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Original Research Article

Detection of Hemoglobinopathies by Electrophoresis in Microcytic Hypochromic Blood Picture – One Year Observational Study

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Abstract:

Introduction: Hemoglobinopathies are lethal inherited genetic haematological disorders requiring lifelong blood transfusions and treatment in their severe and chronic form. These disorders ultimately lead to death if they are undiagnosed or untreated. Highest frequencies of hemoglobinopathies are present in South Asia particularly in India, Pakistan, and Bangladesh. In India, there are an estimated 100,000 thalassemia major patients and nearly 3.5 to 4 million carriers of this disorder and tribal population have a range of 5–40% sickle cell anaemia sufferers.

Aim: Detection of hemoglobinopathies by electrophoresis in microcytic hypochromic blood picture in the population who attended tertiary care centre at Government Medical College and Hospital, Anantapuramu.

Objectives: 1. Detection of frequencies of different variants of hemoglobinopathies. 2. Detection of frequency of Iron deficiency anemia in microcytic hypochromic anemia.

Methods: Observational study was carried out in a hospital in rural area of Anantapuramu on patients who attended tertiary care centre (Both out and in patients) from January 2023 to December 2023. Total 229 cases of mild to severe anemia were included in the study. All cases were subjected to hematological investigations-includes complete hemogram, reticulocyte count, peripheral blood smear, and some standard procedures. Zy bio fully automated 5 – part Cell counter was used for complete blood count. A peripheral blood smear is used for evaluation of morphology of blood cells.

Results: In the study, out of 229 cases, 32 (13.9%) were positive for hemoglobinopathies on HPLC and 197 (86 %) cases were negative. Among 32 hemoglobinopathies, 12 (37.5%) cases were males and 20 (62.5 %) cases were females. On HPLC, beta Thalassemia trait is the most common and accounts for 18 cases (56.25%), delta beta Thalassemia ware 4 cases (12.5%). Next common hemoglobinopathies found were beta Thaassemia major, Hb D Punjab heterozygous, HPFH and compound heterozygous each one accounts 2 cases (6.25 %). Hb E homozygous and delta beta Thalassemia trait accounts one (3.12%) case each.

Conclusion: The systematic review carried out across the India showed that the microcytic hypochromic anemia with hemolytic component refractory to the treatment must undergo the analysis for hemoglobinopathies. The HPLC is probably the best analyzer for detecting the rare hemoglobin variants. In our study we emphasise that the anemic patients who are resistant to the treatment have to be evaluated for haemoglobin disorders by HPLC to detect even the rare varients of hemoglobin.

Keywords: Microcytic, Hypochromic, Anemia, Hemoglobinopathies, Electrophoresis.

Introduction

Hemoglobinopathies are serious inherited genetic haematological disorders which need lifelong management in chronic and most severe form. If undiagnosed or untreated, ultimately these disorders lead to death. Highest incidences of hemoglobinopathies are seen in south Asia especially India, Pakistan, and Bangladesh. It has been estimated that the prevalence of hemoglobinopathies is 1.2/1,000 live births and with approximately 27 million births per year in India [1]. The spectrum of hemoglobinopathies is wide [2]. The studies published in India have documented that thalassemia minor and major form the major volume of hemoglobinopathies [2]. There are an estimated 100,000 thalassemia major patients and nearly 3.5–4 million carriers of this

genetic disorder [5]. Tribal populations in India have a range of 5–40% sickle cell anemia (SCA) sufferers. In Eastern India, variant hemoglobinopathies like HBE are as common as 3–50% of the population. In India, beta-thalassemia is prevalent across the country, with an average frequency of carriers being 3–4% [3]. A higher frequency has been observed in certain communities, such as Sindhis, Punjabis, Gujaratis, Bengalis, Mahars, Kolis, Saraswats, Lohanas, and Gaurs [4]. HbS is highly prevalent in the tribal populations of Southern, Central, and Western states reaching as high as 48% in some communities [5]. HbE is common in the North-eastern states and has a carrier frequency as high as 50%, in some areas [5]. It is found in lower frequencies in the Eastern states of West Bengal, Bihar, and Uttar Pradesh, while HbD is present in about 2% of people in Punjab [5].

