Thalassaemia trait and pregnancy

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SUMMARY The haematological variables, haematinic state, and placental function of more than 2000 pregnant women, heterozygous for either α - or β -thalassaemia genes, were examined during pregnancy. Four features emerged. Firstly, it was possible by discriminant function analysis of haematological variables to distinguish in pregnant patients between the anaemia caused by thalassaemia trait and that caused by iron deficiency. Secondly, patients with thalassaemia become significantly more anaemic in pregnancy, β more than α , but this was mainly due to plasma dilution. From the data percentile curves were drawn for each type of thalassaemia which predicted the patients' expected "normal" haemoglobin throughout gestation. Thirdly, patients with α -thalassaemia had the same incidence of iron deficiency as normal pregnant patients, whereas in those with β -thalassaemia it was four times less common. The incidence of folic acid and vitamin B₁₂ deficiency was the same in all groups. Finally, as assessed by serum oestriol concentration, there did not appear to be any abnormality of placental function or fetal development associated with maternal thalassaemia, and, also, there seemed to be no increase in maternal or fetal morbidity in pregnancy.

Adult male or female carriers of either α - or β -thalassaemia trait are not usually seriously anaemic, although there is some variation between ethnic groups, sexes, and individual patients.¹ The anaemia usually worsens at times of clinical stress, notably infection and commonly (or always) pregnancy. Indeed, female patients may present or be diagnosed for the first time in pregnancy as cases of refractory hypochromic anaemia.

Despite thalassaemia trait being the most common maternal genetic abnormality associated with pregnancy, there is still uncertainty regarding the clinical course, complications, and management, probably because there are few well reported studies.² For example, it is uncertain, because of plasma dilution, whether or not thalassaemia trait can be suspected in pregnancy from an examination of the haematological variables. Also there are few data regarding the pathogenesis of the increased anaemia or the need for iron and folate supplementation. Finally, and most importantly, there is no evidence as to whether or not maternal thalassaemia is associated with an increase in fetal or maternal morbidity.

To investigate these problems we studied 2326 pregnant patients heterozygous for either α -thalassaemia (90%) or for β -thalassaemia (10%)

and compared our findings with those of 7000 pregnant normal subjects.

Patients and methods

PATIENTS

Pregnant women of 16 different ethnic origins temporarily resident in the United Arab Emirates were studied: 46% were from the Middle East, 14% from north Africa, 35% from the Indian subcontinent, and 3% from other countries. Two per cent of the population was Caucasian.

Data were collected over 18 months and computed on more than 9619 pregnancies. Most patients (80%) were normal, but the remainder were carriers of either α - (18%) or β - (2%) thalassaemia genes.

Thalassaemia state

Earlier findings³ had indicated that the α - and β -thalassaemia trait was extremely common among the populations studied. The diagnosis was based on the association of hypochromia, microcytosis, a relative erythrocytosis, and a normal serum ferritin concentration. β -thalassaemia trait was confirmed by finding a raised Hb A₂ concentration and α -thalassaemia by the association of the above haematological variables and normal serum ferritin and Hb A₂ values.¹ Although different molecular

types of α - and β -thalassaemia genes must be operative, it is useful clinically to divide carriers into three main groups depending on their mean corpuscular haemoglobin contents, which is an index as to the type and severity of the genetic defect (Table 1). Table 2 shows the type and proportion of thalassaemia carriers in each group.

Iron deficient subjects

Two hundred and fourteen patients had pure iron deficiency anaemia, which was suspected on haematological grounds and confirmed by finding serum ferritin concentrations of less than 10 ng/ml. All responded to oral iron treatment, and after about eight weeks the mean corpuscular haemoglobin content had risen to more than 27 pg. The haematological variables of these patients at presentation were compared with those of patients with thalassaemia.

METHODS

The routine performance of the haematology laboratory is continuously monitored by the United Kingdom national external quality assessment scheme; in 1983 and 1984 the overall assessment index was 0.5 (good).

Haematological indices were measured on routine edetic acid blood samples using a Coulter Model S, calibrated with Coulter Counter cell control 4C. The data were stored in a computer (Commodore PET II).

Haemoglobin A_2 concentrations were measured by DE chromatography at pH 8.4.⁴ The normal range for the laboratory was $2.8 \pm 0.05\%$, and values above 4.0% were considered diagnostic of β -thalassaemia trait.¹ Serum ferritin concentration was measured using the radioimmunoassay kit (Amersham). In this laboratory values of less than

10 ng/ml had been established as indicative of iron deficiency.³ Serum folate and vitamin B_{12} concentrations were measured by a radioimmunoassay double antibody technique (Amersham) using quoted values as the normal range. The total serum oestriol concentration was also measured by a radioimmunoassay double antibody technique (Amersham).

