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INHERITED HAEMOGLOBIN DISORDERS AND THEIR CORRELATION WITH COMPLETE BLOOD COUNT AND PERIPHERAL BLOOD SMEAR. AN ANALYSIS OF 50 CASES.

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ABSTRACT

Introduction: Inherited hemoglobin disorders present a significant health problem all over the world. Due to migration, this problem is increasing day by day. No definitive data regarding the frequency of hereditary haemoglobin disorders are available in Nepal. Iron deficiency is most widely prevalent in the country and beta thalassaemia is also common. **Objective:** The aim of the study is to determine the relative frequency of inherited haemoglobin disorders. **Methods:** This retrospective cross-sectional study was carried out in the Department of Haematology Mangalam diagnostics center from July 2014 to January 2016. A total of 50 cases were included in this study. Blood samples of specific amount were taken from all these individuals for estimation of CBC, red cell variables and Hb electrophoresis. **Results:** Hb electrophoresis revealed normal Hb pattern in 76% of cases, followed by (Hb S, Hb A and Hb F), Hbs/β+ Thalassaemia (66.6%), (Hb A Hb A2 and Hb F) Heterozygous db Thalassaemia (16.6%). HPFH and HBAS (Sickle Trait) are only 8.3% cases in each. In this study, a total of 24% abnormal Hb pattern were detected. **Conclusion:** The finding of abnormal Hb pattern on Hb electrophoresis in this study among the cases indicated the high frequency of inherited haemoglobin disorders among our population and hence warranted detection of these disorders and subsequent appropriate genetic counseling.

KEYWORDS: Inherited haemoglobin disorders, Hb electrophoresis, Genetic counseling.

I. INTRODUCTION

Inherited hemoglobin disorders especially thalassaemia and sickle-cell disorders were originally characteristic of the tropics and subtropics but these disorders are now common worldwide due to migration.[1,2,3] As programmes such as integrated treatment, carrier detection and genetic counseling are the most effective effort for controlling these disorders cost-effectively, World Health Organization (WHO) has recommended global development of these services and programme.[4] Different types of inherited haemoglobin disorders present a significant health problem all over the world accounting 71% of 229 countries, and these 71% of countries include 89% of all births worldwide.[2,3,4] About 7% of the world's population is a carrier of hemoglobin disorders.[4] Data obtained from Sri Lanka as well as from two northwest states of India also show a noticeable variation in the frequency of beta thalassemia and Hb E.[5,6] Weatherall and Clegg and added that at the younger age group inherited haemoglobin disorder are most commonly detected.[7] Hemoglobin electrophoresis is a blood test that can detect different types of haemoglobin. Hemoglobin is the protein inside red blood cells responsible for transporting oxygen. If it is abnormal in some way, it may cause too little oxygen to reach the tissues and organs. The most common types of normal haemoglobin in the Adults are:

- **Hemoglobin A:** (95 to 98%) This is the most common type of haemoglobin found normally in adults. Some diseases, such as severe forms of Thalassemia, may cause haemoglobin A levels to be low and haemoglobin F levels to be high.

- **Hemoglobin F (foetal haemoglobin):** (0.8 to 2%) This type is normally found in fetuses and new born babies. Haemoglobin F is replaced by haemoglobin A (adult haemoglobin) shortly after birth; only very small amounts of haemoglobin F are made after birth. Some diseases, such as sickle cell anemia, aplastic anaemia, and leukaemia, have abnormal types of haemoglobin and higher amounts of haemoglobin F.

- **Hemoglobin A2:** (2 to 3%) This is a normal type of haemoglobin found in small amounts in adults.

There are more than 350 types of abnormal haemoglobin. The most common are:

- **Hemoglobin S:** (0%) This type of haemoglobin is present in sickle cell disease. Red blood cells become

hard and crescent-shaped. They block small blood vessels and prevent blood from circulating properly.

 Hemoglobin C: (0%) This type of haemoglobin does not carry oxygen well.

 Hemoglobin E: (0%) This type of haemoglobin is found in people of Southeast Asian descent.

 Hemoglobin D: (0%) This type of haemoglobin is present in some sickle cell disorders.

