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Guidelines of Antenatal Thalassaemia Screening

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1 INTRODUCTION

Thalassaemia (-thal) is the commonest single-gene defect in the world. Homozygous α^0-thal is associated with anaemia and pregnancy complications, including hypertension, antepartum haemorrhage, postpartum haemorrhage, heart failure, renal failure and hydrops fetalis[1]. Children with β-thal major need lifelong transfusion and iron chelation. Couples who are both α^0-thal or β-thal minor run 25% risk of having homozygous offsprings.

2 MOLECULAR BASIS OF THALASSAEMIA

Two α genes have been identified on the ζ–α globin gene cluster on chromosome 16. In Hong Kong, deletion of α genes (deletion α-thal) and non-deletional α-thal exist in a ratio of approximately 3 to 1[2]. The majority of deletion α-thal involves deletion of both α genes on the same chromosome (in cis) (α^0-thalassaemia). Occasionally there is loss of the entire ζ–α gene cluster. Loss of one α gene in the gene cluster is designated α^+-thalassaemia, and offsprings of these individuals will not suffer from serious anaemia. The commonest non-deletional α-globin gene mutation in Hong Kong is Hb Constant Spring (Hb CS). This is caused by a termination codon mutation resulting in the production of unstable α globin chain. In general, the degree of anaemia depends on the number of functional α genes left.

β-thal is due to mutation in or around the β gene. Over 90 β gene mutations have been discovered worldwide. A local study revealed that 87% of β-thal was related to four common forms of mutations[3]. The mutations are classified depending on the degree of reduced β globin chain synthesis: β^0 results in total loss of β chain synthesis, while synthesis is only reduced but not absent in β^+. Thus homozygous β^0-thal (β^0/β^0) results in life-long transfusion dependency. (A glossary of terms is included at the end of the Guideline for easy reference.)

3 PREVALENCE

Thalassaemia is prevalent in the Mediterranean, through Africa and the Middle East to the Far East. Using different criteria for screening, the studies on the prevalence in Hong Kong revealed that α-thalassaemia (α-thal) and β-thalassaemia (β-thal) exist in 3 - 5% and 2.5 – 3.1% of the local population respectively [4-9].

With local delivery of 50,000 a year, and an estimate of 4.3% carrier of α-thal and 2.8% carrier of β-thal [9], the number of infants with homozygous α^0-thalassaemia is estimated to be 23; and the number of infants with β-thal major is 10. Because of the risks of adverse pregnancy outcome among the affected pregnancies, screening for at-risk pregnancies is required.

In Hong Kong, thalassaemia screening has been offered to all pregnant women at antenatal booking at every public hospital and every Maternal and Child Health Centre (MCHC), from July 2000 onwards. Currently, it is estimated that over 95% of all deliveries in public hospitals have undergone thalassaemia screening. Corresponding information from the private sector is lacking.

This guideline applies to screening for the majority of the local community, which consists of southern Chinese. For other ethnic populations, the guideline may not apply and full hematological study is needed to detect the at-risk hemoglobin disorders.

4 HAEMATOLOGICAL FINDINGS AMONG THALASSAEMIA CARRIERS (Table 1)

An understanding of the haematological changes in thalassaemia carriers provides basis to develop simple screening criteria. Haemoglobin (Hb) level is usually normal among non pregnant α-thal and β-thal carriers. In α^0-thalassaemia carriers, the MCV is below 80fl. Hb A_2 is normal. Occasional Hb H granules may exist, but these may be absent. Hence α^0-thal carrier state cannot be excluded in patients with low MCV, in spite of normal haemoglobin pattern.

In single α-globin gene deletion (α^+–thal) and α-globin gene mutation (non deletion α^+–thal) such as Hb Constant Spring, MCV ranges between 80 and 85fl. This finding may be significant if the partner carries α^0-thal, because Hb H disease or uncommon forms of thalassaemia genotypes may exist in the offsprings. The current antenatal thalassaemia screening programme is not tailored towards prediction of HbH disease.

