Red cell genetic abnormalities in Peninsular Arabs: sickle haemoglobin, G6PD deficiency, and α and β thalassaemia

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SUMMARY The frequencies of four major red cell genetic defects, sickle haemoglobin (Hb S), glucose 6 phosphate dehydrogenase deficiency (G6PD), and α and β thalassaemia, have been determined in nearly 5000 subjects from the three major Peninsular Arab States, namely Yemen (North and South), the United Arab Emirates, and Oman. All four defects are common with an overall pattern of α thalassaemia > G6PD deficiency > β thalassaemia > Hb A/S. However, the frequencies of these within each state varies and they are, respectively, Oman: 0.389, 0.328, 0.024, and 0.038; the United Arab Emirates: 0.165, 0.087, 0.017, and 0.019; and Yemen: 0.065, 0.062, 0.624, and 0.0095. Two, namely α thalassaemia and G6PD deficiency, are extremely common, but in spite of this there appears to be a lack of observed clinical disease. For example, Hb H disease and Barts hydrops fetalis were not seen and the oxidative haemolytic syndromes are rare.

The Peninsular Arab States (United Arab Emirates [UAE], Yemen, and Oman) are endemic for falciparum malaria. Although the exact frequency of infection is not known, our own observations indicate that Oman has the highest rate, followed by Yemen, then the UAE. Thus, the major red cell genetic defects affording protection against malaria should be present. However, apart from the work of Kamel *et al.*,¹ concerning the frequency of Hb S and G6PD deficiency in relatively small numbers of patients from the UAE, the presence and frequencies of these genes among the other two States have not been reported. Moreover, there is no reported evidence as to the existence or frequency of α or β thalassaemia in this part of the world.

Blood samples of nearly 5000 subjects from the Peninsula have been examined and all four abnormal genes have been found to be present. With respect to Hb S and G6PD deficiency our findings confirm the earlier work of Kamel *et al.*¹ on UAE nationals and also provide their frequencies in Yemeni and Omani subjects. The incidence and expression of α and β thalassaemia have also been determined. Both exist, indeed α thalassaemia reaches endemic levels, a finding which has been

confirmed by examination of over 2000 cord blood samples for Hb Barts (γ 4).

In spite of the very high incidence of carriers for these genetic abnormalities, there appears to be a clinical dichotomy in that the severe thalassaemia syndromes are not seen, and with regard to G6PD deficiency, favism and other oxidative haemolytic syndromes do not appear to be a major clinical problem.

Subjects

The majority of investigations was carried out on routine blood samples from pregnant females attending the Corniche Hospital, Abu Dhabi for routine antenatal care. A breakdown of the ethnic origins is shown in fig 1. Routine blood samples were used to determine the frequencies of Hb S and α and β thalassaemia trait. The frequency of G6PD deficiency was determined in adult males on EDTA samples of patients attending the Al Jazeera Hospital, Abu Dhabi, and the incidence of deficiency in male newborn infants by testing cord blood samples of babies born at the Corniche Hospital. Cord bloods of both sexes were also examined for Hb Barts (γ 4). The haematological parameters were measured within 24 hours and haemoglobin electrophoresis carried out within 48 hours.

FIG 1 Map of the countries of the Arabian Peninsula studied. Saudi Arabia is shown as a reference. The total number of subjects from each country tested were: Yemen 1260 (24.9%); UAE 2750 (54.3%); Oman 1050 (20.7%).

Methods

The performance of the routine haematology laboratory is monitored monthly by NEQAS United Kingdom Quality Control Scheme and during 1983 and 1984 the deviation index was 0.5 (good). Routine blood counts were measured on a Coulter S Plus and the data stored on a computer (Commodore PET Mark II). This had been programmed with the Discriminant Function of England and Frazer,² MCV-RBC-(5xHb)-3.4, to discriminate between hypochromic indices due to the thalassaemia trait and those due to iron deficiency (see below). Hb A₂ was measured by DEAE chromatography.³ The normal range for the laboratory was 2.3 to 3.2±0.15%. Values greater than 3.5% were accepted as being diagnostic of β thalassaemia. Haemoglobin Barts was estimated qualitatively by cellulose acetate electrophoresis, pH 9.2. Sick cell haemoglobin was detected using the "Sickle Dex" commercial kit. The presence of Hb S was confirmed by cellulose acetate electrophoresis at pH 9.2. G6PD deficiency was estimated using the Boehringer kit. The iron status of the patients was determined by measuring the serum ferritin level. In this laboratory a value of less than 10 ng/100 ml has been established as indicative of iron deficiency.⁴

