Serum haptoglobin types in patients with hemoglobinopathies

H. W. MOREIRA¹ and P. C. NAOUM²

¹ Departamento de Análises Clínicas, Faculdade de Ciências Farmacêuticas de Araraquara — UNESP, S. Paulo, Brazil
² Departamento de Biologia, Instituto de Biociências, Letras e Ciências Exatas de São José do Rio Preto — UNESP, S. Paulo, Brazil


Haptoglobin types were determined in 626 individuals living in the State of São Paulo (Brazil). Of these, 484 had Hb AA, 31 major beta thalassemia, 43 minor beta thalassemia, 14 Hb SS, and 54 Hb AS. Frequency distribution of the three most common types observed among patients with type beta thalassemia differed significantly from that observed in the Caucasian group with Hb AA. There was a significant increase in Hp 1–1, which led us to assume that these disorders participate in a selective process acting on haptoglobins and altering the equilibrium of their frequencies. This relationship was not observed when we compared patients with Hb SS and Hb AS with Black patients with Hb AA, although the type most often observed among patients with Hb SS was Hp 1–1. The distributions of Hp groups observed among Caucasian and Black patients with Hb AA were similar to those obtained by other investigators for the South and Southeast regions of Brazil, with the exception of Rio de Janeiro.

H. W. Moreira, Departamento de Análises Clínicas, Faculdade de Ciências Farmacêuticas de Araraquara — UNESP, S. Paulo, Brazil

The occurrence of polymorphism at the haptoglobin locus has motivated many investigations directed at the determination of possible associations between haptoglobin (Hp) and different disorders. Some of these investigations show clearly a significant association; some others show an excess of heterozygotes or a bigger frequency of one kind of homozygotes; others, by the way, show contradictory results, sometimes an association is shown and occasionally this association cannot be demonstrated.

In relation to studies of the Hp types and hemoglobinopathies Cuttillo and Melloni (1974) observed a nonsignificant correlation between beta thalassemia and haptoglobin, and the same was reported by Habib (1982) in a study on Egyptian patients. On the other hand, Ostrowski et al. (1987) indicated a significant association between sickle cell disease and the Hp 1–1 type in American blacks screened for hemoglobinopathies.

In view of these inconclusive reports, it is obviously important to obtain additional data. Thus, the objective of the present study was to determine a possible correlation of Hp types and the gene frequency of their alleles with hemoglobinopathies in patients living in the State of São Paulo, Brazil.

Material and methods

The serum samples used in the present study were obtained from 142 individuals from the cities of São Paulo, Araraquara and São José do Rio Preto, who had been diagnosed clinically and by laboratory tests as patients with hemoglobinopathies, and from 484 healthy individuals from the city of Araraquara, who presented electrophoretic hemoglobin profiles with normal fractions. No kinship existed among the individuals studied. Serum samples were used fresh or stored frozen at −20°C.

Haptoglobin types were characterized by horizontal starch gel electrophoresis using the technique of Smithies (1955). The gel was prepared with 1.42 M Tris-EDTA-borate buffer, pH 8.6, and hydrolyzed starch, and 0.36 M sodium borate buffer, pH 8.6, was used for the electrolytic compartments. The haptoglobin types were disclosed with ortho-dianisidine.

Of the 142 patients with hemoglobinopathies (age range, 3 to 54 years), 31 had major beta thalassemia, 43 had minor beta thalassemia, 14, sickle-cell anemia, and 54, sickle-cell trait. The control group consisted of 484 healthy individuals, aged 8
Table 1. Haptoglobin types in caucoids and negroid control groups

<table>
<thead>
<tr>
<th>Racial group</th>
<th>n</th>
<th>1-1 No.</th>
<th>2-1 No.</th>
<th>2-2 No.</th>
<th>2-1M No.</th>
<th>0-0 No.</th>
<th>Hp*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caucasians</td>
<td>374</td>
<td>51</td>
<td>13.6</td>
<td>187</td>
<td>50.0</td>
<td>135</td>
<td>36.1</td>
</tr>
<tr>
<td>Negroids</td>
<td>110</td>
<td>36</td>
<td>32.7</td>
<td>49</td>
<td>44.6</td>
<td>21</td>
<td>19.1</td>
</tr>
</tbody>
</table>

*Caucoids: Negroids \( \chi^2 = 25.772 \) P < 0.001

Hp\(^1\) and Hp\(^2\) gene frequencies were calculated by gene counting. Deviations from the expected Hardy-Weinberg equilibrium and differences between patients and controls with respect to gene and phenotype distributions were determined using the \( \chi^2 \) test (Scheffler 1969).

