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SPECTRUM OF HEMOGLOBINOPATHIES IN SOUTHERN ODISHA USING HPLC- A 1 AND HALF YEAR INSTITUTIONAL STUDY(ANALYSIS OF 1658 CASES)

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ABSTRACT

Introduction: Hemoglobinopathies are one of the major public health problems in the state of odisha evidenced from the fact that there are 511 transfusion dependant patients registered in Blood bank of MKCG Medical college and hospital, Berhampur. They also cause significant morbidity and mortality in the population. A plethora of variant haemoglobins have been described in the multi-ethnic Indian population. Detection of asymptomatic carriers by reliable laboratory methods is the cornerstone of prevention of this serious health problem. **Aim:** (i) To determine the spectrum of hemoglobinopathies diagnosed by High performance liquid chromatography (HPLC) Bio-rad Variant II. (ii) To report the cases of rare variants of haemoglobin if any. **Material & Methods:** This study was a prospective study done at Department of pathology, MKCG Medical College and Hospital, Berhampur from September 2016 to March 2018. A total of 1658 clinically diagnosed and undiagnosed cases of hemoglobinopathies including suspected cases of hemolytic anemia were subjected to detailed clinical and routine haematological evaluation. **Results:** Sickle cell anemia including both disease and trait constituted the most prevalent form followed by beta thalassemia trait. The rare variants encountered were lepore heterozygous, lepore homozygous, sickle-lepore double heterozygous, hemoglobin E trait, haemoglobin E- beta thalassemia double heterozygous, sickle- haemoglobin D double heterozygous and hemoglobin D homozygous. **Conclusions:** Out of 1658 cases studied, 972 were of hemoglobinopathy (~58.7%). 732 cases of 1658 cases referred to us were of sickle cell anemia (~44.1%).Multidisciplinary approach along with screening, creating public awareness by counselling and mass education can reduce both mortality and morbidity of hemoglobinopathies.

KEYWORDS: Complete blood count, Hemoglobinopathies, HPLC, Sickle cell anemia.

INTRODUCTION

Haemoglobin comprises four globin chains: fetal haemoglobin (Hb F) has two α and two gamma chains (α2γ2) and adult haemoglobin (Hb A) has two α and two β chains (α2β2). Genes in the α-globin and β-globin gene clusters (on chromosomes 16 and 11) control globin-chain production. Due to spontaneous mutation, haemoglobin gene variants are present at low prevalence (carriers 1–1.5/1000) in all sizeable populations.[1] They fall into two broad groups – structural variants that change the amino acid sequence and produce an unusual haemoglobin, and thalassemia that lower or abolish production of globin chains.[2] Hemoglobinopathies can be either quantitative or qualitative.[3] WHO figures estimate that 5% of the world population is a carrier for hemoglobinopathies,[4] and of these, thalassemia syndromes, particularly beta thalassemia major is serious and a major cause of morbidity. The frequency of β-thalassemia in India ranges from 3.5% to 15%, in general, population.[5] Every year 10,000 children with thalassemia major are born in India, which constitutes 10% of the total numbers in the world.[6] Hemoglobinopathies are one of the major public health problems in the state of Odisha, India. They are generally not curable but can be prevented by mass screening, genetic counselling and prenatal diagnosis. Hemoglobinopathies are prevalent in this part evidenced from the fact that there are 511 transfusion dependant patients registered in Blood bank of MKCG Medical college and hospital (MKCG MCH), Berhampur. This paper presents the pattern of hemoglobinopathies amongst the 1658 referral cases came to department of pathology, MKCG MCH for the period from September 2016 to march 2018. The present study was designed with the following aims and objectives in mind: (i) To determine the spectrum of hemoglobinopathies diagnosed by High performance liquid chromatography

(HPLC) Bio-rad Variant II. (ii) To report the cases of rare variants of haemoglobin if any.

MATERIALS AND METHODS

A total of 1658 cases were referred to department of pathology, MKCG MCH were subjected to detailed clinical, routine haematological evaluation. They were diagnosed and undiagnosed, suspected cases of hemoglobinopathy.

2ml. EDTA intravenous blood samples were collected after obtaining informed consent from each patient. Complete blood count using sysmex XT-2000i and peripheral smear examination was done. The sickling test was performed by using freshly prepared sodium metabisulphite solution as reducing agent.[7]