THE DIAGNOSIS OF α AND β THALASSAEMIA TRAIT

As stated, the laboratory computer had been programmed with the Discriminant Function (DF) of England and Frazer.² It had been shown previously⁴ that it was possible in pregnancy, by using this function, to distinguish between a

hypochromia of 18 to 24 pg due to α or β thalassaemia trait and a similar hypochromia caused by iron deficiency, due to the persistence of the relatively higher red cell counts associated with thalassaemia. In thalassaemia the function is between -10 to +15 depending on the type of thalassaemia (α or β), the individual expression of the gene, and the gestational period of the pregnancy. However, in iron deficiency the function ranges from +15 to +30. There is an overlap of 3.5% between α thalassaemia and iron deficiency at less than 20 weeks' gestation, rising to 7% at 40 weeks.

The haematological parameters of each patient were fed from the Coulter to the computer and if the MCH was between 18 and 24 pg the programme was 'activated'. When the function indicated a thalassaemic picture the Hb A₂ and serum ferritin were measured. β thalassaemia was diagnosed by finding a raised Hb A₂ level, while α thalassaemia was diagnosed by finding a normal Hb A₂ and a ferritin greater than 15 ng/100 ml. If the ferritin was less than 10 ng/100 ml the patient was treated with iron and the analyses repeated after six weeks. Because the programme was selective for MCH values between 18 and 24 pg it was known from previous studies^{4,5} that 95% of all β thalassaemia carriers would be detected, but it would only detect α thalassaemia resulting from the deletion of two or more α chain genes (α thalassaemia 1:- α - α). The one gene deletion (α thalassaemia 2:- α / α) only results in a minor decrease in the MCH in the region of 25 to 27 pg and would not be selected.

Results

The frequencies of the four main red cell genetic defects are shown in tables 1, 2, and 3. Each will be considered separately, but overall the frequencies of all abnormalities increase from Yemen through the UAE to Oman.

SICKLE HAEMOGLOBIN (HB S)

A total of 5060 samples was tested (table 1). The frequency of Hb S carriers among the Yemeni was 0.0095 rising to 0.019 in the UAE nationals and still

TABLE 1 Frequency of sickle Hb carriers (Hb A/S) in Peninsular Arabs.

Country	No tested	No positive	Frequency of A/S
Yemen	1260	12	0.0095
UAE	2750	52	0.019
Oman	1050	40	0.038

If Hb S was suspected by a positive 'Sickle Dex' test it was confirmed by haemoglobin electrophoresis.

further to 0.038 among the Omani. All positive sickle tests were confirmed by electrophoresis. It is of some interest that among the total number of 2036* samples which were subsequently examined by electrophoresis, apart from two samples assumed to contain Hb O Arab, no other variants were found. During this survey no patients homozygous for Hb S were detected. One practical problem encountered was that often a positive sample might only give a slight precipitate. This was probably due to the frequent association of α thalassaemia in

patients also heterozygous for Hb S, whereby the proportion of Hb S would be no greater than 20%.⁶ However, even samples containing small amounts of Hb S always gave a positive result.

G6PD DEFICIENCY

The results of the frequency of G6PD deficiency in 850 male subjects are shown in table 2a. Among Yemenis the frequency was 0.062 and in UAE nationals 0.087, but among Omanis it was 0.328. It should be stressed that the samples examined were routine blood samples from patients suffering from conditions other than haemolysis or jaundice.

The above frequencies correlate almost exactly with the incidence of G6PD deficiency found on routine testing of 345 cord blood samples from male neonates of the three countries (table 2b). Only two of the 50 neonates who were deficient later developed neonatal jaundice but this was attributed to ABO blood group incompatibility.

