Original Article

DOI: 10.29054/APMC/19.326

Pattern of Hemoglobinopathies And Thalassemia in Children by Using HPLC At LUMHS Jamshoro Hyderabad Sindh

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ABSTRACT

Objective: To determine the frequency of Hemoglobinopathies in children by using HPLC technique in Hyderabad Sindh. **Study Design:** Descriptive study. **Duration:** During 2015 and 2017. **Methodology:** All children aged below 12 were selected to diagnose thalassemia and haemoglobinothies. EDTA anticoagulant was used to collect a sample of 5 ml blood (intravenous). Red cell indices were evaluated on an autonomous sysmex XN1000 i. hematology analyzer. The HPLC technique was applied to evaluate the hemoglobin in various types such as A2, A, D, S, F and further types. **Results:** Patients' mean age was 8.11±03.36 years. Thalassemia major and minor were highly common as; 36.5% (n=301) and 47.5% (n=301) respectively, after that Sickle cell disorder and trait, Hb-D, Hb-C, Hb-D disease and B-thalassemia with compound heterozygous, with percentages of 1.3%, 7.6%, 0.7%, 1.7%, 0.7% and 3.3% respectively, whereas Hb-E disorder was also seen in 2 subjects. **Conclusion:** We concluded that thalassemia minor, thalassemia major, Sickle cell trait, HbD & HbC trait, and HB-D were most common haemoglobinopatheis in children. HPLC is an excellent diagnostic tool to directly identify the haemoglobin variants in quantification of abnormal and normal haemoglobin fractions with a high precision.

Keywords: Hemoglobinopathies, HPLC technique, Children.

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Submitted for Publication: 12-08-2019 *Accepted for Publication: 30-09-2019*

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Citation: Abbasi S, Qadri F, Jawed M, Ujjan ID, Abbasi S, Jawed K. Pattern of Hemoglobinopathies And Thalassemia in Children by Using HPLC At LUMHS Jamshoro Hyderabad Sindh. APMC 2019;13(2):245-8.

INTRODUCTION

Hemoglobinopathy is a genetic defect, which leads to an abnormal construct of a globin chain. Hemoglobinopathy is single-gene inherited abnormality ; mostly, it is inherited as co-dominant autosomal characteristic.[1] Hemoglobinopathies are the commonest genetic defects in the world, implying structural defects in the globin proteins.[1] The WHO proposed that around 5% of the global population is the carrier of various hereditary hemoglobin disorders.[2] Hemoglobinopathy is the cluster of inherited diseases defined by structural changes in hemoglobin molecule; resulting from either the formation of an unusual haemoglobin chain, like an amino acid substitution, or the under-synthesis of a specified globin chain.[3] Hemoglobinopathies are either produced by thalassemias or abnormal hemoglobin. While anomalous hemoglobin is triggered by the hemoglobin molecule's qualitative structural anomaly and thalassemias is triggered by decreased globin chain synthesis.[4] Worldwide, Hb disorder carriers are projected at 269,000,000.[5] Around 3 percent (0.150 billion) of the global population carry β-thalassemia genes. By far, β-thalassemia is the most prevalent recessively genetically received autosomal monogenic disease with roughly 30,000,000 defective gene carriers and the carrier frequencies ranges between 3% and 17%. Hemoglobin synthesis hereditary disorders such as thalassemias and hemoglobin structure, for example. HbD, HbC, HbS are distributed globally. Thalassemias are likely to be present in each ethnic and racial group, while structural defects of hemoglobin are distributed within some racial and geographic limits. Among the hemoglobin-associated diseases, β-thalassemia remains the most prevalent single gene disease, whereas further hemoglobin diseases, such as HbD, HbS and HbE, alone or in conjunction as well occur in our society .[6] Statistics and facts from the WHO report that 5% of the world's population carries HB diseases.[1] As per the Thalassaemia International Federation, around 0.2 million thalassemia major cases are alive and recorded as getting periodic therapy wordlwide.[7] It has a strong incidence in the nations of the Mediterranean region, India, Central Asia, south-China, Middle East, and the Far East along with nations across the northern coast of South America and Africa.[8] In Pakistan, thalassemia-associated prevalence is around 5 % to 8 %.[9] The projected birth rate of infected babies is 13 per 10000 live births, and an annual birth rate with β-thalassemia major is around 5250 babies .[10] Thalassemia's most prevalent detection techniques are electrophoresis, CBC and techniques of molecular diagnosis like genotyping of PCR and HPL chromatography. In the 20th century, the first technique used for separating colored substances was liquid chromatography. Csaba Horvath coined the word HPLC. It was subsequently renamed "High Performance Liquid Chromatography (HPLC)". Various methods are present to measure Hb A2. These methods include the segregation of haemoglobin from cellulose acetate electrophoresis pH 8.9, microcolumn chromatography, spectrometric elution analysis and HPLC.[11]

