes individuals are normal, and HbE levels are less than 40%.

Eight cases of Sickle cell disease including traits were identified by HPLC. HbS homozygous presented as an S window, with an abnormal HbS values between 70-90%. Values of HbF generally are raised in most cases. One case of delta beta Thalassemia was identified by molecular studies. This variant usually elutes in HbA2 window.[6]

Delta-beta thalassemia and hereditary persistence of fetal hemoglobin (HPFH) constitute a heterogeneous group of disorders characterized by absent or reduced synthesis of adult hemoglobin (Hb A) and increased synthesis of fetal hemoglobin (Hb F). The distinction between HPFH and delta-beta thalassemia is subtle and should be confirmed by alpha-beta-globin chain synthesis ratio and DNA analysis since the distinction between these two conditions is not always possible from routine hematologic analyses. It is important to differentiate between these two conditions especially in antenatal screening because HPFH is clinically asymptomatic, but interaction of δβ-thalassemia with β-thalassemia can result in a severe disorder. [24]

The sensitivity specificity obtained (when comparing Gel Electrophoresis with HPLC) were 100%, 100% respectively. Alkaline and Acid gel electrophoresis results were equable with HPLC. However, co-migration of HbE with HbA2 were seen in two cases which had to be confirmed by HPLC and capillary electrophoresis.

This method is sensitive and specific for the detection of hemoglobinopathies. However, the process is labour intensive. The diagnostic accuracy of this method when compared with HPLC was 96.0%, with a p-value of <0.001 and AUC of 1.00.

Despite having a global prevalence of 5% of the world population,[27] Alpha Thalassemia are easily missed out during routine screening as it goes undetected in blood count parameters and gel electrophoresis.[28]

Routine HPLC and capillary electrophoresis can detect migration of Hb Bart's and HbH hemoglobins as they migrate faster than HbA on alkaline electrophoresis, however alpha thalassemia trait and silent carrier will exhibit a normal electrophoretic pattern and require a thorough family history, and the diagnosis is made by excluding iron deficiency anemia, anemia of chronic disease and other thalassemias.[29]

A definitive diagnosis of alpha or beta thalassemias require globin chain synthesis studies or molecular methods like Allele-specific PCR, Dot Blot analysis, mismatched analysis, and DNA sequencing methods.[30]

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CONCLUSIONS

The mathematical RBC indices (Sehgal's and Mentzer's) had a sensitivity, specificity, and diagnostic accuracy similar with that of HPLC method and were found to be statistically significant. These basic RBC indices (Sehgal and Mentzer's) can be used for screening cases. HPLC forms a rapid, accurate, and reproducible method for early detection and management of hemoglobinopathies and its variants. Gel Electrophoresis produces equable results.

Conflict of Interest: None

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