Sickle cell disease is one of the most common genetic pathologies in the world. Sickle Hemoglobin (Hb S) is an abnormal variant of hemoglobin in which there is substitution of adenine in sixth codon of beta gene (GAG-GTG), thereby encoding valine instead of glutamic acid in sixth position of beta chain. On de-oxygenation of red blood cells, Hb S forms scattered to aggregates of fibers that fill the cell and distorts into sickle shape or elongated forms. Upon oxygenation, reversible sickle cells regain normal red cells shape. Irreversible sickle cells are permanently stabilized in abnormal crescent or oval forms. Sickling of red cells can result into anemia, crises and organ injury.[8,9] Hb S is the most common pathological hemoglobin mutation worldwide. AS individuals are (heterozygotes) are usually asymptomatic and SS individuals (Homozygotes) suffer from sickle cell anemia. It is characterized by homozygous hemoglobin S (Hb S) or Hb S associated to other Hb variants.[1] There is great clinical variation in the clinical manifestations between sickle cell disease patients; several factors are associated with the different presentations. Some determinants are already well established, such as genetic, clinical and laboratory factors, while others, such as psycho-social and nutritional factors, have been less well studied.[10]

Of the genetic factors, the importance of the phenotype of the hemoglobinopathy is well characterized in that individuals doubly heterozygous for sickle cell anemia and those with Hb S/ β0-thalassemia have a more severe clinical profile. On the other hand, carriers of Hb SC together with Hb S/ β +-thalassemia have better outcome, which makes the correct diagnosis of these syndromes an issue of great importance for a better understanding and adequate clinical and therapeutic management of patients.[10]

This study was conducted to identify the burden of Inherited Haemoglobin Disorders by Hemoglobin electrophoresis and correlation with complete blood count and peripheral blood smear.

II. MATERIALS AND METHODS

This retrospective cross-sectional study was carried out in the Department of Haematology, Mangalam diagnostics center from July 2014 to January2016. A total of 50 cases were included in this study.

A total of two milliliter venous blood from antecubital vein was taken in a vacutainer containing three milligram Ethylene Diamine Tetra Acetic Acid (EDTA) for complete blood count and haemoglobin electrophoresis. Complete blood count was done by automated haematology analyzer (BECKMAN COULTER). Before running the sample the analyzer was calibrated daily in the morning by low normal, normal and high normal supplied by the manufacturer. Haemoglobin level, Haematocrit (Hct), Mean Cell Volume (MCV), Mean Cell Haemoglobin (MCH) and Mean Cell Haemoglobin Concentration (MCHC) were recorded for further analysis.

Peripheral blood film was prepared from all the available samples of complete blood count and haemoglobin electrophoresis and examined under microscope for microcytic hypochromic blood picture. Haemoglobin electrophoresis was done by Genio fully automated Gel Electrophoresis System which uses cellulose-acetate-strips.

III. RESULTS

A total of 50 cases irrespective of sex between 6 month to 45 years of age were included in this study. Normal hemoglobin was present in 38/50 (76%) cases and Inherited haemoglobin disorder was present in 12/50 (24%) of the study population. Among 12 patients with Inherited Hemoglobin disorders, maximum were in the age group of 0-10 (50%). In this study male population was predominant than female.

CBC, peripheral blood smear and Hb Electrophoresis findings for these patients are given below in tables.

Distribution of the pattern of Haemoglobin on Hb electrophoresis (n=12).

1	Normal	38 out of 50	76%
2	Inherited haemoglobin disorder	12 out of 50	24%

Demographic data of age with abnormal HB pattern(n=12)

Age Group (In Year)	percentage
0 - 10	50.00 %
11 - 25	08.33 %
26 - 45	41.67 %
> 46	00.00 %

Demographic data of sex with abnormal HB pattern.(n=12)

Sex	percentage
Male	66.67 %
Female	33.33 %

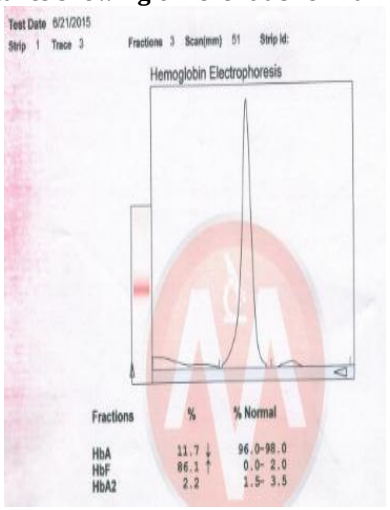
C.B.C. and Peripheral smear findings with abnormal HB pattern. (n=12)

