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COUNTRY REPORT

Two decades of pre-marital screening for beta-thalassemia in central Iran

Mehrdad Zeinalian · Reza Fadaei Nobari · Alireza Moafi · Mansour Salehi · Morteza Hashemzadeh-Chaleshtori

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Introduction

Thalassemia is the most widespread recessive disease worldwide (Ryan et al. 2010; Weatherall and Clegg 2001). Beta-thalassemia is characterized by the deficiency or absence of beta globin production. The condition is clinically mild in the healthy carrier that can be easily detected through routine blood testing, but intermediate or severe in the patients who are born with 25 % chance at each pregnancy from parents who are both healthy carriers.

Thalassemia carriers are today present worldwide with high frequencies mainly in the endemic countries of Africa, the Middle East, the Indian subcontinent, Southeast Asia, and the Mediterranean region (Fucharoen and Winichagoon 2007; AlHamdan et al. 2007; Sarper et al. 2009). Thalassemia is a common disease in Iran with higher frequencies in the north and south parts of the country (Haddow 2005; Samavat and Modell 2004; Moafi et al. 2010).

Thalassemia major leads to serious medical, social, and economic problems for patients and their families, and patient's care represents a considerable financial burden for the public health budget (Verma et al. 2011; Langlois et al. 2008). After a 5-year pilot screening, the Iranian Ministry of Health approved in 1996 a mandatory national screening protocol for premarital testing. This program included a laboratory strategy to identify and counsel couples at risk providing support and care (Samavat and Modell 2004; Iranian Health 2004). Although compared to other regions, the Isfahan province can be considered an area at moderate prevalence; premarital screening was locally started in 1992 and gradually extended to the entire province by 1997 (Zeinalian et al. 2009). This report presents the results of the premarital screening program applied in the Isfahan province during the last two decades (1992–2010).

Material and methods

With more than 4,500,000 people, the Isfahan Province is the second in Iran. It is situated in the central part of the country (Fig. 1). The province is divided into 22 districts with a state center for thalassemia screening and genetic counseling in each and every district. After pilot studies in Isfahan and other districts in 1997, all couples within the province who applied for marriage were screened for beta-thalassemia. Although the screening was mandatory, informed couples were generally willing to be tested and comprehensive guidelines were prepared and distributed to all involved in the program by the Public Health Ministry. According to these guidelines, screening tests included complete blood count first for the male partner and eventually for the female if the male had low blood indices. A standard premarital questionnaire included name, sex, age, address, and telephone number. The subjects could be considered potential carriers of beta-thalassemia if their mean corpuscular volume (MCV) was lower than 80 fL and/or the mean corpuscular hemoglobin (MCH) was less than 27 pg. If both partners of a couple present low blood indices, their hemoglobin A2 will be measured by manual column chromatography as from international recommendations (Stephens et

M. Zeinalian (✉) · M. Hashemzadeh-Chaleshtori
Cellular and Molecular Research Center, Medicine School, Shahrekord University of Medical Sciences, Shahrekord, Iran
e-mail: zeinalian_mehrdad@yahoo.com

M. Zeinalian · R. F. Nobari
Health Deputy, Isfahan University of Medical Sciences, Isfahan, Iran

A. Moafi · M. Salehi
Medicine School, Isfahan University of Medical Sciences, Isfahan, Iran

Fig. 1 Isfahan province location on Iran's map

al. 2012). The beta-thalassemia trait will be considered present if the hemoglobin A2 is more than 4 %. For cases between 3.5 and 4 % with microcytic parameters in both partners, they will be classified as a possible beta-or alpha thalassemia couple. When both partners have low blood indices and their hemoglobin A2 is less than 3.5 %, iron therapy is prescribed for 1–3 months. After this period, if their blood indices are normalized, their condition can be attributed to iron deficiency. If their blood indices are not improved after iron-therapy period and their hemoglobin A2 is above 3.5 %, they will be classified as suspected couple at risk (Iranian Health 2004).

Comprehensive genetic counseling is offered to all proved or suspected couples at risk by teams trained in genetic counseling centers. If the couples decide to marry, they are referred to urban or rural health centers within the support system to assist them in family planning and genetic testing as long as they are fertile. Prenatal genetic testing technology has been established in Iran since 1999 (Najmabadi et al. 2006).

