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DETECTION OF PREVALENCE OF SICKLE CELL DISEASE/TRAIT AND OTHER HEMOGLOBINOPATHIES AMONG PATIENTS OF SOUTH GUJARAT REGION- BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)- 14400 CASES.

Hematology

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ABSTRACT

Introduction: Sickle cell disease (SCD) is the most common inherited disorder of haemoglobin. Present study is carried out to diagnose the prevalence of Sickle Cell Disease/Trait, β-thalassaemia disease/trait and other hemoglobinopathies. **Methods:** A total of 14400 cases were evaluated in a period from January 2017 to December 2017. Suspected Samples of hemoglobinopathies were received from affiliated tertiary hospital and nearby health centres. Total 9381 samples from suspected patients of hemoglobinopathies were received from affiliated hospital and 5009 samples received from health centres. Solubility test was performed in all the samples (9381/9381) from affiliated hospital and it was performed in 3374 samples out of 5006 (655/5009) from nearby health centres. HPLC test was performed using Bio Rad HPLC Variant II in the 9515hospital samples and 5006 samples of nearby health centres. All the reports were analysed and interpreted. **Results:** There were Sickle cell disease (SCD)-631, Sickle cell trait (SCT)-3592, B Thalassemia disease-2, B thalassemia Trait-32, Hb E- 1. So total 4258 cases were diagnosed having hemoglobinopathies. In sickle cell trait (SCT) patients, there was a higher level of HbA2, presence of HbS (less than 50 %) and lower level of HbA. In sickle cell disease patients, there were significantly higher levels of HbA2 and HbF, presence of HbS (more than 50 %) and significantly lower levels of HbA. **Conclusion:** All the patients in tribal area needs to be undergone solubility test and If positive should be confirmed by HPLC test, to rule out Sickle Cell Disease/trait and all the suspected cases needs to be evaluated by HPLC test to rule out other hemoglobinopathies. While analysing HPLC patterns, appearance of HbS, low levels of HbA and high levels of HbF and HbA2 should raise a suspicion for presence of Sickle cell hemoglobinopathy.

KEYWORDS

Solubility test, Sickle Cell disease, HPLC, haemoglobin variants

INTRODUCTION-

Sickle cell disease (SCD) and its variants are genetic disorders resulting from the presence of a mutated form of hemoglobin, hemoglobin S (HbS).[1] It has an autosomal recessive inheritance[2]

In 1957, Vernon Ingram discovered that sickle haemoglobin resulted from a single amino acid substitution in the haemoglobin molecule[3,4]

The disease results from a single base A>T mutation in the triplet encoding the sixth residue of the β-globin chain, leading to a substitution of valine for glutamic acid and the abnormal haemoglobin S (HbS). The primary pathophysiology is based on the polymerization of deoxy HbS with formation of long fibres within the Red Blood Cells (RBCs) causing a distorted sickle shape which eventually leads to increased haemolysis and vaso-occlusion of sickle red cells. However, the clinical presentation of SCD patients is extremely variable and there are several events that may trigger vaso-occlusion. Recent work has shown the importance of red cell dehydration, abnormal adhesion of RBCs to the vascular endothelium, inflammatory events, and activation of all the cells in the vessel and abnormalities of nitric oxide metabolism in the pathophysiology of this multi-organ disease.[5]

In India, sickle cell anemia is more prevalent in tribal population. The first description of sickle haemoglobin in India was by Lehman and Cutbush in 1952 in the tribal populations in the Nilgiri hills in south India[6].

In the same year, Dunlop and Mazumder also reported the presence of sickle haemoglobin in the tea garden workers of Upper Assam who were migrant labourers from tribal groups in Bihar and Odisha.

Since then, many population groups have been screened and the sickle cell gene has been shown to be prevalent among three socio-economically disadvantaged ethnic groups, the scheduled tribes, scheduled castes and other backward classes in India. In Gujarat, the Dhodia, Dubla, Gamit, and Naika tribes have a high prevalence of HbS (13-31%).[8]

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 βthalassaemia trait (6.3 to 13.6 %).[9]

In this study we want to profile various types of haemoglobins and their relative percentage in sickle cell cases versus controls in our study population. Also, we will compare RBC indices such as Hb, HCT, MCV, MCH, MCHC and RDW-CV in sickle cell cases versus controls.

Method

This retrospective study was done in a tertiary care hospital in south Gujarat from January 2017 to December 2017. Our population consists primarily of tribals from south Gujarat.

