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Prenatal Diagnosis of Severe Thalassemia Syndrome in Maharaj Nakorn Chiangmai Hospital

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ABSTRACT

Objective To describe prenatal diagnosis programme for prevention and control of severe thalassemia syndrome which was successfully carried out at Maharaj Nakorn Chiangmai Hospital.

Design A prospective descriptive study.

Setting Maharaj Nakorn Chiangmai Hospital.

Subjects and methods The programme included : 1) screening of pregnant women for thalassemia carriers in order to identify couples at risk of having baby with severe thalassemia syndrome, 2) prenatal diagnosis by serial ultrasonography or fetal blood sampling under ultrasound-guided cordocentesis in risk couples, 3) analysis of fetal blood under HPLC (High Performance Liquid Chromatography) or haemoglobin electrophoresis, 4) genetic counseling of couples with affected fetus and discontinuing pregnancy.

Results From 13th September 1994 to 1st August 1995, 3,310 pregnant women were screened for carriers of important thalassemia by EOFT (Erythrocyte Osmotic Fragility Test) and 1,000 cases (30.2%) gave abnormal results. Among them 115 couples were at risk, 87 of them obtained prenatal diagnosis and 19 of 87 fetuses were severe thalassemia syndrome and were terminated.

Conclusion Prenatal diagnosis programme for thalassemia is an obstetric role in prevention and control of the disease.

Key words : prenatal diagnosis, thalassemia

Thalassemia is the most common haematologic genetic disease in Thailand. About 500,000 Thai people are affected and more than 15 million Thais are carriers. By calculation of abnormal gene prevalence, the couples at risk for having an affected child are 50,000 pregnancies a year. The common type of thalassemia are homozygous beta-thalassemia, beta-thalassemia Hb E disease, Hb Bart's hydrops fetalis and Hb H disease. The affected persons will have low quality of life (blood transfusion, gall stone, etc) and the mothers who have hydropic fetus will suffer from obstetric complications such as pre-eclampsia, antepartum haemorrhage and postpartum haemorrhage. Prevention of the new case is an obstetrician's role to control the disease.

In prevention and control programme of thalassemia, genetic counseling and carrier detection followed by prenatal diagnosis of the risk couple should be in the step. Maharaj Nakorn Chiangmai hospital is the largest tertiary care centre of Northern Thailand which has to face the problem of thalassemia due to a high prevalence of abnormal gene.

Carrier detection of thalassemia in asymptomatic patients can be achieved by different methods such as red blood cell (RBC) indices (MCV), haemoglobin electrophoresis, PCR (polymerase chain reaction) technique and erythrocyte osmotic fragility test (EOFT). In this study, EOFT was chosen to screen the patients because it is a rapid, simple procedure and not expensive or time consuming, and suitable for mass screening.[1,2]

Materials and Methods

Laboratory Tests

Erythrocyte Osmotic Fragility Test (EOFT) is a rapid and simple method for determination of RBC osmotic fragility,[3] using hypotonic solution (0.36% NaCl or glycerine saline solution). At a specific time (90 seconds), rate of haemolysis of normal erythrocyte is more than 50% while in cases of abnormal erythrocyte like thalassemia and thalassemia carriers haemolysis of erythrocyte is less than 50%. In this study, "less than 60% haemolysis at 90 seconds" was used as cut off point of abnormal erythrocytes to decrease false negative test. Laboratory methods for this study was

(1) Reagents : 0.45% glycerine saline solution (0.45% GSS), pH 7.4

(2) Specimens : Venous blood collected in EDTA, centrifuge at 3,000 rpm for 5 minutes, then remove supernatant and collect RBC for the test

(3) Procedure :

(a) Add 20 μl of RBC to 20 ml of distilled water and mix together. When there is complete haemolysis, set a spectrophotometer to record its absorbance at 620 nm as 0 (zero)

(b) Add 20 μl of RBC to 20 ml of 0.45% GSS and mix together. Record the absorbance every 15 seconds until 120 seconds, assume that absorbance at 0 and 15 seconds = 100 (No haemolysis)

$$\% \text{ haemolysis} = \frac{\text{Absorbance at 15 sec} - \text{Absorbance at 90 sec}}{\text{Absorbance at 15 sec}} \times 100$$

If the laboratory test was abnormal (EOFT < 60%, at 90 sec), the sample was further tested for HbA_2 level (by microcolumn DEAE Sephadex A 50 chromatography) to differentiate the patient as alpha-thal 1 trait, beta-thal trait or Hb E trait. (Fig. 1)

