Short communication

Frequency of haemoglobinopathies: a single-centre, cross-sectional study from Islamabad, Pakistan

U. Waheed,[1] H.S. Satti,[1] N. Farooq[2] and H.A. Zaheer[3]

معدّل تواتر الاعتلالات الهيموغلوبينية: دراسة مستعرضة في مركز وحيد في إسلام آباد، باكستان

عثمان وحيد، همايون شفيق ساتي، نجم فاروق، حسن عباس ظهير

الخلاصة: تُعَدُّ الثلاسيميا من أكثر الاضطرابات الوراثية تواتراً في باكستان، إذ يقدَّر أن عدد من يحملونها 8 - 10 ملايين. وفي هذه الدراسة التي أجريَت في مركز واحد تقرير عن تواتر اعتلالات الهيموغلوبينية بين 504 من الحالات المتتالية التي زارت مركز إسلام آباد التشخيصي لإجراء رحلان كهربي للهيموغلوبين في الفترة من تموز/ يوليو 2010 وحتى شباط/ فبراير 2011. وقد أجري رحلان الهيموغلوبين على غشاء من أسيتات السيللوز، تلاها تلوين وتفرُّس لقياس كثافة الأشرطة. وتبين أن 143 شخصاً (28.4٪) لديهم اعتلال الهيموغلوبيني، وأن أكثر هذه الاعتلالات هي خلَّة الثلاسيمية (25.6٪)، يتلوها الثلاسيمية الكبرى (1.4٪) والهيموغلوبين S والهيموغلوبين (1.4٪) D. وقد كان تكرار جينات خلَّة الثلاسيميا 0.256 وتكرار الثلاسيميا الكبرى 0.0139. وتؤيد الدراسة الجهود المتواصلة للكشف الباكر والتعرُّف على خصائص الاعتلالات الهيموغلوبينية من أجل معالجة المواليد المصابين بها في باكستان.

ABSTRACT Thalassaemia is the most frequent hereditary disorder in Pakistan, with an estimated 8–10 million carriers. This single-centre study reported the frequency of haemoglobinopathies among 504 consecutive cases visiting Islamabad Diagnostic Centre for haemoglobin electrophoresis from July 2010 to February 2011. Haemoglobin electrophoresis was performed on cellulose acetate membrane, followed by staining and densitometric scanning of bands. A total of 143 (28.4%) subjects had haemoglobinopathies. The most predominant was thalassaemia trait (25.6%), followed by thalassaemia major (1.4%) and HbS or HbD (1.4%). The gene frequencies for thalassaemia trait and major were 0.256 and 0.0139 respectively. The study provides support for continuing efforts towards early detection and characterization of haemoglobinopathies to control the affected births in Pakistan.

Fréquence des hémoglobinopathies : étude monocentrique et transversale à Islamabad (Pakistan)

RÉSUMÉ La thalassémie est l'affection héréditaire la plus fréquente au Pakistan, avec 8 à 10 millions de porteurs selon les estimations. La présente étude monocentrique a étudié la fréquence des hémoglobinopathies chez 504 cas consécutifs en consultation au Centre diagnostique d'Islamabad pour une électrophorèse de l'hémoglobine entre juillet 2010 et février 2011. Une électrophorèse de l'hémoglobine a été réalisée sur une membrane d'acétate de cellulose, suivie par une coloration puis une analyse densitométrique des bandes. Au total, 143 patients (28,4 %) étaient atteints d'hémoglobinopathies. Le trait thalassémique était l'hémoglobinopathie la plus fréquente (25,6 %), suivie par la thalassémie majeure (1,4 %) et l'HbS ou l'HbD (1,4 %). Les fréquences géniques du trait thalassémique et de la thalassémie majeure étaient de 0,256 et 0,0139 respectivement. La présente étude confirme la nécessité de poursuivre les efforts vers un dépistage et une caractérisation précoces des hémoglobinopathies afin de prévenir la naissance d'enfants porteurs au Pakistan.

[1]Department of Biochemistry, Quaid-i-Azam University, Islamabad, Pakistan (Correspondence to U. Waheed: usman.waheed07@gmail.com).
[2]Islamabad Diagnostic Centre, Islamabad, Pakistan.
[3]National Programme Manager, National Blood Transfusion Programme, Ministry of Health, Islamabad, Pakistan.

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Introduction

Haemoglobinopathies are the most prevalent genetic defect worldwide, with an estimated 269 million carriers [1]. Globally, the populations of certain regions are at higher risk of having a haemoglobinopathy [1,2]; while approximately 5% of the world's population carries a gene for sickle-cell anaemia or thalassaemia, the percentage of carriers can reach 25% in some regions [3]. A majority of the haemoglobinopathies are not clinically apparent but some produce serious life-threatening diseases and constitute a significant health care burden. These are quantitative (thalassaemia syndromes) or qualitative (variant Hb) [4–7]. Thalassaemia syndromes are subclassified based on the gene involved, i.e. α and β. These α- and β-thalassaemias are further sub-divided into α+, β+ or αo, βo depending on whether some (+) or no (o) globin protein is produced as a result of the causative mutation.

In Pakistan, where the prevalence of thalassaemia is about 5%–8% [8,9], thalassaemia continues to be a health care challenge and burden on affected families and the health care delivery system. The disease runs in families where intermarriages among relatives are common. It is very important to have reliable detection and identification methods for Hb variants and β-thalassaemia trait (heterozygous) because this can lead to the prevention of more severe disorders such as thalassaemia major (homozygous) in infants [10]. In recent times successful implementation of national thalassaemia screening programmes in neighbouring countries such as the Islamic Republic of Iran and Turkey have shown a steady decrease in newly registered thalassaemia cases [7,11].