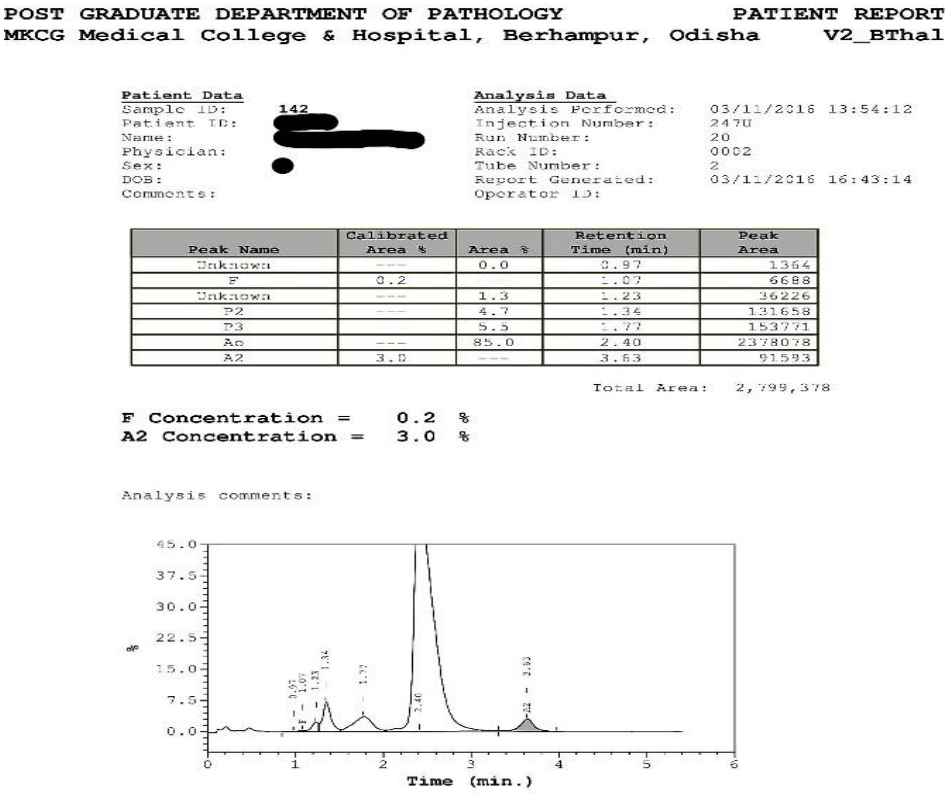
The instrument, known as BIO-RAD 'VARIANT II' (beta thalassemia short program) utilizes the principle of HPLC. An Hb A2/F calibrator and two levels of controls (BIO-RAD) were analysed at the beginning of each run. The total area acceptable was between 1 and 3 million. The software delivers a printed report showing the chromatogram, with all the haemoglobin fractions eluted. The integrated peaks are assigned to manufacturer-defined "windows" derived from specific retention time (RT).This RT is the time that elapses from the sample injection to the apex of the elution peak, of normal haemoglobin fraction and common variants.[8] [Table 1].

Table 1: Manufacturer assigned windows for Bio-Rad variant HPLC system.

Peak name	Window (min)	Retention time (min)
F window	1.00-1.30	1.15
P2 window*	1.30-1.60	1.45
P3 window*	1.60-1.90	1.75
A0 window	1.90-3.30	2.60
A2 window	3.30-3.90	3.60
D window	3.90-4.30	4.10
S window	4.30-4.70	4.50
C window	4.90-5.30	5.10

*Shows P2 and P3 are minor peaks associated with Hb A. HPLC=High performance liquid chromatography

The "windows" are established ranges in which common variants have been observed to elute using the variant beta-thalassemia short program. The printed chromatogram shows all the haemoglobin fractions eluted, the RT, the areas of the peaks, and the values (%) of different haemoglobin components [Figure 1]. If a peak elutes at a RT that is not predefined, it is labelled as an unknown. Each analytical cycle, from sampling to printing of results takes about 6.5 min.[8]


POST GRADUATE DEPARTMENT OF PATHOLOGY PATIENT REPORT
MKCG Medical College & Hospital, Berhampur, Odisha V2_BThal

Patient Data
Sample ID: 142
Patient ID:
Name:
Physician:
Sex:
DOB:
Comments:

Analysis Data
Analysis Performed: 03/11/2016 13:54:12
Injection Number: 247U
Run Number: 20
Rack ID: 0002
Tube Number: 2
Report Generated: 03/11/2016 16:43:14
Operator ID:

Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
Unknown	---	0.0	0.97	1364
F	0.2		1.07	6688
Unknown	---	1.3	1.23	36226
P2	---	4.7	1.34	131658
P3		5.5	1.77	153771
Ao	---	85.0	2.40	2378078
A2	3.0	---	3.63	91593

Total Area: 2,799,378

F Concentration = 0.2 %
A2 Concentration = 3.0 %

Analysis comments:

Figure 1: (a) Chromatogram of normal haemoglobin.