Results

The distribution of haptoglobin types in the control group divided into racial groups (Table 1) showed that Hp 2–1 was the predominant type among Caucasians and Blacks, followed by Hp 2–2 among Caucasians and Hp 1–1 among blacks. On this basis, the Hp\(^1\) gene frequency differed in a highly significant manner between the two racial groups. The Hp\(^1\) gene frequency observed among Caucasians was equivalent to that detected among the Caucasian populations of Europe and America, whereas the Hp\(^1\) gene frequency observed among Blacks followed the distribution occurring in African groups. This demonstrates the value of the Hp\(^1\) gene as a racial marker.

Table 1 also shows that other haptoglobin types differing from the three most common ones were also observed, with unaptoglobinemia (Hp 0–0) occurring in 0.3 \% of the Caucasians and in 2.7 \% of the Blacks. With respect to the rare modified Hp 2–1 type, which is more common among Blacks, only one case was detected in the present group of Blacks.

The haptoglobin types observed among the patients with hemoglobinopathies (Table 2) show that among the patients with major beta thalassemia, with predominance of Hp 1–1, followed by Hp 2–1 and Hp 2–2, Hp\(^1\) gene frequency was significantly different when compared with that detected in the control Caucasian group. Patients with minor beta thalassemia, despite the evidence for higher Hp 2–1 percentages followed by Hp 1–1 and Hp 2–2, also differed significantly in the Hp\(^1\) gene frequency when compared with their control group. This suggests the occurrence of an increase in the Hp\(^1\) gene in view of the haptoglobin distribution among individuals with major or minor beta thalassemia, which differed significantly from that observed in the group of Caucasians with normal hemoglobin.

Among patients with hemoglobin variants (Table 2), those with Hb SS showed a higher percentage of Hp 1–1, followed by equal percentages of Hp 2–1 and Hp 2–2. On this basis, calculation of Hp\(^1\) gene frequency showed nonsignificant differences in relation to the control Black group. Patients with Hb AS had a higher percentage of Hp 2–1 followed by Hp 1–1 and Hp 2–2, also showing nonsignificant differences in relation to the control Black group.

The hemoglobinopathies groups and controls are well complying with the Hardy-Weinberg equilibrium, although patients with major beta thalassemia and Hb SS showed an excess of homozygotes (48.4 and 35.7 \%) and patients with minor beta thalassemia and Hb AS showed an excess of heterozygotes (48.8 and 48.2 \%).

Discussion

The State of São Paulo is a Federal Unit located in the Southeast Region of Brazil with 24,708,946 inhabitants distributed among 571 cities and 304 villages over a surface of 247,320 km\(^2\) (I.B.G.E. FOUNDATION 1982/83). The formation of this population has been well defined: over a period of 327 years, the population basically consisted of natives, Portuguese colonizers, African slaves, and hybrids resulting from racial miscegenation. From 1827 to 1932, the region received 2.6 million immi-
Table 2. Distribution of haptoglobin types and the statistical comparison between the phenotype distributions observed in the hemoglobinopathies and the respective control group

<table>
<thead>
<tr>
<th>Study group</th>
<th>1-1</th>
<th>1-1</th>
<th>2-2</th>
<th>2-1M</th>
<th>0-0</th>
<th>Hp'</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>MBT</td>
<td>31</td>
<td>15</td>
<td>10</td>
<td>32.3</td>
<td>6</td>
<td>19.3</td>
</tr>
<tr>
<td>mBT</td>
<td>43</td>
<td>11</td>
<td>21</td>
<td>48.8</td>
<td>9</td>
<td>21.0</td>
</tr>
<tr>
<td>SS</td>
<td>14</td>
<td>5</td>
<td>4</td>
<td>28.6</td>
<td>4</td>
<td>28.6</td>
</tr>
<tr>
<td>AS</td>
<td>54</td>
<td>16</td>
<td>26</td>
<td>48.2</td>
<td>10</td>
<td>18.4</td>
</tr>
</tbody>
</table>

MBT : Caucasian control  
\[ \chi^2 = 25.351 \]  
P < 0.001

mBT : Caucasian control  
\[ \chi^2 = 6.432 \]  
P < 0.04

SS : Negroid control  
\[ \chi^2 = 1.352 \]  
P < 0.70

AS : Negroid control  
\[ \chi^2 = 0.219 \]  
P < 0.90

* Hp 2-1M and Hp 0-0 were excluded

MBT = Major beta thalassemia  
mBT = Minor beta thalassemia  
SS = Sickle-cell anemia  
AS = Sickle-cell trait

With grants, 36% of whom were Italians, 15% Portuguese, 14% Spaniards, 4% Japanese, 20% Germans, Arabs, Austrians, Russians, and Poles, and 11% of other origins (Ellis Jr. 1949). Obviously, these groups dispersed in an uneven manner throughout the state, although the ethnic diversity that gave origin to the present São Paulo population shows little differences, except for a few isolated nuclei.