Subjects

Prenatal diagnosis programme for prevention and control of severe thalassemia syndrome was conducted at the antenatal care clinic (ANC) of Maharaj Nakorn Chiangmai Hospital since 13th September 1994. The data was collected

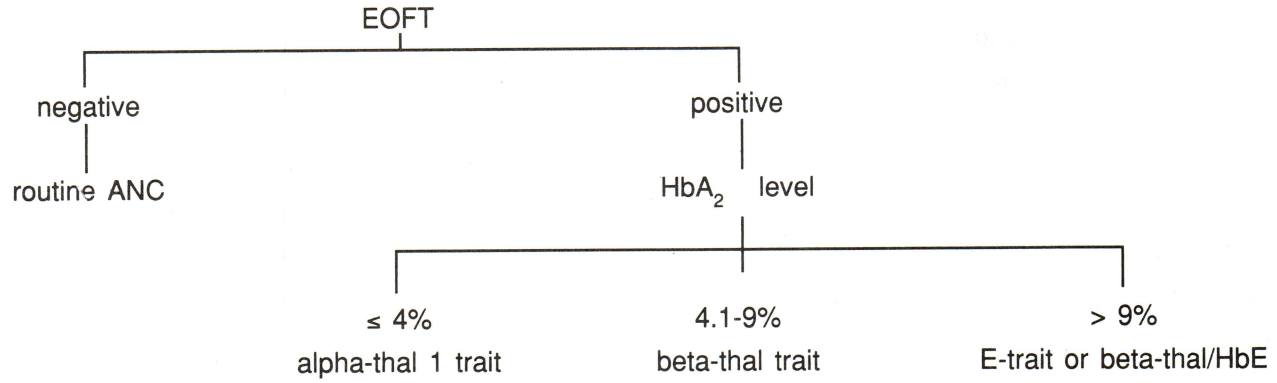

Fig. 1. Algorithm for thalassemia screening.

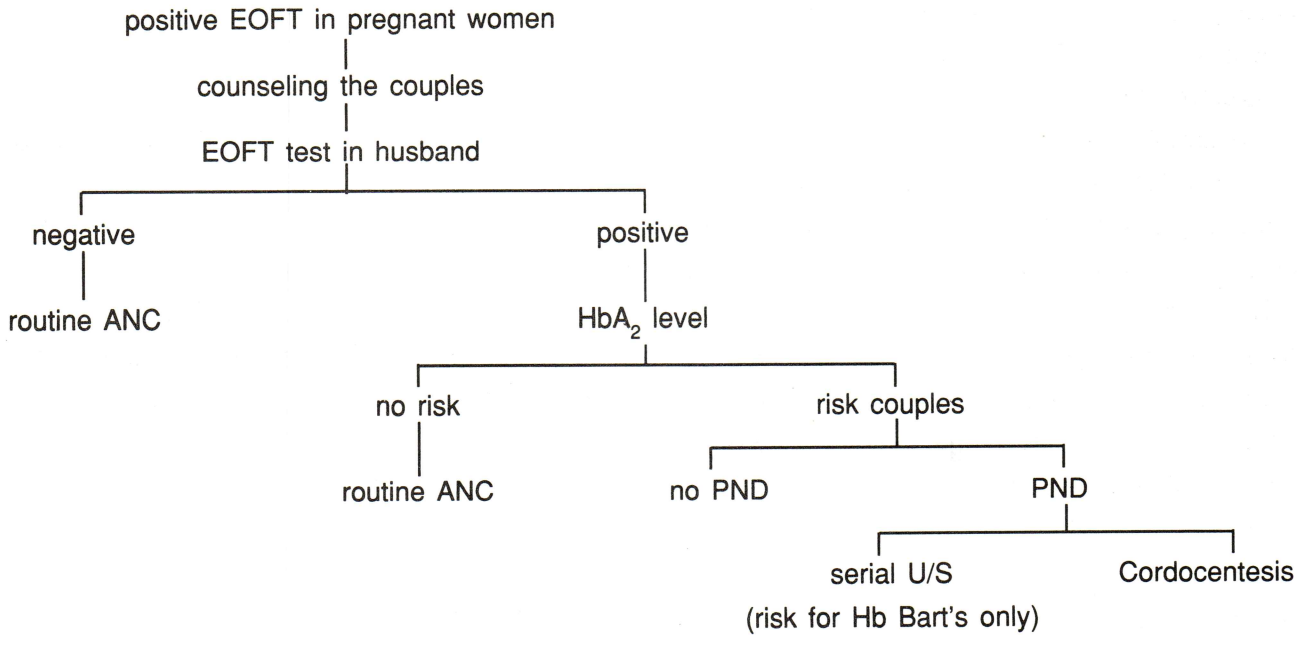

Fig. 2. Algorithm for counseling and management of risk couples.

from 13th September 1994 to 1st August 1995.