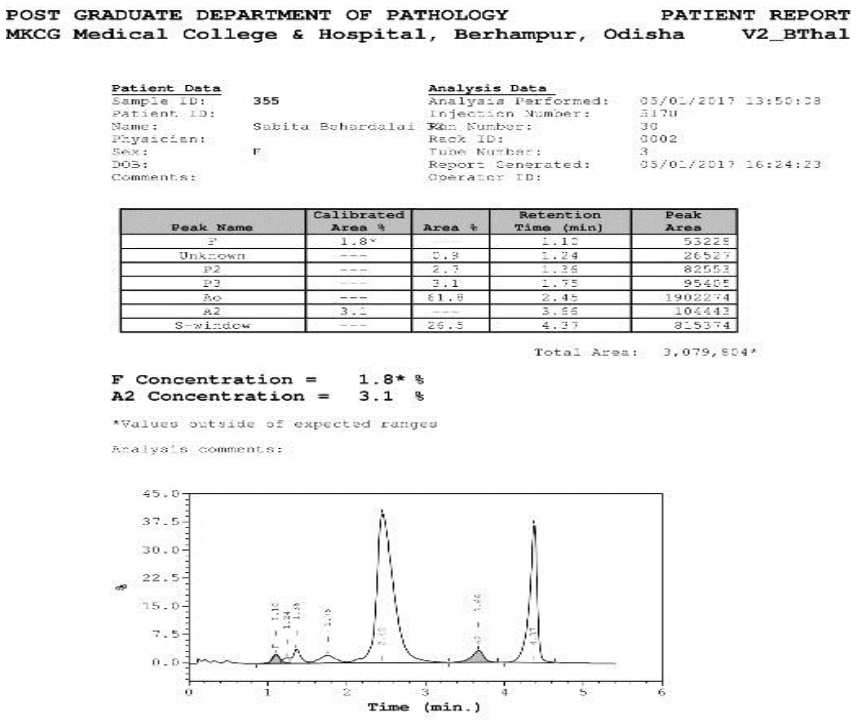
RESULTS

Laboratory records of 1658 patients screened for suspected hemoglobinopathies were analysed. Nine hundred and seventy-two (58.7) showed different abnormal haemoglobin variants. The age group ranged from 3 months to 77 years with a maximum number of patients with hemoglobinopathy in the paediatric age group (till 16 years). Criteria for suspecting hemoglobinopathy in these cases included: results of screening tests such as various discriminant functions so obtained on haematology cell counters, findings obtained from peripheral smear examination, family history, and relevant clinical signs and symptoms suggestive of hemoglobinopathy. The different hemoglobinopathies found are as shown in Table 2.

Table 2: Spectrum of hemoglobinopathies.

Name	No. of cases	%
"No-Hemoglobinopathies" Group	686	41.38
Sickle cell anaemia homozygous	331	19.97
Sickle cell trait	402	24.25
Beta thalassemia trait	104	6.27
Beta thalassemia intermedia	4	0.24
Beta thalassemia major	36	2.17
Hb H	4	0.24
Delta-beta thalassemia heterozygous	3	0.18
Sickle-beta thalassemia	74	4.46
Lepore homozygous	2	0.12
Lepore heterozygous	4	0.24
Sickle-lepore double heterozygous	2	0.12
Hb E Trait	3	0.18
Hb E-beta thalassemia double heterozygous	1	0.06
Hb D homozygous	1	0.06
Sickle-Hb D double heterozygous	1	0.06
Total	**1658**	**100**

We found that a maximum number of patients were of sickle cell trait (24.25%) and sickle cell disease (19.97%). Sickle cell trait was considered in patients with Hb A 50-75%, Hb S 25-40% and Hb F<1% [Figure 2]. They had mild anemia, normochromic normocytic with mild anisopoikilocytosis, and few target cells. The patients with Hb S 70-90%, Hb F 10-30% and Hb A 0-10% were diagnosed as sickle cell disease [Figure 3].


POST GRADUATE DEPARTMENT OF PATHOLOGY PATIENT REPORT
MKCG Medical College & Hospital, Berhampur, Odisha V2_BThal

Patient Data
Sample ID: 355
Patient ID:
Name: Sabita Beharadalai
Physician:
Sex: F
DOB:
Comments:

Analysis Data
Analysis Performed: 05/01/2017 13:50:38
Injection Number: 3170
Run Number: 30
Rack ID: 0002
Tube Number: 3
Report Generated: 05/01/2017 16:24:23
Operator ID:

Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
F	1.8*	---	1.12	53228
Unknown	---	0.8	1.24	26527
P2	---	2.7	1.26	82553
P3	---	3.1	1.75	95405
Ao	---	61.8	2.45	1902274
A2	3.1	---	3.56	104443
S-window	---	26.5	4.37	815374

Total Area: 3,079,804*

F Concentration = 1.8* %
A2 Concentration = 3.1 %

*Values outside of expected ranges

Analysis comments:

Figure 2:

POST GRADUATE DEPARTMENT OF PATHOLOGY PATIENT REPORT
MKCG Medical College & Hospital, Berhampur, Odisha V2_BThal

Patient Data		Analysis Data	
Sample ID:	468	Analysis Performed:	08/02/2017 15:11:57
Patient ID:		Injection Number:	6447
Name:	samual nayak 21	Run Number:	34
Physician:		Rack ID:	0003
Sex:	F	Tube Number:	10
DOB:		Report Generated:	08/02/2017 15:19:43
Comments:		Operator ID:	

Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
P1	---	0.5	0.78	2582
F	23.7*	---	1.13	548148
Unknown	---	0.8	2.17	19734
Ao	---	2.2	2.31	54829
A2	2.5	---	3.67	71350
S window	---	72.6	4.33	1843589

Total Area: 2,540,229

F Concentration = 23.7* %
A2 Concentration = 2.5 %

*Values outside of expected ranges

Analysis comments:

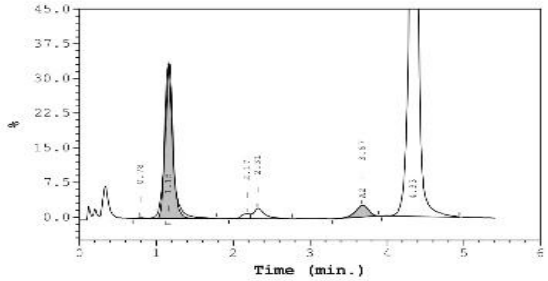


Figure 3:

Beta thalassemia trait was diagnosed based on high levels of Hb A2 (4-8%). These patient presented with mild anemia, low mean corpuscular volume (MCV <80fl), and low mean corpuscular haemoglobin [Figure 4]. Fetal Hb was not increased. Hb A2 of 3.5-3.9% was considered as borderline and were advised iron study with repeat HPLC after iron therapy.