For β thal carriers, MCV is consistently below 80 fl. Hb A2 is raised[10]. The presence of low MCV with raised Hb A2 is diagnostic of β thal. However, Hb A2 level may remain normal among β–thal carriers with iron deficiency. Hence β–thal trait cannot be excluded in women with low MCV and normal Hb A2 levels, unless iron deficiency is excluded.

Individuals with compound α and β thal trait also have MCV below 80fl. HbH inclusion bodies may be absent. Hb H granules should therefore not be used as a means to detect concomitant α^0-thal in β-thal carriers[11]. DNA analysis to exclude α^0-thal would be necessary for such individuals.

Table 1: Haematological parameters of thalassaemia carriers

	Hb (g/dL)	**MCV** (fL)	**A2** (%)	**HbH bodies**
* α^0-thal (Pregnant females) n = 408	10.82 (8.3 – 13.7)	68.8 (61.1 – 78.9)	2.39 (2 – 3.24)	occasional or absent
* α^0-thal (Males) n = 423	13.84 (10 – 17)	68.45 (58.8 – 79.5)	2.37 (2 – 3)	occasional or absent
** α^+-thal (Males and non pregnant females) n = 70	13.5 (11.7-15.8)	81.5 (72.6-92.5)	2.7 (2.4 – 3)	Absent
* β thal minor (Pregnant females) n = 478	10.21 (6.9 – 13.2)	66.1 (54-78)	5.48 (3.6-6.7)	Absent
* β thal minor (Males) n = 570	13.17 (8.0-16.0)	65.2 (52.6-77.5)	5.54 (4.0-6.9)	Negative
* α-β thal (Males and females) n = 26	13.00 (9.3-16.6)	71.81 (64.7-78.9)	5.55 (4.8-6.5)	occasional (absent in 11 cases)

Notes:

1. Table is compiled from data provided by Professor V Chan from the Division of Molecular Medicine, Department of Medicine, the University of Hong Kong (*), and by Dr. E Ma from the Division of Hematology, Department of Pathology, the University of Hong Kong (**)

2. Hb, MCV, A_2 are tabulated as mean values, with range indicated in brackets.

5 PARAMETER FOR THALASSAEMIA SCREENING IN PREGNANCY

Given the heterogeneity of thalassaemia, a simple screening test for every variety of thalassaemia has not been discovered. A practical approach is to screen for the common and clinically important thalasssaemias in the locality. The goal is to identify the couples at risk and to detect in early pregnancy the severe forms of the disorder in the fetus, ie, homozygous α^0-thalassaemia and β-thal major, in order to avoid morbidity and mortality.

Both MCV and MCH have been identified as appropriate measures to screen for α- and β-thal. MCV is more often used for the purpose, while MCH has the theoretical advantages over MCV because it is more stable over time. Both parameters are highly accurate. Although red cells stored in room temperature tend to swell over time so that the sensitivity of MCV may decrease when stored blood is screened, this may not be a concern in Hong Kong as our samples need not travel long distances. In Hong Kong, MCV has been adopted for screening thalassaemias.

Different cutoffs in MCV have been used in various screening programmes [5,9]. Most have adopted a cutoff between 76 fL and 82 fL. With MCH, 27pg seems to be the most acceptable cutoff. By genotyping archived samples from school children up to MCV of 85 fL, Ma et al [12] found that an MCV cutoff of 80 fL or MCH cutoff of 27 pg could detect all α^0-thal carriers and β-thal carriers. The authors recommended taking these cutoffs as criteria for thalassaemia screening in Hong Kong. Since both MCV and MCH tend to increase in pregnancy, the cutoffs may not apply to antenatal screening without loss in sensitivity. Currently the joint antenatal thalassaemia screening programme between public hospitals and the MCHC adopts an MCV cutoff of 80 fL, while some local haematology laboratories adopt a cutoff of 82 fL.

6 TIMING OF SCREENING

Early experience of antenatal thalassaemia screening involved mainly pregnancies before 18 weeks. Early screening allows adequate time for further workup and counselling of the couples. In case termination of pregnancy needs to be considered, timely diagnosis of thalassaemia major in the foetus is desirable. Since Hb Bart's hydrops foetalis carries risks to the mother, antenatal thalassaemia screening should be offered to all pregnant women with unknown thalassaemia status, regardless of gestational age.

