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

By

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Disclaimer

- This presentation reflects only my personal opinion not that of my faculty in the purpose of independent scientific discussion.

- No conflict of interest
-β-thalassemia is encountered in polymorphic frequencies in almost all Arab countries with carrier rates ranging from 1 to 11% and a varying number of mutations.

-The most widespread mutation in Egypt, Lebanon, Syria, Jordan, Tunisia and Algeria is the **IVS-I-110 (G>A)**.

-In the Eastern Arabian Peninsula, the Asian Indian mutations (IVS-I-5 (G>C), codons 8/9 (+G) and IVS-I (−25 bp del) are more common.

Hamamy et al, J Community Genet (2013)
-The populations of many Arab countries are characterized by marriage at a young age, large family sizes and advanced maternal and paternal ages.

-Consanguineous marriage is customary in most of Arab communities and intra-familial unions currently account for 20–50 % of all marriages.

- (Teebi and Teebi, Community Genet 2005)
Challenges in providing Comprehensive and up-to-date care and prevention services

- paucity of resources,
- presence of other competing priorities of communicable and non-communicable disorders
- insufficient number of trained health professionals in this field
- inadequate data on the real magnitude, health and economic burden of hemoglobinopathies.
Prevention programs in the Arab Communities

Community genetic services mainly include population screening programs such as NBS for sickle cell anemia, and premarital screening for carriers of β-thalassemia.
Community programs for premarital screening to detect hemoglobinopathies carriers have been initiated in a number of Arab countries including Bahrain, Jordan, Palestinian territories, Tunisia, Iraq, Oman, Saudi Arabia, and UAE.

These programs maintain the autonomy of decision for the couple after delivery of results, including the decision to proceed with the marriage plan, reproduction decisions, and decisions to go through PND.
WHAT ABOUT EGYPT?

Egypt has a population of **104,269,501** citizens

NO

National Prevention Program for Thalassemia
Magnitude of the problem in Egypt

- β-thalassemia is the most frequent hemoglobinopathy in Egypt.

- An estimated 1,000 infants born each year with β-thalassemia major among the national 1.5 million live births.

- The estimated average financial cost for β-thalassemia management in Egypt is $10 million/year and is on the increase.

(Elbeshlawy et al, 2009)
Registered cases of Homozygous Beta Thalassemia in big centers of Egypt in 2013 (n=9258)
Follow Up Cases in Hematology Clinic of pediatric hospital of CU (n=10968)

- Thalassemia: 47%
- SCD: 11%
- ITP: 9%
- Hemophilia: 6%
- Aplastic: 6%
- Gaucher: 21%

Thalassemia: 47%
SCD: 11%
ITP: 9%
Hemophilia: 6%
Aplastic: 6%
Gaucher: 21%
Birth Rates of Thalassemia

Fall in the thalassaemia major birth rate in four countries

![Graph showing fall in thalassaemia major birth rate over years in four countries: UK, Italy, Greece, and Cyprus. The graph displays the percentage of expected births over a period from 1972 to 1994.](image)
Birth Rates of Thalassemia in Egypt
Carrier Rates in Different Egyptian Studies

- 1.2 (1,000) (Nafei 1992)
- 9.25 % (410) (Rizk et al. 2005)
- 9 % (1,000) (El-Beshlawy et al. 2007)
- 5–9 % (El-Beshlawy and Youssry 2009)
- 9–10 % (Teebi 2010)
Original Article

Spectrum of Beta Globin Gene Mutations in Egyptian Children with β-Thalassemia

MR El-Shanshory ¹, AA Hagag ¹, SS Shebl ¹, IM Badria ¹, AH Abd Elhameed ², ES Abd El-Bar ², Y Al-Tonbary ³, A Mansour ³, H Hassab ⁴, M Hamdy ⁵, M Alfy ⁶, L Sherief ⁷ and E Sharaf ⁸
The most common mutation in 200 Egyptian children with beta thalassemia in 16 governorates

<table>
<thead>
<tr>
<th>IVS Region</th>
<th>Mutation</th>
<th>Frequency</th>
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<tbody>
<tr>
<td>IVS I-110</td>
<td>(G&gt;A)</td>
<td>48%</td>
</tr>
<tr>
<td>IVS I-1</td>
<td>(G&gt;A)</td>
<td>24%</td>
</tr>
<tr>
<td>IVS II-848</td>
<td>(C&gt;A)</td>
<td>9%</td>
</tr>
<tr>
<td>IVS II-1</td>
<td>(G&gt;A)</td>
<td>7%</td>
</tr>
<tr>
<td>IVS I-6</td>
<td>(T&gt;C)</td>
<td>40%</td>
</tr>
<tr>
<td>IVS I-5</td>
<td>(G&gt;C)</td>
<td>10%</td>
</tr>
<tr>
<td>IVS II-745</td>
<td>(C&gt;G)</td>
<td>8%</td>
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</table>
B-thal alleles in Egypt (n= 349)

