Evaluation of haptoglobin phenotype 0-0 in cirrhotic and non-cirrhotic hospital populations¹

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SYNOPSIS The haptoglobin phenotypes of 3,332 individuals, consisting of 2,930 caucasians and 402 negroes living in the greater Boston area, were determined. Of these, 3,222 were hospitalized medical patients fully documented regarding diagnoses. One hundred and twenty-eight of the total population studied were shown to exhibit starch gel anhaploglobinaemia (3.7%). Re-evaluation on acrylamide gel of 118 0-0 samples revealed that the majority (94%) were derived from patients exhibiting hypohaploglobinaemia rather than anhaploglobinaemia.

Haptoglobins are α_2 glycoproteins which form stable complexes with free haemoglobin (Polonovsky and Jayle, 1939 and 1940). Smithies (1955) using starch gel electrophoresis demonstrated three distinct serum haptoglobin phenotype patterns. These were designated 2-1, 2-2, and 1-1, and are the expression of three genotypes produced by a single pair of allelomorphous genes (Smithies and Walker, 1956). Later, it was observed using the starch gel technique that sera from a small percentage (<3%) of healthy adult caucasians exhibited a total absence (anhaploglobinaemia) of serum haptoglobin proteins (Allison, Blumberg, and Ap Rees, 1958). Family studies have demonstrated that in some instances such a phenomenon can be explained on a genetic basis (Harris, Robson, and Siniscalco, 1958; Matsunaga, 1962).

Illnesses accompanied by intravascular haemolysis (Brus and Lewis, 1959; Owen, DeGruchy, and Smith, 1960; Reerink-Brongers, Prins, and Krijnen, 1962) and/or liver disease with hepatocellular dysfunction (Jayle and Boussier, 1955; Owen, MacKay, and Got, 1959) can result in hypohaploglobinaemia which is characterized by a reduction of the serum haptoglobin to a

level below the accepted normal range of 40 to 180 mg%, expressed as haemoglobin-binding capacity (Nyman, 1959; Bayani-Sioson, Lauch, Sutton, Neel, Horne, and Gershowitz, 1962). If depletion is severe (ie, haemoglobin-binding capacity of less than 20 mg%) then electrophoresis on starch gel may show an apparent absence of haptoglobin-haemoglobin protein complexes (anhaploglobinaemia). Such sera are classified 0-0 phenotype. By using either a freeze-drying or ultrafiltration technique as a means of concentrating serum proteins, investigators (Whitten, 1961; Murray, Robinson, and Visnich, 1966) using starch gel have been able to determine the haptoglobin phenotype of apparent anhaploglobinaemic sera. The true haptoglobin phenotype of most sera classified 0-0 (starch gel) can, however, be ascertained without prior concentration using an acrylamide gel electrophoresis technique (Nandi and Lewis, 1970). The present paper reports a study of the haptoglobin phenotype distribution within a hospital population and the result of a re-examination on acrylamide gel of 117 patients with the 0-0 starch gel haptoglobin phenotype classification.

Clinical Material

A comprehensive drug surveillance programme is at present being conducted in five Boston hospitals (Slone, Jick, Borda, Chalmers, Feinleib,

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Muench, Lipworth, Bellotti, and Gilman, 1966). Nurse monitors collect information concerning vital statistics (including admission and discharge diagnoses), drug experience, and the occurrence or not of adverse reaction on all patients entering certain general medical wards. This information is then stored on magnetic tape and is available for programmed analysis. As a part of this survey, a blood sample is collected from each patient so that a genetic profile can be defined (Slone, Gaetano, Lipworth, Shapiro, Lewis, and Jick, 1969). Determination of haptoglobin phenotype on starch gel electrophoresis is included among the battery of tests routinely performed. The excess serum left over following completion of genetic testing is frozen at -25°C and is available for further study. Of 3,310 consecutive patients admitted to the monitored medical wards, 3,222 were fully documented regarding race, diagnosis, haptoglobin phenotype, and titre. Because of racial differences in haptoglobin phenotype distribution a division into caucasian and negro was made. These populations were then subdivided into cirrhotics and non-cirrhotics. 'Normal' sera from an additional 110 caucasians (LSH staff members) were used for control purposes.