Considering that the control sample for the present study was from the city of Araraquara, in the geographical center of the State of São Paulo, and that the groups of individuals with hemoglobinopathies were from three different cities in the state, we proposed to investigate whether our results could be considered valid as control for the entire state and to determine the relationship of the present study to others carried out in Brazil.

The data about haptoglobin type distribution in our Caucasian and Black control groups did not differ from those obtained in a study on the city of São Paulo (Hoxter et al. 1965) or from those obtained in Southern Brazil (Tondo et al. 1963; Schwantes et al. 1967). These values are probably valid for the Southern and Southeastern regions of Brazil, except for Caucasians in Rio de Janeiro (Rocha et al. 1972), who have their own peculiar anthropological characteristics with miscegenation higher than other states.

When we compared the Hp' gene frequency and distribution observed in the present groups of Caucasians and Blacks as representative of the general population of Araraquara (State of São Paulo), with those obtained in two cities of the Northern region of Brazil (specifically the Amazon region), significant differences were observed (P < 0.02 to Belem and P < 0.04 to Manaus). This was probably due to the characteristics of miscegenation of the Amazon population with natives. Indeed, Amazon Indians must have high Hp' gene frequencies since Salzano and Sutton (1965) detected high frequencies (0.90) among the Guaharibos of Venezuela, and Santos and Guerreiro (1986) also observed high frequencies (0.91) in a tribe of the Brazilian Amazon region. A larger number of tribes of this region, however, need to be studied to confirm this frequency.

The anaptoglobinemia observed in the Caucasian and Black groups considered as control was as expected for these two groups—known to be free of hemolytic processes—since Hp 0-0 is rare among European adults (Giblett 1961), whereas its frequency is significant among inhabitants of North Africa, reaching 40% in some areas (Allison and Barnicot 1960). This percentage, however, should be considered with caution in view of the high local incidence of hepatic disease, malaria and hemoglobinopathies, which are associated with a more intense hemolytic process. Among American Blacks with normal hemoglobin, anaptoglobinemia frequencies close to 3% were observed (Giblett and Steinberg 1960), in agreement with the values obtained for our Black control group.

Haptoglobin type distribution among the present individuals with hemoglobinopathies was analyzed, taking into account that beta thalassemia, even though it is currently detected both in Caucasians and in Blacks because of the high level of misce-
nation characteristic of the Brazilian population, is highly prevalent and possibly of mutational origin among Caucasians, and especially among Mediterranean populations. The hemoglobin S gene, though frequent in our Black population but also present among Caucasians, possibly originates from African groups (Lehmann and Hunstman 1974; W.H.O. 1983; Honig and Adams III 1986). For this reason, we compared the patients with major and minor beta thalassemia with the Caucasian controls and the patients with hemoglobin AS and SS with the Black control group.

The nonsignificant differences observed here between patients with Hb SS and Hb AS in relation to the Black group are similar to those obtained by Mehta and Jensen (1960) when they compared 32 patients with Hb SS with 92 patients with Hb AA.

High frequencies of anaptoglobinemia are known to occur among patients with sickle-cell anemia and patients with major beta thalassemia. In our study, however, we observed only one such case among the subjects with minor beta thalassemia, one case among subjects with Hb SS, and two cases among subjects with Hb AS. Except for the patient with minor beta thalassemia, the anaptoglobinemia observed in the others must have been associated with intravascular hemolysis.

In an attempt to discuss our results, we may consider the fact that Hp³ gene frequency has been well characterized as varying from 0.36 to 0.41 for European and American Caucasians and from 0.51 to 0.41 for Africans. Thus, any significant variation in this value may represent evidence that the system is not in equilibrium.

On the other hand, the Hp² allele is the result of partial duplication of the Hp¹ gene (Smithies et al. 1962), and it is surprising that the new and longer polypeptide Hp² is stable and active and has spread, with higher frequencies than its older Hp¹ allele in some populations (Norton et al. 1966).

By comparing the individuals with major and minor beta thalassemia with the Caucasian control group, we detected a statistically significant difference between them. Furthermore, in both thalassemia groups, the Hp¹ gene frequency was higher than in the control group and also higher than expected for Caucasian populations. Thus, we may assume that these pathologies may participate in a selective process acting on the haptoglobin systems by changing the equilibrium of their frequencies.

Even though patients with Hb SS and Hb AS had lower Hp¹ gene frequencies than the Black control group, the differences were not statistically significant. However, it is interesting to note that the type most often observed among patients with Hb SS was Hp 1–1, possibly indicating the same participation as suggested for the thalassemia patients.

On this basis, we believe that more extensive studies should be conducted to confirm or exclude this association among patients with thalassemia or individuals with hemoglobin variants.

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References


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