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Screening for Thalassaemia in Pregnant Women: A Laboratory Perspective

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ABSTRACT

Aims:To investigate whether in Malaysia, a mean corpuscular volume (MCV) less than 80 fl and a mean corpuscular haemoglobin (MCH) less than 27 pg will identify carriers in pregnant women with severe forms of thalassaemia, α-thal 1 (α^0) and classical β (β^0)-thalassaemia. The results from this study will aid the implementation of a national program to screen for thalassaemia. **Methods**: For classical β (β^0)-thalassaemia, blood samples collected in EDTA from 153 pregnant women were taken for full blood counts and haemoglobin subtyping by automated blood counting and high performance liquid chromatography (HPLC) respectively. For α-thal 1 (α^0), the full blood counts were obtained from archives of 30 pregnant women who were genotyped positive for the α-thal 1 (α^0) during prenatal diagnosis for Hb Barts hydrops fetalis. The effects of storage on MCV, MCH and Hb A_2 were determined by tests done daily for 3 weeks. **Results**: By correlating red cell indices with high performance liquid chromatography and genotypic data, we show that mean corpuscular volume (MCV) <80 fl and mean corpuscular haemoglobin <27pg is able to detect all heterozygous carriers of α-thal 1 (α^0) and classical β (β^0)-thalassaemia. On storage, the MCV of heterozygous carriers with classical β (β^0)-thalassaemia rose at 1% a day after 24 hours reaching a mean of 80 fl by day 15. However, the MCH and Hb A_2 were stable for 3 weeks. **Conclusion**: A mean corpuscular volume (MCV) <80 fl and mean corpuscular haemoglobin <27pg should be recommended as cut-off values for screening of carriers of α-thal 1 (α^0) and classical β (β^0)-thalassaemia. In blood samples, not processed within a day, MCH with a cut-off value of 27 pg is the recommended choice for screening of carriers.

Keywords: Screen, thalassaemia, pregnant, MCV, MCH

INTRODUCTION

Thalassaemia is a disorder of haemoglobin synthesis, resulting from quantitative reductions in globin chain synthesis of human haemoglobin [1]. Those with diminished synthesis of β globin chains are termed β-thalassaemia. In classical β-thalassaemia (β^0)-thalassaemia, there is total absence in the synthesis of the β globin synthesis leading to the homozygous state (β^0/β^0) or β-thalassaemia major to total absence in the predominant haemoglobin subtype in the adult Hb A ($\alpha_2\beta_2$). Beta thalassaemia major presents in patients in the first year of life and requires life-long blood transfusion and iron chelation therapy. Stem cell transplantation

provides a possible cure available to a limited few from HLA compatible donors. In α-thal 1 (α^0) there is absence in the synthesis of the globin chains, while in the homozygous state for thal 1 ($\alpha^0/(\alpha^0$) or Hb Barts hydrops fetalis, no adult (Hb A, $\alpha_2\beta_2$; Hb A_2, $\alpha_2\delta_2$) and fetal ($\alpha_2\gamma_2$) haemoglobins are synthesized. Hb Barts hydrops fetalis, a fatal condition is incompatible with life where the gestation ends with death *in-utero*, stillbirth or death of the baby soon after birth commonly within 6 hours. Mothers carrying a fetus with Hb Barts hydrops fetalis may have hypertension, preeclampsia, postpartum haemorrhage and a difficult birth. Thalassaemia is a public health problem in Malaysia [2]. The thalassaemia major syndromes produce severe life threatening anaemia and it is imperative to identify carriers of these severe forms of thalassaemia.

Laboratory haematologists have a major role in laying down the screening strategy. The availability of automated blood counting and high performance liquid chromatography (HPLC) has permitted an efficient stepwise diagnostic strategy for the presumptive identification of carriers of α thal 1 (α^0) and classical β (β^0)-thalassaemia [3, 4]. The first step in screening for thalassaemia involves a scrutiny of the blood counts and red cell indices generated by an automated blood counter. A thalassaemia carrier or a thalassaemia minor is characterized by persistent microcytosis and hypochromia in the absence of anaemia or accompanied by mild anaemia. The second step, involves the quantification of the Hb subtypes present. Automated cation-exchange HPLC has emerged as the method of choice for accurate and precise quantification of Hb subtypes [3, 4, 18]. The hallmark for the presence of a classical beta thalassaemia trait is an elevation of Hb A_2 > 4% [4, 5, 6]. The α-thal 1 (α^0) carrier is presumptively identified by exclusion of β-thalassaemia trait and iron deficiency as a cause of the hypochromic and microcytic red blood cell indices. The α-thal 1 (α^0) carrier status can only be accurately identified by its genotype as determined by DNA studies [3, 4, 7, 8, 9, 10].

