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Fourteen-Year Experience of Prenatal Diagnosis of Thalassemia in Iran

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Prenatal diagnosis · Prevention · Screening · Thalassemia · Traits

Abstract
For 14 years, Iranian scientists have worked to develop a national thalassemia prevention program. Although historically abortion was considered unacceptable in Iran, intensive consultations led to the clerical approval of induced abortion in cases with β-thalassemia major in 1997, and a nationwide prevention program with screening, counseling and prenatal diagnosis (PND) networks has been developed. This paper reports the experience from one of the two national PND reference laboratories. As one of the oldest reference laboratories, we performed a total of 906 PND in 360 couples at risk for thalassemia from 1990 to 2003. Direct and indirect mutation detection methods were applied for all cases. In total, 22 mutations were tested routinely, and an additional 30 rare mutations were identified. 208 fetuses were found to be normal, 215 fetuses had β-thalassemia major, and 435 fetuses were carriers of the trait. In 40 cases, we only defined one allele. In 8 cases, we were unable to provide any diagnosis, corresponding to 0.9%. Our data support the functionality of the Iranian β-thalassemia prevention program. The success of this system in Iran, a multiethnic and Islamic-based country, would mean that it might be applied as an adaptive system for neighboring and other Islamic countries.

Introduction

The hemoglobinopathies (thalassemias and sickle cell disorders) are the commonest human single gene disorders. It is estimated that about 7% of the world population carry a potentially pathological globin gene variant, and over 300,000 affected infants are born each year [1], about 80% with a sickle cell disorder and 20% with thalassemia major [2].

Similar to many other Middle Eastern countries, β-thalassemia poses a major health problem in Iran. Within an ethnically diverse population of over 65 million people, there are nearly two million thalassemia carriers [3]. In the absence of any intervention, ~1,000 infants with β-thalassemia major might be born annually. Every effort is made to provide affected infants with appropriate treatment, and it is estimated that over 15,000 patients are currently under care. With the exception of bone marrow transplantation, which is available only for

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a limited number of patients with a fully compatible sibling donor, there is no definite cure for β-thalassemia. Consequently, at present, patients must rely on regular blood transfusion and iron chelation therapy. Despite treatment, many still suffer major complications including impairment in growth and sexual maturation, diabetes and cardiac iron loading. As a result, there is a growing need for preventive measures.

Population-based prenatal diagnosis (PND) of β-thalassemia was first introduced in Sardinia and Cyprus. This was followed by whole countries (Italy and Greece) implementing the approach. However, the World Health Organization recognized at an early stage that the greatest challenge is to introduce these approaches in large communities comprising ethnic groups at various risk levels [4].

In practice, a crucial component of a prevention program is the availability of PND, because it helps carrier couples in family planning. PND was initiated in the private sector in Iran at the end of the 1980s. At that time the common view was that within Islam, interruption of affected pregnancies was unethical and socially unacceptable, and it was customary to inform families with affected children of the 1:4 risk incidence in every subsequent pregnancy, with the expectation that this would discourage further pregnancies. In 1997 mandatory premarital screening for thalassemia was introduced, together with non-directive genetic counseling. At that time PND was available only as a private service, had not been endorsed by the health authorities and was too expensive for most families. Hence most carrier couples detected by screening had to choose between the available alternatives, none of which was entirely satisfactory: (1) breaking off the engagement and finding another partner (who could very well turn out to be another carrier), (2) marrying and not having children and (3) marrying and accepting the 25% risk in every pregnancy. In fact, however, many carrier couples asked for PND, despite the lack of approval of induced abortion, allowing motivated scientists to demonstrate its feasibility and reliability, and advancing the knowledge on the patterns of mutations in Iranian subpopulations.

Once the high demand for PND became clear, together with the evidence that it was feasible in the Iranian population, the next step was to work towards the legalization of induced abortion.

Iranian scientists therefore met with religious leaders to put forward the peoples' need for PND as an adjunct to the national carrier-screening program. As a result, in 1997, the religious authorities issued the permission to interrupt pregnancies before the 16th week of gestation, provided PND had demonstrated that the fetus had a severe congenital disorder.

The organization and outcomes of the first 5 years of the national thalassemia screening program have already been reported by Samavat and Modell [5]. The thalassemia program is a strategy for identifying and counseling carriers of genetic disorders, especially recessively inherited ones. Screening was included as part of existing mandatory premarital blood tests. Couples at risk were offered genetic counseling. The laboratory component of the program is supplemented by a PND network with five peripheral laboratories and two reference laboratories. If the peripheral laboratories are unable to detect the mutations, the cases are referred to the reference laboratories for further analysis. The activities of the network are funded by the government, and the costs of the tests are covered by insurance companies. Here we report the experience of one of the two national reference laboratories.