Collection of data

Most patients were studied throughout pregnancy, during labour, and after birth. Data were derived from patients receiving accepted normal antenatal care with iron and folic acid supplementation, and no attempt was made to select out the patients with thalassaemia and ensure that the iron and folate concentrations etc were normal. Instead, the overall changes of the haematological variables were monitored as if for normal pregnant mothers. To assess haematinic requirements ferritin, folate, and vitamin B_{12} concentrations were determined on about 1000 subjects on initial examination (normal subjects and thalassaemia carriers) and again at 36 weeks or later. Fetal development was assessed retrospectively by examining the total serum oestriol concentrations, which had been measured between 28 weeks and term, in over 300 patients who proved subsequently to have thalassaemia.

Results

DIAGNOSIS OF THALASSAEMIA IN PREGNANCY

To determine whether it was possible to distinguish in pregnancy between hypochromic anaemia due to thalassaemia and that due to iron deficiency the discriminant function index of England and Fraser⁵ was calculated from the patients' variables using the formula:

$$MCV - RBC - (5 \times Hb) - 3.4$$

Table 1 Grouping of patients into three types based on mean corpuscular haemoglobin content

	Mean corpuscular haemoglobin content (pg)	Thalassaemia type	Probable defect
Grade I	27-25	α^2 -thalassaemia	One α gene deleted
Grade II	24-23	α^1 -thalassaemia	One α gene deleted
Grade III	22-18	β -thalassaemia	One β gene deleted

Table 2 Incidence and type of thalassaemia in the patients studied*

Mean corpuscular haemoglobin content (pg)	Grade of hypochromia			Total
	I 27-25	II 24-23	III 22-18	
Number of patients (%)	1759 (18)	808 (8.4)	208 (2.2)	2775 (28.6)
% with α -thalassaemia	100	88	74	
% with β -thalassaemia	—	12	26	

*Total number of patients studied = 9619.

where MCV = mean corpuscular volume, RBC = red blood cell count, and Hb = haemoglobin concentration. Fig. 1 shows the data as composite histograms. These compare the discriminant functions of thalassaemic patients with mean corpuscular haemoglobin contents of 19–22 pg and 23–24 pg with those of subjects with proved iron deficiency associated with the same degree of hypochromia at 20 and 40 weeks' gestation. For patients with severe hypochromia (19–22 pg) the function for patients with thalassaemia was -10 to $+15$, whereas that of iron deficient patients was $+15$ to $+30$. There was a small overlap of the functions of about 4.0% at less than 20 weeks, which increased to 7% at 36 weeks or more. For patients with mean corpuscular haemoglobin values of 23–24 pg the degree of overlap was slightly greater: 6.0% at less than 20 weeks

Fig. 1 Comparison between the discriminant functions of pregnant patients with α - and β -thalassaemia trait (mean corpuscular haemoglobin content 19–22 pg and 23–24 pg) (unshaded) with those of pregnant patients with pure untreated iron deficiency anaemia (shaded) with the same degree of hypochromia. The discriminant functions of patients with thalassaemia fall between -10 and $+15$ while those associated with iron deficiency fall between $+15$ and $+30$. For hypochromia of 19–22 pg the overlap is 3.7 and 6.5% respectively, whereas for hypochromia of 23–24 pg it is 5.6 and 13.4%.

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rising to 14% at more than 36 weeks. With a mean corpuscular haemoglobin content of 25 pg and above the separation was less clear and the use of the function became unreliable (data on request). In pregnancy the critical point or value to distinguish between the function associated with thalassaemia and that of iron deficiency was $+15$, which is strikingly different from the value of 0 found in the non-pregnant state.⁵ This variance results from the relative lowering of the absolute erythrocyte count in pregnancy due to expansion of the plasma volume.

HAEMATOLOGICAL VARIABLES DURING PREGNANCY

Fig. 2 shows the variation in the mean haemoglobin concentration during pregnancy in normal subjects and in patients with thalassaemia and Table 3 shows the mean variation of all haematological variables. Although the haemoglobin concentrations in patients with thalassaemia are considerably lower than those of normal subjects when values are expressed as a ratio of the haemoglobin concentration at less than 20 weeks' gestation, the net change is the same in all four groups. This indicates that the increased anaemia is due mainly to expansion of the plasma volume with little in the way of increased haemolysis. The finding that the absolute reticulocyte counts of normal and thalassaemic patients showed no significant variation during gestation supports this conclusion. (Table 4).