On microcolumn chromatography and cellulose acetate electrophoresis, a level of Hb A2 above 3.4 percent is observed in β-thalassemia carriers. Unstable haemoglobins may be correlated with a rise in HbA2 and RBC indices in these situations can possibly remain incompetent with typical trait of β-thalassemias. Several HPLC tools are currently accessible for the quantitation of HbA2. HPLC is an accurate and a sensitive technique for abnormal haemoglobin, Hb-F and Hb A2 detection. Due to its reliability and speed, it is considered as a technique of choice for screening thalassemias. For the identification of β-thalassemia carriers and prevalent anomalous haemoglobin (Hb E, Hb C, Hb S), an automatic HPLC system is presently accessible.[11] Reliable techniques of identification and detection for β-thalassaemia trait (heterozygous) and Hb variants are very essential as it can result in prevention of further severe diseases such as thalassaemia major in children.[12] Hyderabad, Sindh has limited evidence in terms of thalassemias and hemoglobinopathies patterns and therefore it is worthy to investigate these diseases using a greater part of subjects referred towards a medical diagnostic lab. The aim of this research is to use HPLC diagnostic method in Hyderabad Sindh to evaluate the frequency of hemoglobinopathies among children. Early diagnosis may prevent disease transmission and lessen the population's financial burden.

METHODOLOGY

Study Design: Descriptive study.

Settings: Pathology Department of Liaquat university of Medical and Health Sciences, Jamshoro/Sindh Pakistan.

Duration: During 2015 and 2017.

Methods: For the diagnosis of thalassemia and Hemoglobinothies, all the babies (study participants) were referred to diagnostic laboratories LUMHS. All the cases diagnosed with Thalassemia and Hemoglobinothies, Fanconi's anemia and with Iron deficiency anemia were excluded. EDTA anticoagulant was used to collect a sample of 5 ml blood (intravenous). RBC indices were evaluated on an autonomous sysmex XN1000 i. hematology analyzer. Hb A2/F and further variants were evaluated using the HPLC technique of human blood's chromatographic segregation, by using variant-II β-Thalassemia short program of Bio-Rad laboratories. All the data was recorded in the proforma.

Data Analysis Procedure:

The data were analyzed using 20 version of SPSS. For quantitative data, the mean and SD were computed. For qualitative information, frequency and proportion were calculated. The $\chi 2$ test was performed and a P-value < 0.05 was considered as significant.

RESULTS

A total of 2400 children were investigated in this research and 301 of them had hemoglobinopathies. Patients ' mean age (in years) was 8.11±03.36, with a minimum 6 months and a maximum 11 years of range. Male gender 59.8% was in majority, while females were 40.2%. As per religion distribution, almost all subjects were Muslims (96.0 %), while reaming 04.0 % were Hindu. With a strong family history seen in our research; most cases possessed a family history positive (83.4 %), while only 16.6 % had a family history negative. Table 1

Table 1: Demographic characteristics of the patients n=301

Characteristics	Frequency (%)
Age Mean ± SD	8.11±03.36 years
Gender	
Female	121(40.2%)
Male	180(59.8%)
Religion	
Hindo	289 (96.0%)
Muslim	12(4.0%)
Family history	
Positive	250(83.4%)
Negative	50(16.6%)

After HPLC diagnosis, out of 301 cases, major- and minor-thalassemia were highly prevalent as; 47.5 percent and 36.5 percent respectively, followed by HB D trait, β-thalassemia, Sickle cell disease, Sickle cell trait, HBC trait, HBD disease and with heterozygous compound, with a proportion of 7.6 %, 3.3 %, 1.3 %, 1.7 %, 0.7 %, and 0.7 % respectively, whereas Hb-E disorder was also seen in 2 subjects. Table:2

Table 2: Hemoglobinopathies by using HPL chromatography technique (n=301)

Diagnosis	Frequency	Percent
Thalassemia minor	143	47.5
Thalassemia major	110	36.5
Sickle cell disease	4	1.3
Hb D trait	23	7.6
Hb C trait	2	0.7
Sickle cell trait	5	1.7
HB-E disease	2	0.7
HB-D DISEASE	2	0.7
Beta thalassemia with compound heterozygous	10	3.3
Total	301	100.0

