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ORIGINAL ARTICLE

Etiological Profile of Hereditary Hemolytic Anemia among Patients Presenting in Tertiary Care Hospital of Lahore

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ABSTRACT

Background: Hereditary hemolytic anemia is a frequent genetic condition that can have varied levels of morbidity and death. These genetic diseases raise the burden on the patient as well as society and pose a serious threat to public health.
Aim: To determine the frequency of various causes of hereditary hemolytic anemia among patients in a tertiary care hospital.
Methodology: It was a descriptive cross sectional study. Patients (n=252) were enrolled through probability convenient sampling. The study was conducted at Pathology Laboratory, Jinnah Hospital, Lahore. Blood samples were taken from all patients for complete blood count, peripheral smear, reticulocyte count, indirect bilirubin, serum lactate dehydrogenase, and direct antiglobulin test. Median corpuscular fragility (MCF) >4.45g/l NaCl was considered positive. Quantitative enzyme-based assays of Glucose 6 phosphate dehydrogenase were determined spectrophotometrically by using Pointe scientific G6PD reagent. Data were entered and analyzed using SPSS v25.0. A p-value of 0.05 was used to define statistical significance after post-stratification analysis using the chi-square test.
Results: age range in this study was from 1 to 60 years with a mean age of 29.3±9.5 years. According to causes of heredity hemolytic anemia distribution, Thalassaemia major 137(54.4%) was the most common cause followed by Thalassaemia intermedia at 41(16.3%), Sickle Cell Anemia at 34(13.5%), Heredity spherocytosis as 31(12.3%) and Glucose-6 phosphatase deficiency as 9(3.6%).
Practical Implication: As there is a high incidence of anemia among our population and there is lack of local data that specifically addresses this health issue thus current study was planned. This study highlighted that educating the entire medical community could help lower the prevalence of thalassemia major and emphasise the need of diagnosing the thalassemia trait and provide premarital and prenatal counselling.
Conclusion: It was concluded that majority of hereditary hemolytic anaemias were hemoglobinopathies, which place a heavy strain on families and society. The occurrence of these conditions can be reduced in large part by prevention.
Keywords: Heredity Hemolytic Anemia, Heredity Spherocytosis, Thalassaemia Trait and Prention.

INTRODUCTION

A wide range of genetically and phenotypically variable illnesses that are brought on by an increase in the rate of RBC oxidation are collectively referred to as hereditary hemolytic anaemia (HHA)[1,2]. The degree of this destruction determines the severity of the anaemia or the timing of the onset of hemolysis.

While mild hemolysis can be undiagnosed, but severe hemolysis can cause life-threatening anemia, which can lead to cardiac complications[2,3]. Population screening counseling genetic counselling, and prenatal diagnosis can all help prevent HHA, which bears a significant burden on patients, their families, and eventually communities[3-5].

Literature has shown that the prevalence of various causes of HHA varies in different regions and ethnic groups[4-7] but studies regarding its etiological spectrum are scarce on international as well as national levels. Preethi et al. reported β-thalassaemia major as the most common cause of HHA with a frequency of 57.5% followed by β-thalassemia trait at 15%, sickle cell anemia at 12.5%, and hereditary spherocytosis as 10%[5].

While another study conducted by Venkataswamy reported the β-thalassemia trait as the most frequent cause of HHA at 28.26%, followed by β-thalassemia major at 16.45%, hereditary spherocytosis at 13.3%, sickle Thalassaemia 9%, sickle cell disease 8%, Thalassaemia intermedia 6.2% and glucose-6-phosphatase deficiency as 6.29%[4,8].

However, no local literature is available in this regard resulting in a lack of information about the disease burden in our population. The goal of this study is to identify the range of etiological causes for HHA as well as frequency among patients who present in the pathology department of the tertiary care hospital.

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The international literature has shown considerable variation in the etiological profile along with scarcity[9] on the local level warrants a study in the local population to study the magnitude of various causes of HHA. Thus this study provided the information to give an insight into the burden of hemoglobinopathies causing HAA which will help the clinicians to keep a high level of suspicion and evidence-based screening to enable early intervention and diagnosis to lower death and morbidity.