Fig. 3 shows the haemoglobin concentrations of the four groups expressed as percentile curves. The ranges of values in each group varied considerably, but the degree of variation—that is, the limits between the 10th and 90th percentiles—is roughly of the same magnitude for each group during gestation. The value of these curves in practice is illustrated by the following case history:

Case History: A 26 year old Scottish girl presented at the hospital in December 1983. Results of biochemical investigations were as follows: haemoglobin concentration 12.7 g/dl with mean corpuscular haemoglobin content of 24 pg and red cell count of $4.9 \times 10^{12}/l$. The Hb A₂ concentration was normal at 2.7%, and the ferritin concentration was normal at 23.8 ng/100 ml. A diagnosis of α -thalassaemia was suspected and this was confirmed by finding an α : β ratio of 0.72:1. She became pregnant, and in March 1984 presented for antenatal care at 18 weeks' gestation. Her haemoglobin concentration was 11.4 g/dl with the other variables unchanged; this is just below the 50th percentile for her α -thalassaemia. She was given iron and folic acid and was not seen again until she was

Fig. 2 Comparison of the variation of the mean haemoglobin concentrations of pregnant patients with thalassaemia trait with those of normal pregnant patients throughout gestation. Only mean values are shown and for clarity the ranges have been omitted, but for each grade of hypochromia the differences are highly significant at each gestational age. The three grades of hypochromia loosely represent the severity of the thalassaemia. The progression of these haemoglobin values does not represent individual patients, but the mean of the group.

Table 3 Variations in mean or red cell indices with Gestational stage in normal subjects and patients with thalassaemia (grade I-III)

	Haemoglobin concentration (g/dl)	Ratio of haemoglobin concentration: value at <20 wk	Red blood cell count ($\times 10^{12}/l$)	Mean copuscular volume (fl)
Normal subjects (MCH 28+ pg (n = 6844))				
20 wk	12.69	1.0	4.245	86.50
20-24 wk	11.79	0.93	3.911	88.21
25-26 wk	11.67	0.91	3.842	89.25
31-34 wk	12.04	0.94	3.954	88.83
35-40 wk	12.43	0.97	4.118	88.58
Grade I (MCH 25-27 pg (n = 1759))				
20 wk	12.27	1.0	4.544	80.56
20-24 wk	11.46	0.93	4.261	80.54
25-26 wk	11.21	0.91	4.177	80.94
27-30 wk	11.23	0.92	4.167	81.10
31-34 wk	11.41	0.93	4.223	81.11
35-40 wk	11.51	0.93	4.288	81.00
Grade II (MCH 23-24 pg (n = 808))				
20 wk	11.36	1.0	4.830	71.65
20-24 wk	10.43	0.91	4.449	71.55
25-26 wk	10.31	0.90	4.368	71.89
27-30 wk	10.45	0.92	4.403	73.05
31-34 wk	10.31	0.90	4.389	72.28
35-40 wk	10.58	0.93	4.510	72.72
Grade III (MCH 18-22 pg (n = 208))				
20 wk	10.63	1.0	5.090	65.17
20-24 wk	9.779	0.92	4.692	65.49
25-26 wk	10.05	0.92	4.751	66.31
27-30 wk	9.422	0.94	4.464	66.65
31-34 wk	9.837	0.88	4.694	65.88
35-40 wk	10.17	0.96	4.801	66.56

MCH = mean corpuscular haemoglobin content.

Table 4 Mean absolute reticulocyte counts ($\times 10^{12}/\text{dl}$) of normal subjects and patients with thalassaemia (grade III, mean corpuscular haemoglobin content 18–22 pg) during gestation

	Gestation (wk)				
	20–24	25–26	27–30	31–34	35–40
Normal subjects (n = 426)	0.85	0.73	0.71	0.8	0.9
Patients with thalassaemia (n = 331)	0.87	0.76	0.73	0.84	0.93
p	0.1	0.1	0.2	0.1	0.15

Fig. 3 Percentile curves of haemoglobin concentrations of normal pregnant women and pregnant patients with thalassaemia (grade I–III) during gestation. Curves were drawn from the data of measured values for each group. Patients were not selected, but were receiving normal antenatal care. In practice, we found that haemoglobin values falling below the 10% percentile were usually associated with a secondary cause—for example, urinary tract infection or iron deficiency. An illustration of the value of such normograms is depicted by the case history described in the text.

was 30 weeks' pregnant. Her haemoglobin concentration had fallen to 9.0 g/dl at the 10th percentile for her α -thalassaemia. The mean corpuscular haemoglobin content was unchanged but she admitted not taking iron. Her ferritin concentration was reduced to 3.1 ng/100 ml. She was given intramuscular iron and in six weeks the haemoglobin concentration had

risen to 11 g/dl, at her original 50th percentile point. Therefore, although anaemic, the haemoglobin was now normal for her thalassaemic state. She has since delivered a healthy baby boy without any complications.