Subjects and Methods

Subjects

In the thalassemia prevention program, marriage registrars refer prospective couples to a designated local laboratory for premarital screening. At first, red cell indices for the prospective husband are checked. If there is a microcytosis condition (MCV <27 pg), the wife is also tested. If both are microcytic, hemoglobin A2 concentrations are checked. If both bear the β-thalassemia trait (hemoglobin A2 >3.5%), they are referred to the locally designated health house for genetic counseling. Those who marry after counseling are followed up by their local health house. As part of the national thalassemia screening program, hematologically determined couples were offered counseling, mutation detection and PND. All couples were interested in having PND, and the only limiting factor is a financial one: the couples who refrain from these services are those who are unable to afford the tests. In our experience, the majority of the couples are interested in having PND, and all couples whose fetuses have been diagnosed with thalassemia major have been inclined to interrupt the pregnancy; only in 3 cases abortion was not performed, primarily because gestational age was past the legal limit of 16 weeks, and illegal attempts were unsuccessful or had failed.

Methods

DNA Extraction. We performed DNA extraction from chorionic villi using a salting-out procedure [6]. First, the samples were screened for a panel of 22 relatively common β-globin mutations using an assay based on polymerase chain reaction and reverse hybridization to oligonucleotide arrays immobilized on test strips [5]. This powerful technique covers more than 80% of the known β-globin alleles in Iran in a single amplification and hybridization step, allowing even very small amounts of DNA (e.g. prenatal sam-

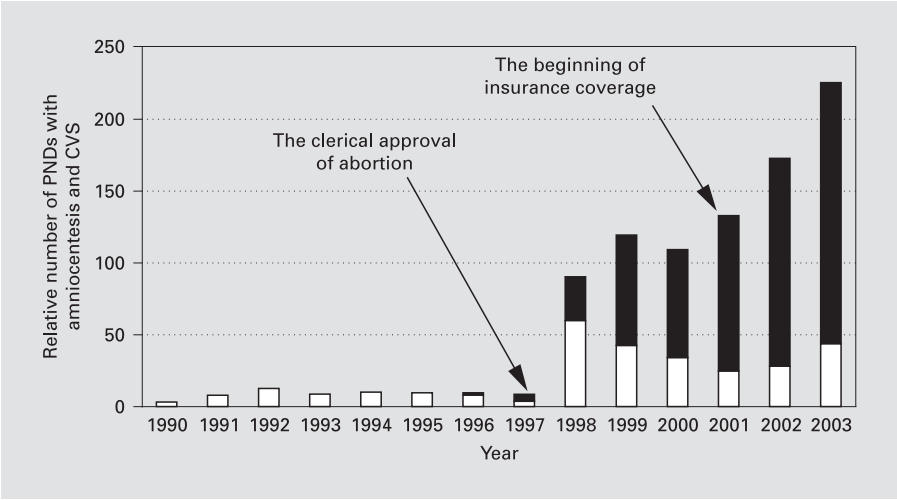

Fig. 1. The comparative number of CVS and amniocentesis cases performed from 1990 to 2003. The white portion of each bar indicates amniocentesis cases while the black portion corresponds to CVS cases. The clerical approval in 1997 and the beginning of insurance coverage in 2001 are demonstrated.

ples) to be rapidly and comprehensively typed [7]. The samples which remained negative for these prevalent mutations were subjected to DNA sequencing of the entire β-globin gene including the 5′-untranslated region.

DNA Sequencing. The amplified β-globin gene was sequenced by automated fluorescent sequencing, using either the ABI PRISM 3100 Genetic Analyzer and ABI PRISm Big Dye Terminator version 2.0 Ready Reaction Cycle Sequencing Kit (Applied Biosystems, Foster City, Calif., USA) or the Long READER 4200 Analyzer (LI-COR, Lincoln, Nebr., USA) and SequiTherm EXCEL II DNA sequencing Kit-LC for 25–41 cm gel (Epicentre, Madison, Wisc., USA).

Table 1. The number and percentage of phenotypes detected in PND cases

Phenotype	Cases detected	
	n	%
Major	215	23.7
Trait	435	48.0
Normal	208	22.9
Trait or normal	30	3.3
Trait or major	10	1.1
Unidentified	8	0.9

Results

The majority of couples were from the North, Central and Southwest (47, 21 and 20%, respectively). Four percent were from the South and West, respectively, and the remaining 4% had Southeast, Northeast and Northwest origin. Seven hundred and eighteen couples were referred for PND; 358 couples proceeded to have pregnancy, and a total of 906 PND were performed for them (fig. 1).

