Diagnosis of α-thalassaemia trait from Coulter Counter ‘S’ indices

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Summary A number of patients of Mediterranean and Asian origins were found to have unexplained microcytic hypochromic red blood cells. Iron deficiency and β-thalassaemia trait were both satisfactorily excluded in all of them. The haematological indices of these patients, obtained on a Coulter Model ‘S’ Counter, were found to be very similar to those seen in obligatory heterozygotes for α-thalassaemia. It is postulated that these patients were also carriers for α-thalassaemia. Subsequent investigation of some of these patients showed the characteristically reduced rates of α-chain synthesis seen in this condition. The discriminant function of England and Fraser (1973) may be of help in diagnosing this state. α-Thanassaemia trait should be considered in all patients of ‘high-risk’ ethnic origins with a blood picture suggestive of β-thalassaemia trait but in whom the levels of Hb A2 and Hb F are within normal limits.

α-Thalassaemia is common in the Far East. The incidence of the heterozygous state has been estimated to be as high as 30% in Northern Thailand (Na-Nakorn and Wasi, 1970). This condition has also been described in Arabs (Ali, 1969), Turks (Aksoy and Erdem, 1968), and Indians (Chauhan et al., 1970; Walford and Deacon, 1976). Raven and Tooze (1973) demonstrated Hb H inclusions in several Mediterranean, Asian, and Negro immigrants in South London and stated that one case of α-thalassaemia could be expected for every two with β-thalassaemia in these communities. These findings are similar to those of Pearson et al. (1974), who reported that the incidence of β-thalassaemia and α-thalassaemia trait was 5-0% and 2-4% respectively in unselected Greek Americans. The genetic control of α-chain synthesis is extremely complex, and there is uncertainty regarding the number of α-chain loci in humans. One interpretation, based on the assumption that there are two α-chain loci in most populations (Lehmann, 1970), is that α-chain production is controlled by four genes. Suppression of two of the four genes results in a condition designated as α-thalassaemia-1. Suppression of only one gene results in a minor reduction in α-chain production, leading to a milder state, α-thalassaemia-2. The frequency of the Hb Bart’s hydrops fetalis syndrome in neonates—a condition where there is complete absence of all four α-chain genes (Weatherall et al., 1970)—is 1 in 4 among siblings, which is compatible with the hypothesis that both parents of these affected infants are heterozygous for α-thalassaemia-1 (Wong, 1970). Parents who have children with Hb H disease—where three of the four genes are affected—must be obligatory carriers for the α1 or α2 state, and evidence of this has been provided by family studies (Wasi et al., 1974; Na-Nakorn et al., 1969) in the Far East. Since depression of α-chain synthesis affects all major and minor components of normal adult haemoglobin in heterozygotes for α-thalassaemia, the levels of Hb A2 and Hb F are not raised. Hb H inclusions may be found in some of these carriers but only after an exhaustive search, and this test is not a practical screening procedure. α-Thalassaemia heterozygotes are, therefore, not easy to detect by routine haematological methods. This paper reports the haematological findings in patients of Mediterranean and Asian origins in whom iron-deficiency and β-thalassaemia trait were both excluded but who had hypochromic microcytic red cells. It is postulated that these patients, who have similar haematological indices to those in β-thalassaemia trait when measured on a Coulter Model ‘S’ Counter, are carriers for α-thalassaemia. Values obtained in these suspect patients were compared with those obtained in obligatory heterozygotes for

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α-thalassaemia, ie, parents of patients with Hb H disease.

Patients

Sixty-six subjects with a microcytic hypochromic blood picture of varying severity associated with a mild anaemia and abnormal haematological indices were studied. A number of these patients were siblings or close relatives, and all were of Mediterranean or Asian origin. (Although α-thalassaemia has been described in Negroes, there is evidence to suggest that the genetic control differs from that in other ethnic groups (Lehmann, 1970) and hence they were not included in this study.)

The levels of Hb A₂ and Hb F were within normal limits in all 66 subjects. The rate of globin chain synthesis was measured in nine of them; the α:β ratio was reduced (ie, < 0.9) as is found in α-thalassaemia heterozygotes. These nine patients were grouped with 14 other subjects who were parents of patients with Hb H disease in order to define the haematological indices of α-thalassaemia-1 and -2 trait. Twenty iron-deficient patients who had haemoglobin levels between 8-0 g/dl and 12-0 g/dl and 20 patients with β-thalassaemia trait were also studied to compare values found in these conditions with those in the α-thalassaemia traits (Table).