The objective of the study was to determine the frequency of various causes of hereditary hemolytic anemia among patients presenting to a tertiary care hospital.

METHODOLOGY

It was a descriptive cross sectional study. Patients (n=252) were enrolled through probability convenient sampling. The study was conducted at Pathology Laboratory, Jinnah Hospital, Lahore after permission from AIMC Ethical Committee. Study population was patients with hereditary hemolytic anemia. Patients with age 5 months to 60 years of both genders were included in this study. Patients with Coombs-positive hemolytic anemia and drug-induced hemolytic anemia were excluded. Blood samples from each patient were taken for complete blood count, peripheral smear, reticulocyte count, indirect bilirubin, serum lactate dehydrogenase, and direct antiglobulin test. The Hb concentration of all patients was estimated by doing a complete blood count on Hematology Analyzer Sysmex KX21. A microscopic examination of the peripheral smear of blood samples was performed. Reticulocyte count was done manually by using a brilliant cresyl blue solution. Indirect bilirubin was calculated as the difference between total and direct bilirubin performed by spectrophotometry on Beckman coulter AU480. Serum lactate dehydrogenase was performed by spectrophotometry on Microlab 300.

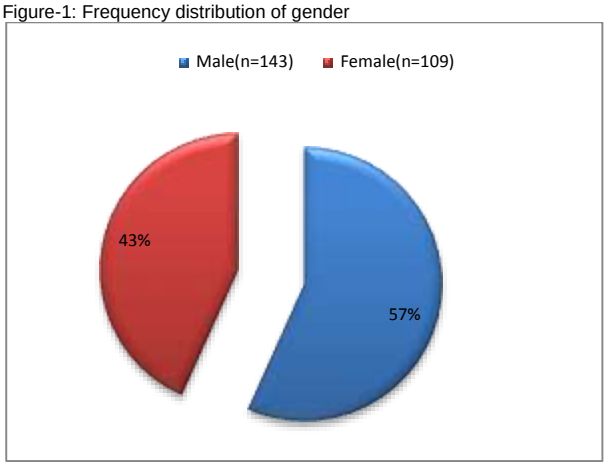
Direct antiglobulin test was performed manually by using polyclonal antihuman globulin serum by Imu Med. Osmotic fragility was performed by using serial hypotonic saline solution and mean osmotic fragility at the wavelength of 540nm was determined. Median corpuscular fragility (MCF) >4.45g/l NaCl was considered positive. High-performance liquid chromatography (HPLC) was done on the Biorad variant. Quantitative enzyme-based assays of Glucose 6 phosphate dehydrogenase were determined spectrophotometrically by using the Pointe scientific G6PD reagent. All data was collected on preformed proforma.

Statistical analysis: SPSS v25.0 was used to enter and analyze the data. The mean and standard deviation of the numerical variable age were calculated. The frequency and percentages for qualitative characteristics including gender and the causes of hemolytic anemia were determined. Age and gender-specific data were stratified. A p-value of 0.05 was used to define statistical significance after post-stratification analysis using the chi-square test.

RESULTS

A total of 252 patients with hereditary hemolytic anemia were included. The age range in this study was from 1 to 60 years with a mean age of 29.3±9.5 years. The frequency distribution of gender and age is shown in Figures-1 and 2 respectively.