Of interest is the pattern of haemoglobin variation during gestation associated with thalassaemia. The

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haemoglobin concentration decreased less as the grade of thalassaemia became more severe. For example, in the 23–24 pg group the decrease at 25–26 weeks (mean change in haemoglobin concentration -0.8 ± 0.5 g/dl) was less than that in the normal subjects (-1.1 ± 0.99 g/dl); in the 19–22 pg group the haemoglobin value increased: (mean change $+0.3 \pm 0.12$ g/dl). The same change in pattern is seen in the red blood cell count (Table 3).

HAEMATINIC STATE

Table 5 shows the changes in the ferritin concentrations during pregnancy in normal subjects and patients with α - and β -thalassaemia. At term the incidence of iron deficiency in patients with α -thalassaemia is the same as that of normal subjects, but in those with β -thalassaemia it is four times less common, occurring in no more than 4% of patients. In some patients with thalassaemia and severe iron deficiency (serum ferritin 5 ng/ml) the discriminant function, as expected, fell into the critical area. There was no precise correlation, but if patients were over deficient there was usually a fall in the mean corpuscular haemoglobin content below 28 pg.

Serum folate concentrations at less than 20 weeks' gestation showed no difference between the normal subjects and patients with thalassaemia (α or β) (Table 6), and by term, although the degree of deficiency was high (11%), it was the same for each group. Similarly, there was no difference in the overall vitamin B₁₂ concentrations and incidence of deficiency between normal subjects and patients with thalassaemia (Table 6).

PLACENTAL FUNCTION

The total plasma oestriol values of 325 patients with

β - or α -thalassaemia are shown in Fig. 4, where they are compared with our own normal non-thalassaemic range. No difference was found between the two groups. This was a retrospective study—in other words, the values were obtained from the records of patients who were subsequently shown to be thalassaemia carriers.

Discussion

We believe that this report represents the largest study of the variation of the haematological state, the haematonic requirements, and the placental function in pregnancy of patients with thalassaemia. Although the patients studied originated from the Middle East, north Africa, and Indian subcontinent, it is probably correct to assume that the clinical expression of the types of thalassaemia seen are comparable with the clinical expression of the different types of molecular α - and β -thalassaemia seen in Mediterranean and Far Eastern people.

Several points have emerged. Firstly, it is possible to distinguish with a high degree of certainty between the hypochromia caused by α - and β -thalassaemia and that caused by pure iron deficiency anaemia in pregnancy. Patients with thalassaemia have consistently increased red cell counts compared with iron deficient pregnant patients despite expansion of plasma volume. Although the discriminant function index is only a guide to the cause of the hypochromia, it does provide a rapid simple indicator as to the need for further investigation. Moreover, as we have not found any significant decrease in the Hb A₂ concentration due to frank iron deficiency anaemia (normal subjects 2.8 g/dl $\pm$ 0.05% ; iron deficiency 2.75 g/dl $\pm$ 0.06%), and not surprisingly, during gestation, it should be possible

Table 5 Serum ferritin concentrations (ng/ml) of normal subjects and patients with thalassaemia

Gestational stage	Normal (n = 726)			β -thalassaemia (n = 71)			α -thalassaemia (n = 526)		
	Mean	Range	Deficient* (%)	Mean	Range	Deficient (%)	Mean	Range	Deficient (%)
20 wk	29.7	1.6–79.6	(6.3)	36.8	1–130	(2.1)	31.3	0.5–79	(5.6)
36+ wk	20.4	0–63.1	(22.4)	31.2	3–89	(4.1)	22.3	1.6–63	(17.4)

*Deficient = ferritin concentration <10 ng/ml.