Methods

Routine haematological methods used were those described by Dacie and Lewis (1975). The discriminant function (DF = MCV — (Hb x 5) — RBC — 3·4) of England and Fraser (1973) was calculated from indices obtained on a Coulter Model ‘S’ calibrated in a manner similar to that of these workers. Haemoglobin electrophoresis was carried out routinely on cellulose acetate at pH 8·9 (Marengo-Rowe, 1965) to exclude abnormal haemoglobins. Hb A₂ and Hb F were estimated in all patients as were serum iron and total iron binding capacity levels. Haemoglobin electrophoretic studies were repeated in some of these patients after a therapeutic course of oral iron. Iron deficiency was excluded in nine by bone marrow examination. Hb H bodies were looked for in a number of patients after incubating freshly collected blood in brilliant cresyl blue. These inclusions were also looked for in bone marrow aspirates from some of the patients. The rates of globin-chain synthesis (Lingrel and Borsook, 1963) were measured in nine patients suspected of being heterozygous for α-thalassaemia from blood data. Hb Bart’s was looked for by electrophoresis on starch gel (Smithies, 1959) in the cord blood at delivery in infants born to seven of our patients during the course of this study.

Results

The obligatory carriers could be easily separated into two categories. Nine of them showed major changes; MCV 59 — 68 fl (mean 64·7) and MCH 19 — 22·6 pg (mean 21·1) and relatively high RBC counts 5·05 — 6·25 × 10¹²/l (mean 5·50). In contrast, the other five parents showed only mild changes; the MCV and MCH in them ranged from 73 fl and 23·6 pg to near normal levels (mean 75·4 fl and 25·4 pg respectively). The morphological changes in the red cells were much more severe in the first group than in the second, and hypochromia, microcytosis, and target cells were conspicuous features. It is generally accepted that heterozygotes with the more severe haematological abnormalities have α-thalassaemia-1 while those with milder changes have α-thalassaemia-2 (see p.1).

Of the nine other patients in whom the rates of globin chain synthesis were measured, the α:β chain ratio was between 0·6 and 0·8 in seven and was 0·84 in two. The haematological indices and red cell morphology of the patients with the lower ratios were similar to those of the obligatory carriers who were more severely affected and thought to have α-thalassaemia-1 trait. The mean MCV, MCH, and RBC count in these patients were 64·9 fl, 21·0 pg, and 5·75 × 10¹²/l respectively, which are similar to the values obtained in our patients with β-thalassaemia trait, ie, 64·6 fl, 21·1 pg, and 6·01 × 10¹²/l. The blood picture of two patients with α:β chain ratios of 0·84 was less abnormal and like that seen in α-thalassaemia-2 (Fig. 1).

The haematological indices of the remaining 57 suspect patients are shown in Figure 2. In 49 microcytosis and hypochromia were marked and red cell counts were relatively high. The mean values for MCV, MCH, and RBC counts were 65·6 fl, 21·4 pg, and 5·56 × 10¹²/l respectively. (Four patients in this group were siblings of patients confirmed by chain synthesis to have α-thalassaemia-1.) In the remaining eight patients, the changes were milder but nevertheless significant, and the mean values were 72·9 fl, 23·7 pg, and 5·06 × 10¹²/l for MCV, MCH, and RBC counts respectively.

Hb Bart’s was found in the cord blood in three out of seven infants born of patients included in this
study. In two infants, born to mothers whose blood picture showed only minor abnormalities, the level of Hb Bart's was 2.7% and 2.9%. In the third infant the level was 7.9%; the mother of this child, who was Chinese, had a blood picture indicative of α-thalassaemia-1 trait.

Fifteen of the 16 patients known to have α-thalassaemia-1, ie, seven patients with α:β ratios of less than 0.8 and the nine severely affected obligatory carriers, gave negative values for the discriminant function. Thirty-seven of the 49 (75%) other patients with the more severe haematological changes also gave negative values. However, only one of the confirmed α-thalassaemia-2 traits and one of the eight suspect subjects with milder haematological changes gave a negative value for this function. These findings, together with those found in our patients with β-thalassaemia trait and those with iron deficiency, are shown in Figure 3.