Before 1999, genetic counseling for thalassemia was aimed at convincing couples at risk not to marry. After 1999, prenatal diagnosis and genetic counseling have become available in Iran leading to approximately 1,000 fewer thalassemia major births per year in the whole of the country (Iranian Health 2008).

In particular, a network of 97 rural health centers, 166 urban health centers, 22 screening laboratories, and 22 genetic counseling clinics are assigned to provide testing, counseling and care services for all potential couples in the Isfahan Province.

All data have been collected according to the local ethical regulation and anonymously analyzed using SPSS-19 and Microsoft Excel softwares.

Results

Out of the 703,082 couples screened in this period, 1,317 (0.19 %) were classified as at risk (i.e., both partners were beta thalassemia carriers with Hb A2>3.5 %) while 4,412 (0.63 %) remained suspected (at risk) (Table 1 and Fig. 2).

Suspected couples were followed up to 2009 providing prenatal genetic testing to about 94 % of them and confirming their status in 19 % of the couples as carrier couples of beta-thalassemia trait. Using this information, the rate of thalassemia trait in screened couples was calculated to be about 0.31 % ((0.63 %×0.19)+0.19 %).

During the first 8 years (before setting up genetic testing), the average rate of marriage renounce among presumed couples at risk was 45 % while it dropped to 13 % after

Table 1 Summary of premarital screening program for beta-thalassemia in central part of Iran

Year	Number of screened couples	Screened couples BOTH with thalassemia trait		Suspected couples		Number of marriage refusal	Percent of marriage refusal	Number of under care carrier couples	Number of couples with PND1
		Number	Percent	Number	Percent				
1992	15,252	21	0.14	67	0.44	53	60.2	24	0
1993	16,218	23	0.14	86	0.53	61	56	52	0
1994	17,256	25	0.14	104	0.60	59	45.7	94	0
1995	18,274	24	0.13	121	0.66	66	45.5	131	0
1996	19,625	28	0.14	134	0.68	85	52.5	159	0
1997	34,015	65	0.19	217	0.64	142	50.4	196	0
1998	36,967	72	0.19	231	0.62	139	45.9	201	0
1999	38,422	76	0.20	245	0.64	94	29.3	207	0
2000	39,627	77	0.19	238	0.60	58	18.4	215	0
2001	40,572	81	0.20	246	0.61	61	18.7	221	2
2002	41,156	85	0.21	259	0.63	55	16	234	4
2003	42,356	82	0.19	268	0.63	61	17.4	252	7
2004	43,659	85	0.19	272	0.62	56	15.7	286	15
2005	45,122	91	0.20	288	0.64	45	11.9	306	21
2006	46,792	95	0.20	285	0.61	29	7.6	337	45
2007	52,080	105	0.20	296	0.57	50	12.5	376	70
2008	51,158	91	0.18	314	0.61	45	11.1	442	314
2009	53,182	85	0.16	323	0.61	24	5.9	584	450
2010	51,349	106	0.21	418	0.81	49	9.4	661	560
total	70,3082	1,317	0.19	4,412	0.63	1,232	21.5		

setting up genetic testing. For confirmed couples at risk, marriage renouncing dropped from 60.2 % in the first year to 9.4 % in the last year of the study (Fig. 3).

In total, 661 couples at risk were followed by the health system until the end of 2010. By the end of our study, genetic testing was performed for 560 couples (85 %) against 6.9 % in the first year of testing (2005) (Fig. 4). Five hundred eleven couples (77 %) were tested within the premarital screening program, 124 (19 %) were parents of the current patients who were still fertile and seeking healthy offspring, and 26 (4 %) had been detected before starting the program (they had been screened passively by healthcare system). During the last 5 years of the program, the pregnancy of 92 fetuses affected with beta-thalassemia major was terminated by therapeutic abortion.

In total in the Isfahan Province, 109 beta-thalassemia major cases have been born from the start of the national premarital screening in 1997 and 2010. So, the birth prevalence has decreased from 41.3 cases per 100,000 live births in 1997 to 1.5 cases per 100,000 live births at 2010 (Fig. 5). The most prevalent reasons for the births of the affected children were: marriage before the start of the program (51 %), culture-related causes (15 %), wrong counseling (9 %), and mistakes in the healthcare organization (6 %) (Table 2).