METHODS

Classical β (β^0)-thalassaemia Trait

Peripheral blood samples from one hundred and fifty-three pregnant women were obtained randomly following informed consent at their first visit to the antenatal clinic at Hospital Universiti Kebangsaan Malaysia, Kuala Lumpur by venepuncture. Three and half milliliters (3.5 ml) of venous blood collected in EDTA generated full blood counts on an automated blood counter (Coulter STKS, Coulter Corporation, 11800 Miami, Florida 33196-2500, United States of America) and for Hb subtyping on a HPLC automated Hb analyzer (Variant, BioRad 2000 Alfred Nobel Dr., Hercules, CA 94547, United States of America). Two milliliters blood collected in a plain tube was used for estimation of serum ferritin levels.

α-thalassaemia 1 Trait

Data for full blood counts were generated from archived results from previous studies done on 30 pregnant women who had been genotyped positive for α-thal 1(α^0) and requested for prenatal diagnosis for Hb Barts hydrops fetalis at the Haematology Unit, Department of Pathology, Faculty of Medicine Universiti Kebangsaan Malaysia.

RESULTS

Classical β-thalassaemia Trait

The presumptive identification of a case of classical β-thalassaemia trait is the presence of an elevated Hb A_2 level >4%. This cut-off value was determined by earlier studies on the BioRad Variant HPLC Hb analyzer [6]. Six were identified as classical β-thalassaemia trait with all having MCV <80 fl and MCH <27 pg. (Table 1). All the patients had haemoglobin levels < 10.5 gm/dl with two of the patients having serum ferritin levels < 3 μg/L (normal 10-150 μg/L). The mean MCV and MCH were 65.7 fl and 21.2 pg respectively.

α-thal 1 (α^0)-thalassaemia Trait

All the patients had MCV <80 fl and MCH < 27pg, Hb A_2 < 4% and haemoglobin levels < 10.5 gm/dl (Table 1). The mean MCV and MCH were 67.1 fl and 20.9 pg respectively. The iron status of these patients was not available.

Storage of Samples

The MCV showed a progressive rise reaching a MCV of 80 fl by day 14. The MCH and Hb A_2 were steady for 3 weeks (Table 2).

Algorithm for Screening of Thalassaemia

The data of the study enabled a critical appraisal of the algorithm presented as a strategy for thalassaemia screening in Malaysia [11] (Figure 1). In pregnant women, the MCV < 80 fl and MCH < 27 pg are valid cut-off values for screening thalassaemia.

Table 1. Hb, MCV, MCH and Hb A_2 in pregnant women with thalassaemia trait

	α thal 1 (α^0) N=30	β-thal N=6
Hb gm/dl	9.7 ± 1.9	9.4 ± 1.8
MCV fl	67.1 ± 5.9	65.7 ± 5.5
MCH pg	20.9 ± 1.5	21.2 ± 2.1
Hb A_2 %	2.7 ± 0.2	5.5 ± 0.4

Table 2. MCV, MCH and Hb A_2 in beta thalassaemia trait in stored blood samples (stored at 4°C)

	MCV fl	MCH pg	Hb A_2 %
Day 1	62.6	20.5	5.6
Day 7	69.6	20.8	5.4
Day 14	80.8	20.8	5.5
Day 21	84.0	20.8	5.4