Table 6 Serum folate and vitamin B₁₂ concentrations in normal subjects and in patients with thalassaemia

	Gestational stage (wk)	Normal (n = 784)			β -thalassaemia (n = 97)			α -thalassaemia (n = 214)		
		Mean	Range	Deficient* (%)	Mean	Range	Deficient (%)	Mean	Range	Deficient (%)
Folate concentration (μ g/l)	20	3.6	0.7–15.5	(9.2)	3.1	0.4–12.1	(8.3)	3.3	0.3–13.1	(7.2)
	36+	2.9	0.5–12.1	(11.2)	3.2	0.6–14.1	(10.7)	3.1	0.2–11.6	(10.1)
Vitamin B ₁₂ concentration (ng/l)	20	276	52–794	(7.8)	296	43–1100	(6.7)	278	27–760	(6.3)
	36+	231	30–562	(37.6)	228	32–761	(33.4)	237	31–961	(32.0)

*Folate deficiency <2.0 μ g/l; B₁₂ deficiency <150 ng/l.

Fig. 4 Total plasma oestriol concentrations in 325 pregnant patients with α - or β -thalassaemia trait grade III (mean corpuscular haemoglobin content 18–22 pg). The upper and lower normal values are shown as the continuous line. In all but four patients the reason for measuring the oestriol concentration was for suspected placental or fetal abnormality, not for anaemia. The data were obtained from records of patients with mean corpuscular haemoglobin contents of 18–22 pg at the time when the oestriol was measured; the diagnosis of thalassaemia was established subsequently.

to diagnose β -thalassaemia conclusively and α -thalassaemia by exclusion. We have ignored the likelihood that some of the patients considered as having α -thalassaemia might have $\delta\beta$ thalassaemia, but the incidence is likely to be low and also the precise type of thalassaemia is not clinically important in this study.

The increased anaemia associated with thalassaemia carriers depends on the severity of the thalassaemia, a guideline to which is the mean corpuscular haemoglobin content at presentation, but during gestation it is largely due to an increase in the plasma volume. There is no evidence for increased haemolysis. We found, however, that as the expression of the type of the thalassaemia became worse—that is, the mean corpuscular haemoglobin content decreased—the pattern of haemoglobin change was quite different and was associated with an increase in the haemoglobin concentration at around 28 weeks. This could be due to an increased production of red cells at this time or, alternatively, due to a smaller change in the plasma volume. Although the latter hypothesis is difficult to prove, it is attractive and may be determined by the control

of the intravascular fluid volume department, which is influenced by the osmotic control of the total intracellular haemoglobin content.

The percentile curves showing the haemoglobin concentrations for each grade of thalassaemia during gestation have proved to be of practical value. For example, the mean haemoglobin concentration for any particular type of thalassaemia at presentation should be accepted as the norm for that patient provided there is no other complication. Thereafter, it should remain at or near the same percentile throughout gestation, and any decrease will be due to a secondary factor—for example, iron deficiency or infection.

The studies on the haematinic state have shown that there are no differences in the degree of deficiency of serum folate or serum B_{12} between patients with thalassaemia and normal subjects, and with regard to folic acid normal supplementation would appear adequate. It is worthwhile emphasising that the observed and often severe laboratory folate or B_{12} deficiency may go unsuspected because of the lack of macrocytosis of erythrocytes or hypersegmentation of neutrophils. The lack of macrocytosis

may be due to restriction of red cell size in patients with thalassaemia; the hypersegmentation of neutrophils is unexplained. As others have found, however, there is a dichotomy between the in vitro values and the in vivo evidence for megaloblastosis in pregnancy.⁶ The remarkably high incidence of vitamin B₁₂ deficiency found in our subjects is probably due to ethnic or dietary variations.

The reason for the differences in the iron states of patients with α - and β -thalassaemia is not yet clear. It is thought that β -thalassaemia is associated more with dyserythropoiesis than α -thalassaemia, thus providing a greater stimulus for iron absorption. Results of preliminary studies, however, in which serum α -hydroxybutyrate dehydrogenase, an index of dyserythropoiesis, has been measured in patients with α - and β -thalassaemia with severe hypochromia (mean corpuscular haemoglobin content 19–21 pg), show no significant difference (White *et al*, unpublished observations). Although it appears that patients with β -thalassaemia are “protected” from developing iron deficiency, it does occur and it would seem sensible to supplement the 1 g of iron required for each pregnancy. It is also noteworthy that none of the β -thalassaemia carriers were iron overloaded.

Finally, the most important observation is that maternal thalassaemia, as assessed from the oestriol values, does not appear to have any effect on placental function of fetal development. Moreover, there was no increase in maternal morbidity—that is, rates of caesarian section, pre-eclampsia and so on.

In summary, the results of this study suggest that

maternal thalassaemia per se should not give rise to concern and patients should receive normal antenatal care with no active intervention to increase the haemoglobin concentration so long as it remains within the normal expected value for that type of thalassaemia. Once thalassaemia has been diagnosed in pregnancy, then it is assumed that genetic counselling of the parents would take place.

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