The first case of β-thalassemia/Hb E disease in India was reported by Chatterjea et al. [6] and β-thalassemia/sickle cell disease by Nail et al. [7] Subsequently, other variants of hemoglobins were reported-C, D, F, G, H, J, K, L, M, Q (India), G, etc. [5] Hemoglobins S, D, and E were observed to be quite common: Hb S has been found mostly in tribal communities, Hb D in Gujaratis and Punjabis and Hb E in Bengalis, Assamese and Nepalese. Sickle Cell Disease (SCD) is another hemoglobin disorder that requires lifelong management and contributes to infant and childhood morbidity and mortality. Sickle cell syndromes include (SCD, HbSS), also called SCA, as well as disorders due to sickle cell gene combined with another hemoglobinopathy such as Hb C, E, or beta-thalassemia. In India – First described in the Nilgiri Hills of Northern Tamil Nadu in 1952 [8], the sickle cell gene is now known to be widespread among people of the Deccan plateau of central India with a smaller focus in the north of Kerala and Tamil Nadu [5]. Extensive studies performed by the Anthropological Survey of India [14] have documented the distribution and frequency of the sickle cell trait which reaches levels as high as 35% in some communities.

Materials and Methods

Observational study was carried out on patients (Both out and in patients) who attended Pathology laboratory in a tertiary care centre, Anantapuram from January 2023 to December 2023. Total 229 cases were included in this study population with mild to severe anemia. All cases were subjected to a series of haematological investigations - includes complete hemogram, reticulocyte count, peripheral blood smear. All patients were analysed by Zy bio fully automated 5 – part Cell counter. A peripheral blood smear is used for evaluation of morphology of blood cells by pathologist. Simultaneously all patients were evaluated for iron profile and hemoglobinopathies by Hb analyser - D 10 HPLC - BIO RAD company, which is fully automated, simple, rapid, cost effective and easily operable.

Results

The study was conducted on 229 patients, which includes all age groups and both genders, were subjected for investigations like iron profile and revealed a mix of IDA (86.02%) and anemia of chronic disease and simultaneously all of these cases were subjected to HPLC electrophoretic analysis.

Out of the 229 cases, 197 (86.02%) cases were IDA; remaining 32 (13.97%) cases were found to have one or the other form of hemoglobinopathies. Out of 32 cases of HPLC positive hemoglobinopathies 22 (68.75%) cases were children, remaining 10 (31.25%) cases were adults and 20 (62.5%) cases were females and remaining 12 (37.5%) cases were males which imply that females are affected frequently than the males.

In our study beta thalassemia trait topped the list with 19 (8.29%) cases out of 229 cases. Next to the beta thalassemia trait, delta beta thalassemia was second commonest hemoglobinopathy with 6 (2.62%) cases. The third most common hemoglobinopathies detected were beta thalassemia major and HbE. Thalassemia major accounts 0.8% (Two cases) and Hb E also constituted about 0.8% (two cases). We also found that single (0.43%) case of each Hb D Punjab, delta beta thalassemia and HPFH. We have found no other hemoglobinopathies in our study period.

Out of 32 cases of hemoglobinopathies, 8 cases have shown severe anemia, 10 cases have shown moderate anemia and 14 cases have shown mild anemia with decreased MCV and MCH in all cases.

Table 1: Distribution of patients according to group

Group	Number (%)
Cases negative on electrophoresis	197 (86)
Cases positive on electrophoresis	32 (13.97)
Total	229

Table 2: Distribution of Anemias

Anemia	Female	%	Male	%	Total No of cases
Iron deficiency anemia	123	53.71%	74	32.31%	197
Hemoglobinopathies	20	8.73%	12	5.24%	32

Table 3: Distribution of hemoglobinopathies according to gender

Type of hemoglobinopathies	Female, n (%)	Male, (%)
Beta thalassemia trait	12 (5.24%)	7 (3.05)
Beta thalassemia major	-	2 (0.87%)
Hb D Punjab heterozygous	1 (0.43%)	-
HPFH	1(0.43%)	-
Delta beta thalassemia	-	1 (0.43%)
Delta beta thalassemia trait	4 (1.74%)	2 (0.87%)
Hb E homozygous	2(0.87%)	-

