Ahaptoglobinaemia and Predisposition to Iron-Deficiency Anaemia*

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Ahaptoglobinaemia in a sample from northeastern Brazilian was found to be significantly associated with a low hematocrit (Krieger *et al*, 1965; Morton, Krieger, and Mi, 1966). Further analysis showed no explanation for the association. Individuals with abnormal haemoglobin genotypes SS, SC, and CC were excluded but the association with low hematocrit remained significant (Azevêdo, Krieger, and Morton, 1969). Because malnutrition and infections by parasites were severe in this population, iron-deficiency anaemia was probably the cause for the low hematocrit. A possible role of ahaptoglobinaemia in the development of iron-deficiency anaemia was considered at this time and chosen as a working hypothesis for the present paper.

Material and Methods

Coloured schoolchildren from Bahia state, northeast Brazil, were sampled for clinical aspects of anaemia. To avoid acquired changes of haptoglobinaemia (Shinton, Richardson, and Williams, 1965) a brief medical examination was carried out to rule out children with body temperatures over 36.9° C, infected tonsils, jaundice, or respiratory infections. For every anaemic child thus selected a non-anaemic control matched for age, race, and sex, was chosen. Race was classified as light mulatto, medium mulatto, dark mulatto, and negro, according to our previous criteria (Krieger *et al*, 1965). The controls were also examined clinically and rejected by the same criteria. Five millilitres of venous blood were collected in dry oxalate and immediately refrigerated.

Two to eight hours after collection, 4 ml of blood were centrifuged and the plasma refrigerated for electrophoresis on the following day. Quantitative haemoglobin, hematocrit, and mean corpuscular haemoglobin concentration (MCHC) were estimated and a reticulocyte count made on the remaining aliquot of whole

blood. Horizontal electrophoresis for haptoglobin was carried out at room temperature using a discontinuous buffer system (Poulik, 1957). The haptoglobin zymogram was stained by the modified benzidine method (Higashi and Lubs, 1966). Those samples with insufficient haptoglobin levels to allow phenotyping were classified as ahaptoglobinemias.

Partial analysis of the material was done with an IBM 1130 computer at the University of Bahia.

Results

One hundred and one anaemic children and their controls were sampled from 24 schools. There were 73 boys and 28 girls in each group. Their mean age was 10.2 years. Nineteen percent of the sample was light mulatto, 54% medium mulatto, 25% dark mulatto, and 2% negro.

Reticulocyte counts were found to be too low to justify haemolytic anaemia as a cause of both the low hematocrit and of the ahaptoglobinaemia.

The means for haemoglobin, hematocrit, and MCHC in the anaemic group were 10.97, 34.31, and 31.65 g%, while in the controls they were 13.14, 39.50, and 33.50 g% respectively. The differences between the means of the anaemic group and the control group were highly significant ($p < 0.001$).

Haptoglobin phenotypes and gene frequencies for anaemics and controls are given in Table I. For each group there was no significant deviation from the expected phenotype frequencies under Hardy-Weinberg equilibrium ($\chi^2_1 = 1.33$; $p > 0.10$).

Five ahaptoglobinaemics were found among the anaemic children and 8 among the controls. There was no evidence of lower hematocrit, lower haemoglobin levels, or lower MCHC in the ahaptoglobinaemic children (Table II).

To investigate further the possibility of haptoglobin phenotype effect on the development of severe anaemia, we selected a subsample of anaemic children with hematocrit equal or below the group

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TABLE I

HAPTOGLOBIN PHENOTYPES AND GENE FREQUENCIES IN ANAEMIC AND CONTROL CHILDREN, AND EXPECTED NUMBERS UNDER HARDY-WEINBERG EQUILIBRIUM