El Beshlawy & Gahfar 2010
Objectives for the need of Prevention of Thalassemia In Egypt

♦ High frequency of carriers of β thalassemia.
♦ High rate of births of severe thalassemia patients with consanguineous and non-consanguineous marriages.
♦ β-thalassemia major creates a need for life long regular blood transfusion and iron chelation.
♦ With the lack of curative treatment by BMT except for small percentage of the cases prevention of birth affected children is an option for couples at risk
Prevention of Thalassemia In Egypt

• Carrier Screening Limited Studies.

• Prenatal Diagnosis conducted on a limited and voluntary basis.

• Genetic Counseling Centers are very few.

• Population awareness is improving.
In 2015 Data of patients with thalassemia were collected from:

1-Hematology & Oncology Units of Delta region including (Tanta, Al-Minoufiya, Al-Zagazig, Al-Mansoura & Alexandria)
Registered patients with thalassemia in Delta Hematology Units in 2015 = (2532)

<table>
<thead>
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<th>Thalassemic centers</th>
<th>Total (n=2532)</th>
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<tbody>
<tr>
<td></td>
<td>N</td>
</tr>
<tr>
<td>El Zagazig</td>
<td>644</td>
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<tr>
<td>Al Alexandria</td>
<td>620</td>
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<tr>
<td>El Mansoura</td>
<td>557</td>
</tr>
<tr>
<td>Tanta</td>
<td>451</td>
</tr>
<tr>
<td>El Menoufyaia</td>
<td>260</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>2532</strong></td>
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Expected number of thalassemic patients in 2020 in Delta region.

### Populations in Delta region

<table>
<thead>
<tr>
<th>Year</th>
<th>No.</th>
<th>%</th>
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<tbody>
<tr>
<td>2010</td>
<td>1506</td>
<td>0.005</td>
</tr>
<tr>
<td>2015</td>
<td>2532</td>
<td>0.008</td>
</tr>
<tr>
<td>2020</td>
<td>3500</td>
<td>0.010</td>
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<td>3500</td>
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\( \chi^2 \) 358.785*, \( p < 0.001 \)
Scientific Goals

1. Establishment of Delta Thalassemia Research Center for Control of the increasing number of patients with thalassemia

2. Establishment of thalassemia network between Egyptian Thalassemia centers
Scientific Goals

3. Carrier screening will include:

a) screening immediate family members of thalassemia patients
b) Secondary school students and young adult;
c) Premarital screening.

4. Organize training programs
Carrier rates among secondary school students

• Carrier rate among 500 secondary school students in urban areas is 6.2%

• Carrier rate among 500 secondary school students in rural areas is 7.6%
To see clearly you might only have to change the perspective

Antoine de Saint-Exupéry
What about Prenatal Diagnosis of Thalassemia?
Prenatal diagnosis of β-thalassemia was accomplished for the first time in the 1970s. PND is recognized as an important option for the prevention of serious genetic diseases for couples with an increased genetic risk. There are several ethical, legal, social and religious implications regarding pregnancy termination of an affected foetus. Some of these implications are unique to Arab countries.
Prenatal diagnosis of beta-thalassemia in Egypt: implementing accurate high-tech methods did not reflect much on the outcome.

Elgawhary S, Elbaradie Sahar MY, Rashad WM, Mosaad M, Abdalla MA, Ezzat G, Wali YA, Elbeshlawy A.