Table 4: Hemoglobin Variants with types and Frequencies

S.N.	Author, Year of Publication, No. of cases(n)	Hemoglobin Variants										
		βTT	βTM/I	Hb D Punjab	Hb E	Hb Q India	Hb S	Hb J Meeru	Hb D Iran	Hb Lepore	HPFH	Thal + Variant
1	Sachdev et al. [1], Jan. 2010 (327)	232 (8.9)	56	13	7	5	3	1	1	1	-	8
2	Chandrashek-har and Soni [2], May 2011 (543)	206 (37.9)	14 (3.1)	5 (0.8)	229 (42.1)	-	29 (6.7)	1 (0.1)	-	1 (0.1)	_	52 (9.4)
3	Shrivastav et all Sep 2013 (1615)	839 (11.5)	308 (4.2)	58 (0.8)	21 (0.2)	4 (0.06)	299 (4.1)	-	-	2 (0.03)	8 (0.11)	13
4	Ankur et all Aug, 2021 (858)	586 (21.0)	149 (5.3)	13 (4.04)	24 (0.8)	-	27 (1.0)	-	-	6 (0.2)	15 (0.5)	
5	Present study	12	2	1	2	-	-	-	-	-		

Discussion

The study was conducted on 229 patients attending the tertiary care hospital, Govt Medical College, Anantapuramu between January 2023 and December 2023. Initially all cases were evaluated by hemogram and peripheral smear which showed reduced hemoglobin, MCV and MCH with microcytic hypochromic cells, respectively. All 229 patients were subjected to further investigations like iron profile and revealed a mix of IDA (86.02%) and anemia of chronic disease. Subsequently, all of these cases were subjected to HPLC electrophoretic study. Out of the 229 cases, thirty two (13.97%) were found to have one or the other form of hemoglobinopathy and remaining 197 (86.02%) cases were IDA. In the present study, out of 32 cases of HPLC positive hemoglobinopathies 22 (68.75%) cases were children, remaining 10 (31.25%) cases were adults which is concordant with the review of fourteen Indian studies conducted by Kaustubh Kharche, Arvind Bhake (2023) [2]. In the present study, out of 229 cases, 20 (8.73%) cases were females and remaining 12 (5.24%) cases were males which imply that females are affected frequently than the males. In the study conducted by Utkarsha singh et al (2023), out of 178 cases positive on electrophoresis 73 (41.0%) were females showing high incidence of beta-thalassemia trait [5]. A study conducted by Chopra and coworkers (2008), out of 258 abnormal cases, 136 (53%) were males and 122 (47%) were females, thus giving slight male preponderance [9].

Among these, out of 229 cases beta thalassemia trait topped the list with 19 (8.29%) cases which is similar to Raj kumar V, Ande Penchalaiah (2020) (10), Upasana joneja et al in 2018[11], Meenakshi Mohapatra et al (2017) [1], Biswas AK and Philip J (2016) [12], Sachdev et al (2010) [13]. Next to the beta thalassemia trait, delta beta thalassemia is

second commonest hemoglobinopathy with 6 (2.62%) cases in our study which is unlike that of other studies. Similar to this study, in most series iron deficiency and thalassemia trait were the commonest causes of microcytic hypochromic blood picture [14]. Mach-Pascual S and colleagues found iron deficiency anemia to be the leading cause of microcytosis (45.2%) [15]. Sinha, et al found a high incidence of IDA (40.57%) among 262 people presenting with microcytic hypochromic anemia on peripheral blood smear [14]

The third most common hemoglobinopathies that were detected in our study are beta thalassemia major and HbE. Thalassemia major accounts 0.8% (Two cases) cases which is concordant with a study conducted by Bhalodia et al (2015) [13] and Hb E also detected third most common hemoglobinopathy constituted about 0.8% (two cases) which is similar to studies conducted by Ankur et al (2021) (D 14) and Ramana et al (2017) [13]. We also found that single (0.43%) case of Hb D Punjab which is similar to the study conducted by Bhalodia et al (2015) [13], single case of each delta beta thalassemia and HPFH.

We have found no other hemoglobinopathies in our study period though they were found in other studies. Out of 32 cases of hemoglobinopathies, 14 cases have shown mild anemia, 10 cases have shown moderate anemia and 8 cases have shown severe anemia with decreased MCV and MCH in all cases.

Conclusion

HPCL is necessary for specific diagnosis of hemoglobinopathies. This is a rapid, simple, comprehensive, reliable, and cost-effective method to detect hemoglobinopathies. As hemoglobinopathies are common and distribution varies across the four regions of the India, all anemic patients should be screened by HPLC, prenatal diagnosis and counseling to reduce the burden of hemoglobinopathies.

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