Methods

HAPTOGLOBIN PHENOTYPE

Starch gel electrophoresis (Smithies, 1955)

Haptoglobin phenotyping was routinely carried out in batches of 15 samples at a time on a horizontal starch gel block (10 cm $\times$ 18.5 cm $\times$ 0.25 cm) in a Buchler tank. In order to provide an adequate level of haemoglobin-haptoglobin

complex for phenotyping, one drop of a 400 mg% solution of free haemoglobin was added to 5 drops of each serum sample before introduction into the gel in filter paper wicks (Lewis, MacLennan, and Miller, 1969). A continuous borate buffer system (0.027 M for the gel and 0.30 M for the bridge) was used. Optimum migration was obtained using an electric current of 1.5 ma/cm width (complementary voltage 140). Electrophoresis was continued for 18 hours overnight after which the gel was cut into three slices (Lewis and Yudowitz, 1966). The top surface of the middle slice was flooded with 5% o-tolidine in 90% acetic acid. After 10 minutes colour development was induced by washing the gel surface with 1 vol% solution of hydrogen peroxide. Optimal clarity and colour intensity of phenotype patterns occurs within 15 minutes.

Acrylamide gel

A two-phase horizontal acrylamide gel system was used (Nandi and Lewis, 1970).

Results

Table I shows the distribution of haptoglobin phenotypes (starch gel) excluding those categorized as 0-0 in caucasian and negro populations. A significant difference exists in the distribution of phenotypes when an overall comparison is made between caucasians and negroes ($P < 0.001$) in that there is an excess of 1-1 and a deficiency of 2-2 phenotype in the negro population. In contrast, no significant difference emerges when caucasian normals are compared with either caucasian non-cirrhotics or cirrhotics. Again, cirrhotic and non-cirrhotic negroes have similar patterns of distribution.

Table II gives the incidence of 0-0 (starch gel) phenotype pattern associated with the populations listed in Table I. A significant excess of patients with the 0-0 phenotype occurs in both the caucasian and negro cirrhotic groups.

Table III lists the principal diagnoses associated with the 127 patients exhibiting the 0-0 pattern on starch gel electrophoresis. Fifty-five of the 110 caucasians had a diagnosis on discharge of cirrhosis of the liver. Of the 55 caucasian non-cirrhotics, 17 were described as either alcoholic or having acute hepatitis. Four of the 17 negroes had cirrhosis of liver, three of acute or chronic alcoholism, and four of sickle cell anaemia.

Table IV shows the true haptoglobin phenotype distribution as revealed by acrylamide gel of 118 sera samples categorized as 0-0 on starch gel. One 'normal' and six of the 50 caucasian cirrhotics remained 0-0 following this examination. In contrast, none of the 51 caucasian non-cirrhotics with an 0-0 starch gel pattern remained so following re-examination. Comparison between

Population	Haptoglobin Phenotype			Total
	2-1 (no.) ¹	2-2 (no.)	1-1 (no.)	
Caucasian				
Normal ²	46 (42)	45 (41)	18	109
Non-cirrhotic	1,146 (47)	938 (38)	358 (15)	2,442
Cirrhotic	128 (47)	104 (39)	36 (14)	268
χ^2 (4df) = 1.31, $P > 0.85$				
Negro				
Non-cirrhotic	171 (47)	86 (24)	102 (29)	359
Cirrhotic	11 (42)	7 (27)	8 (31)	26
χ^2 (2df) = 0.28, $P > 0.85$				
Caucasian v negro (overall haptoglobin phenotype distribution)				
χ^2 (2df) = 59.6, $P < 0.001$				

Table I *Distribution of haptoglobin phenotypes (starch gel) in caucasian and negro cirrhotic and non-cirrhotic populations excluding those classified 0-0*

¹Figures in parentheses indicate percentages.

²Hospital staff (LSH).