Abstract
The clinical severity of thalassemia major makes it a priority genetic disease for prevention programs through prenatal diagnosis for carrier couples. Incorporation of automated DNA sequencing that enables the characterization of mutations not detected by other mutation specific detection procedures was a prime goal of this work. Automated DNA sequencing was offered on fetal tissues in 30 pregnancies during the year 2005. The pregnancies were at high risk for homozygosity or compound heterozygosity for beta-thalassemia based on mutation analysis of both parents before prenatal diagnosis. Both parents have beta-thalassemia trait. Fetal samples were collected by chorionic villus sampling (CVS) in the first trimester and by amniocentesis in the second trimester. The point mutations were characterized by PCR (ARMS). The absence of the expected fragment with all the mutant ARMS primers insinuated an uncharacterized DNA segment that was further subjected to direct automated fluorescent DNA sequencing in an attempt to know if the fetus was affected by parents' mutations. If no mutation was detected using the PCR ARMS, the sample was further analyzed using direct automated fluorescent DNA sequencing. The mean gestation when carrying out the invasive procedure was 14 (10-18) weeks. All mothers had a previous affected pregnancy, and 13 had two or more previous affected pregnancies. Pregnancies were: 8 carrier fetuses (trait) and 22 affected fetuses in which 2 were homozygous and 20 double heterozygous. Fourteen parents of affected fetuses preferred to continue pregnancy and the babies were born as diagnosed. The other 8 parents decided on termination and DNA of the abortuses proved to be as previously diagnosed by DNA sequencing. The use of PCR amplification and direct sequencing have permitted the accurate characterization of unidentified alleles and successfully solved 100% of the examined samples. However, it has resulted in minor changes of the outcome as the majority of couples preferred continuation of pregnancy.

PMID: 18728973 DOI: 10.1080/08880010802313509 [Indexed for MEDLINE]
PND was offered to 30 pregnancies. The mean gestation when carrying out the procedure was 14 (10-18) weeks. All mothers had a previous affected pregnancy and 13 had two or more previous affected pregnancies.

**Results:** 8 carrier fetuses (trait) and 22 affected fetuses in which 2 were homozygous and 20 double heterozygous.

(Elgawhary et al, 2008)
Fourteen parents of affected fetuses preferred to continue pregnancy and the babies were born as diagnosed.

The other 8 parents decided on termination (selective termination of pregnancy rate was 36.3 %) and DNA of the abortuses proved to be as previously diagnosed by DNA sequencing.

The study was carried out in 2005.
Prenatal diagnosis for thalassaemia in Egypt: what changed parents' attitude?

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¹Pediatric Hematology Department, Cairo University, Cairo, Egypt
²Fetal Medicine Department, Cairo University, Cairo, Egypt
³Clinical Pathology Department, Cairo University, Cairo, Egypt
⁴Institute of Women’s Health and Centre for Health Informatics and Multiprofessional Education (CHIME) and University College London Hospitals NHS Foundation Trust Pathology Division, University College London, London, UK
PATIENTS AND METHODS

-Seventy-one pregnant mothers at risk for beta-thalassemia 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 PCR-amplification refractory mutation system techniques was conducted in all cases.
Results

59.2 % were carriers

33.8 % were affected:

11.3% homozygous & 22.5% double heterozygous

7 % were normal fetuses
Percentage of β globin gene mutation in the samples obtained

- IVS 1.10 homozygous: 5.5%
- IVS 1.10 heterozygous: 5.5%
- IVS 1.6 homozygous: 2.7%
- IVS 1.6 heterozygous: 2.7%
- IVS 2.745 homozygous: 2.7%
- IVS 2.745 heterozygous: 2.7%
- Codon 27 homozygous: 2.7%
- Codon 27 heterozygous: 2.7%
- Codon 39 homozygous: 5.4%
- Codon 39 heterozygous: 5.4%
- Negative: 7%

39%
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 thalassemia major affected sibling.

Three children have been successfully transplanted.
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 religious fatwa, which permits termination of pregnancy up to 120 days of fetal life was discussed with the couple.

- Following this, **all mothers with affected fetuses (33.8%),** opted to terminate their pregnancies.
The Muslim world league- high Islamic council- fatwa 12th session dated 15-22 Ragab 1410 H (1990)

It is proved before 120 days of pregnancy, counted from the conception, and confirmed by a report from a committee formed of competent trustworthy physicians and on the basis of medical and laboratory findings the fetus is growthly malformed with an untreatable severe condition and if he or she stays and is born on time, his or her life will be vicious and painful for him or her and for his or her family, then it is allowed to abort it on the basis of the parents requisition.
The *fatwa* declares that an abortion may take place if a committee of physicians has determined that the fetus is severely malformed and its birth would have a seriously negative effect on itself and its family.

Furthermore, the abortion for this untreatable and unmanageable malformation must be carried out before the one hundred and twentieth day after conception.
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.

From an Islamic perspective, it is considered ethical to perform an abortion because of fetal abnormality incompatible with life.

(Petrou M., TIF 2003)
In kingdom of Saudi Arabia

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.