7 EDUCATION AND COUNSELLING AS AN INTEGRAL PART OF SCREENING

Current practice of screening necessitates informed consent from patients. Education and information for patients is required before informed consent can be obtained. These may be provided to patients through various means, including information pamphlets, group counseling, video display, posting on website or explanation in person. The information appropriate for the purpose is listed in **appendix 1**.

Detailed pre-test counselling to patients is usually not necessary unless the patient is particularly anxious. In cases of screen-positives or known carriers, further counselling is appropriate to allay anxiety and to prepare patients and their families psychologically. While admitting the chance that the fetuses may be affected adversely, the couples should understand that the pregnancies are more likely to be normal and termination of pregnancy before further diagnostic procedure is not necessary.

8 FURTHER WORKUP FOR SCREEN-POSITIVES AND LOW MCV COUPLES (Appendix 2)

Screen-positives are women whose MCV and/or MCH fall below the cutoff value. Among ethnic Southern Chinese, the majority has one of the following conditions: α^0-thal, β-thal trait or iron deficiency. Tests for Hb H inclusion bodies and elevation in Hb A2, and iron study, should be performed. Presence of Hb H inclusion bodies and elevation in Hb A2 (>3.5%) are diagnostic of α^0-thal and β-thal respectively. Co-existent β-thal cannot be totally excluded if there is iron deficiency, and α^0-thal cannot be excluded in any individual with low MCV or low MCH, absent Hb H inclusion bodies, and normal ferritin level.

Workup on the partners begins with MCV and/or MCH estimation for screening of α^0-thal and β-thal trait. Save for some special situations to be discussed later, levels above the respective cutoff virtually excludes serious thalassaemia affecting the foetus. If the partners are screen-negative, no further workup is necessary.

Partners who are screened positive should be tested for presence of Hb H inclusion bodies, elevation of Hb A2, and iron study. The purpose is to identify whether the partners have thalassaemia affecting the same globin gene as the screen positive women, ie, whether they are α-α couples or β-β couples. Since 7% of β thal carriers were found to be compound α- and β-thal heterozygotes[13], couples discordant for α- and β thalassaemia (α-β couples) should be counselled and offered DNA study to exclude co-existence of α thal in the β thal partner.

According to a review of the screening programme between public hospitals and MCHC, testing was required in 0.89% (133 out of the 15013 couples) of the pregnancies screened at MCHC between July 2000 and June 2001.

Due to the high prevalence of thalassaemias in Hong Kong, clinically important thalassaemias due to rare thalassaemia genotypes has been reported[14-16]. If resources allow, partners of known thalassaemia carriers, regardless of MCV, may be referred to haematologists for investigation of rare thalassaemias and identification of pregnancies at risk of Hb H disease.

For α-α couples, β-β couples and α-αβ couples, detailed counselling and further prenatal testing should be offered. Since α^0-thal cannot be excluded in individuals with low MCV despite normal Hb and iron studies, low MCV couples (with normal iron studies and normal Hb pattern) should be managed as potential α-α couples. Explanation on the significance of α-α couples, β-β couples and α-αβ couples may be carried out by most practicing obstetricians (**appendix 3**).

Experienced personnel and laboratories should perform further prenatal testing for these couples. Currently the workup involves special ultrasound examination, or sampling of amniotic fluid cells, chorionic villi or foetal blood. The risk of miscarriage (fetal loss) is around 0.5% following amniocentesis, and around 1% following chorionic villus sampling or fetal blood sampling. Trained personnel can safely and reliably perform these procedures. The samples should then be sent to established laboratories experienced in handling fetal samples and running the special DNA studies.

9 SUMMARY

9.1 Thalassaemia is highly prevalent in Hong Kong. Antenatal thalassaemia screening provides an opportunity for early detection of Hb Bart's disease and β-thal major.