Figure-1: Frequency distribution of gender



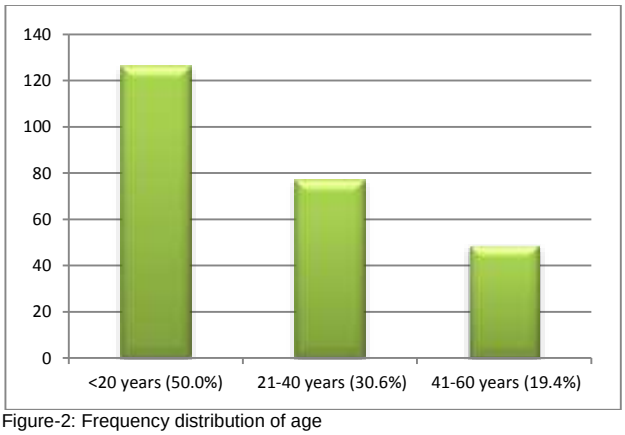


Figure-2: Frequency distribution of age

The distribution of causes of hereditary hemolytic anemia is shown in Figure-3. Thalassaemia major was the most common cause followed by Thalassaemia intermedia, Sickle Cell Anemia, Hereditary spherocytosis, and Glucose-6 phosphatase deficiency.

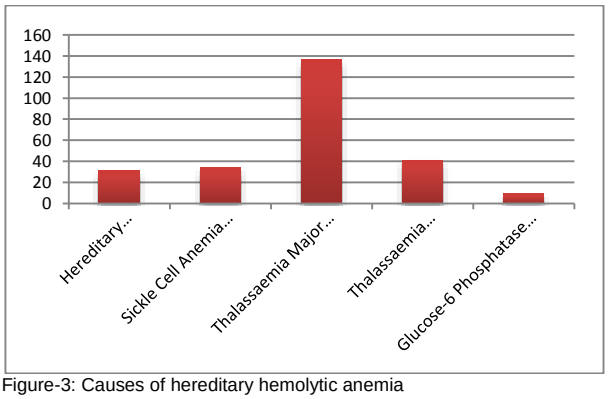


Figure-3: Causes of hereditary hemolytic anemia

According to the stratification of causes of hereditary hemolytic anemia concerning gender, there was no significant difference between males and females (p=0.271) as shown in table-1.

Table 1: Stratification of causes of heredity hemolytic anemia with respect to gender

Gender	Cause of hereditary hemolytic anemia					Total	p-value
	Hereditary Spherocytosis	Sickle Cell Anemia	Thalassaemia Major	Thalassaemia Intermedia	Glucose-6 Phosphatase Deficiency		
Male	22(15.4%)	15(10.5%)	76(53.1%)	25(17.5%)	5(3.5%)	143(100%)	0.271
Female	9(8.3%)	19(17.4%)	61(56%)	16(14.7%)	4(3.7%)	109(100%)	
Total	31(12.3%)	34(13.5%)	137(54.4%)	41(16.3%)	9(3.6%)	252(100%)	

According to the stratification of causes of hereditary hemolytic anemia concerning age, there was no significant difference between age groups (p=0.370) as shown in table-2.

Table-2: Stratification of causes of heredity hemolytic anemia with respect to age

Age groups	Cause of hereditary hemolytic anemia					Total
	Hereditary Spherocytosis	Sickle Cell Anemia	Thalassaemia Major	Thalassaemia Intermedia	Glucose-6 Phosphatase Deficiency	
<20 years	11(8.73%)	6(4.76%)	91(72.22%)	14(11.11%)	4(3.17%)	126(100%)
21-40 years	12(15.58%)	18(23.37%)	32(41.55%)	11(14.28%)	4(5.19%)	77(100%)
41-60 years	8(16.32%)	10(20.40%)	14(28.57%)	16(32.65%)	1(2.04%)	49(100%)
Total	31(12.3%)	34(13.5%)	137 (54.4%)	41(16.3%)	9(3.6%)	252(100%)

P value 0.370

DISCUSSION

Hereditary hemolytic anemia encompasses a broad and diverse range of illnesses, some of which are somewhat common and others that are extremely rare. Worldwide, hemoglobinopathies are more common than hereditary hemolytic anemia. A healthy carrier of a hemoglobin problem makes up more than 5% of the global population[6].

In India, the prevalence of the thalassemia trait and sickle cell disease, respectively, ranges from 3–17% and 1-44%[7-9]. In

Southern India, the –thalassemia gene carrier rate ranges from 1-3%, and in Northern India, it varies from 3-15%[10-12].