VARIABLES	RBC	HB	HCT	MCV	MCH	MCHC	RDW	PERIPHERAL SMEAR		
Range	4.5-6.3	13-18	39-52	80-100	27-32	31-38	12-14.	nRBC	Target Cells	MC/HC
SN	10^6/µl	g/dl	%	fl	pg	g/dl	%			
1	3.8	10.8	30	72	27	34.5	15	A	P	P
2	2.43	5	13.3	54.8	20.7	37.9	17.5	A	P	P
3	3.2	8.8	22.8	68	25	38	14.5	A	P	P
4	5	13.3	37.4	78	26.6	35.5	14.2	A	A	A
5	3.27	6.3	22.7	69	19.3	27.8	18.7	A	P	P
6	2.8	4.6	12.4	50.7	20.2	37.4	18	280	P	P
7	1.34	4.8	12.7	95	35.8	37.9	14.4	A	P	P
8	5.88	11	32.6	55	18.7	33.6	15.8	A	A	A
9	2.9	5.7	17.8	61	19.7	32.1	19.7	250	P	P
10	4.87	10.6	32.9	68	21.8	32.3	19	310	P	P
11	3.5	6.5	23.8	70	19.4	27.9	18.4	A	P	P
12	3.4	6.8	24.5	72	19.6	28	17.2	A	P	P
Mean or Avg.	3.53	7.85	23.5	67.8	22.8	33.5	16.8	3/12(25%)	10/12(83.3%)	10/12(83.3%)

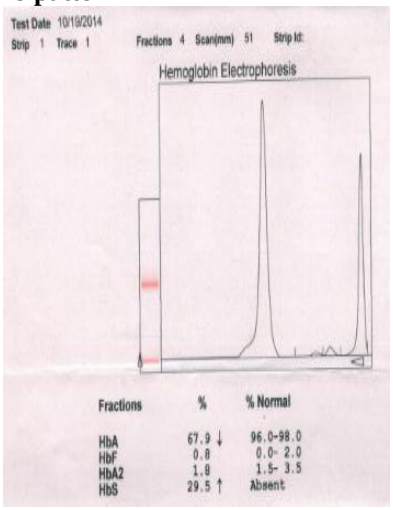
Distribution of the pattern of inhreritated Haemoglobin disorder on Hb electrophoresis (n=12).

VARIABLES		HB ELECTROPHORESIS				DIAGNOSIS
		HbA	HbF	HbA2	HbS	
SN	Range	96.0-98.0	0.0-2.0	1.5-3.5	Absent	
1		-	98.2	1.6	0.2	H.P.F.H.
2		1.2	43.5	28.6	6.7	Hbs/β+ Thalassaemia
3		93.2	4.5	2.1	0.2	Hbs/β+ Thalassaemia
4		6.5	10.4	79.3	3.8	Hbs/β+ Thalassaemia
5		21.8	75.5	1.6	-	Heterozygous db Thalassaemia
6		1.6	95.6	2.3	0.5	Hbs/β+ Thalassaemia
7		7.1	87.6	3.9	1.4	Hbs/β+ Thalassaemia
8		11.7	86.1	2.2	-	Heterozygous db Thalassaemia
9		6.8	90.8	1.5	0.8	Hbs/β+ Thalassaemia
10		16.9	78.6	2.3	2.2	Hbs/β+ Thalassaemia
11		67.9	0.8	1.8	29.5	HBAS (Sickle Trait)
12		0.9	92.6	4.5	2	Hbs/β+ Thalassaemia

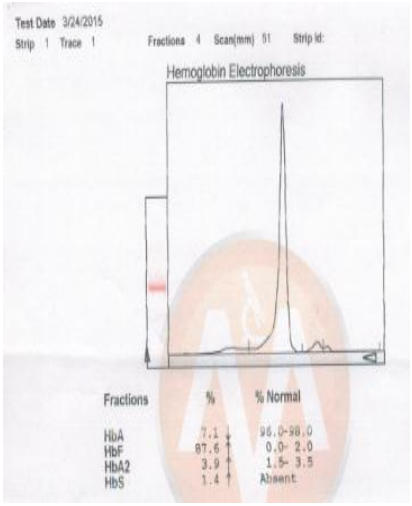
Pictures showing different abnormal Hb pattern



Heterozygous db Thalassemia



HbAS(Sickle Trait)



HbS/b Thalassemia

IV. DISCUSSION

Hb disorders are recognized as one of the most common inherited diseases worldwide. Among the hemoglobinopathies, sickle cell disease and β-thalassemia have the greatest impact on morbidity and

mortality, affecting millions of individuals worldwide.[10,15]