Hb inclusion bodies were found in 16 of the 46 patients studied (35%). These inclusions were found only in the patients who had markedly abnormal blood pictures and then only after a prolonged search in some cases. No advantage was gained by examining bone marrow aspiration material from those patients in whom this procedure was carried out.

Discussion

Of the genetic defects which result in a specific inhibition of globin chain synthesis, β-thalassaemia trait is usually diagnosed by the demonstration of a raised Hb A2. In α-thalassaemia trait, however, it is difficult to make the diagnosis by routine haematological methods.

Pornpatkul et al. (1969), in a large study of obligatory carriers, found that the more severely affected heterozygotes designated α-thalassaemia-1 could be separated quite easily from the milder α-thalassaemia-2 traits and from normal subjects. They also reported that while normal haematological values were often found in α-thalassaemia-2 traits, as a group these patients had significantly lower mean red cell indices than normals. The majority of our patients had the more severe changes of α-thalassaemia-1 and only 10 had the milder, though clearly abnormal changes. This by no means reflects the relative incidence of α-thalassaemia-1 and -2 since a number of α-thalassaemia-2 heterozygotes with near normal blood pictures will certainly have been overlooked. Although it seems that our two patients with α:β chain ratios of 0.84 had α-thalassaemia-2 (Schwartz, 1974), it is impossible to state categorically that the other eight patients with these milder changes had α-thalassaemia-2 trait or indeed carried an abnormal gene at all. Moreover, patients in this group with

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**Fig. 1** Haematological indices in β-thalassaemia trait in subjects confirmed to be heterozygous for α-thalassaemia (ie, α:β chain ratio <0.9) and in the parents of patients with Hb H disease.

**Fig. 2** Haematological indices in subjects suspected to be heterozygous for α-thalassaemia.


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<table>
<thead>
<tr>
<th>α thalassaemia traits</th>
<th>β thalassaemia traits</th>
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Fig. 3 Distribution of discriminant function derived from indices obtained on a Coulter Counter ‘S’ in iron deficiency, β-thalassaemia trait, and in subjects both confirmed and suspected to be heterozygous for α-thalassaemia.

...
obtained in members of a family previously described to be heterozygotes for Hb G α-Philadelphia, a condition where an α-chain variant may be linked to an α-thalassaemia gene (French and Lehmann, 1971). Our results confirm that the discriminant function may be of some value in diagnosing α-thalassaemia-1 trait. β-thalassaemia trait and iron deficiency should, of course, be excluded. The limitations of this formula, i.e., that it must not be used in patients with iron deficiency polycythaemia or clinical states with haemodilution such as splenomegaly, pregnancy, etc. (Engeland and Fraser, 1973; Hegde et al., 1975) should be borne in mind.

Na-Nakorn and Wasi (1970) showed that the level of Hb Bart’s correlated well with the severity of α-thalassaemia and that infants with 1-2% Hb Bart’s develop α-thalassaemia-2 and those with 5-6% have α-thalassaemia-1. The levels obtained in three of the infants we studied would imply that their parents, who had characteristic haematological abnormalities, were, therefore, heterozygous for this condition.

Approximately 1 in 100 000 red cells may show Hb H inclusion bodies in α-thalassaemia-1 trait. These are rarely found in α-thalassaemia-2 or in normals (Wasi et al., 1974). Our experience confirms this observation. However, we feel that the prolonged search involved makes this an impractical test as a screening procedure. The modification using blood rich in reticulocytes and young red cells, suggested by Raven and Tooze (1973), may be more rewarding.

In conclusion, it is therefore important that α-thalassaemia trait should be considered in all patients of ‘high-risk’ ethnic origin with a refractory microcytic hypochromic blood picture after iron deficiency and β-thalassaemia trait have been excluded. Pearson et al. (1974) suggested that the combination of microcytosis, normal levels of Hb A2 and Hb F, and normal iron studies indicated α-thalassaemia trait when the same haematological findings were documented in a first-degree relative. The discriminant function may serve as a useful screening tool to recognise the majority of patients heterozygous for α-thalassaemia-1. A few of these could well be carriers for Hb Constant Spring in addition to α-thalassaemia but more elaborate investigation would need to be carried out to characterise these patients. However, until an immunological method like that described by Wasi and Pravatmuang (1973) is in common usage, α-thalassaemia trait may be suspected from the characteristic haematological indices obtained on a Coulter Counter ‘S’.

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