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Mini Review

Antenatal Screening and Diagnosis of β-thalassemia in High-Risk Population of Sardinia

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Abstract

Sardinia, an Italian island in the Mediterranean sea with a population of 1,7 millions, has one of the highest incidence of β-thalassemia with 10-12% of carriers.

Since 1977 we have been offering a capillary screening program and prenatal diagnosis to couples following non-directive genetic counselling.

Thanks to the implementation of new molecular analysis methods, we shifted from prenatal diagnosis performed in 2nd trimester of pregnancy to 1st trimester. In order to avoid termination of pregnancy in case of affected fetuses following prenatal diagnosis we introduced preimplantation genetic testing, thus increasing notably the acceptance rate of antenatal screening and diagnosis by couples at high risk.

In this paper, we report the continuous experience of our prenatal centre in Sardinia in 9,324 antenatal diagnoses of β-thalassemia, the accuracy and safety related to the prenatal procedures we employed, the termination of pregnancy rate and the couples' acceptance of the different approaches as well as analyze the screening programs available to the Sardinian population.

Molecular screening by Polymerase Chain Reaction (PCR) based methods, prenatal diagnosis by Chorionic Villous Sampling (CVS) with very low fetal loss rate (0,3%) and Preimplantation Genetic Diagnosis (PGD) were the most common techniques applied in our prenatal centre and they have brought to a drastical reduction of newborns affected by β-thalassemia to only 3-5 per year.

Keywords: Thalassemia; Molecular screening; DNA; Prenatal and preimplantation genetic diagnosis; Termination of pregnancy

Abbreviations

Chorionic Villous Sampling (CVS), Quantitative Fluorescent Polymerase Chain Reaction (QF-PCR), Preimplantation Genetic Diagnosis (PGD), Transabdominal CVS (TA-CVS), Termination of Pregnancy (TOP), Prenatal Diagnosis (PD)

Introduction

β-thalassemia is the most common inherited autosomal recessive disease in the world with more than 200,000 million carriers and 250,000 affected babies are born every year. The disease is widespread mostly in the Mediterranean region, North Africa, Middle East, Far East and East Asia. However, due to the complex migration trends, β-thalassemia is also diffused worldwide [1].

Sardinia, an Italian island in the Mediterranean sea with a population of 1,7 millions is at high risk incidence of β-thalassemia, with 10-12% of carriers which means that nowadays about 1 of 50 couples are at risk of genetic transmission of this disorder. In absence of prevention by antenatal genetic screening and diagnosis and with the progressive denatality in Sardinia, the affected newborns are estimated to be about 60-70 per year [2].

People affected by β-thalassemia are characterized by severe haemolitic anemia, hepatosplenomegaly and characteristic skeletal malformations such as osteoporosis and dysmorphic facies. Without prevention and therapy their survival and life quality is highly compromised and generally difficult.

Affected people necessitate regular blood transfusion and iron chelation. Bone marrow transplantation from an HLA identical sibling is the only possibility to cure the disease with a success rate of 90% [3].

β-thalassemia is characterized by absent to minimal hemoglobin A (0-30%), variable hemoglobin A_2 (2-5%), and hemoglobin F (95-70%). More than 200 different molecular defects have been described and 95% of them cause β-globin gene joint mutations [4].

In this paper we describe the prevention of β-thalassemia performed in our Ob/Gyn Department in Cagliari, Sardinia by antenatal molecular screening, prenatal and preimplantation genetic diagnosis (PGD) in the last 45 years from 1977 to 2022 using different diagnostic approaches.

Identification of Carriers and Molecular Screening

A voluntary β-thalassemia screening program in Sardinia was launched in 1974-75 involving mass media, family doctors, obstetricians, pediatricians, geneticists, midwives and students of

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Table 1: Frequency of β-thalassemia mutations in Sardinia.

Mutations		%
β -39	(C->T)	95,7
β -6	(-A)	2,2
β -76	6	0,7
β I-110	(G->A)	0,5
β II-745	(C->G)	0,4
β -87	(C->G)	0,2
β I-6	(T->C)	0,2
β 1	(-G)	0,1
β II -1	(G->A)	0,1
β I -1	(G->A)	0,03

all ages in order to inform the whole population on its transmission and prevention and to provide blood testing in order to identify the carriers and the molecular mutations [5].