Sickle cell disease should be considered as both a qualitative and quantitative genetic disorder because it is caused by the presence of an abnormal Hb variant – Hb S. Homozygosity for Hb S or sickle cell anemia is the most common genotype; the other causative genotypes include compound heterozygous states of Hb S with Hb C (Hb SC) or β -thalassemia variants (Hb S / β 0-thalassemia and Hb S/ β +-thalassemia).[10,15]

Diagnosis of specific sickle cell diseases is accomplished by integrating clinical and hematological parameters along with laboratory Hb analysis. Combining these elements to prop-erly diagnose Hb disorders is essential for the treatment of anemia, primary prevention and genetic counseling for underlying disorders. In the majority of patients, the presence of a hemoglobinopathy can be diagnosed with sufficient accuracy for clinical purposes from knowledge of the patient's ethnical background and clinical history (including family history) and the results of the physical examination combined with relatively simple blood tests. Initial investigations should include determination of Hb concentration and red cell indices. A detailed examination of a well-stained blood film should be carried out. Other important basic tests are Hb electrophoresis or chromatography and the measurement of Hb A2andHb F.[10]

In our study we found that inherited haemoglobin disorder was common in male (66.6%) compared to female (33.33%) with M:F ratio 2:1. Similar results were seen in Kamble et al[11] and Rao et[12] study. Our study showed that maximum number (50%) were in group of 0-10 years of age. Similarly in a study done by Kamble et al 63% of patients were below 5 years.[11] and in study done by Rao et[12] patients with inherited hemoglobin disorders were in the range of 5-15 years. In the same way Weatherall and Clegg added that inherited haemoglobin disorder are most common in younger age group.[13] Modell and Darlison (2008) also found a result which is consistent with the present study.[1]

In this study, patient with inherited haemoglobin disorder showed average hemoglobin concentration 7.85 gm/dl. Minimum hemoglobin concentration was 4.6 gm/dl and maximum hemoglobin concentration was 13.3 gm/dl. Similar results were found in a study done by Shrikhande et al[14] where average Hb observed in males was 7.11 gm/dl while in females was 6.75 gm/dl.

We found that patient with inherited haemoglobin disorder had average RBC (3.53-10ˆ6/µl), Haematocrit (23.5%), MCV(67.8 fl) and MCH(22.8 pg) which were low than normal whereas average RDW was high (16.8%).

The peripheral blood smear among patients with Inherited hemoglobin disorders (12 patients) revealed microcytic hypochromic picture in 10 cases (83.3%). These cases also had (25%) nRBC, and10 cases (83.3%) with target cells.

Types of Hb Disorder on the basis of Hemoglobin electrophoresis was endorsed in this study. Among 50 cases, majority were normal (76%), followed by (Hb S, Hb A and Hb F), Hbs/β+ Thalassaemia (66.6%), (Hb A Hb A2 and Hb F) Heterozygous db Thalassaemia (16.6%). HPFH and HBAS (Sickle Trait) were only 8.3% cases in each. Weatherall and Clegg (2001)[13] had reported similar result and mentioned that over 700 structural haemoglobin variants have been identified and only three reach high frequencies which are Hb S, Hb C, and Hb E. Other study done by Kamble M et all[11] observed 61.6% cases of Hb SS and 38.4% cases of Hb AS.

According to Silvana F et all[10] over the last two decades various authors have reported the occurrence of falsely elevated Hb A2 in screening for Hb variants (Hb S, Hb C, Hb D, Hb E and Hb Lepore) and for AT. Some of these authors have cautioned about the risk of interpretation errors and the false diagnosis of HbS/ β 0-thalassemia. He also mentioned in his study that Hb A2 values seen in their patients were falsely elevated due to interference of the methodology used in the presence of structural Hb variants and AT, and as such the patients can be diagnosed as having sickle cell anemia, and not Hb S/ β 0-thalassemia, even though we recognize a limitation by the fact that we did not perform molecular analyses to conclusively exclude the diagnosis of β -thalassemia. But in our study HbA2 levels were in normal range in most of the cases. So we carry on with the diagnosis of Hbs/β+ Thalassaemia but we also did not perform molecular analyses to reach conclusive diagnosis.

V. CONCLUSION

In South East Asia, inherited haemoglobin disorders are one of the most common inherited disorders and put tremendous burden to the national budget. Most of the carriers remain asymptomatic and pass relatively normal life. If any case with decreased Hb, MCV and increase RDW with peripheral blood smear findings of microcytic hypochromic picture with target cell Inherited Haemoglobin disorder should be considerd as differential diagnosis and Hb electrophoresis should perform to rule out Inhreritated Haemoglobin disorder.

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