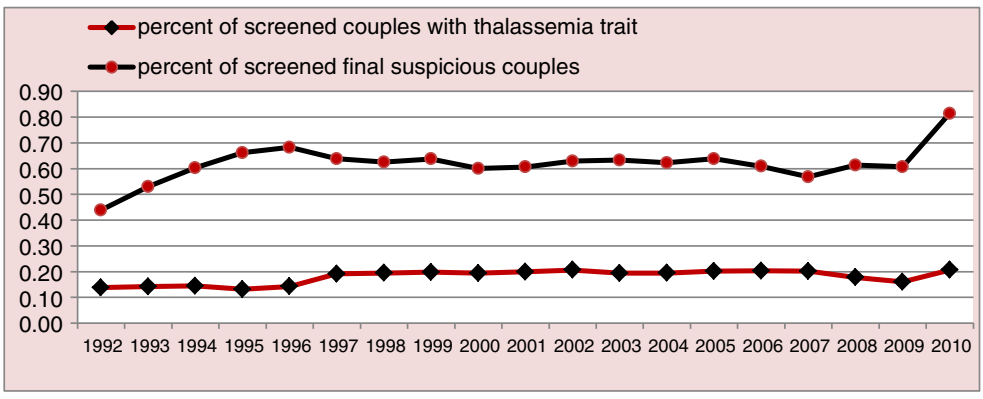

Fig. 2 Comparative frequency of screened couples with beta-thalassemia trait and suspected couples in Isfahan Province within 1992–2010

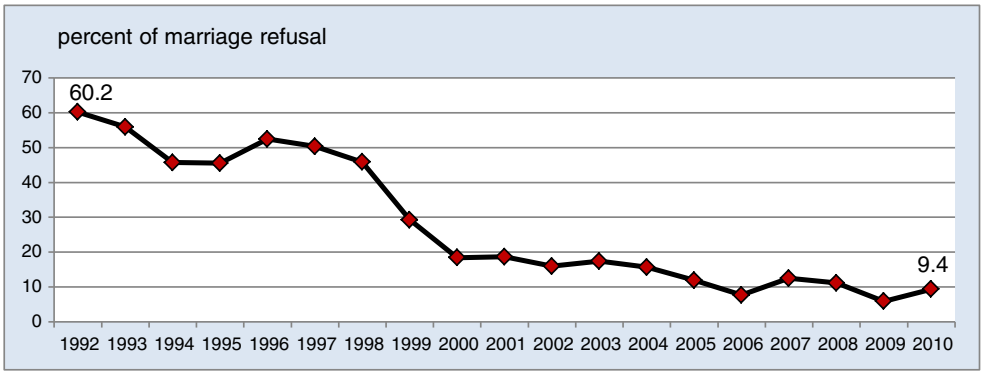

Fig. 3 Marriage refusal rate among screened couples with thalassemia trait and suspected couples in Isfahan Province within 1992–2010

Discussion

Screening in Iran

According to an Iranian law from 1997, screening and genetic counseling documents are needed for an official marriage registration. As a consequence, the Iranian premarital screening program for β-thalassemia started in 1997, preceded only by a few Middle East countries like Bahrain (1985) and Turkey (1995). In contrast with the most of endemic European countries where screening is since the 1970s in most cases an optional service (Alswaidi and O'Brien 2009), premarital screening programs as a mandatory rule has been applied from 2000 in the majority of the Middle East's countries.

In Isfahan, the compliance of obligatory premarital screening has been almost complete since only three cases of marriage without premarital screening have been reported in the period between 2001 and 2011. Due to such a strict rule, a large number of couples have renounced marriage during the first years of the program, before genetic testing became available in Iran. In a previous study over the period between 1997 and 2001, out of 2.7 million couples screened all over Iran 10,290 were suspected at risk (0.4 %) and in 29 % of the cases the couple renounced to marry (Samavat and Modell 2004).