9.2 Antenatal thalassaemia screening aims at identifying α-α couples, β-β couples or α-αβ couples whose off-springs are at risk of having Hb Bart's disease and β-thal major. It should be offered to all pregnant women, regardless of gestational age, in particular, for prenatal detection of Hb Bart's disease.

9.3 Information on the significance of thalassaemia should be provided to pregnant women as an integral part of the antenatal thalassaemia screening programme.

9.4 Measurement of MCV and/or MCH is appropriate for antenatal thalassaemia screening. An MCV cutoff of < 80 fL, or an MCH cutoff of <27 pg is recommended for antenatal thalassaemia screening.

9.5 Thalassaemia screening by measuring MCV and /or MCH should be offered to partners of pregnant women who are screen-positive.

9.6 In areas with high prevalence of thalassaemia (such as Hong Kong), haemoglobin study to identify presence of occasional H granule and elevation in Hb A2, and iron study should be arranged for pregnant women who are screen-positive. Haemoglobin study and iron study for the partners should also be arranged for women known to be α^0-thal or β-thal carriers.

9.7 Couples identified as α-α couples, β-β couples and α-αβ couples should be counselled and offered prenatal diagnostic tests to exclude Hb Bart's disease and β-thal major affecting the foetus.

9.8 Prenatal work-up for low MCV couples, α-α couples, β-β couples and α-αβ couples should be carried out by personnel and laboratories with experience in the procedures.

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This guideline was produced by the Hong Kong College of Obstetricians and Gynaecologists as an educational aid and reference for obstetricians and gynaecologists practicing in Hong Kong. The guideline does not define a standard of care, nor is it intended to dictate an exclusive course of management. It presents recognized clinical methods and techniques for consideration by practitioners for incorporation into their practice. It is acknowledged that clinical management may vary and must always be responsive to the need of individual patients, resources, and limitations unique to the institution or type of practice. Particular attention is drawn to areas of clinical uncertainty where further research may be indicated.

Appendix I

Information for Pretest Education

1. Thalasaemia is one of the commonest hereditary blood disorders.

2. Incidence in Hong Kong: about 5% of local population carries the gene responsible for alpha thalassaemia (alpha thal); about 3% for beta thalassaemia (beta thal).

3. Most of these carriers are healthy (thalassaemia minor) and they would not know that they are carriers until appropriate blood tests have been performed for them.

4. The carrier status is inherited. It will not change with age.

5. If two carriers marry, their children have 25% chance of having serious anaemia (thalassaemia major). Children with β-thal major will gradually develop severe anaemia a few months after birth. Infants with α-thal major die in late gestation or shortly after birth. The pregnant women carrying α-thal major fetuses may develop serious illness such as high blood pressure and convulsion in the latter part of pregnancy or after delivery.

6. Even if the partners are not carriers, pregnant carriers of thalassaemia require vitamin supplement to maintain generation of blood in her body.

7. The great majority of thalassaemia carriers in pregnancy can be detected by simple blood tests (screening tests for thalassaemia), which can be performed together with other antenatal blood tests. Similar blood tests may be arranged for the partners of screen-positives, to ascertain whether the partners are also thalassaemia carriers.

