Prenatal diagnosis for thalassaemia in Egypt: what changed parents’ attitude?

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ABSTRACT

Objectives To present the current status of the prenatal diagnosis services and results from the largest thalassaemia center in Egypt treating 3000 patients. Traditionally, prenatal diagnosis has not been successful in reducing the births of affected children in Egypt, because the majority of women undergoing prenatal diagnosis continued to have affected pregnancies.

Methods Seventy-one pregnant mothers at risk for β-thalassaemia underwent prenatal diagnosis by chorionic villus sampling (n = 57) or amniocentesis (n = 14) between 11 to 14 weeks of gestation. Molecular characterization of fetal DNA by reverse dot blot hybridization and polymerase chain reaction-amplification refractory mutation system techniques was conducted in all cases.

Results Twenty-four women (33.8%) were found to have affected fetuses; 100% of these women opted to terminate the pregnancy. The change in attitude towards termination of pregnancy was related to in-depth counseling of the religious aspects towards prenatal diagnosis and termination of pregnancy. Forty-eight women (66.2%) with normal or carrier fetuses for β-thal requested human leukocyte antigen typing of the fetal material to determine if the fetus was a human leukocyte antigen match for their existing thalassaemic siblings.

Conclusion This study demonstrates that prenatal diagnosis is feasible and acceptable in Egypt, a Muslim country, provided an in-depth discussion, which also addresses the religious considerations of prevention, is held with the couples. © 2012 John Wiley & Sons, Ltd.

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INTRODUCTION

β-Thalassaemia represents a major public health problem in Egypt. The carrier rate varies between 5.5% to ≥9%; it is estimated that there are 1000/1.5 million per year live births born with β-thalassaemia.1

In spite of optimal treatment being available, only a few patients can afford it. Unfortunately, most patients suffer from complications of blood transfusions, mainly transfusion-transmitted viral infections and iron overload. Prevention by carrier detection and prenatal diagnosis is needed in populations with high incidence of the disease, such as Egypt.2 Several prevention programs based on carrier detection and early prenatal diagnosis have been applied in at risk populations in the Mediterranean areas.3-8

β-Thalassaemia is very heterogeneous at the molecular level, and more than 200 mutations have been reported so far.9,10 Seven mutations account for more than 70% of β-thalassaemia alleles in Egypt.2,11 The application of the molecular biology techniques, polymerase chain reaction-amplification refractory mutation system (PCR-ARMS) for the common gene mutations allows for rapid screening for β-thalassaemia alleles in Egyptian patients; DNA sequencing is used for uncharacterized alleles.12

Educational programs for the patients and their parents have been conducted on a limited scale.13 Although prenatal diagnosis of β-thalassaemia has been previously conducted in Egypt in at-risk pregnancies, the majority of the couples continued the affected pregnancy despite knowledge of the hazards of an affected child in Egypt.14,15

In this study, we present the current status of prenatal diagnosis services and results for β-thalassaemia in the Children Hospital of Cairo University.
PATIENTS AND METHODS
During the years 2007 to 2010, 71 pregnancies at risk for β-thalassaemia because of previously affected children were referred to the molecular diagnosis unit for genotype characterization. Mothers from the 11th week of gestation were referred for prenatal diagnosis.

FETAL TISSUE SAMPLING
Couples were counseled about the fetal sampling procedures and the small risk of miscarriage following the procedure.16

Following parental consent, fetal sampling was carried out either by chorionic villos sampling (CVS) at 12 weeks or by amniocentesis in women who presented in the second trimester.

MOLECULAR STUDIES
DNA was first extracted from parental and siblings peripheral blood samples using the QiAmp DNA blood minikit. Mutation identification of samples was performed by the reverse dot blot hybridization technique (RDB). For RDB a panel of primers and probes (n = 22) was used (β-Globin Strip/Assay MED kit, Vienna Lab).17,18 Results were confirmed by the ARMS technique.19

DNA was extracted from cleaned chorionic villos or amniotic fluid cells, followed by PCR of the β-globin gene.14 When fetal and maternal mutations were identical, a combination of two variable number tandem repeats (YNZ22 and APOB) was used to check for any maternal contamination.20 When prenatal diagnosis indicated normal fetal status or carrier for thalassaemia, human leukocyte antigen (HLA) typing was performed at the parents request to identify if the fetal HLA matched the affected child, and if so, arrangements were made for cord blood collection at delivery for potential cord blood transplantation.

RESULTS
Prenatal diagnosis was successfully conducted in the 71 pregnancies; only in one case the procedure needed to be repeated because of insufficient DNA in the fetal sample.

Table 1 shows the β-thalassaemia mutations carried by the parents. There were eight β-thalassaemia mutations that required testing, the most frequent was the severe IVS110[G > A] (51.4%) followed by the mild IVS 1-6[T > C] (21.8%). Table 2 shows that there were 16 parental risk combinations. The most common was homozygous IVS 1-110[G > A] (29.6%), followed by IVS110[G > A] /IVS1-6[T > C] (23.9%).