* The total number of samples electrophoresed comprised those that gave a positive sickle test and also those on which the Hb A₂ had been quantified in a case of suspected thalassaemia.

TABLE 2 Frequency of G6PD deficiency in Peninsular Arabs.

Country	No tested	No deficient	Frequency
<i>(a) Adult males</i>			
Yemen	146	9	0.062
UAE	582	51	0.087
Oman	122	40	0.328
Tests carried out on routine blood samples.			
<i>(b) Male neonates</i>			
Yemen	73	4	0.054
UAE	222	20	0.091
Oman	50	26	0.32

Tests carried out on routine cord samples. Two neonates became jaundiced due to ABO incompatibility.

THE THALASSAEMIAS

Earlier studies had shown that it was common for subjects from the Peninsular states to show a blood picture of hypochromia (MCH < 27 pg) associated with an erythrocytosis (RBC > 4.5 × 10¹²/dl).^{4,5} The overall degree of hypochromia seen in the three countries was Yemen 22%, UAE 43%, and Oman almost 70% (table 3). Since the frequency of iron deficiency in the three countries is low (6%) and

TABLE 3 Frequency of 'hypochromia' and α thalassaemia 1 and β thalassaemia trait in Peninsular Arabs.

Country	Total No	Hypochromia		β thalassaemia trait Hb A ₂ > 3.5%		α thalassaemia 1 trait Hb A ₂ < 3.5 Normal ferritin		Ratio of carriers (α 1: β)
		MCH < 27 pg (%)	MCH > 24 pg (%)	No	Frequency	No	Frequency	
Yemen	721	21.9	9.0	17	0.024	47	0.065	2.7
UAE	4221	43.1	18.2	72	0.017	696	0.165	9.7
Oman	790	69.4	41.4	19	0.024	308	0.389	16.2

The Hb A₂ level was only measured on samples with an MCH of < 24 pg. This will detect nearly all high Hb A₂ β thalassaemia carriers with the exception of some Omanis (fig 2), but will only detect α thalassaemia 1 (two genes deleted), α thalassaemia 2 (one gene deleted), which must be of the same or greater frequency, only causes a minor decrease in the MCH (25 to 27 pg).

TABLE 4 Haematological parameters associated with α and β thalassaemia in Peninsular Arabs.

Ethnic group	β thalassaemia				α thalassaemia 1			
	No	MCH (pg) (Mean)	MCV (fl) (Mean)	Hb A ₂ (%) (Mean)	No	MCH (pg) (Mean)	MCV (fl) (Mean)	Hb A ₂ (%) (Mean)
Yemen	17	21.4	68.2	5.2	47	22.2	68.2	2.3
UAE	72	21.2	67.9	5.3	696	22.1	69.3	2.2
Omani	19	22.4	72.3	4.8	308	22.1	70.1	2.7

uniform⁴ the major cause of hypochromia must be either α or β thalassaemia. The frequency of these genes was determined in 5732 samples as described

in the Methods section and the results are shown in table 3.

β thalassaemia

The frequency of β thalassaemia carriers was Yemeni 0.024, UAE nationals 0.017, and Omani 0.024 (table 3). Superficially the expression of the gene(s) is identical in all three countries in terms of the mean MCH, MCV, and Hb A₂ levels (table 4). However, the distribution of MCH associated with β thalassaemia shows different patterns (fig 2) which might indicate that different genetic variants with underlying different molecular lesions exist among the states. Alternatively, and more likely, it is due to interactions between α and β thalassaemia.

α thalassaemia

The calculated frequencies for α thalassaemia 1 in the three countries are shown in table 3. Evidence to substantiate these frequencies was the finding that the frequency of Hb Barts (γ_4) in cord blood of neonates was almost identical in number and ethnic distribution (table 5, fig 3). The haematological parameters associated with α thalassaemia are given for completeness in table 4. However, they will not be as meaningful as those for β thalassaemia since the exact genotype of the thalassaemia is not known. For instance, it is not certain how much, if any, α thalassaemia 1 will be associated with an MCH greater than 24 pg, and conversely whether the α thalassaemia 2 genotype causes a low MCH.