The most commonly identified point mutation (95,7%) in Sardinia was c.118C>T [4] (Table 1).

Since parental mutations had to be investigated on fetal material, the molecular definition of the parents' ß-globin genotype was necessarily required.

Couples at risk were mostly identified and classified as composed of ß-thalassemia carriers on the base of whole blood count and haemoglobin electrophoresis.

The ß-globin gene defects were detected by:

- Reverse hybridization (Nuclear Laser Medicine, ß-globin test) able to identify, in a single step, up to 25 mutations in Mediterranean population [6].

- Sanger sequencing of the amplified ß-globin gene, to identify other less common variants.

- Amplification Refractory Mutation System - a primer-specific amplification [7].

- Most rarely, Multiplex Ligation Probe Amplification, to find out gene deletions and duplications [8].

Since 1994 we performed prenatal diagnosis by Chorionic Villous Sampling (CVS) and the villi were carefully cleaned under inverted microscope from the maternal decidua and blood clots. After a sample incubation at 56°C for 2 hours with a proteinase K digestion mix, fetal DNA was isolated using QIAmpDNA mini kit (Qiagen) with an automatic system (QiaCube, Qiagen) maternal cell contamination on prenatal samples was ruled out by short tandem repeat testing (Quantitative Fluorescent Polymerase Chain Reaction (QF-PCR)), Compact Devyser v3) [9].

DNA fetal samples were analysed by the same techniques described for the carrier study, but using two different procedures to limit misdiagnosis.

Molecular Analysis by Preimplantation Genetic Diagnosis (PGD)

For PGD analysis of one embryo cell or of more cells from blastocysts we used molecular techniques for the detection of mutations based on PCR multiple steps to amplify the region of β-globin gene [10]. Two nested PCR reactions for producing DNA fragments were subsequently used for the analysis. Therefore, by minisequencing reaction we identified the β-globin gene mutations. Automatic sequencing, mini-sequencing, micro satellites analysis for linkage and genotyping by TaqMan analysis were also used [11,12].

Antenatal Diagnosis

In 1977 the first prenatal diagnosis of β-thalassemia in Europe was performed in our prenatal centre in Cagliari using Fetal Blood Sampling by Placentacentesis and globin chains synthesis analysis in the 2nd trimester of pregnancy [13].

Later on, in 1981-82 we performed Fetoscopy and Cordocentesis by globin chain analysis [13].

In 1983, following the molecular improvement of PCR-based methods and thanks to new invasive procedures we shifted to Amniocentesis [14] and in 1984 we performed first trimester CVS. In the beginning, we carried out CVS using a transcervical rigid biopsy forceps [15]; but, since 1986 till present, CVS has been done only transabdominally [16] by free-hand technique under continuous ultrasound monitoring and it has been better accepted by patients [17].

Due to the strong request by couples, since 2002 we also offered PGD [10] by embryo biopsy and later on by blastocyst biopsy, only to infertile carrier couples initially and subsequently to fertile patients as well. An Italian Law adopted in 2004 prohibited PGD for several years but we were enabled to perform it again in 2014 thanks to the Italian Constitutional Supreme Court [18]. PGD is a more sophisticated method which can be carried out at the earliest stage of embyo development and it requires In Vitro Fertilization, transfer of non affected embryos and intracytoplasmatic sperm injection.

In Table 2 we report the results of fetal diagnosis in 9015 cases using different prenatal invasive techniques. Sampling failure, fetal loss rate and misdiagnosis were very low.

The majority of women (98,2%) decided to interrupt voluntarily the pregnancy of affected fetuses following non-directive genetic counselling (Table 3).

Table 2: Continuous invasive fetal diagnoses of ß-thalassemia in Sardinia in 9015 cases (1977-2022).

Techniques	No	Years	Gestational Age (weeks)	Failure (No)	Fetal loss (%)	Misdiagnosis
Placentacentesis	981	1977-83	18-24	10	5,2	2
Fetoscopy	67	1983-85	18-24	2	5,6	-
Cordocentesis	120	1984-85	18-24	1	2,1	-
Hepatic Vein Puncture	3	1984-86	18-24	-	-	-
Cardiocentesis	3	1984-86	18-24	-	-	-
Amniocentesis	203	1982-83	16-18	6[2]	2,6	-
Transcervical CVS[1]	572	1983-86	9-13	1[2]	4,2	1
Transabdominal CVS[1]	7066	1986-22	6-24	-	0,4	-

[1]Chorionic Villus Sampling

[2] Due to DNA failure and completed by Fetal Blood Sampling in 1984

Table 3: Fetal diagnosis of β-thalassemia 1977-2022.

