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Study of Haptoglobin Polymorphism and its Significance in Human Leukemias

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Abstract—*Serum haptoglobin polymorphism was studied by the horizontal acrylamide gel electrophoretic technique in 156 leukemia patients, 377 other cancer patients and 210 non-cancer patients. The results revealed Hp 1-1, Hp 2-1 and Hp 2-2 phenotypes. There were a large number of samples which did not reveal any of the haptoglobin phenotypes and were called Hp 0 because they were ahaptoglobinemic. The frequency of Hp 1-1 was found to be significantly greater in leukemia patients when compared to the other cancer group. The possible association between Hp^1 and leukemia is discussed and the probable cause of the high frequency of Hp 0 is explained.*

INTRODUCTION

HAPTOGLOBIN (Hp) is a hemoglobin binding α_2 globulin fraction of the serum and is genetically determined by two autosomal codominant allelic genes Hp^1 and Hp^2 which express themselves in three phenotypes recognised as Hp 1-1, Hp 2-1 and Hp 2-2 [1–3]. These three common phenotypes and other rare ones like Hp 2-1 M (modified) have been reported to occur in varying frequencies in populations of different ethnic origin [4, 5]. The samples which did not reveal any haptoglobin were called hypohaptoglobinemic or ahaptoglobinemic and designated as Hp 0. The plasma Hp level is reported to be very low in a variety of hemolytic diseases and also in several liver disorders [6]. The Hp has some protective effect in preventing the loss of plasma hemoglobin and this observation stimulated the studies on the association between the frequency of Hp types and various diseases. Peacock [7] reported a high frequency of Hp^1 gene in leukemia patients and indicated their possible susceptibility to leukemogenic agents. In order to test this hypothesis an attempt was made to study the frequency of Hp types in leukemia patients attending the Tata Memorial Hospital, Bombay, and to compare them with those of other cancer patients and also of those who were sick but had no cancer detected in them. Some of the important observations that emerged from this study are presented in this communication.

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MATERIALS AND METHODS

The intravenous blood samples were collected aseptically from 156 leukemia patients comprising of 93 chronic myeloid leukemia (CML), 20 acute myeloid leukemia (AML), 17 chronic lymphatic leukemia (CLL), 26 acute lymphatic leukemia (ALL) and serum was separated and preserved at −20°C until further study. Sera from 377 patients suffering from cancer other than leukemia and also 210 sera from those who were sick but had no cancer were included in the study to serve as positive and negative controls respectively. The sex and the age of the leukemia patients were also recorded.

The serum samples were complexed with hemoglobin (300 mg/100 ml serum) and allowed to stand for 30 min prior to electrophoresis. The haptoglobins were typed by the horizontal thin layer acrylamide gel electrophoretic technique using 5% cynogum 41, with discontinuous buffer system of Polik [8] at pH 8.6. The runs were made for 3–4 hr at 100 V and 20–25 mA at 4°C. The gels were stained with benzedine H_2O_2 and the haptoglobin phenotypes were recorded. The samples which were ahaptoglobinemic did not reveal any haptoglobin bands and were therefore called Hp 0. These samples were re-run to confirm them.

RESULTS

The electrophoretic study revealed the three common phenotypes of haptoglobin: Hp 1-1, Hp 2-1 and Hp 2-2 (Fig. 1). Table 1 presents the actual observed numbers of different phenotypes of the haptoglobin together with their percentages and Hp^1 gene frequency in patients with different types of leukemia. For the calculation of percentages and gene frequency, the subjects with ahaptoglobinemia (Hp 0) were omitted. Since no significant difference was observed in the occurrence of different phenotypes among the various leukemia groups, all the leukemia cases were pooled together for further analysis. Table 2 presents the observed and expected values of haptoglobin phenotypes in 156 leukemias, 377 other cancers and 210 non-cancer patients which included respectively 20, 6 and 3 ahaptoglobinemic patients who were omitted from the calculations of percentages and Hp^1 gene frequency, in all the three groups. The observed and expected values did not significantly differ in all the three groups. It is evident that the percentage frequency of Hp 1-1 phenotype and the Hp^1 gene frequency were markedly higher in leukemia group when compared to both the other groups. The number of individuals with Hp 1-1 in the leukemia group was twice the number in the other cancer group and almost thrice that in the non-cancer group.

In order to find the difference in the frequency of occurrence of Hp^1 and Hp^2 genes, they were actually counted in all the three groups and subjected to the Chi-square test of significance. It revealed a highly significant difference in the occurrence of the Hp^1 gene between leukemia and other cancer groups ($\chi^2=5.34$; $P<0.02$, 1 d.f.) and no significant difference between leukemia and non-cancer groups ($\chi^2=2.4$, $P<0.10$, 1 d.f.). Similarly when other cancer and non-cancer groups were tested, no significant difference was observed ($\chi^2=0.41$, 1 d.f.).