DISCUSSION

This research was performed to evaluate the pattern of hemoglobinopathy and thalassemia in babies based on HPLC diagnosis, and we discovered minor and major thalassemia to be the most prevalent as; 47.5 percent and 36.5 percent, respectively. Conversely, Sachdev R et al[13] noted that the total

number of variants of hemoglobin detected was 12.5%. The characteristic of β-thalassemia was the biggest subgroup of anomalous hemoglobin (8.9%). Hosseini S, et al[14] also observed that thalassemia major was most prevalent, which is in favor of our study. While in contrast, β-thalasmia trait was revealed to be the most prevalent in some studies,[15,16] this inconsistency could be because of geographical distribution of hemoglobinopathy in the study population. Khera et al.[17] also noted 110 cases with hemoglobinopathy, with most cases having thalassaemia as 87 (79.1%). Consistently, Hussain J et al[18] from Pakistan, documented that among hemoglobin diseases, β-thalassemia was the most prevalent disorder among 87 (38.30%) cases, after that sickle cell disorder among 73 (32.16%) cases and β-thalassemia among 42 (18.5%) cases. Other hemoglobin diseases such as sickle cell trait, β-thalassemia intermedia, HbE disorder and sickle cell/ β-thalassemia, were collectively identified among 25 (11.0%) cases.

We also observed HB D trait, β-thalassemia with compound heterozygous, Sickle cell disease, HB-D disease, and Sickle cell trait, with percentage of 7.6%, 3.3%, 1.7%, 1.3%, 0.7% and 0.7%, whereas Hb-E disorder was noticed among 2 cases and HB C trait also among 2 cases. Conversely, Khera et al.[17] documented 23 (20.9%) of other hemoglobinopathies including HbS, HbE and HbD. Rao S et al[16] documented 247 (30.8%) cases of 800 with abnormal hemoglobin variants including HbD, HbE, HbS, Hb-Lepore, Hb Q-India, Sb- thalassaemia / HbFH, HbJ-Meerut, HbD-Iran and HbH disease. Similarly, Uddin MM et al[19] stated that β-thalassemia trait (21.3%) was the commonest hemoglobinopathy in Bangladesh, after that E/ β-thalassemia (13.5%) and HbE disorder (9.2%).

A research performed in a reference laboratory found that 327 (12.6%) of hemoglobinopathies patients (out of 1200) and most cases possessed thalassaemia trait (8.9%), whereas other hemoglobinopathies identified in their research were thalassaemia intermedia, β-thalassaemia major, HbF, HbD, HbQ, HbE-b, HbE.[13] Gupta PK et al,[20] reported that following the application of HPLC for the hemoglobinopathies diagnosis, 14.3% cases exhibited various hemoglobin variants and 66.4% had β-heterozygous thalassaemia, 10.9% had sickle cell trait, 3.7% had β-homozygous thalassaemia (HbF 25–91%), 2.2% had homozygous sickle cell anaemia and 1.5% had compound heterozygous state of sickle-β+thalassaemia. these above-stated studies on abnormal hemoglobinopathies differ as per findings in some palaces, the likely explanation for this variation in rates and the hemoglobin variants' large number may be credited to the reality that their trials were performed in referral center of hematology, while in our research all cases had already been diagnosed with thalassemia and hemoglobinopathy and IDA cases were excluded. Thalassemias and further variants of hemoglobin were limited to specific geographic regions, religion, tribes and caste, particularly where marriages were limited to the same regions and community. They are today prevalent all over the world.[21] The likely reason for this is elevated migration of individuals from one area to another.

CONCLUSION

We concluded that thalassemia major, Hb D trait, thalassemia minor, Sickle cell trait, Hb C trait and Hb-D were highly frequent haemoglobinopatheis among children. HPLC is a powerful and an excellent diagnostic tool to directly detect the haemoglobin variants in quantification of abnormal and normal haemoglobin fractions with a high precision. It is automated and gives a superior resolution in addition to an easy internal sample preparation. It is valuable especially if there is a higher prevalence of β-thalassemia traits in population.

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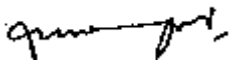
Dr. Ikram Din Ujjan Professor, Pathology Department Liaquat University of Medical and Health Sciences/Jamshoro	Helping in Manuscript Writing	
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