Bio-Rad CDM System PATIENT REPORT
Bio-Rad Variant V-II Instrument #1 V2_BThal

Patient Data		Analysis Data	
Sample ID:	Joyshree Panda	Analysis Performed:	17/09/2016 12:38:40
Patient ID:	1	Injection Number:	210
Name:	Joyshree Panda	Run Number:	5
Physician:		Rack ID:	0009
Sex:	F	Tube Number:	1
DOB:	17/09/2016	Report Generated:	17/09/2016 12:49:14
Comments:		Operator ID:	

Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
F	0.7	---	1.04	8868
Unknown	---	1.1	1.20	16362
P2	---	3.5	1.33	50215
P3	---	4.1	1.73	57771
Ao	---	89.0	2.47	1272613
A2	5.2*	---	3.60	80100

Total Area: 1,425,929

F Concentration = 0.7 %
A2 Concentration = 5.2* %

*Values outside of expected ranges

Analysis comments:

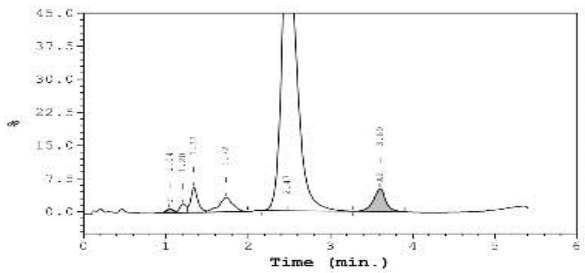
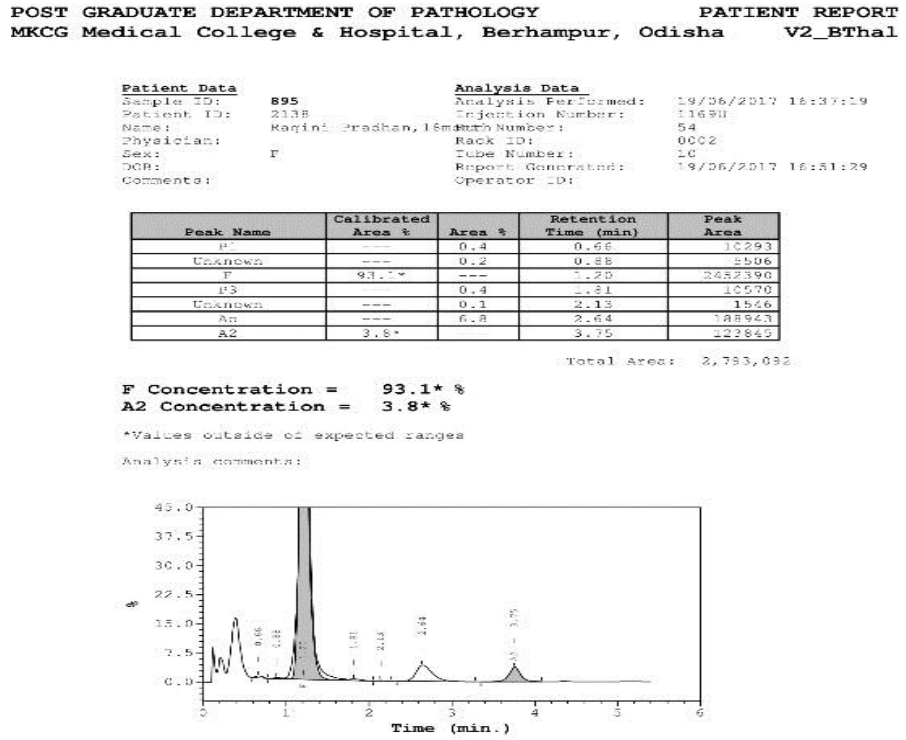


Figure 4:

36 cases of beta thalassemia major were reported. All these patients had raised Hb F values (75%-98%). Clinically presented with severe pallor, blood transfusion dependency and moderate to marked splenomegaly [Figure 5]. 4 cases of beta thalassemia intermedia were diagnosed. They had variable degree of anemia with anisopoikilocytosis and microcytic hypochromic blood picture. Hb F were raised with a variable reduction in Hb A and patients are not transfusion dependent [Figure 6].



POST GRADUATE DEPARTMENT OF PATHOLOGY PATIENT REPORT
MKCG Medical College & Hospital, Berhampur, Odisha V2_BThal

Patient Data
Sample ID: 895
Patient ID: 2138
Name: Ragini Pradhan, 18m
Physician:
Sex: F
DOB:
Comments:

Analysis Data
Analysis Performed: 19/06/2017 16:37:29
Injection Number: 11690
Run Number: 54
Rack ID: 0002
Tube Number: 10
Report Generated: 19/06/2017 16:51:29
Operator ID:

Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
P1	---	0.4	0.66	10293
Unknown	---	0.2	0.88	5506
F	93.1*	---	1.20	2452390
P3		0.4	1.81	10570
Unknown	---	0.1	2.13	1546
A0	---	6.8	2.64	178943
A2	3.8*		3.75	123845