In contrast to a study conducted by Balgir RS7 in Orissa, where sickle cell disease was more common, thalassaemia syndromes were more frequently observed in one study[9]. Among hemoglobinopathies and inherited hemolytic anaemias, the thalassemia trait constituted the most frequent cause. Hb electrophoresis was recommended for all incidentally discovered features on peripheral smears, but was only carried out in 50% of the instances, showing doctors' lack of awareness.

The majority of patients with thalassaemia major are below the age of 5 months and present with hepatosplenomegaly and severe anaemia (6g/dl). These patients were all dependent on blood transfusions. The homozygous condition in these patients may have been avoided with parental counselling, premarital testing, and disease knowledge.

Patients with thalassemia intermedia have milder clinical trajectories. Thalassaemia intermedia has received very little research attention, and its actual prevalence is unknown. Patients with thalassemia intermedia may inherit in a homozygous or heterozygous manner[13].

Homozygous patients have high HbF levels at an early age, but heterozygous patients exhibit higher HbA2 levels and close to normal HbF levels at a later age. These patients' haemoglobin levels varied from 6 to 11g/dl and only a small number of them required frequent transfusions. Their HbF levels were lower than those of patients with thalassaemia major. All of these results were consistent with the earlier research[13].

The overall prevalence of the sickle cell gene is 5%. In India, Orissa (9% of the population) has the greatest incidence of sickle cell disease, followed by Assam (8.3%), Madhya Pradesh (7.4%), Uttar Pradesh (7.1%), Tamil Nadu (7.1%), and Gujarat (6.4%). During the extended family screening of sickle cell anemia patients[14], cases of sickle cell trait were found.

Similar to the findings of a study, sickle cell anaemia had a milder clinical presentation with less severe anaemia, the presence of splenomegaly up to the age of 15, and a requirement for blood transfusion in only 23.4% of individuals[15]. The study was similar to the reported elevated HbF levels[16]. This may account for the disease's milder clinical course and haematological findings in this study vs the prior studies[17-18].

Sickle cell thalassaemia compound heterozygosity develops from the inheritance of the sickle cell gene and the thalassaemia gene from each parent. As in this study, the condition might not manifest until late infancy or perhaps later.

Congenital hemolytic anemias like Hereditary Spherocytosis are very prevalent. Hereditary spherocytosis is the most prevalent hemolytic anemia, according to a Japanese study[19]. In addition to thalassemia major, hereditary spherocytosis was the third most common cause of hereditary hemolytic anemia in this study. Hereditary spherocytosis has not been widely reported in the literature[20-21]. The range of ages at the presentation—from one year to 34 years—indicates the disease's varying penetrance.

The most prevalent red blood cell enzymopathy is G6PD deficiency. Drug-induced hemolytic anemia is a significant clinical symptom[22]. Even in this study, 16.3% of patients experienced acute hemolysis after taking sulpha medications for a variety of causes. The goal of the future is to prevent the occurrence of major thalassemia. To do this, high-risk couples must undergo carrier screening, genetic counselling (premarital and preconception), and prenatal diagnosis using PCR-based techniques including reverse dot-blot hybridization, amplification refractory mutation system, and DNA sequencing on chorionic villus sampling. In the second trimester, HPLC on cord blood is rarely performed in facilities[23].

Limitations of study: Financial constrains and limited resources with no genetic workup and no follow-ups added to limitations. It was a single centre study.

CONCLUSIONS

It was concluded that families and society are severely impacted by hemoglobinopathies, which make up the majority of hereditary hemolytic anaemia cases. The occurrence of these conditions can be reduced in large part by prevention. Since few doctors are aware of the thalassaemia trait, educating the entire medical community could help lower the frequency of thalassaemia major and emphasise the importance of diagnosing the thalassaemia trait and provide premarital and prenatal counselling.

Author's contribution: NR&SK: Overall supervision and Write up and literature review, **SK&SSC:** Literature review help in write-up, **SZ&SS:** Write-up with proof reading.
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