All pregnant women who first came to ANC received a paper counseling for thalassemia screening. Two ml of blood was collected and tested for EOFT followed by HbA_2 level in abnormal EOFT cases. Once the test was positive, the patient and her husband were asked to come for intensive counseling. The counseling included the screening tests in husbands, the chance to have an affected child and the options of prenatal diagnosis. (Fig. 2)

The risk couple for having an affected child was the couple who both were alpha-trait/both were beta-trait/or one was beta-trait while the other was Hb E trait. They may be risk for having Hb Bart's hydrops fetuses, homozygous beta-thalassemia or beta-thalassemia Hb E child depending on their types of carrier states.

After identifying and counseling the risk couples, prenatal diagnosis was offered. The couples who at risk for having Hb Bart's hydrops fetalis may choose prenatal diagnosis (PND) by either cordocentesis at 18-22 weeks or serial ultrasonography (every 2-3 weeks from 20-32 weeks). The couple who at risk for having homozygous beta-thalassemia or beta-thalassemia Hb E child were offered cordocentesis. The aim of serial sonography was an early detection of hydrops fetus which usually occurred in late 2nd or early 3rd trimester. Ultrasound findings included placental thickening (> 5 cm), fetal ascites, edematous skin, cardiomegaly and hepatomegaly. Cordocentesis was performed under ultrasound-guided, 2 ml of fetal blood was collected and analysed by HPLC technique (High Performance Liquid Chromatography) or Hb electrophoresis. The patients were followed up by routine antenatal care if the results of PND were negative for disease and pregnancy was terminaled if the fetuses were affected.

Pregnant women who have negative EOFT, or positive EOFT with negative EOFT husbands (no risk) and the couples who were assumed different kinds of carrier state (one was alpha-trait and the other was beta-trait or Hb E trait) were followed up by routine antenatal care.

Results

During the study period, there were 5,038 pregnant women attended antenatal care clinic, 3,310 women accepted for the screening and 1,000 gave abnormal EOFT (30.2%). Using HbA_2 level to group the patients, 494 were assumed alpha-thal 1 trait, 273 were beta-thal trait and 233 were Hb E trait or beta-thalassemia Hb E disease.

Five hundred and ninety-five couples came for intensive counseling and 593 husbands accepted the screening and gave positive EOFT 216 cases. After matching the result of pregnant women and her husbands, there were 115 risk couples for having thalassemia child ; 66 were at risk for Hb Bart's hydrops fetalis, 20 were at risk for homozygous beta-thalassemia and 29 were at risk for beta-thalassemia Hb E disease. Prenatal diagnosis was done in 87 couples ; 54 cases by cordocentesis and 33 cases (who were at risk for Hb Bart's hydrops fetalis) by serial ultrasonography. The indications for cordocentesis included risk for Hb Bart's hydrops fetalis 23 cases, risk for homozygous beta-thalassemia 13 cases and risk for beta-thalassemia Hb E 18 cases.

From this prenatal diagnosis programme 19 affected fetuses were detected. Analysis of fetal blood from cordocentesis showed 15 affected cases ; 5 cases were homozygous beta-thalassemia, 2 cases were beta-thalassemia Hb E disease and 8 cases were Hb Bart's. Serial ultrasonography could detect 4 hydrops fetuses