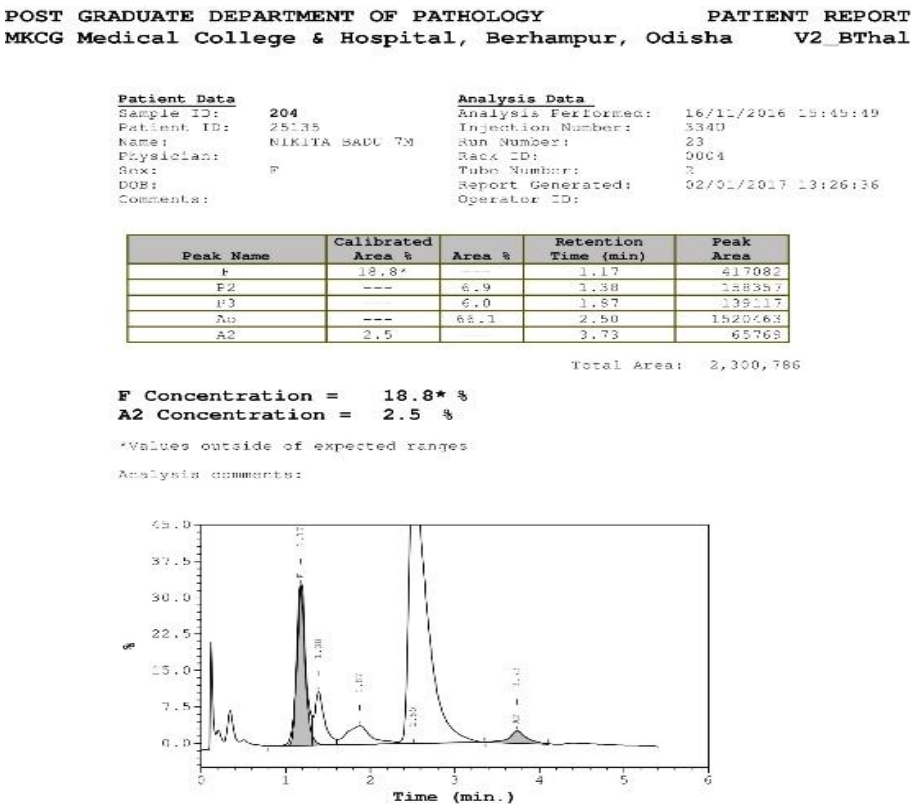
Total Area: 2,783,092

F Concentration = 93.1* %
A2 Concentration = 3.8* %

*Values outside of expected ranges

Analysis comments:

Figure 5:



POST GRADUATE DEPARTMENT OF PATHOLOGY PATIENT REPORT
MKCG Medical College & Hospital, Berhampur, Odisha V2_BThal

Patient Data
Sample ID: 204
Patient ID: 25135
Name: NIKITA BADU 7M
Physician:
Sex: F
DOB:
Comments:

Analysis Data
Analysis Performed: 16/11/2016 19:45:19
Injection Number: 3340
Run Number: 23
Rack ID: 0004
Tube Number: 3
Report Generated: 02/01/2017 13:26:36
Operator ID:

Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
F	18.8*	---	1.17	417082
P2	---	6.9	1.38	158357
P3		6.0	1.87	139217
A0	---	68.1	2.50	1520463
A2	2.5		3.73	65769

Total Area: 2,300,786

F Concentration = 18.8* %
A2 Concentration = 2.5 %

*Values outside of expected ranges

Analysis comments:

Figure 6:

74 cases (4.46%) were diagnosed as Hb S- beta thalassemia double heterozygotes [Figure 7].

POST GRADUATE DEPARTMENT OF PATHOLOGY PATIENT REPORT
MKCG Medical College & Hospital, Berhampur, Odisha V2_BThal

Patient Data		Analysis Data	
Sample ID:	232	Analysis Performed:	30/11/2016 15:04:02
Patient ID:	12676	Injection Number:	372U
Name:	SULATA SWAIN 25	Run Number:	25
Physician:		Rack ID:	0001
Sex:	F	Tube Number:	6
DOB:		Report Generated:	01/12/2016 12:21:35
Comments:		Operator ID:	

Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
F	4.0*	---	1.11	70345
P2	---	2.5	1.37	47128
P3	---	2.1	1.77	40272
Unknown	---	0.4	2.17	8271
Ao	---	46.0	2.52	870510
A2	4.1*	---	3.68	89692
S-window	---	40.5	4.37	765506

Total Area: 1,891,725

F Concentration = 4.0* %
A2 Concentration = 4.1* %

*Values outside of expected ranges

Analysis comments:

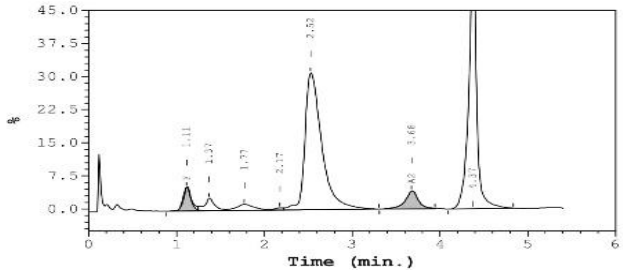


Figure 7:

2 cases and 4 cases of Lepore homozygous [Figure 8] and heterozygous [Figure 9] reported respectively. HPLC of Hb Lepore demonstrates characteristic hump in Hb A2 peak.