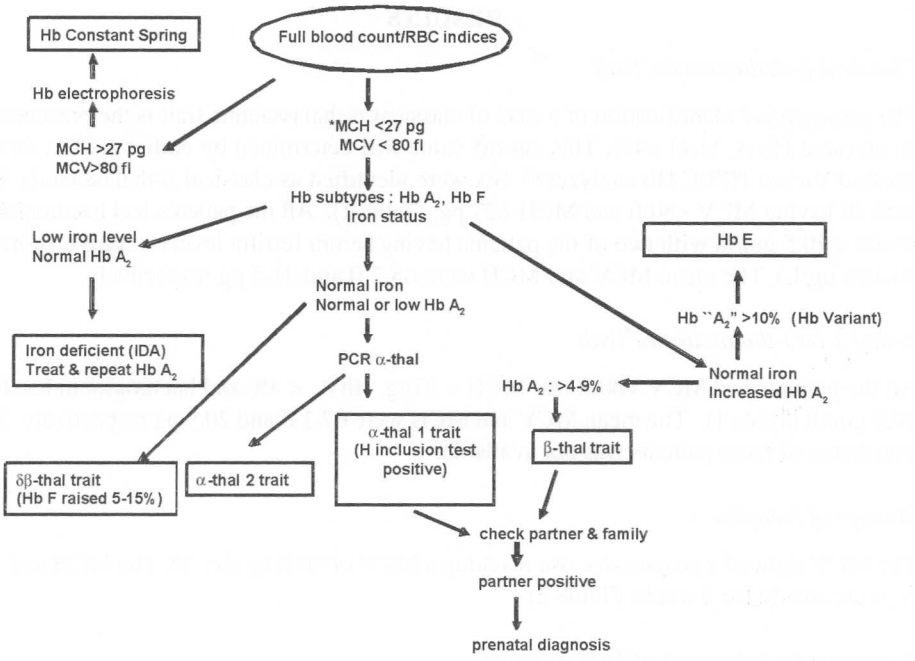

Figure 1. Algorithm: Screening for thalassaemia

DISCUSSION

Thalassaemia defined as a heterogeneous group of inherited autosomal recessive disorders of haemoglobin synthesis is characterized by the absence or reduced output of one or more globin chains of haemoglobin. Theoretically for any autosomal recessive disorder, the incidence of the homozygote could be reduced to zero with proper preventive measures to identify heterozygote carriers of the disease [12]. It remains a public health problem in Malaysia as there has been no national policy in place to screen for thalassaemia [2]. Since the late 1970s, programs of population screening of adults at child-bearing age, genetic counselling and prenatal diagnosis have been introduced in the Mediterranean region. All such programs are marked by intensive population education conducted mostly through mass media, including newspapers, radio, television and magazines. Screening in the Mediterranean has been voluntary, where at the beginning it involved primarily couples with an ongoing pregnancy. With increasing knowledge, this screening was extended to non pregnant couples and single adults in the child-bearing age group. This resulted in the prevention of over 94% births of beta thalassaemia major [12]. In Iran, a similar program for premarital screening of thalassaemia was commenced in 1996. Over 5 years, 10,000 couples were screened for thalassaemia resulting in 70% reduction in the expected annual births of affected infants with beta thalassaemia major [13].

The first step in the current screening strategy for thalassaemia carriers is based upon a scrutiny of the erythrocyte indices, MCV and MCH. The British Committee for Standards

in Haematology (BCSH) recommends testing for β-thalassaemia and α^0-thalassaemia when MCH is <27 pg and <25 pg respectively [4]. The hallmark for classical β-thalassaemia is an elevation of Hb A_2 and α^0-thalassaemia trait is presumptively identified by hypochromic microcytic red cell indices, a Hb A_2 level < 3.5% and the presence of a positive H inclusion test [1,4]. It is a laborious process to scan for H inclusions. In addition, faulty incubation of erythrocytes may give rise to false positive results. In a carrier of α-thal 1 (α^0), the H inclusions are present as 1 inclusion containing cell in 1000-10,000 cells [3]. A negative H inclusion test does not exclude the presence of α-thal 1 (α^0) but the presence of typical H inclusion is helpful in making the presumptive diagnosis more informative. Accurate identification requires DNA studies to identify the α-thal 1(α^0) [7].

We critically appraised the red cell indices in carriers of classical β-thalassaemia and α-thal 1 (α^0)-thalassaemia and report that a MCV < 80 fl and MCH <27 pg identifies pregnant women accurately. The need for adequate quality assurance of automated blood counters to produce accurate and precise results cannot be over-emphasized. Blood samples from carriers of beta thalassaemia showed a progressive rise in the MCV during storage and thus, this parameter cannot be used in samples that cannot be processed within a day. This information is important in national screening programs where blood samples collected need to be transported to a centre for analysis. In addition, the MCV has been reported to rise from 2 to 4% in pregnancy and will aggravate the effects of storage[14]. The MCH and Hb A_2 estimations remain stable for over 3 weeks, making these two parameters the choice for samples that cannot be processed within a day. The BCSH also places reliance on the MCH value in the United Kingdom [4].

It is well known that a MCV > 80 fl which is considered normal by screening criteria, does not preclude the presence of single gene mutations which include single globin gene deletions, point mutations in globin genes [15]. These conditions however may result in 5thalassaemia intermedia but not thalassaemia major. Microcytosis and hypochromia are common findings in full blood counts in pregnancy. Iron deficiency anaemia (IDA) occurs when there is not enough iron to support erythropoiesis. It is the most common cause of anaemia diagnosed during pregnancy [16, 17]. IDA results in anaemia, microcytosis and hypochromia. In Malaysia, both thalassaemia and IDA can be present in a pregnant woman. It is a myth that iron should never be given to thalassaemia patients. Women with β thalassaemia trait may become iron deficient.

This report supports the cut-off values of MCV <80 fl and MCH < 27pg to be applicable for the screening of classical beta thalassaemia and α-thal 1 (α^0) thalassaemia in pregnant women [4, 13, 14, 16, 18,19]. These cut-off values provide the strategy to identify carriers of severe forms of thalassaemia so as to enable genetic counseling and prenatal diagnosis to be offered to couples who are carriers of thalassaemia. The majority of pregnant women have a blood test done at first antenatal visit to assess the haemoglobin status. A scrutiny of red blood indices from the automated blood count results will provide the first step in the identification of carriers of severe forms of thalassaemia. It is imperative to screen the partner for thalassaemia when a pregnant woman is identified as a thalassaemia carrier. Appropriate genetic counselling and prenatal diagnosis can then be made available to the couple and other family members also screened for thalassaemia [12, 20]. In Malaysia, it is recommended that all pregnant women with a MCH cut-off value <27 pg be screened for thalassaemia and iron deficiency.

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