Lastly, it is interesting to note the different

FIG 2 Distribution of MCH associated with α (----) and β (—) thalassaemia in the three countries examined.

FIG 3 Frequency and distribution of Hb Barts (γ_4) in cord blood of neonates from the three countries. The frequencies are shown in table 5. The MCV of Omani neonates was significantly lower than the other two countries irrespective of whether Hb Barts was present or not. However, there was no statistical difference in the mean Hb levels of neonates from the three states: Oman 18.5 ± 0.4 g/dl; Yemen 18.1 ± 0.35 g/dl; UAE 18.4 ± 0.39 g/dl.

TABLE 5 Frequency of Hb Barts in cord blood of Peninsular Arabs.

Country	No tested	Hb Barts	%	Calculated α thalassaemia 1 (%)
Yemen	131	23	7.5	6.9
UAE	572	87	15.2	14.0
Oman	97	52	53.6	34.4*

* The difference in the Omani between the calculated percentage of α thalassaemia 1 (34.4) and the percentages of cords containing Hb Barts (53.6) probably indicates that the $-a/a\alpha$ genotype in the Omani results in a more severe expression of α chain synthesis than is seen in the Yemeni or UAE nationals.

distribution of the MCV of neonatal cord cells among the three ethnic groups (fig 3). Firstly, Hb Barts was only associated with MCVs below 105 fl but, secondly, it can be seen that there is a marked difference in the volume of the cells depending on the ethnic origin. We have noticed in adult Peninsular Arabs marked variations in red cell morphology similar to hereditary spherocytosis, ovalocytosis, and elliptocytosis, but the findings from the MCVs of cord blood are the first evidence that differences of membrane structure might also exist between these ethnic groups.

Discussion

The origins of the people of the Peninsular Arab States are not precisely known due to a paucity of historical documentation, but what history there is has been well summarised by Kamel and colleagues.¹ It appears that Yemen and Oman were established states going far back in time, and there is some evidence that the people of the United Arab Emirates originated from either one or both of these states. Also, both Yemen and Oman historically have been active trading colonies, notably with the East African countries and Western Europe. However, it is generally accepted that there has been ethnic isolation due to geographical or cultural restriction of at least Oman and Yemen, and this is borne out in part when one examines the frequencies of major red cell gene defects, which indicate both inbreeding and selection with regard to α thalassaemia and G6PD deficiency.

Although it is not surprising to find that these abnormal genes are present in this malarious part of the world, it is surprising that they are found at such high frequencies when examined in the context of an observed lack of clinically associated disease(s).

The frequency of carriers for sickle haemoglobin, which confirms earlier preliminary findings,⁷ clarifies the uncertainty as to the exact incidence of this gene among Peninsular Arabic people. The origin of

the sickle gene is likely to originate from black East Africans⁸ centuries ago.

The homozygous state (sickle cell disease) in the true Peninsular Arab is uncommon in our experience, the predicted frequencies being Yemen 1 in 40 000, UAE 1 in 20 000, and Oman 1 in 4000. These frequencies have been determined from the carrier frequencies but do not take into account the high degree of consanguinity which exists. This would increase the frequency of the homozygous state and it may well be that sickle cell disease is associated with a high infant mortality. Fourteen homozygous Arabic patients have been examined over a two year period (six UAE, six Omani, two Yemeni) who appear to present two types of clinical picture. Nine patients had a clinical course and haematological picture very similar to African blacks, while six of them (four Yemeni, two UAE) presented with features of severe haemolytic anaemia (haemoglobins 5 to 7 g/dl, reticulocytes 30 to 40%, and 30 to 40% irreversible sickled cells on blood films). However, observations over a five to eight year period have shown very little, if any, evidence of infarctive crises. All six had marked splenomegaly and hypersplenism. It is likely that the latter group have another erythrocyte defect (? membrane) resulting in a different pathological state. None of the 14 patients had Hb F levels higher than 10% and thus must be genetically different from the Saudi Arab who commonly has Hb F levels greater than 30% associated with homozygous sickle cell disease.⁹ Also, none of the patients had evidence of interaction with α thalassaemia or G6PD deficiency.