POST GRADUATE DEPARTMENT OF PATHOLOGY PATIENT REPORT
MKCG Medical College & Hospital, Berhampur, Odisha V2_BThal

Patient Data		Analysis Data	
Sample ID:	186	Analysis Performed:	16/11/2016 13:46:32
Patient ID:	65826	Injection Number:	316U
Name:	MAnoj pradhan 15	Run Number:	23
Physician:		Rack ID:	0002
Sex:	M	Tube Number:	4
DOB:		Report Generated:	02/01/2017 13:06:12
Comments:		Operator ID:	

Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
Unknown	---	0.6	0.89	9850
F	90.1*	---	1.21	1504688
P3		0.8	1.75	13511
A2	10.6*	---	3.56	207999

Total Area: 1,736,048

F Concentration = 90.1* %
A2 Concentration = 10.6* %

*Values outside of expected ranges

Analysis comments:

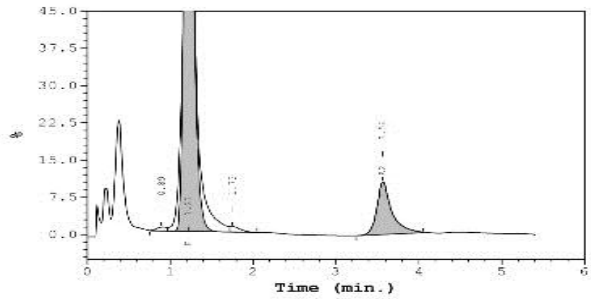


Figure 8:

POST GRADUATE DEPARTMENT OF PATHOLOGY PATIENT REPORT
MKCG Medical College & Hospital, Berhampur, Odisha V2_BThal

Patient Data		Analysis Data	
Sample ID:	218	Analysis Performed:	24/11/2016 14:02:47
Patient ID:		Injection Number:	352U
Name:	jhunu pradhan 45	Run Number:	24
Physician:		Rack ID:	C002
Sex:	F	Tube Number:	4
DOB:		Report Generated:	02/01/2017 13:09:14
Comments:		Operator ID:	

Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
Unknown	---	0.0	0.95	825
F	3.1*	---	1.11	76157
Unknown	---	0.7	1.24	17327
P2	---	3.2	1.37	84076
P3	---	3.2	1.78	83651
Unknown	---	0.0	2.17	729
Ao	---	76.2	2.46	1992783
A2	11.8*	---	3.50	360353

Total Area: 2,615,893

F Concentration = 3.1* %
A2 Concentration = 11.8* %

*Values outside of expected ranges

Analysis comments:

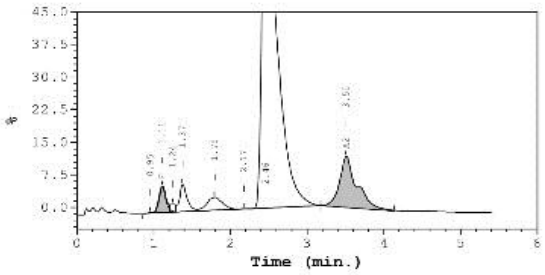


Figure 9:

2 cases were diagnosed as sickle-Lepore double heterozygous [Figure 10].

POST GRADUATE DEPARTMENT OF PATHOLOGY PATIENT REPORT
MKCG Medical College & Hospital, Berhampur, Odisha V2_BThal

Patient Data		Analysis Data	
Sample ID:	220	Analysis Performed:	24/11/2016 14:16:01
Patient ID:	45075	Injection Number:	354U
Name:	Tutu swain 53	Run Number:	24
Physician:		Rack ID:	0002
Sex:	M	Tube Number:	6
DOB:		Report Generated:	24/11/2016 16:26:27
Comments:		Operator ID:	

Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
F	17.3*	---	1.16	420380
P2	---	1.4	1.35	36502
P3	---	1.0	1.76	26671
Unknown	---	0.8	2.17	21013
Unknown	---	0.8	2.33	21260
Ao	---	12.3	2.58	318860
A2	9.4*	---	3.56	283260
S-Window	---	56.4	4.37	1457960

Total Area: 2,585,886

F Concentration = 17.3* %
A2 Concentration = 9.4* %

*Values outside of expected ranges

Analysis comments:

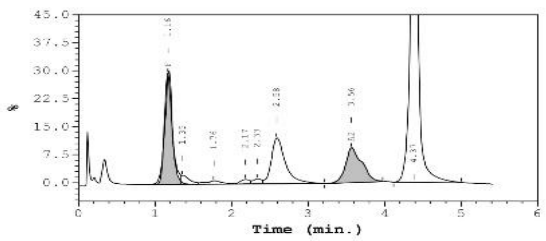


Figure 10:

4 cases were reported as Hb H [Figure 11].

POST GRADUATE DEPARTMENT OF PATHOLOGY PATIENT REPORT
MKCG Medical College & Hospital, Berhampur, Odisha V2_BThal

Patient Data		Analysis Data	
Sample ID:	804	Analysis Performed:	19/05/2017 14:37:24
Patient ID:	847	Injection Number:	1853
Name:	Sasanjali Sahu, 13Y	Run Number:	56
Physician:		Rack ID:	3003
Sex:	F	Tube Number:	1
DOB:		Report Generated:	19/05/2017 15:46:18
Comments:		Operator ID:	

Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
F	0.5		1.10	9915
Unknown	---	0.3	1.29	9089
P2		2.1	1.38	48486
P3	---	2.2	1.82	50555
A0	---	93.7	2.46	2161686
A2	1.7*	---	3.70	43213