In another study in southern Iran on about 1.04 million couples between 1995 and 2004, 0.4 % of them were identified as carriers and 37 % did not marry after screening (Karimi and Rasekhi 2002).

Moreover, due to uncertain situations regarding health insurance and high costs related to genetic testing, the option not to marry has gone on during the years also after the introduction of molecular analysis and prenatal diagnosis.

Many reports have shown that molecular analysis needs a thorough knowledge on the spectrum of mutations present in the area. Specific mutations are particularly prevalent in Sardinia (11–34 %) (Guiso et al. 1996), Sicily (10 %) (Lukens 1993), Greece (5–15 %) (Lukens 1993), and also in Iran (4–10 %) (Haghshenas and Zamani 1997).

Previous studies have estimated the prevalence of the β-thalassemia trait in Iran above 10 % around the Caspian Sea and the Persian Gulf, and about 4–8 % in other areas (Haghshenas and Zamani 1997).

Overall, in a report of the Iranian Ministry's of health from 2008, the average frequency of β-thalassemia trait for the whole of the country has been estimated to be 3.6 % (Iranian Health 2008). This would mean that without premarital screening the national birth prevalence would be at least 1,300 affected newborns per year (Iranian Health 2008). On the other hand, due to the high rate of consanguineous marriages in the Iranian population (30–80 %), predicting the

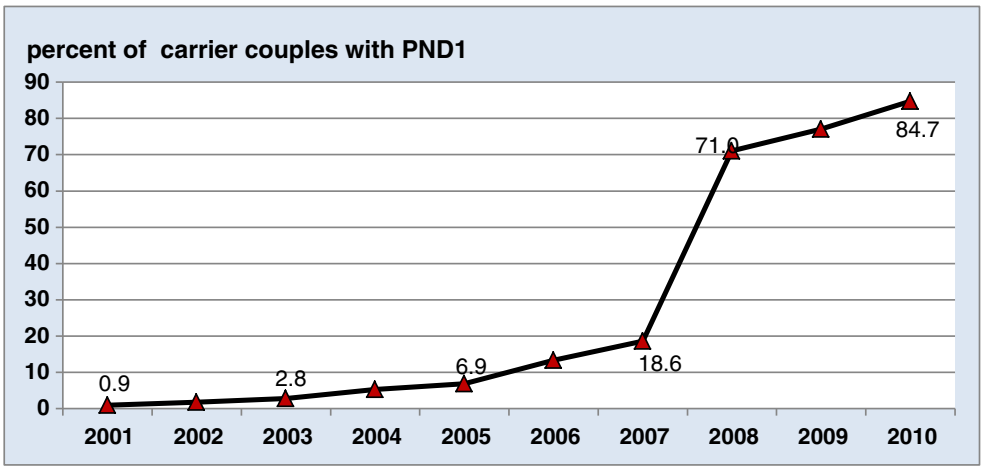

Fig. 4 Frequency of genetic testing (PND1) among under-care couples with thalassemia trait in Isfahan Province

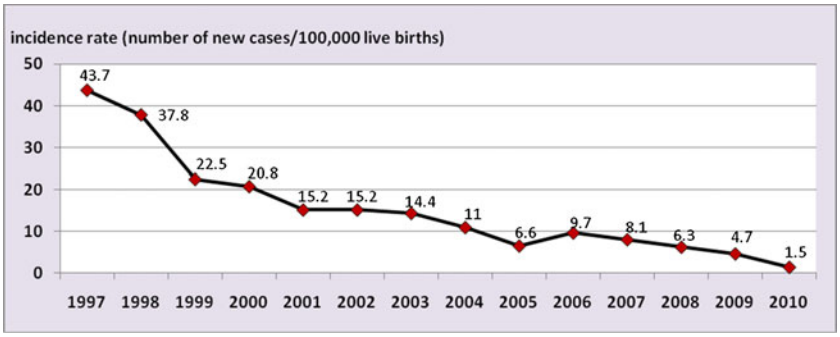

Fig. 5 Incidence rate of major beta-thalassaemia in Isfahan Province within 1997–2010

prevalence for beta-thalassemia trait in Iran becomes more complex.