We received samples of venous blood in EDTA bulb from affiliated tertiary hospital and nearby health centres of all suspected cases of hemoglobinopathies . A total of 14400 cases were evaluated.out of which Solubility test was performed using solvent of sodium disulphilde.Amog that positive samples HPLC test was performed using Bio Rad HPLC Variant II in the 9515hospital samples and 5006 samples of nearby health centres along with controls. All the reports were analysed and interpreted using cbc parameters the following criteria were used

Table 1: Criteria Used For Differentiating HPLC Patterns.

Hemoglobin	Disease
A>S	Sickle cell trait, sickle alphathalassemia
S, F and no A	Sickle cell anemia, Sickle-beta thalassemia
S> A and F	Sickle-beta thalassemia
A>C	HbC trait
C, F and no A	HbC disease, HbC-beta thalassemia
C>A	HbC-beta thalassemia

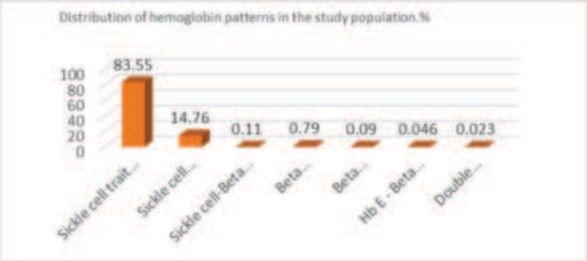
RESULTS

A total of 14400 cases were evaluated. Solubility test was performed in all the samples (9381) from affiliated hospital and in 3374 samples out of 5006 from nearby health centres. HPLC studies performed on total 4266 samples.

Table 2 : Distribution Of Hemoglobin Patterns In The Study Population.

HB pattern	Total	Percentage(%)
Sickle cell trait (SCT)	**3590**	83.55
Sickle cell disease (SCD)	**630**	14.76
Sickle cell-Beta thalassemia (SBT)	**5**	0.11
Beta Thalassemia trait (BTT)	**34**	0.79
Beta thalassemia disease (BTM)	**4**	0.09
Hb E - Beta Thalassemia	**2**	0.046

Double heterozygous state of HbS and HbD	**1**	0.023
Total	**4266**	100

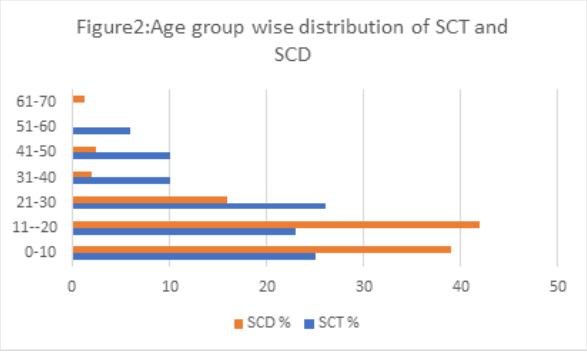


Study showed Sickle cell trait accounts for the majority of cases, representing 83.55% (3590 cases) of the sample and Sickel cell disease constitutes 14.76% (630 cases).

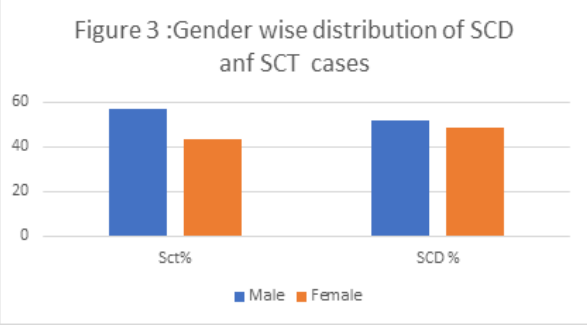
While 34 were of beta thalassemia trait accounts for 0.79% (34 cases)and beta thalesemmia minor 0.09%(4 cases).

Present study showed 83% patients diagnosed with sickle cell trait were below the age of 40 and 17% patients were above 40 yrs age. Most commonly affected age group were 0 to 10 yrs and 11-20 yrs age group with 26% and 24% respectively.

94& patients with Sickle cell disease are below the age of 40 and 6% were above 40 yrs age group. Most commonly affected in age group was 11-20yrs and 0 to 10 yrs with 42% and 39% respectively. (Figure 2)



Present study showed male predominance in both sickle cell disease and trait with 56.64 and 51.5 % respectively. (figure 3)



we analysed the HPLC patterns in patients and controls. Sickle cell trait patients demonstrated a significantly higher level of HbA2 and HbS and significantly lower level of HbA as compared to control group. The difference in HbF levels were not statistically significant in our study. The mean level of HbA, HbA2, HbF and HbS in sickle cell trait patients as compared with control group is given in Table 4

Table 4: The Mean Level Of HbA, HbA2, HbF And HbS In SCT And SCD Patients As Compared With Control Group.