Population	Haptoglobin Phenotype		Total
	0-0 (no.) ¹	Others (no.)	
Caucasian			
Normal	1 (0.9)	109 (99.1)	110
Non-cirrhotic	55 (2.2)	2,442 (97.8)	2,497
Cirrhotic	55 (16.9)	268 (83.1)	323
0-0 v others	χ^2 (2df) = 175.1, $p < 0.001$		
Normal v non-cirrhotic	χ^2 (1df) = 0.34, $p > 0.5$		
Normal v cirrhotic	χ^2 (1df) = 17.5, $p < 0.01$		
Non-cirrhotic v cirrhotic	χ^2 (1df) = 163.8, $p < 0.001$		
Negro			
Non-cirrhotic	13 (3.5)	359 (96.5)	372
Cirrhotic	4 (12.3)	26 (87.7)	30
Non-cirrhotic v cirrhotic (Fisher exact test)	$p = 0.03$ (one-tailed test)		

Table II Distribution of haptoglobin 0-0 phenotype (starch gel) in caucasian and negro non-cirrhotic and cirrhotic populations

¹Figures in parentheses indicate percentages.

Diagnosis	Caucasian Negro	
Cirrhosis of liver (including Laennec's, alcoholic, idiopathic, and nutritional)	55	4
Non-cirrhotic		
Alcoholism (acute and chronic)	11	3
Acute hepatitis (serum, infective, and alcoholic)	6	0
Cardiovascular and pulmonary diseases (including rheumatic and arteriosclerotic heart diseases, hypertension, congestive cardiac failure)	9	4
Sickle cell anaemia	1	4
Neoplastic (including carcinomas of various organs, lymphomas, leukaemias, and Hodgkin's disease)	11	0
Miscellaneous (including endocrine, renal, haematological, neurological, and psychiatric disorders)	17	2
Total	110	17

Table III Distribution of 127 patients classified haptoglobin phenotype 0-0 on starch gel electrophoresis within several broad diagnostic categories

Population	Total	Haptoglobin Phenotype (%)						
		2-1		2-2		1-1		0-0
		Observed	Expected ¹	Observed	Expected ¹	Observed	Expected ¹	Observed
Caucasian								
Normal	1	0		0		0		1
Non-cirrhotic	51	19 (37)	23.9	30 (59)	19.7	2 (4)	7.4	0
Cirrhotic	50	17 (39)	20.6	22 (50)	17.0	5 (11)	6.4	6
Non-cirrhotic (observed) v non-cirrhotic (expected) χ^2 (2df) = 10.3								
Cirrhotic (observed) v cirrhotic (expected) χ^2 (2df) = 2.4								
Negro								
Non-cirrhotic	12	5	5.7	7	2.9	0	3.4	0
Cirrhotic	4	0	1.9	3	1.0	1	1.1	0

Table IV Haptoglobin phenotype distribution of sera designated 0-0 on starch gel after re-evaluation on acrylamide gel

¹Expected values based on non 0-0 distribution (for each group) of Table I

the observed distribution of the re-allocated samples and their expected distribution calculated from the distribution of haptoglobin phenotype in the non 0-0 phenotype population (Table I) reveals an excess of patients with 2-2 phenotype in both caucasian cirrhotic and non-cirrhotic starch gel 0-0 samples. A comparison of the distribution of the re-evaluated 0-0 haptoglobin phenotypes of negro cirrhotic and non-cirrhotic sera was not made because of the smallness of the sample sizes.

Discussion

The overall pattern of haptoglobin phenotype distribution in normal, non-cirrhotic, and cirrhotic caucasian populations living in the greater

Boston area were found to be identical when subjects with an 0-0 phenotype (starch gel) were excluded. Similarly, no significant difference in phenotype distribution emerged when negro-cirrhotics and non-cirrhotics were compared. The differences in phenotype distribution between caucasians and negroes reported by others were confirmed (Allison *et al.*, 1958; Giblett, 1959; Allison and Barnicot, 1960). The incidence of apparent anhaploglobinaemia (starch gel) in caucasian and negro cirrhotic populations (17% and 12%) was very much higher than that found in the respective non-cirrhotic populations (2.2% and 3.5%).

Because of the relative insensitivity of the starch gel electrophoresis technique for haptoglobin phenotyping, it is possible for a serum derived from a patient with a profound degree of hypohaptoglobinaemia to be categorized

'anhaptoglobinaemic'. Unfortunately, such a classification carries with it an inference of genetic amorphism.