According to the prevalence of carrier couples with beta-thalassemia trait among screened couples in our study (~0.31 %), we can calculate an expected prevalence of β-thalassemia trait in general population about 5.6 % for Isfahan Province ($\sqrt{0.31\%}$), but it would be less than this percentage taking into account an estimated consanguinity of 30–40 % for Isfahan's population (Mehrabi and Zeyghami 2005; Hashemipour et al. 2007).

Genetic testing

The introduction of genetic testing has gone through quite a few difficulties. The first genetic laboratory in Isfahan became available in 2000, in the private sector. However, prior 2007 genetic testing remained accessible to a few couples only because of discrepancies in price between the private and public sectors, and insurance companies paying only a fraction of the private sector's costs.

Table 2 Frequency of causes that major beta-thalassemia patients were born within 1997–2010 in Isfahan Province

Causes	Number of cases	Percent
Marriage before starting program	56	51.4
Culture related	16	14.7
Genetic counseling inadequacy	10	9.2
Healthcare inadequacy	7	6.4
Screening testing errors	5	4.6
Economic related	3	2.8
Non-referring from marriage registration offices	2	1.8
Loss to follow up of carriers	2	1.8
Delay to genetic testing	2	1.8
Genetic testing errors	4	3.7
Unknown	2	1.8
Total	109	100.0

Although genetic testing was officially free in the public sector, only a few labs were functional and could accept only a few couples.

On the other hand, only a few teams were available for genetic counseling in the Province until 2007, and some districts had no genetic counseling center (Iranian Health 2004). So, just a few carrier couples were referred for genetic testing.

By the beginning of 2007, steps were taken to promote quantity and quality of genetic counseling centers. So, all districts within the province had at least a genetic counseling center with a trained team that included a physician and an expert counselor by the end of 2007. Moreover, financial contracts were signed between the Iranian Health Ministry and some genetic laboratories in the private sector to facilitate acceptance of genetic testing by the at risk couples. This process was accelerated when the first public genetic laboratory was established in the Isfahan Province in 2008. As a result, the rate of genetic testing increased from 6.4 % in the first of 2007 to 85 % at the end of 2010.

The considerable number of suspected couples at risk imposes an additional burden on the healthcare system, even though most of them (over 80 % based on our study) will not require special services after genetic testing. All together, the number of suspected couples compared with the real carrier couples confirmed by high HbA2 (more than 3.5 %, according to the national guideline) was to high during the first decade of beginning the program, probably due to genetic counseling inadequacy and incomplete iron-therapy of suspected couples. We have been however able to better select suspected couples and decrease false positive during recent years. So, the proportion of screened suspected couples to confirmed carrier couples has decreases from 6.7 in 2004 to 3.9 in 2009 and 2010.

Effects on birth prevalence

In one study in Southern Iran, the birth prevalence of beta-thalassemia major was 253 cases per 100,000 births in 1995 and 82 per 100,000 births in 2004 (Karimi et al. 2007).

Based on our results, the birth prevalence of major beta-thalassemia in the Isfahan Province was much less than in Southern Iran, but it decreased by about 29 fold at the end of 2010 compared with 1997, when the premarital screening program was introduced in the Iranian Healthcare System. Our analysis revealed however 109 affected births between 1997 and 2010 and indicated five major reasons for the occurrence of new cases:

1. culture-related causes in 16 cases (30.2 %), leading to refusal to have genetic testing
2. genetic counseling inadequacy in 10 cases (18.9 %)
3. inadequate healthcare, due to negligence in seven cases (13.2 %)
4. screening testing errors in five cases (9.4 %)
5. economic-related causes in three cases (5.7 %) although couples would have chosen for genetic testing and therapeutic abortion (Wong et al. 2011)

Our data reveal that no new cases were born because of inadequate genetic counseling or healthcare after 2004.

The five births attributable to screening testing errors were born before 2000, during the first years of setting up the program. Quality control for the screening labs has solved this problem. The three cases due to economic problems occurred before 2005, when government funds for genetic testing were not available at the present level. Unfortunately some problems are still present in insurance coverage of genetic testing particularly within the private sector.

Conclusions

Although the Iranian premarital screening program for thalassemia has attained excellent results in control and prevention, much remains to be done to improve the program.

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