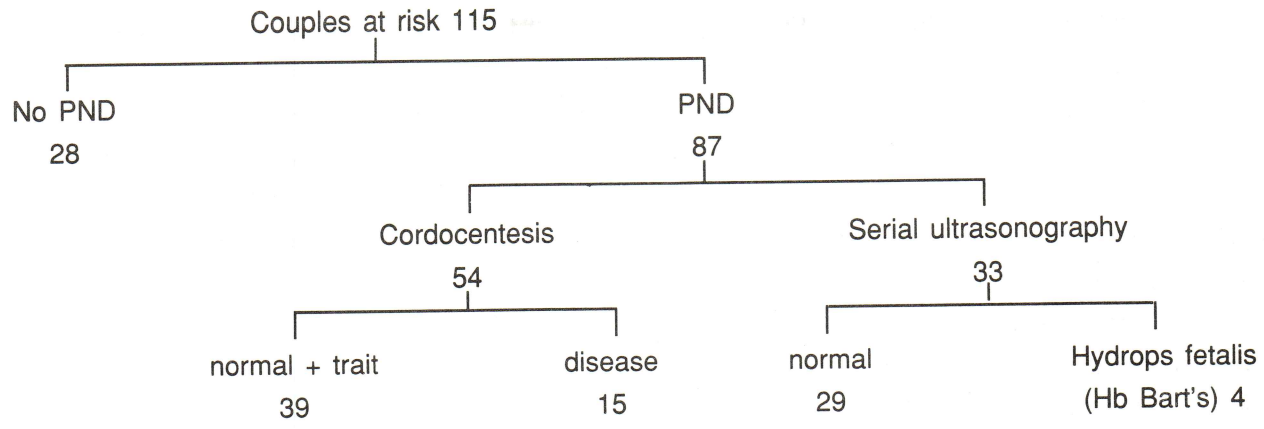

Fig. 3. Results of the programme.

who all were Hb Bart's (confirmed by fetal blood analysis). (Fig. 3)

Discussion

Thalassemia is a common haematological disease in Thailand. Prenatal diagnosis programme for severe thalassemia syndrome was conducted to prevent and control the disease. The programme included screening for thalassemia carriers in pregnant women to identify risk couples, prenatal diagnosis for risk couples and termination of pregnancy for affected fetuses.

EOFT was used to screen for thalassemia carriers because it is a simple, rapid, inexpensive and not time consuming procedure and suitable for mass screening. One technician can do up to 150 tests per day. Abnormal EOFT was found in 1) thalassemia and thalassemia carriers (both alpha-trait and beta-trait) 2) iron deficiency anaemia 3) sickle cell anaemia 4) chronic renal failure and 5) lead poisoning.[2-4] In this screening programme EOFT was tested in pregnant women who were healthy. We can exclude chronic renal failure and lead poisoning by history taking, physical examination and laboratory tests. It was assumed that no sickle cell anaemia in this study because of very low incidence in Thai population. Thus, when EOFT test was abnormal we have to differentiate thalassemia carriers, thalassemia and iron deficiency anaemia. Thalassemia can be diagnosed by clinical pictures, peripheral blood smears and Hb typing. Iron deficiency anaemia can be diagnosed by low haemoglobin concentration and abnormal laboratory tests (MCV, serum iron, TIBC). But in thalassemia carriers, they are not anaemia, no thalassemic facies and peripheral blood smears are usually normal. So, in this study we assumed that the study group were thalassemia carriers if they have abnormal EOFT and normal haemoglobin level. However we included both thalassemia and thalassemia carriers into the study group because both can transfer abnormal genes to the fetus.

"Less than 60% haemolysis at 90 seconds" was used as cut off point of abnormal erythrocytes in this study because a screening test should have low false negative rate. If we use lower cut off point, some thalassemia carriers will have normal EOFT and cannot be detected. However, for the cut off point used in this study some patients who were normal may had abnor-

mal EOFT (false positive test).

Flatz and Flatz studied one-step osmotic fragility test in 250 healthy Thai people and concluded that in the Thai group examined iron deficiency is rare and most increased osmotic indices (time to 50% haemolysis more than 90 seconds) not caused by beta-thalassemia were due to alpha-thalassemia 1.[1] We can detect beta-thalassemia trait and Hb E-trait by elevated HbA_2 level (> 4%) but in alpha-thalassemia 1 trait HbA_2 level are normal. When the patients had normal HbA_2 level, they might be alpha-thalassemia 1 trait or not. Due to the high prevalence of alpha-thalassemia 1 trait in Northern Thai people and Flatz and Flatz report, we assumed that "abnormal EOFT and normal HbA_2 level" patients were alpha-thalassemia 1 trait. This conclusion may not be used in other population because of the different prevalence. However, detection of alpha-thalassemia 1 gene in this group (by PCR technique) to determine false positive test of EOFT in detection alpha-thalassemia 1 trait is further studied.

Using only HbA_2 level has a pitfall. When HbA_2 level elevated the patients were assumed to be beta-thal trait despite they might be both alpha and beta trait. We found some couples that one was assumed to be beta-thal trait and the other was assumed to be alpha-thal 1 trait, so prenatal diagnosis was not offered and later the fetuses developed hydrops. From these cases we advise that PCR technique should be used to detect alpha-thal 1 gene in all abnormal EOFT results.

In our study, we screened 3,310 pregnant women and found 115 couples at risk for having an affected child. After counselling, prenatal diagnosis was done in 87 couples and 19 affected fetuses were diagnosed and terminated. From this study we can prevent 19 affected cases which would cost a lot of expenditure for treatment if pregnancy continue.

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