Examination using acrylamide gel electrophoresis of 55 cirrhotic sera samples (51 caucasians and four negroes) exhibiting 0-0 phenotype pattern on starch gel shows that all but six were from patients with severe hypohaptoglobinaemia. The six 0-0 (acrylamide gel) sera were all from the caucasian cirrhotics.

The present study was confined to an examination of only one serum from each patient. The degree of hypohaptoglobinaemia in liver disease can vary from time to time, depending on the state of hepatic decompensation and the presence or not of either biliary obstruction or haemolysis (Kallai, Keler-Bačoka, Blažević, and Knežević, 1966). It would require the demonstration of persistent anhaptoglobinaemia on acrylamide gel over an extended period of time to give even presumptive evidence of genetic amorphism, particularly in those patients with diseases associated with hypohaptoglobinaemia. Such a feature, however, together with the demonstration of anhaptoglobinaemia in one or more healthy blood relative, would be of inestimable value in establishing the existence of true amorphism.

Sixty-eight patients (55 caucasian and 13 negroes) with 0-0 classification on starch gel came from the non-cirrhotic population. Many of these had diseases associated with excessive red cell destruction (eg, sickle cell anaemia) or impaired liver function (eg, acute hepatitis, chronic and acute alcoholism). All such patients were shown to have a small quantity of haptoglobin protein in their sera thereby supporting the presence of hypohaptoglobinaemia rather than true anhaptoglobinaemia. One 'normal' subject exhibiting the 0-0 phenotype on starch gel remained so following examination on acrylamide gel. It is therefore possible that this individual carries the amorphic gene. Further investigation as suggested above, would be required to establish this point.

The distribution of haptoglobin phenotypes (acrylamide gel) of the starch gel 0-0 samples show an excess of phenotype 2-2. This finding is more marked when the caucasian non-cirrhotics are considered. This is in agreement with previous studies which have shown that patients with haptoglobin phenotype 2-2 are more liable to develop hypohaptoglobinaemia than those with 2-1 or 1-1 (Parker and Bearn, 1963; Sutton and Karp, 1964; Murray *et al.*, 1966). Of particular interest, five patients with the sickle cell anaemia in our series (one caucasian and four negroes), although classified 0-0 on starch gel, could be phenotyped on acrylamide gel. Allison *et al.* (1958) using starch gel showed an unusual distribution of haptoglobin phenotypes in an African population in Nigeria (32.3% with phenotype 0-0; 53.5%, 1-1; 11.1%, 2-1; and

3.0%, 2-2). It has been suggested that the excess of 0-0 phenotypes found in this geographic area is partly due to the presence of endemic malaria and sickle cell anaemia (Giblett, 1969). A further examination of the sera from Africans with a starch gel 0-0 phenotype pattern, using the acrylamide gel technique, might give valuable information concerning the contribution made by hypohaptoglobinaemia to this unusual phenotype distribution.

During our study, 92% of the starch gel 0-0 phenotype samples were available for re-examination on acrylamide gel. The information obtained allows a more accurate estimate to be made than hitherto concerning the incidence of anhaptoglobinaemia among the American caucasian and negro populations studied. Starch gel anhaptoglobinaemia has been reported to be present in 0.3% (Allison *et al.*, 1958) of normal caucasians and in 3.5% of American negroes (Giblett, 1969). We observed in a total caucasian population of 2,930 (consisting primarily of the hospitalized patients) an incidence of acrylamide gel anhaptoglobinaemia of 0.24% (seven patients). It must be emphasized that six of these were patients with cirrhosis of the liver. Of the 402 American negroes studied, 4.2% (17) exhibited anhaptoglobinaemia on starch gel. Re-evaluation on acrylamide gel of 94% (16) of these patients revealed the presence of haptoglobin proteins in every sample. The overall occurrence of acrylamide gel anhaptoglobinaemia was, therefore, less than 0.25%. This study supports the premise that true genetic anhaptoglobinaemia is a rare phenomenon. Most sera classified 0-0 on starch gel are derived from patients with hypohaptoglobinaemia.

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