We found that 59.2% of the fetuses were carriers, 33.8% were affected, while only 7% were normal. Of the affected cases, 22.5% were double heterozygous and 11.3% homozygous. Figure 1 shows the RBD results for the six fetal diagnoses confirmed by ARMS technique in Figure 2.

Parents’ views towards termination of pregnancy
All at risk couples with affected fetuses were counseled and offered termination of pregnancy. An in-depth discussion was held with the couple, which also addressed the religious aspects of termination of pregnancy. The religios fatwa, which permits termination of pregnancy up to 120 days of fetal life when the fetus is found to have a severe condition, was discussed with the couple (Figure 3). Following this, all mothers with affected fetuses (33.8%), opted to terminate their pregnancies.

All couples with normal or carrier fetuses (66.2%) asked for fetal HLA typing. In 30% of cases, fetal HLA matched that of the existing thalassaemia major affected sibling. Three children have been successfully transplanted.

Follow up results on babies was concordant with the prenatal diagnosis result. There were no fetal structural abnormalities detected and no fetal or maternal complications observed during the follow up.

DISCUSSION
Thalassaemia is a public health problem in many third world countries and because of the lack of definitive cure for thalassaemia major and poor life expectancy, several countries are applying programs for disease prevention. This involves screening of the populations for carrier detection, identifying couples at risk, and providing counseling and prenatal diagnosis. In our centre, the DNA genotype was
identified in 95.8% of cases by RDB and PCR-ARMS analysis, the uncharacterized alleles (4.2%) were detected by DNA sequencing. Molecular heterogeneity has been reported among Egyptian β-thalassaemia patients and 22 different beta mutations have been reported so far in Egypt. Similar results were found in our study;

Figure 1 Results of mutation analysis by RBD. Strips 1 and 6 represent normal CVS. Strip 2 represents a carrier (Heterozygous) CVS showing mutation IVS 1-110 as and the wild type band. Strips 3, 4, and 5 represent homozygous CVS showing mutation IVS1-110 with the absence of the corresponding wild type bands.

Figure 2 PCR products on 2% agarose gel electrophoresis. Each two successive lanes represent the results of one patient. Lanes 1, 3, 5, 7, 9, and 11 contain the amplified product (390 pb) of the normal allele of IVS I-110 (N) for six patients. Lanes 2, 4, 6, 8, 10, and 12 contain the amplified product (390 pb) of the mutant allele of IVS I-110 (M) for six patients. Lanes 1 to 12 contain also the internal control amplified fragment (861 pb). Lane 13 negative control (–ve). Lane 14 X 174 molecular weight DNA marker digested by Hae III (Promega-USA). Lanes 1 and 2: normal CVS. Lanes 3 and 4: carrier CVS. Lanes 5 and 6: CVS (homozygous for IVS I-110). Lanes 7 and 8: CVS (homozygous for IVS I-110). Lanes 9 and 10: CVS (homozygous for IVS I-110). Lanes 11 and 12: Normal CVS.
Interestingly, in 39% of parental genotypes, at least one parent carried the mild IVS 1-6[T > C] thalassaemia mutation. All families requesting prenatal diagnosis had at least one affected child, who was transfusion dependent and attending the Cairo University thalassaemia centre. However, these families were counseled about the possible milder nature of an affected child when IVS 1-6[T > C] was present, particularly in the homozygous state; however, they all chose to undergo prenatal diagnosis and terminated affected pregnancies. The follow-up of the children born after prenatal diagnosis shows that the methods we used are accurate and reliable for application in our setting. We were particularly concerned because the fetal diagnosis results showed an under-representation of normal results, with an over-representation of affected and carrier results. This is probably because of the relatively low numbers tested; it is likely that we will see these numbers change when we next analyze our data. No fetal or maternal complications were observed among our cases; however, the numbers are too small to evaluate the risk of miscarriage in our setting.

Parents’ attitude
We found that there has been a change in parental attitude towards prenatal diagnosis and termination of pregnancy. In a previous report from Egypt, only 36% of pregnant mothers with affected fetuses elected to terminate their pregnancy despite being counseled about the hazards of having an affected child and the often poor prognosis in Egypt. These couples were well aware of the complications of thalassaemia major as they already had at least one affected live child.14

It is often difficult for people to distinguish between their religious and traditional or cultural beliefs, and this can lead to misconceptions about the permissibility or prohibition of interventions. In the context of prenatal diagnosis, a fundamental question is therefore whether Islam permits termination of pregnancy if the fetus is affected by a serious genetic disorder. The views of Islamic jurisprudence on this topic are unanimous, in that abortion is prohibited after ensoulment occurs in the fetus, which is considered to be after 120 days. From an Islamic perspective it is considered ethical to perform an abortion because of fetal abnormality incompatible with life.25

During genetic counseling for the couples found to have an affected fetus, an in-depth discussion was carried out about the acceptability of termination of pregnancy, including the High Islamic Council Fatwa, which states that prenatal diagnosis followed by abortion is permissible if performed by 120 days of pregnancy.26 The couples were left to make their own decision on whether to continue the pregnancy or not. Genetic counseling was nondirective and followed the ethical principles of genetic counseling.27 In our experience, couples who were hesitant in using prenatal diagnosis and termination of pregnancy were relieved to learn that Islam permits termination of pregnancy under special circumstances.