The frequency of G6PD deficiency is high, especially in the Omani, but there is an obvious lack of the clinical syndromes associated with oxidative haemolysis, especially favism. The latter may be due to the lack of fava beans or fresh legumes in the Arabic diet. Preliminary studies on the Omani variant show it to be a variant with 'zero' activity different from common African types (L Luzzatto, 1984, personal communication). As such, one might have expected oxidative haemolysis to be common in the male population but what data exist indicate that it is not the case.

For example, with respect to the neonate, 16% of males we examined were deficient, yet only two out of 358 (0.5%) developed neonatal jaundice, in both cases due to ABO blood group incompatibility. In a separate study of the cause of neonatal jaundice requiring exchange transfusions in Arabs, three cases out of 20 (14%) were found to be G6PD deficient (S Al-Jawad, 1984, personal communication). However, none of these had the haematological features of oxidative haemolysis.

In children, haemolysis is also uncommon. For example, in 1983 to 1984, out of a total of 694 acute paediatric admissions to the Al Jazeera Hospital, Abu Dhabi, only six (0.9%) had oxidative haemolytic anaemia due to G6PD deficiency. In four it was associated with antibiotic therapy (Bacitracin (R)) and two had viral infections (S Abdulla, 1984, personal communications). In another study¹⁰ it was found that 25% of the 60 Arabic children admitted to the Central Hospital, Abu Dhabi, with acute haemolytic anaemia were G6PD deficient, but there was no evidence that this was the underlying cause in every case.

Finally, in the adult, no case of drug induced oxidative haemolysis has been brought to our attention and none of the 100 adult hospital patients who were found to be deficient had, or gave any history of, haemolytic anaemia. Favism also appears extremely rare, only one case being brought to our attention. We have no direct evidence as to the origin of the variant but it does not show increased activity in immature cells and as stated is unlikely to be similar to the black African variant(s).

We also have no data regarding the origin of the β thalassaemia gene(s) detected. It has only reached a low frequency and may well have come from mixture and inbreeding with Mediterranean countries. For example, Portugal colonised both Yemen and Oman in the 17th century. However, although superficially the expression of the carrier state is very similar to that found in North Africans, North Arabians, and Indians,⁵ the distribution patterns of the MCH (fig 2) may indicate that the southern Arabs have a different molecular variant unique to them.

The presence and high frequency of α thalassaemia, which is the most common genetic defect found, is unexplained since the evidence that it protects against malaria is still circumstantial.¹¹ The frequencies of α thalassaemia are among the highest recorded in the world and, accepting that α thalassaemia 2 (one gene deleted) is the primary mutation, then it reaches a frequency of approximately 0.78 in Omanis. This probably indicates the inbreeding and selection of a benign gene. It is suggested that the frequencies are too high to be explained by a 'founder effect'. Interestingly, the difference between the frequencies of α thalassaemia and those of G6PD deficiency, sickle haemoglobin, and β thalassaemia would suggest that the former is a much 'older' genetic defect. As with β thalassaemia there is no evidence as to the genetic mutation which is responsible. Nevertheless, there are data, albeit indirect, that the 'type(s)' of α thalassaemia in this area may be different. For example, at the Corniche Hospital over the last five years, account-

ing for 35 000 to 40 000 births, no single case of Barts hydrops fetalis (---) has been seen. Also, in the last two years no case of Hb H disease (--- α) has been found, both of which indicate that the α thalassaemia haplotype --/ does not exist in the Arabic population we have studied. Lastly, in our survey of Peninsular Arabs, no single patient had the haematological parameters associated with the homozygous state of α^T thalassaemia which has been detected. This genetic variant is common in Saudi Arabs¹² and, we suggest, confined to this ethnic group.

In summary, four common genetic defects have been found in the Peninsular Arabs. The relative frequencies overall are α thalassaemia > G6PD > β thalassaemia > Hb S. The α thalassaemia and G6PD variants are likely to be endogenous variants confined to these people whereas β thalassaemia and Hb S are probably exogenous genes. The variable frequencies between the three states probably reflect geographical isolation, inbreeding, and selection. Lastly, there is some evidence to indicate differences in erythrocyte membrane structure which may be genetic and certainly warrant further studies.

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