HB pattern	HBA	HBA2	HBF	HBS	OTHERS
Normal	**95-98**	**2.5-3.4**	**1**	-	
Sickle cell trait (SCT)	**50.6**	**3.8**	**0.9**	**39.2**	
Sickle cell disease (SCD)	**3.4**	**5.7**	**17.6**	**70.5**	
Sickle cell-Beta thalassemia (SBT)	**4.1**	**2.2**	**30.2**	**61.8**	
Beta Thalassemia trait (BTT)	**83.4**	**5.5**	**0.5**	**0.5**	
Beta thalassemia disease (BTM)	**84**	**5.0**	**0.7**	-	
Hb E - Beta Thalassemia	**2.4**	**70.6**	**26.6**	-	
Double heterozygous state of HbS and HbD	**3.2**	**2.6**	**2.5**	**40**	**35.6 (Hb D)**

We analysed the HPLC patterns in patients having sickle cell disease, demonstrated a significantly higher level of HBA2 and HbS and HBF and significantly lower level of HBA,

While patients having thalassemia show higher level of HBF and HBA2 while lower level of HBA. Double heterozygous sickle and thalassemia showed higher level of HBF and HBS

Table 4 : Analysis Of Haematological Parameters In SCT, SCD And Other Hemoglobinopathy

HB pattern	HB	MCV	MCHC	RBC count	HCT	RDW
Sickle cell trait (SCT)	9.3	77.4	25.4	4.19	31.5	17.5
Sickle cell disease (SCD)	8.6	75.5	33	3.2	34,42	22.7
Sickle cell-Beta thalassemia (SBT)	10	65.3	33.7	3.9	32.4	18.8
Beta Thalassemia trait (BTT)	10	67.2	34	4.5	44	15.6
Beta thalassemia disease (BTM)	8.8	64.3	33.6	3.6	40.3	24.4
Hb E - Beta Thalassemia	9.8	80.8	32.8	3.8	38.3	18.6
Double heterozygous state of HbS and HbD	8.3	78	34	3.9	39.2	16.6

Analysis showed significant lower level of HB, haematocrit in sickle cell disease and trait while RDW is high.

DISCUSSION

The Indian population comprises numerous castes and tribal groups, each revealing different genetic traits. Several studies reported that Gujarat has higher frequency of β-thalassemia and sickle cell disease[3,4,9,10] Therefore prospective studies are essential to identify high risk communities. Premarital screening of college students not only gives prevalence in different caste groups but also helps in counseling and prenatal diagnosis programs for prevention of birth of an affected child.[10]

Our study demonstrated higher percentage of Sickle cell trait versus sickle cell disease which is in concordance with other studies.[13-16]

Looking at the age distribution, we conclude that sickle cell trait and disease are mor common in age group below 40 which is compatible the other studies.[5,6,17]

Our study reflected that in sickle cell trait patients, there is a significantly higher level of HbA2 and HbS and significantly lower level of HbA as compared to control group. The difference in HbF levels were not significant in our study. This is in accordance with studies done by Jawahar et al[5] Shirley L et al[17,] and Eman A et al.[18]

In sickle cell disease patients, there were significantly higher levels of HbA2, HbF and HbS and significantly lower levels of HbA as compared to control group. This is consistent with various studies done by Eman A et al[19], Cotton F et al[20]and Mondal and Mandal et al[21]

Other variants detected in this study were thalassemia major, thalassemia minor, sickle β thalassemia, double heterozygous state of HbS and HbE, double heterozygous state of HbS and HbD.

HPLC has been established as a sensitive, specific, and accurate technique for the identification and quantification of different Hb fractions. However, it is limited by its inability to detect α thalassemia and normal HbA2 β thalassemia. Hb variants that elute with the same retention time also cannot be separately identified by HPLC, thus during interpretation of chromatograms, nutritional anemias must always be taken into account , HPLC must be followed by molecular studies, such as polymerase chain reaction (PCR), amplifi cation refractory mutation system (ARMS) etc[21]

Both sickle cell trait and sickle cell disease patients had significantly lower levels of Hb and haematocrit as compared with controls. This corelates with various studies done by Jawarkar A et al[6]Walke et al[20] and Chikhlikar et al[21]

The difference in RBC counts was not statistically significant in our study between controls and both sickle cell trait and disease patients which is in concordance with Jawarkar A et al[6]. Which is in contrast with studies by Chikhlikar et al[23] Pathak et al,[24] and Yasmin et al[25]

The value of RDW CV was significantly higher in both sickle cell trait and sickle cell disease patients versus controls. This was similar in study done by Chikhlikar et al[23]

CONCLUSION

We conclude that sickle cell hemoglobinopathies are very common in our population which consists of a large proportion of tribals. Sickle cell trait is more common than disease in our geographic area which has to do with consanguineous and same caste marriages and lack of prenatal counselling and detection. Apart from appearance of HbS on HPLC, low levels of HbA and high levels of HbA2 should raise a suspicion for presence of Sickle cell hemoglobinopathy. There was statistical difference in levels of Hb, HCT, MCH and RDW-CV between cases and controls. High index of suspicion should be maintained when these parameters are on lower side, especially in population who is prone to have sickle cell disorders such as tribals.