Total Area: 2,322,352

F Concentration = 0.5 %
A2 Concentration = 1.7* %

*Values outside of expected ranges

Analysis comments:

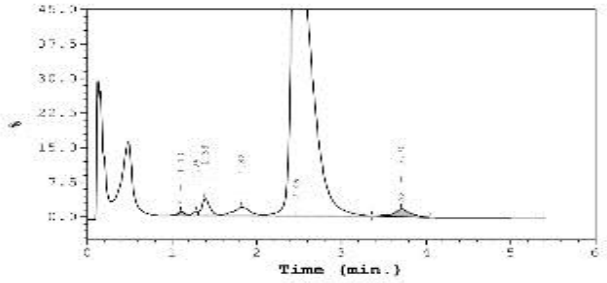


Figure 11:

3 cases of Hb E trait [Figure 12]. Patient were asymptomatic with microcytic hypochromic RBCs, target cells and erythrocytosis on CBC. There was 1 case of Hb E – beta thalassemia double heterozygous [Figure 13] with patient presenting with moderate to severe microcytic hypochromic anemia.

POST GRADUATE DEPARTMENT OF PATHOLOGY PATIENT REPORT
MKCG Medical College & Hospital, Berhampur, Odisha V2_BThal

Patient Data		Analysis Data	
Sample ID:	219	Analysis Performed:	24/11/2016 14:09:24
Patient ID:	35985	Injection Number:	1531
Name:	[illegible]	Run Number:	24
Physician:		Rack ID:	3002
Sex:	M	Tube Number:	5
DOB:		Report Generated:	24/11/2016 15:23:08
Comments:		Operator ID:	

Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
F	3.4	---	1.08	10170
Unknown		0.4	1.27	12907
P2	---	2.6	1.37	87767
P3		4.2	1.82	139443
A0	---	61.5	2.45	1917277
A2	26.4*		3.74	812452

Total Area: 2,629,951

F Concentration = 0.4 %
A2 Concentration = 26.4* %

*Values outside of expected ranges

Analysis comments:

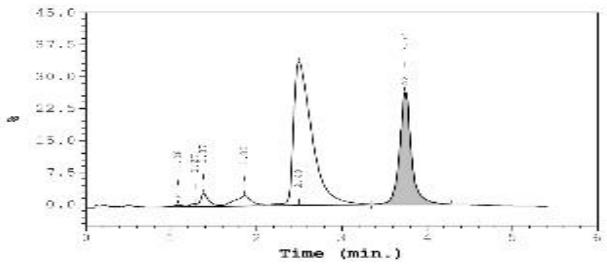


Figure 12:

POST GRADUATE DEPARTMENT OF PATHOLOGY PATIENT REPORT
MKCG Medical College & Hospital, Berhampur, Odisha V2_BThal

Patient Data		Analysis Data	
Sample ID:	716	Analysis Performed:	27/09/2017 15:21:31
Patient ID:	01029609	Injection Number:	4540
Name:	AAJALI BEHERA, 17YR	Run Number:	67
Physician:		Rack ID:	0002
Sex:	F	Tube Number:	1
DOB:		Report Generated:	27/09/2017 16:36:56
Comments:		Operator ID:	

Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
D1		0.1	0.82	1,989
F	24.2*		1.07	537919
P3	---	2.3	1.85	52150
Ac	---	6.1	2.53	135624
A2	60.7*	---	3.72	1526608

Total Area: 2,304,201

F Concentration = 24.2* %
A2 Concentration = 60.7* %

*Values outside of expected ranges

Analysis comments:

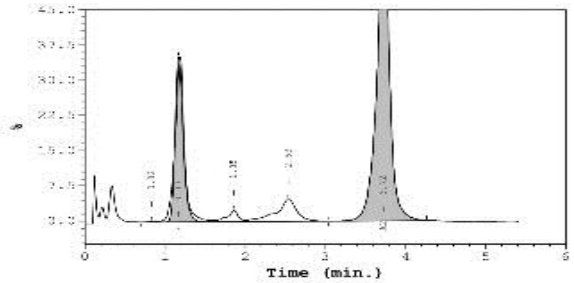


Figure 13:

There were 1 case each of Hb D homozygous (Hb D Punjab) [Figure 14] and Sickle – Hb D double heterozygous [Figure 15] (confirmed by family studies). Hb D Punjab has Retention time window of 3.90-4.30, which in homozygous gets coalesced with Hb A2 retention time window to form one wide graph.

POST GRADUATE DEPARTMENT OF PATHOLOGY PATIENT REPORT
MKCG Medical College & Hospital, Berhampur, Odisha V2_BThal

Patient Data		Analysis Data	
Sample ID:	894	Analysis Performed:	19/06/2017 15:30:01
Patient ID:	28134	Injection Number:	1168U
Name:	Himanshi Chatoi, 4Yrs	Run Number:	54
Physician:		Rack ID:	0002
Sex:	M	Tube Number:	9
DOB:		Report Generated:	19/06/2017 16:46:13
Comments:		Operator ID:	

Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
Unknown	---	0.5	0.92	17.2
F	4.1*	---	1.13	15160*
P2	---	0.2	1.40	9402
P3	---	3.5	1.88	137249
Ac	---	9.4	2.60	369789
A2	70.0*	---	3.64	3223377
S-window	---	0.8	4.35	21915