The objective of the present study was to determine the pattern of haemoglobinopathies diagnosed at the Islamabad Diagnostic Centre from July 2010 to February 2011. This is one of the largest private sector diagnostic centres in Islamabad city, Pakistan.

Methods

Sample

Five hundred and four (504) cases of anaemia referred from different peripheral hospitals and private clinics from July 2010 to February 2011 were analysed in this study. The research protocol was approved by the medical research review board of Islamabad Diagnostic Centre. All the patients involved in this study were briefed about the objectives of the study and informed consent was obtained from each individual.

Data collection and analysis

Details of patients' age and sex were recorded. The minimum time elapsed since last blood transfusion, if any, was 3–4 months before the blood sample was taken for analysis.

For each patient a 3 mL intravenous blood sample was collected in EDTA-containing vacutainer blood collection tubes. The samples were subjected to testing within 2 hours of sampling using a fully automated blood cell counter (Sysmax KX-21). Haemoglobin electrophoresis was carried out using a commercially available electrophoresis kit comprising of cellulose acetate membrane in tris-EDTA-borate buffer at pH 8.8 (Fisher Biotech). The band densities were measured through a TurboScan digital densitometric analysis system (Fisher Biotech). An *HbA2* value > 3.5% was considered as a cut-off point for beta-thalassaemia trait. The red blood cell indices were compatible with thalassaemia trait in all cases where the *HbA2* gene was raised.

The gene frequency of different haemoglobinopathies was estimated using the Hardy–Weinberg equilibrium $p^2 + 2pq + q^2 = 1$, where p is the frequency of the A allele in the population and q is the frequency of the a allele in the population. The frequencies of genotypes in the population are given by: p^2 for genotype AA, $2pq$ for genotype Aa, q^2 for genotype aa.

Results

Out of the total 504 cases, 226 were male (44.8%) and 278 were female (55.2%), with median ages 22.6 years and 24.2 years respectively. Overall, 361 subjects (71.6%) had a normal haemoglobin pattern, while a raised *HbA2* haemoglobinopathy was seen in 143 (28.4%) cases (Figure 1). Among these, 129 (25.6%) had the thalassaemia trait gene, 7 had thalassaemia

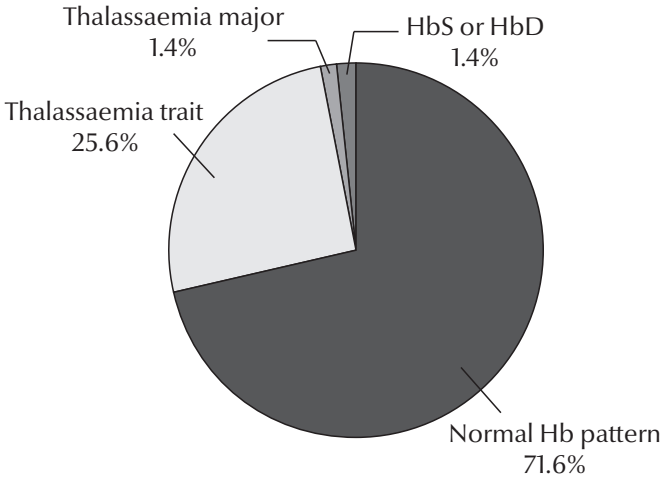

Figure 1 Percentage of normal haemoglobin (Hb) and haemoglobinopathies detected in 504 cases of anaemia at a referral centre in Islamabad, Pakistan

Table 1 Spectrum of 143 haemoglobinopathies detected in 504 cases of anaemia at a referral centre in Islamabad, Pakistan

Haemoglobinopathy	No. of cases	%	Cumulative %	Gene frequency
Thalassaemia trait (+/–)	129	90.2	90.2	0.256
Thalassaemia major (–/–)	7	4.9	95.1	0.0139
HbS or HbD	7	4.9	100.0	0.0139
Total	143	100.0	–	–

HbS = sickle cell haemoglobin; HbD = haemoglobin D variant (Punjab).

major (1.4%) and the remaining 7 patients were found to have HbS or HbD (1.4%). The frequencies of different haemoglobinopathies and their respective gene frequencies are shown in Table 1.

Discussion

The overall frequency of haemoglobinopathies in this study was 28.4%, which is comparable to a previously presented series of 2000 cases from a referral laboratory in the region revealing that 28.2% cases presented with haemoglobinopathies [12]. The results of this study also support the finding that thalassaemia is the most frequent form of haemoglobinopathy in Pakistan [13]. The cumulative percentage of thalassaemia genes among individuals having haemoglobinopathies was 95.1% in this study, compared with 90.1% in another earlier study [12].

This high frequency of thalassaemia genes reflects the high regional and geographical prevalence [8,10]. The estimated thalassaemia gene frequency in Pakistan is around 5%–8%, with 8–10 million carriers and 6000 children born with thalassaemia major every year [8,9]. This is partly because of the high ratio of consanguineous cousin marriages and poor access to education and health facilities [14], and also due to lack of a national thalassaemia screening and prevention programme in Pakistan. In Pakistan, the concept of thalassaemia prevention is gaining momentum and a new bill on thalassaemia prevention has been put forward in the National Assembly. Our results provide support for these continuing efforts towards early detection and characterization of haemoglobinopathies for the control and prevention of affected births.

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