IMAGES

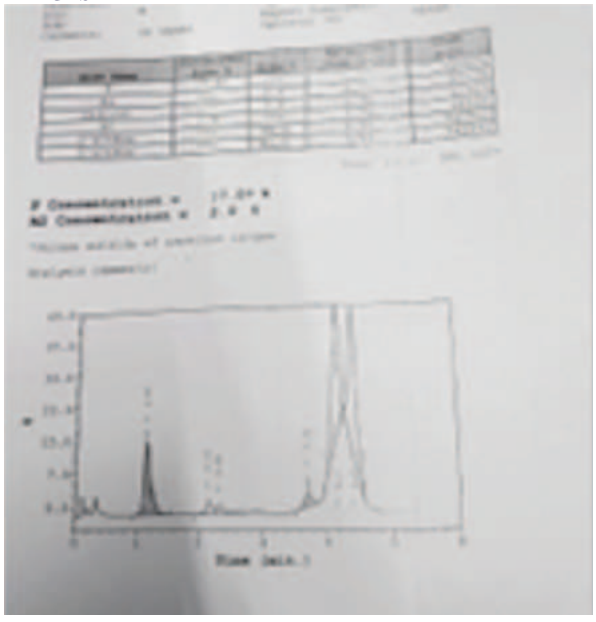


Figure 4: Double heterozygous HBS and Thal

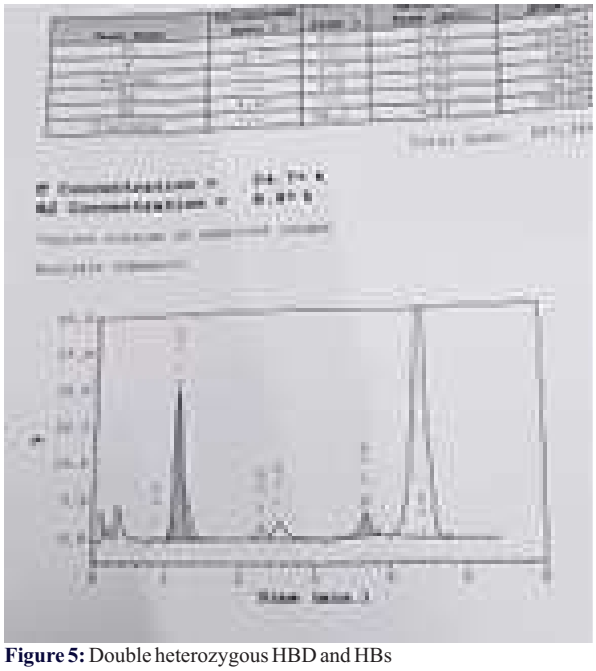


Figure 5: Double heterozygous HBD and HBs

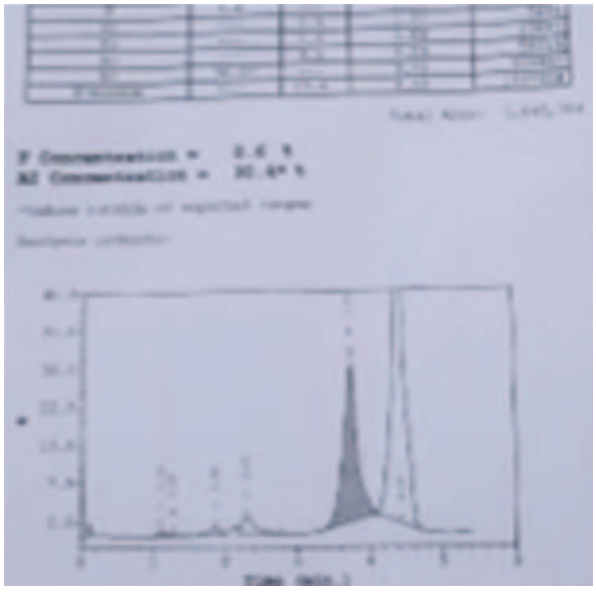


Figure 6: Sickle cell trait

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