In other studies, religion has often been found to be an important factor in decision making. In a study of Saudi families who had children affected with a hemoglobinopathy, education about religious ruling significantly affected parents attitude towards requesting prenatal diagnosis and termination of pregnancy; no other factors were found to influence the outcome.28 In another study involving individuals from four faith communities in the UK, including British-Pakistani Muslims, religion and faith were generally considered to be an important factor in the decision-making process of prenatal diagnosis and selective termination of pregnancy; however, the perceived severity of the condition was found to have a more important role. This study included African-Caribbean individuals, amongst whom there is a high prevalence of sickle cell disorders and a wide clinical spectrum of severity.29 In our study the possibility of a mild condition did not hinder couples from requesting termination of pregnancy with an affected fetus. An additional study on the attitude towards prenatal diagnosis and termination of pregnancy for thalassaemia in pregnant Pakistani women in the North of England found that religion was an important factor in the decision making about termination of pregnancy, and information on Islam’s stance on termination of pregnancy enabled the parents to consider termination of pregnancy more favorably.30

The Iranian National Thalassaemia Prevention program similarly showed that the population wanted prenatal diagnosis. This led to intensive widespread ethical discussion, and a religious fatwa was issued permitting termination of pregnancy, resulting in a government decision to permit...
termination of pregnancy before 16 weeks from the last menstrual period if the fetus is affected.31

All the couples in our study were identified retrospectively, when they already had at least one affected child. In communities where family size is small, retrospective identification of the couples and offer of prenatal diagnosis is unlikely to reduce the prevalence of thalassaemia major. However, when family size is large, retrospective counseling may lead to a stop in reproduction or to prenatal diagnosis, and this can reduce the affected birth rate by up to 50%.32 It has been observed in other populations that informed couples at risk for thalassaemia often limit their family to two healthy children.31,33 However, this effect is waning by falling family size.

Retrospective prevention as practiced in Egypt will not make a significant impact on reducing the prevalence of affected individuals. For prenatal diagnosis and selective termination of affected fetuses to have a large impact on reducing the affected births, prospective identification of carriers is necessary by population screening, which is effectively being conducted in some Mediterranean countries34 and Iran.31

At present a national program using prenatal diagnosis is available in very few Muslim countries. Our experience with our couples, although small, may be used as an example for developing control programs in other such countries.

Hematopoietic stem cell transplantation is a curative therapy for thalassaemia major because umbilical cord blood is an alternative source of hematopoietic cells35 and related donor cord blood transplantation is a safe and effective option for patients with hemoglobinopathies.36 All eligible couples asked for HLA typing. All couples said their aim was to have a healthy child, but if their fetus was HLA compatible with their existing affected child they would request cord blood collection and storage. Three of our thalassaemia patients successfully underwent cord blood transplantation from the related compatible siblings.

REFERENCES

CONCLUSION
Accurate prenatal molecular diagnosis of thalassaemia, together with a comprehensive in-depth genetic counseling session with the couple addressing the religious Islamic aspects of prevention, changed the families’ view towards the concept of prenatal diagnosis and selective termination of pregnancy. The option of using cord blood transplantation for affected siblings was an additional incentive for at-risk couples to seek prenatal diagnosis. Parents at risk for thalassaemia intermediary genotypes still requested prenatal diagnosis and terminated affected fetuses. The results and discussion in this study may help in the establishment of a prevention program for thalassaemia in Egypt. To reduce the prevalence of thalassaemia major in Egypt, a national prevention program inclusive of prospective carrier screening is essential.

WHAT’S ALREADY KNOWN ABOUT THIS TOPIC?
• Prenatal diagnosis for thalassaemia is not common in Egypt. Prospective carrier identification is not common in Egypt. In previous studies in Egyptian mothers who underwent prenatal diagnosis in Egypt, the majority continued an affected pregnancy.

WHAT DOES THIS STUDY ADD?
• Genetic counselling of couples addressing the Islamic views towards prenatal diagnosis and termination of affected pregnancies, including discussion on the religious fatwa, significantly affected the parents’ attitude towards accepting termination of pregnancy. Comprehensive counselling provides a chance for couples to overcome what they regard as restrictions imposed by religion.