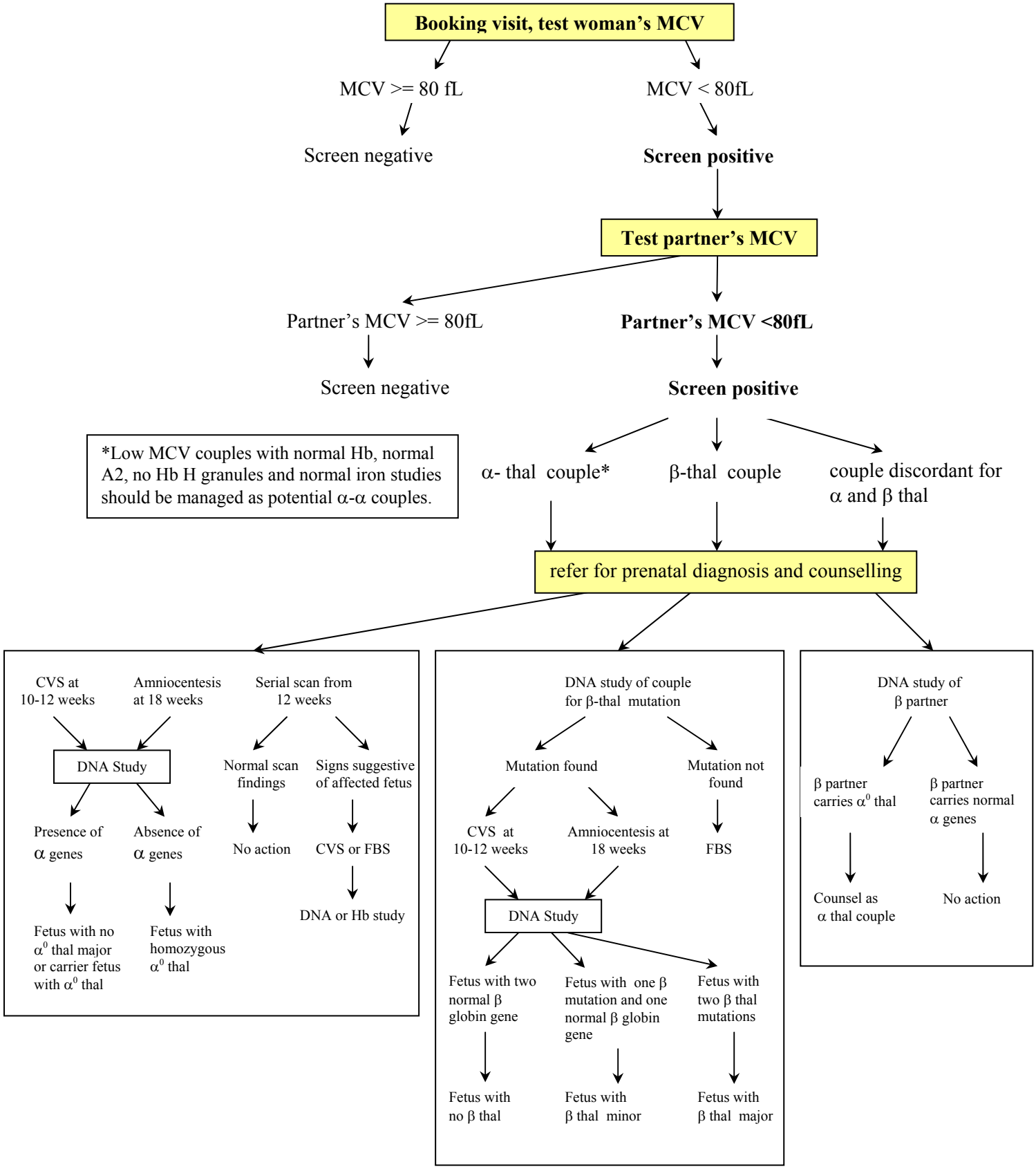
8. In some of the couples who are screen-positive, further blood testing (diagnostic tests) may be required to confirm or exclude whether they are thalassaemia carriers.

9. If both the husband and wife are thalassaemia carriers, prenatal diagnostic tests may be arranged for the pregnancy to exclude thalassaemia major in the fetus.

10. Prenatal diagnostic tests would be performed by personnel and laboratory with experience in the procedure.

Appendix 2

Work flow for antenatal thalassaemia screening



Appendix 3

Information for low MCV couples, alpha-alpha couples, beta-beta couples and alpha-alpha beta couples:

1. Thalasaemia is one of the commonest hereditary blood disorders.

2. Except for vitamin supplement (with folic acid) during pregnancy, no drug treatment is required for thalassaemia minor.

3. Thalassaemia minor does not weaken the body physically or mentally. It will not progress to thalassaemia major.

4. (For low MCV couples)
   Blood tests so far cannot confidently exclude that both you and your partner are alpha thalassaemia minor carriers. Further tests for both of you are required.