The number of ahaptoglobinemic serum samples were found greater in leukemia than in other cancer and non-cancer groups (Table 2). When the ahaptoglobinemic number in the leukemia group was tested against those of other cancer and non-cancer groups separately, they revealed very highly significant chi-square values ($\chi^2=34.78$, $P<0.001$, 3 d.f. and $\chi^2=22.81$, $P<0.001$, 3 d.f.), while the other cancer group showed no significant difference when tested against the non-cancer group ($\chi^2=1.13$, 3 d.f.).

Table 3 presents the distribution of leukemia within different age groups. The age of CML patients ranged from 6 to 84 yr, AML from 15 to 55 yr, CLL from 50 to 75 yr and ALL from 2 to 50 yr. The distribution of the cases according to the age, when presented graphically (Fig. 2) showed distinct peaks except for CML, which had a plateau extending from 30 to 50 yr without any peak. The ALL showed a peak at 20 yr, AML at 40 yr and CLL at 60 yr.

The sex ratio of males to females among the leukemia patients was 3:1 (118/38) which is in agreement with the observations of Paymaster [9]. The sex difference in the incidence of leukemia is difficult to explain.

DISCUSSION

Attempts are being made to find an association of genetic markers with the malignant diseases with a view to detect people with high risk [10, 11]. The haptoglobin, a geneti-

Table 1. Haptoglobin phenotypes, percentages and Hp^1 gene frequency in leukemias

Leukemia type	Total No. examined	No. found with Hp	Haptoglobin phenotypes 1-1	2-1	2-2	Hp^1 gene frequency	No. found without Hp
CML	93	85	5 (5.9)*	23 (27.05)	57 (67.05)	0.19	8 (9.4)
AML	20	16	1 (6.25)	5 (31.25)	10 (62.50)	0.22	4 (20.0)
CLL	17	13	0 (0.0)	5 (38.5)	8 (61.5)	0.19	4 (23.54)
ALL	26	22	1 (4.5)	6 (27.3)	15 (67.2)	0.18	4 (12.82)
Total:	156	136	7 (5.15)	39 (28.68)	90 (66.17)	0.195	20* (12.82)

*20 ahaptoglobinemic samples were omitted from calculations.
Figures in parentheses indicate percentages.

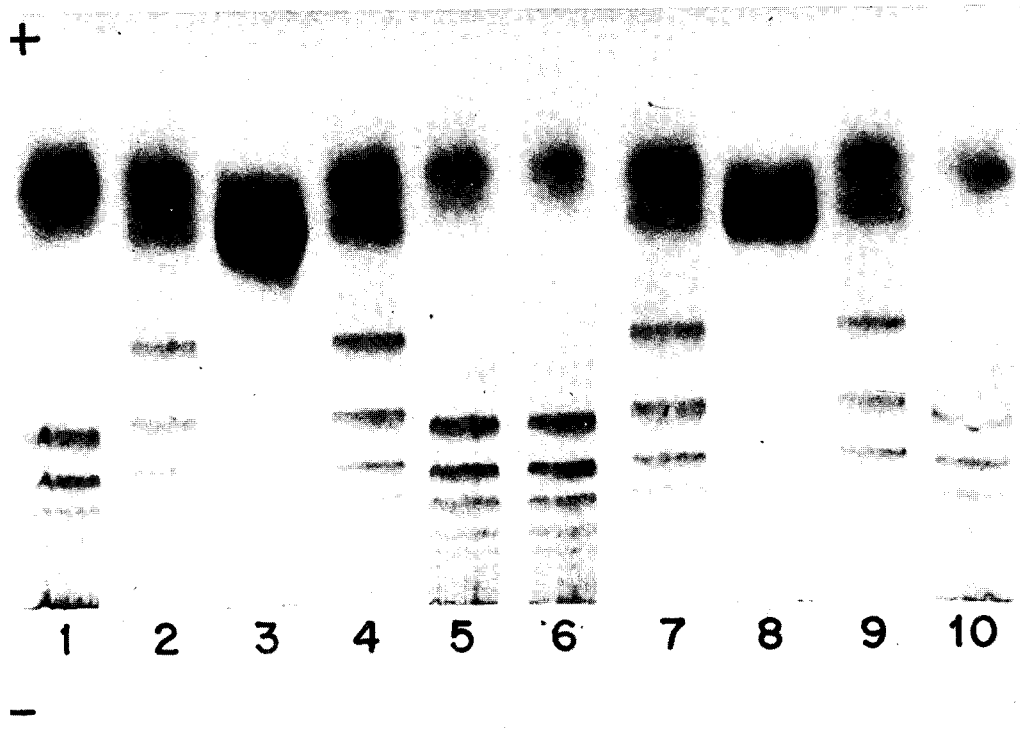

Fig. 1. *Common haptoglobin phenotypes.*
1, 5, 6 *and* 10—Hp 2-2 *type*
2, 4, 7 *and* 9—Hp 2-1 *type.*
3 *and* 8—Hp 1-1 *type.*