Pheno- types	Anaemic Children		Controls	
	Obs.	Exp.	Obs.	Exp.
Hp 1-1	33	31.5 (p ²)	29	28 (p ²)
Hp 2-1	40	44.6 (2pq)	41	44.4 (2pq)
Hp 2-2	19	17.6 (q ² + 2qr + r ²)	20	19 (q ² + 2qr + r ²)
HP 2 ^m -1	4	2.3 (2pr)	3	1.7 (2pr)
Total	96	96	93	93
<i>Gene frequencies</i>				
Hp 1 = 0.4062			Hp 1 = 0.4355	
Hp 2 = 0.5729			Hp 2 = 0.5484	
Hp 2 ^m = 0.0208			Hp 2 ^m = 0.0161	
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Test for Hardy-Weinberg equilibrium: $\chi^2 = 1.93$			Test for Hardy-Weinberg equilibrium: $\chi^2 = 1.33$	

mean (34.31%). There were 36 children in this group none of whom were ahaptoglobinemic. A χ^2 test with Yates' correction for small numbers (Fisher, 1948) showed no difference between the occurrence of the phenotypes Hp 1-1, Hp 2-1, Hp 2-2, and Hp 1-2^m in the most anaemic children and their own controls ($\chi^2_3 = 1.71$; $p > 0.50$).

Discussion

The role of haptoglobin in iron preservation in humans is uncertain. Haptoglobin binds $\alpha\beta$ dimers (Bunn, 1967) to form a stable complex which is removed mainly by the liver (Keene and Jandl, 1965) and is unable to pass the glomerular membrane (Lausell and Nyman, 1957). The breakdown of the complex allows no return of either haemoglobin or haptoglobin into the circulation (Noyes and Garby, 1967). Therefore, reutilization of iron is assumed. In ahaptoglobinaemic subjects the 24-hour excretion of iron and the renal excretion of haemoglobin do not differ from normal (Whitten, 1962) suggesting no specific disadvantage in having hap-

globin absent from the plasma. On the other hand, other proteins such as albumin and β -globulin have certain roles in iron preservation by combining with heme and forming methemalbumin and hemopexin which are not excreted (see Giblett, 1969).

The marked shortening of red blood cell survival in iron-deficiency anaemia (Layrisse, Linares, and Roche 1965; Husser, Rieber, and Berman, 1967; Loria *et al*, 1967) does not lead to an appreciable increase in free hemoglobin in the plasma able to bind all the available haptoglobin (Layrisse *et al*, 1965). Diez-Ewald and Layrisse (1968), demonstrated a correlation between the proportion of abnormal erythrocyte in peripheral blood and shorter survival of the red blood cells. Assuming that the proportion of abnormal erythrocyte reflects the severity of anaemia and consequently the red blood cell survival, it was of interest to measure the haptoglobin levels in cases of extremely severe anaemia. Six children were observed with haemoglobin levels below 6 g% and none had ahaptoglobinaemia. Finally, the haptoglobin levels in 12 children with severe iron-deficiency anaemia (Hb g% from 3.5 to 7.6) studied by Layrisse *et al* (1965), were normal in all but one case and allowed electrophoretic phenotyping in all of them.

Summary

Haptoglobin phenotypes of 101 school children with clinical and laboratory records of anaemia were compared with a control sample matched for age, race, and sex. The gene frequencies for the alleles Hp 1, Hp 2, Hp 2^m, and Hp 0 were similar in both groups. There was no association between low hematocrit and ahaptoglobinaemia. This data does not support the hypothesis that ahaptoglobinaemia predisposes to iron-deficiency anaemia, nor that the shorter survival of red blood cells demonstrated in this type of anaemia is sufficient to cause ahaptoglobinaemia.

TABLE II

HAEMATOLOGICAL DATA AND MEAN AGE OF ANAEMIC AND CONTROL CHILDREN DISTRIBUTED BY HAPTOGLOBIN PHENOTYPES

Phenotypes	Haemoglobin (g%)		Hematocrit (%)		MCHC		Mean Age		Number of Children	
	Anaemics	Controls	Anaemics	Controls	Anaemics	Controls	Anaemics	Controls	Anaemics	Controls
Hp 1-1	9.83	13.37	31.49	39.05	30.66	33.96	10.00	9.62	33	29
Hp 2-1	11.27	12.96	35.17	39.35	31.93	32.96	10.15	10.41	40	41
Hp 2-2	11.60	13.07	36.00	39.37	32.00	33.22	10.63	10.50	19	20
Hp 2 ^m -1	11.70	12.63	36.00	42.17	32.42	30.03	9.00	10.66	4	3
Hp 0-0	13.04	13.52	38.40	39.65	33.94	34.09	10.40	9.62	5	8
Group means	10.97	13.14	34.31	39.50	31.65	33.30	10.16	10.16	101	101
Test between groups	t = 8.42; p < 0.001		t = 8.04; p < 0.001		t = 4.39 p < 0.001					

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