5. (For alpha-beta couples)
   Blood tests so far have shown that you and your partner are carriers of different types of thalassaemias. Because of the limitation of the simple tests, further tests are required to confidently exclude alpha thalassaemia carrier state for you or your partner.

6. If your partner also has thalassaemia minor of the same variety (alpha thal or beta thal), 25% of the offspring will have thalassaemia major.

7. (For alpha-alpha couples, alpha-alpha beta couples and low MCV couples)
   Infants with alpha thalassaemia major may die before or shortly after birth. The pregnant women carrying an affected fetus may develop serious illness such as high blood pressure and convulsion.

8. (For beta/beta couples)
   Babies with beta thalassaemia major will gradually develop severe anaemia a few months after birth. They will need life-long blood transfusion and special treatment (iron chelation) to minimize accumulation of iron in the organs due to repeated transfusion, otherwise they will die from consequences of iron overload.

9. For fetuses at risk of thalassaemia major, prenatal diagnostic tests may be arranged to exclude thalassaemia major affecting the fetus. These tests would be performed by personnel and laboratory with experience in the procedure. The tests are 100% definitive in most cases.

Glossary of terms

based on Weatherall & Clegg, The Thalassaemia Syndormes, 4th Edition, 2001

Thalassaemia: A group of genetic disorders (hereditary anaemia) characterized by a reduced rate of production affecting one or more globin subunits of the haemoglobin molecule. They are divided broadly into α, β, γ, $\delta\beta$, δ and $\varepsilon\gamma\delta\beta$ varieties, depending on which globin chain(s) is under-produced.

α^0-thalassaemia (α-thal-1): Refers to the condition in which both α-globin genes on the same chromosome (in cis) are deleted. The heterozygous genotype can also be designated as - -/$\alpha\alpha$.

α^+-thalassaemia (α-thal-2): Refers to the condition when one of the two α-globin genes is inactivated. The genotype is designated -$\alpha/\alpha\alpha$ when one of the α-globin genes is deleted, or $\alpha^T\alpha/\alpha\alpha$ when one of the α–globin genes is inactivated by a mutation (sometimes termed non-deletional α^+–thal).

β^0-thalassaemia: Refers to the condition in which no β-globin chain is produced.

β^+–thalassaemia: Refers to the condition in which some residual β-globin chain is produced (but less than normal).

β^{++}–thalassaemia: Refers to the condition in which the defect in β-globin chain production is particularly mild.

Thalassaemia carrier / trait: Refers to individuals who are heterozygous for a thalassaemia mutation. These individuals are usually clinically asymptomatic but may give rise to offsprings affected by severe thalassaemia if the partner is also a carrier of the same type.

HbH disease: Refers to individuals who are compound heterozygous for α^0-thalassaemia and α^+–thalassaemia, the latter of which can be deletional or non-deletional. Typical genotypes are therefore either three α-globin gene deletion (--/-α), or α^0-thalassaemia in association with non-deletional α-globin gene mutation (--/$\alpha^T\alpha$), for example HbCS (--/$\alpha^{cs}\alpha$). The clinical phenotype in these individuals is mild, and they are usually not transfusion dependent, although recent evidence shows that non-deletional HbH disease is associated lower haemoglobin, more splenomegaly and hence splenectomy, and more iron overload with age.

Hb Bart's hydrops fetalis: Refers to homozygous α^0-thalassaemia (i.e. four α-globin gene deletion) and is usually associated with intrauterine death or stillbirth.

β-thalassaemia major (Cooley's anaemia): Refers to individuals who are homozygous for β^0-thalassaemia or compound heterozygous for β^0 and β^+–thalassaemia. These individuals usually present below the age of 1 year with severe anaemia that necessitates regular transfusion. They suffer from numerous disease complications due to iron overload, and have a limited life expectancy.

β–thalassaemia intermedia: Refers to individuals who, although usually carrying two β-thalassaemia alleles, either β^+/β^+ or β^0/β^+, are clinically less severely affected than β^0-thalassaemia major. It could also be due to inheritance of other genetic factors that ameliorate the disease phenotype.