(Alkuraya FS et al, Prenatal diag 2001)
In the West Bank and Gaza
130 prenatal samples assessing β-thalassemia major in foetus from January 1999 to July 2005 were drawn. Samples showed 25 (19.2 %) cases of β-thalassemia major, The 25 affected fetuses were aborted according to the wishes of the parents indicating a very good acceptability for PND of β-thalassemia in afflicted families

(Ayesh et al. 2005)
Abstract
Religion is believed to have a significant impact on individuals from minority ethnic groups when making decisions about prenatal genetic screening, prenatal diagnosis and termination of pregnancy. This study aimed to explore the views of individuals from South-Asian and African-Caribbean communities towards termination of pregnancy for sickle cell disorders and thalassaemia major and the influence of (1) faith and religion, (2) perceived severity of the conditions, and (3) religious and community leaders.

METHODS: The study explored the views of (1) individuals from four faith communities (Pakistani Muslims, Indian Hindus, Indian Sikhs, African-Caribbean Christians), using eight focus groups, and (2) parents of children with sickle cell disorders and thalassaemia major, using two focus groups and three interviews.

RESULTS: Participants' accounts suggest that they generally considered religion and faith as an important factor in the decision-making process, but the perceived severity of the condition would play a more important role. Religious and community leaders were believed to have little role to play in the decision-making process. CONCLUSION: The findings emphasise the importance of recognizing diversity within different faith groups and moving away from stereotypical views based on people's ethnicity or religion, and to consider the beliefs and preferences of individuals.
Preimplantation Genetic Diagnosis For the Prevention of Thalassemia
The Egyptian IVF Center Experience
Obtaining a biopsy from the embryo for genetic testing before its transfer into the uterus
First: Confirm the mutation for the both parents and the affected child

Reverse dot blot Hybridization (RDBH) & Real time PCR assay and sequencing
Second: start an ICSI cycle
Third: Balstomere biopsy for genetic testing

- Single cell DNA Amplification.
  REPLI-g single cell kit, or PICOLEX kit
- Mutation Detection.
  “Reverse dot blot Hybridization” confirmed by “Real time PCR” and “Sequencing”.
Results of PGD for thalassaemia
The Egyptian IVF-ET Center

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<tbody>
<tr>
<td>Number of cycles</td>
<td>46</td>
</tr>
<tr>
<td>Embryo transfer cases</td>
<td>35</td>
</tr>
<tr>
<td>Oocytes retrieved</td>
<td>$11.4 \pm 3.2$</td>
</tr>
<tr>
<td>Embryos subjected to genetic testing</td>
<td>$7.8 \pm 2.3$</td>
</tr>
<tr>
<td>Embryo free of thalassaemia</td>
<td>$2.5 \pm 0.5$</td>
</tr>
<tr>
<td>Clinical pregnancies</td>
<td>15 (4 sets of twins)</td>
</tr>
<tr>
<td>Miscarriage</td>
<td>5</td>
</tr>
<tr>
<td>Ongoing pregnancy</td>
<td>3</td>
</tr>
<tr>
<td>Babies born</td>
<td>14</td>
</tr>
</tbody>
</table>

_HLA typing was done for 12 patients_
Case I

Wife (hetero-IVSII-745)  

Husband (hetero-IVSII-745)

Natural conception

Thalassemia major  
Affected child  
Homozygous IVSII-745

carrier child (hetero-IVSII-745)

Female baby (hetero-IVSII-745)

Male baby (hetero-IVSII-745)

Oocytes  
10

Tested blastomeres  8
Normal -
Heterozygous  3
Homozygous  2
uncertain  3

PGD
Case VII

Wife (hetero-IVSI-1)  Husband (hetero-IVSI-6)

Affected child
Double heterozygous
IVSI-1/IVSI-6

Natural conception

PGD

Oocytes 14
Tested blastomeres 12
Normal 3
Heterozygous 3
Double heterozygous 5
No result 1

3 Normal embryos tested for HLA compatibility
2 Normal embryos HLA compatible
-PGD is not an easy option for families, and counselling services are needed to advise, prepare and support them through all the difficult stages that the technology entails.

-The cost of this technology is a real barrier against its wide application in Egypt.

The most important perceived advantage of PGD was the avoidance of termination of an affected pregnancy

(Farra et al. 2008)
Take Home message
-There has been a change in parental attitude towards prenatal diagnosis and termination of pregnancy.

-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.
life must be understood backwards

but;

It must be lived forward

Soren Aabye Kierkgaard