Total Area: 3,919,045*

F Concentration = 4.1* %
A2 Concentration = 70.0* %

*Values outside of expected ranges

Analysis comments:

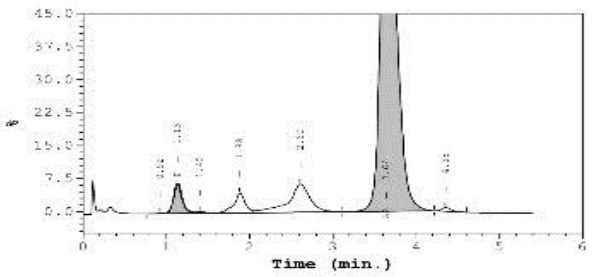


Figure 14:

POST GRADUATE DEPARTMENT OF PATHOLOGY PATIENT REPORT
MKCG Medical College & Hospital, Berhampur, Odisha V2_BThal

Patient Data		Analysis Data	
Sample ID:	1378	Analysis Performed:	04/12/2017 12:10:16
Patient ID:	401166	Injection Number:	17120
Name:	Dipaa Behera,28yrs	Run Number:	75
Physician:		Rack ID:	0001
Sex:	F	Tube Number:	5
DOB:		Report Generated:	04/12/2017 15:22:23
Comments:		Operator ID:	

Peak Name	Calibrated Area %	Area %	Retention Time (min)	Peak Area
F	3.8*	---	1.09	77791
P2	---	2.6	1.52	52284
P3	---	1.8	1.70	36775
A0		31.9	2.41	671107
Unknown	---	0.4	3.12	8273
A2	3.7*		3.57	80258
D-window	---	19.2	4.08	403860
S window		32.7	4.52	688023
Unknown	---	1.0	4.90	21820
C-window	---	3.1	5.15	65133

Total Area: 2,107,036

F Concentration = 3.8* %
A2 Concentration = 3.7* %

*Values outside of expected ranges

Analysis comments:

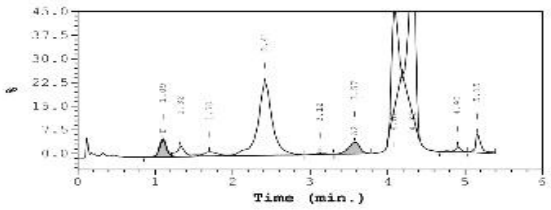


Figure 15:

DISCUSSION

Prevalence of wide range of hemoglobinopathy in the population of southern Odisha indicate that hemoglobinopathies are not uncommon at birth and their related complications. This scenario of hemoglobinopathies reflects that the population of the state of Odisha is genetically heterogeneous one and so many ethnic elements have absorbed into the main stream of people along with the original inhabitants with varied genetic heritages, resulting in population diversity with the passage of time.[9] We attempted this study to see to observe various hemoglobinopathies in southern odisha.

Appropriate laboratory tests are required for diagnosis and confirmation of these disorders. The identification of Hb variants by conventional techniques are often presumptive.[10] HPLC offers the distinct advantage over classic Hb electrophoresis as it can more accurately identify and quantitate abnormal Hbs.[11] We found the maximum number of cases were of sickle cell anemia both heterozygous and homozygous followed by beta thalassemia trait. This calls for the need of antenatal screening and screening of marriageable age groups. This will help in the prevention of sickle cell disease and thalassemia major in the offspring.

Three cases of HB E trait identified in our study was clinically normal, but peripheral blood smear revealed microcytosis with target cells and erythrocytosis. Hb E trait is more common in ethnic groups from Assam. A multicentre study revealed that Hb E trait was mainly seen in Dibrugarh in Assam (23.9%) and Kolkata in West Bengal (3.92%) among six ethnic groups from Assam, the prevalence of Hb E trait varied from 41.1% to 66.7%.[12] detection of this variant is important because when combined to beta thalassemia or sickle cell anemia, it give rise to moderate to severe anemia. In our study, we found one case of Hb E – beta thalassemia double heterozygous.

Hb Lepore is a structural haemoglobin variant coded for by a hybrid gene formed by the fusion of delta and beta genes.It has a similar retention time (RT) value as Hb A2 on HPLC. Values greater than 10% suggest the presence of variant hemoglobin. In the homozygous state, Hb A and Hb A2 are absent and the haemoglobin is made up of Hbs F and Lepore only. The level of Hb Lepore ranges from 8 to 30% with a mean value of approximately 15%, the remainder of the haemoglobin being Hb F. In the heterozygous state the haemoglobin contains Hbs A, Lepore, A2 and a variable amount of Hb F. The reported level of Hb Lepore is between 5 to 15%, with a mean level around 10%. The mean level of Hb A2 is about 2% and the reported values for Hb F range from between 1-14%.[13]

Limitation of this technique is higher capital and reagent costs including high skill and experience required to interpret the results. Another limitation is various hemoglobinopathies elute similar retention time so cannot be ruled out by HPLC alone. A disclaimer should always accompany the report and findings must be supported by CBC findingd, family history, hemoglobin electrophoeresis if required, and sickling tests and advised for molecular studies.

CONCLUSION

To conclude, HPLC is an ideal method for routine diagnosis of hemoglobinopathies. We found a plethora of hemoglobinopathy in southern odisha. Continuous awareness programmes, mass screening of the population especially child bearing age and school going children will help in reducing the burden of disease. This can in turn with proper genetic counselling help in reducing morbidity and mortality.

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