Table 2. Distribution of haptoglobin phenotypes, percentages, observed and expected values, Hp^1 gene frequency in different groups of patients

Patient group	Total No. examined	No. found with Hp	Haptoglobin phenotypes 1-1 Obs.	1-1 Expt.	2-1 Obs.	2-1 Expt.	2-2 Obs.	2-2 Expt.	Hp^1 gene Frequency	No. found without Hp*
Leukemia	156	136	7 (5.15)	5.17	39 (28.68)	43.58	90 (66.18)	88.12	0.195	20 (12.82)
Other cancers	377	371	9 (2.4)	6.86	83 (22.4)	87.18	279 (75.2)	277.0	0.137	6 (1.6)
Sick but no cancer	210	207	4 (1.93)	4.85	54 (26.10)	53.65	149 (71.97)	148.6	0.153	3 (1.43)

*Samples without Hp are omitted from calculations.
Figures in parentheses indicate percentages.

Table 3. Distribution of leukemia patients according to age

Leukemia Type	No. of patients	0–5	6–10	11–20	21–30	31–40	41–50	51–60	61–70	71–80	81–90	Age range
CML	93	0.0	3 (3.23)*	10 (10.75)	23 (24.73)	22 (23.65)	19 (20.43)	9 (9.68)	6 (6.45)	0.0	1 (1.08)	6–84
AML	20	0.0	0.0	4 (20.0)	4 (20.0)	8 (40.0)	3 (15.0)	1 (5.0)	0.0	0.0	0.0	15–55
CLL	17	0.0	0.0	0.0	0.0	0.0	1 (5.88)	11 (64.70)	3 (17.65)	2 (11.77)	0.0	50–75
ALL	26	3 (11.5)	5 (19.2)	8 (30.8)	4 (15.4)	4 (15.4)	2 (7.7)	0.0	0.0	0.0	0.0	2–50

*Figures in parentheses indicate percentages.

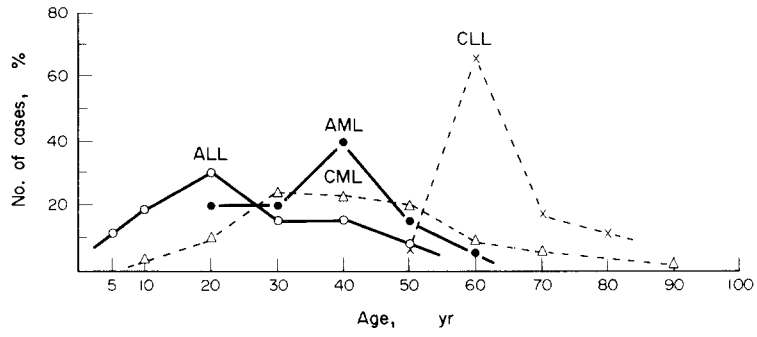

Fig. 2. Percentage of distribution of leukemia cases in different age groups.

cally determined polymorphic character, has been studied here to find the association of any of the Hp^1 and Hp^2 genes with the types of leukemia, but it did not reveal any special affinity to either of the haptoglobin alleles. When the leukemia samples were pooled together and Chi-square test of significance was run between leukemia and the other cancer group, it revealed a significantly high frequency of the Hp^1 gene in the leukemia group when compared to the other cancer group.

Ahaptoglobinemia (designated phenotypically Hp 0) is a characteristic feature of most newborn infants [12] and a variety of diseases in which there is either hepatocellular damage or increased intravascular hemolysis [6]. Both these causes are likely to prevail in the leukemic patients and therefore there is significantly more ahaptoglobinemic patients with leukemia than with both other cancer and non-cancer groups. Since there is an advantage for Hp 1-1 and Hp 2-1 individuals who could bind more hemoglobin than those with Hp 2-2 type, the leukemic patients with Hp 1-1 and Hp 2-1 are at an advantage over Hp 2-2 individuals [13, 14]. The quantitative study of haptoglobin in leukemia patients might be of clinical importance because once the serum haptoglobin is depleted the renal tubules might be affected resulting into other complications.

The significantly high frequency of the Hp^1 gene over the Hp^2 gene in the leukemia group when compared with the other cancer group ($P<0.02$) indicates the higher susceptibility of the Hp^1 gene to leukemogenic agent than the Hp^2 gene as suggested by Peacock [7]. No significant difference observed between the leukemia and non-cancer group might be suggestive of the role of some other factors which have a more direct effect on the neoplastic development. Since the haptoglobins belong to the family of immunoglobulins, the immune system might also be responsible to manifest this association. Haptoglobin, therefore, might not be the only factor to determine resistance or susceptibility to leukemia in man. However, the study of more samples might reveal a clearer picture and the study of the immunological status in the leukemic patients and their family members might throw more light on this problem.

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