The chart shows an abrupt increase in the annual number of cases since 1998 following the approval of induced abortion by the clergy and the consolidation of the screening program. In total, 612 tests have been performed by chorionic villus sampling (CVS) and 294 by amniocentesis. The steady annual increase in the number of cases investigated by CVS versus amniocentesis is also apparent in this figure. In all cases both direct [amplification refractory mutation system (ARMS) and reverse blot strip assay] and indirect methods (RFLP) were used (a 'belt and braces' approach). DNA sequencing was performed when no mutations were detected, and RFLP data were not informative. In 208 cases, the diagnosis was normal, in 215 it was homozygous for β-thalassemia, and 435 cases were carriers of the β-thalassemia trait. In 40 cases it was only possible to define one allele: 30 of these were either carriers of the trait or normal, while 10 were either carriers of the trait or had β-thalassemia major. We were unable to provide a diagnosis in 8 cases. Table 1 demonstrates the number of phenotypes detected and their percentage. ARMS and reverse dot blot hybridization were applied to detect 22 mutations, and direct sequencing identified an additional 30 rare mutations. IVS II-1 was the most common mutation detected, representing 33.3% of all β-thalassemia mutations. Other common mutations, IVS I-5, IVS I-110, Fr36/37, CD8 (–AA), and CD30 (G→C) comprised 33% of mutations. About 61% of the couples referred for PND procedures were consan-

guineous. Nevertheless, in 21% of these couples the mutations detected were different.

Discussion

Screening and PND of β-thalassemia has been applied successfully in the Mediterranean countries. Their success has been based on the excellence of public facilities for antenatal detection. In Sardinia, with a homogeneous population of 1.5 million people [8] the birth prevalence of β-thalassemia is less than 10% of expectation. However, in Iran it is necessary to develop a corresponding service for a larger population (>65 million people) with seven major ethnic groups distributed throughout a vast area of 1.6 million km^2, encompassing a wide spectrum of mutations, in comparison to the small and homogenous Sardinian population where two alleles [CD39 and CD6 (–A)] account for nearly 98% of the mutations. In the Iranian population IVS II-I, IVS I-5, IVS I-110, Fr36/37, CD8 (–AA), and CD30 (G→C) comprise only about 66% of mutations [9]. In about 20% of consanguineous couples referred for PND, the two mutations have been found to be different, which by itself indicates high prevalence and heterogeneity of mutations in our community. This heterogeneity has posed great challenges in developing an appropriate molecular diagnostic setup. The efforts of the past years to establish the spectrum of β-thalassemia mutations in different ethnic groups of the Iranian population [9–17] have greatly assisted us to adapt mutation detection techniques to PND. By applying direct and indirect methods such as ARMS, reverse dot blot hybridization, sequencing and RFLP we have been able to reduce the assessment time to a few days.

We have performed PND for 94.6% of referrals with 100% accuracy. In 4.4% accuracy was only 50% (only one allele detected), and for the remaining 0.9% no diagnosis was made. The DNA of parents with inconclusive results is being studied to localize the mutations which are assumed to be in the regulatory elements of the β-globin gene.

The clerical approval of abortion for pregnancies with confirmed β-thalassemia major genotype in 1997 and its subsequent legalization enabled us to start prenatal procedures officially, and as figure 1 indicates in 1998 referrals for PND increased sharply. This sudden increase would not have been possible unless an organized national screening network, proper public awareness and laboratory setup had been prepared in advance. This trend is continuing, with an approximate mean increase of 36 cases annually. This increase can be attributed to improvements in the above-mentioned elements combined with insurance coverage, which started in 2001 and has been crucial to permit families to use PND.

The ratio of CVS to amniocentesis has increased each year. This reflects both the development of antenatal medicine techniques and growing public awareness of the importance of performing PND in time to receive results before abortion is prohibited. Nevertheless, some couples still present in the second trimester and consequently undergo amniocentesis. There is still room for increasing public awareness.

Screening and antenatal diagnosis programs have been widely applied in Mediterranean populations and have contributed to the reduction in the number of children affected with thalassemia in the respective societies [16, 17]. Our data show that the Iranian prevention program may be equally efficient, and that it is reasonable to anticipate an early decline in the number of β-thalassemia patients in the country. The Iranian program is consistent with the country's religious and multiethnic background and can be proposed as a model for Islamic communities and large countries with diverse populations.

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