	No
Women	9015
Normal fetuses	2247
Health carriers	4523
Affected fetuses	2245[1]

[1]98,2% women opted for termination of pregnancy following non-directive genetic counselling

Table 4: Pre-implantation Genetic Diagnosis in Sardinia.

	No
Women	309
Cycles	342
Stage of biopsy 3 (blastomere)	195
Stage of biopsy 5 (blastocysts)	114
Miscarriages	9
Clinical pregnancies	67

Table 5: Acceptability of PGD versus Prenatal Diagnosis by CVS.

Group	No. Women	Experience		Accepting PGD	
		PD	TOP	No. Women	% YES
1	60	YES	YES	60	100
2	60	YES	NO	18	30
3	60	NO	NO	15	25

Palomba ML. Hum Reprod 1994
CVS – Chorionic Villous Sampling
PD – Prenatal Diagnosis
PGD – Preimplantation Genetic Diagnosis
TOP – Termination of Pregnancy

Table 6: Acceptance of prenatal diagnosis of β-thalassemia in Sardinia according to the invasive procedures.

Technique	Acceptance (%)
Fetal blood sampling	93.2
Amniocentesis	96.4
Chorionic villus sampling	99.3

Cao A. Prenat Diagn 1987

The results of 309 PGD procedures are reported in (Table 4).

All couples at high risk for β-thalassemia received informative genetic counselling based on the ethical principle of respect for authonomy. Informed written consent about initiating and managing the pregnancy was also obtained by the couples.

Discussion

Our continuous experience in applying β-thalassemia prevention programs in the high-risk population of Sardinia demonstrates their substential and permanent efficacy and safety as well as the high acceptance rate of the various diagnostic approaches offered to couples. The prevention programs adopted in Sardinia have contributed considerably to the decrease of the birth rate percentage of affected newborns [4].

In the 70's, with 15,000-18,000 deliveries per year in Sardinia, about 120-130 newborns were affected by β-thalassemia. Nowadays, with natality of about 9,000-10,000 deliveries yearly and if prevention programs and prenatal and preimplantation genetic diagnosis were not available, the estimated birth percentage of affected newborns would be 60-70 per year. However, by applying efficient screening strategies, fetal diagnosis and PGD, only 3-5 children are born [19].

Since 1986, the only invasive diagnosis offered to carrier couples has been transabdominal CVS (TA-CVS) with the strong reduction of fetal loss related to the procedure (0,3%). TA-CVS was increasingly preferred to the other invasive techniques available; also, compared to transcervical CVS it is less traumatic and has less infections, less fetal loss rate and is generally better accepted by women (17). To those women who were referred to our prenatal centre after 13 weeks of gestation, we also offered 2nd trimester TA-CVS that has proven to be very safe and efficient [20].

PGD has also led to the strong acceptance of TA-CVS by the population, mostly in couples that have already had a termination of pregnancy (TOP) for an affected fetus [21] (Table 5).

Several experimental treatments were performed in the past by in utero hematopoietic stem cell transplantation, but the results were not satisfying [22,23].

Using CD45+ cells with lentoviral-delivered β-globins in utero gene therapy demonstrated an improvement of blood transfusion results in children [24] but gene treatment in the postnatal period is still only at an experimental stage [25].

New "omics" studies of the placenta and the amniotic fluid could explain several metabolomic mechanisms involved in early phenotype prediction [26].

First trimester TA-CVS with very low fetal loss rate related to the procedure [17,27] and without misdiagnosis since 1986, significantly increased the number of couples requesting invasive techniques [28] as reported in Table 6.

Certainly, the high request (98,2%) of a traumatic experience such as TOP of an affected fetus chosen by women after non-directive genetic counselling was a major ethical issue. It was only partially overcome by offering PGD as an option to all couples [29].

This complex prevention was made possible in Sardinia thanks to capillary educational and informative healthcare programs, using haematological and molecular screening of carriers. The shift to offering prenatal and preimplantation genetic diagnosis in an earlier gestational period and the multidisciplinary collaboration efforts among obstetricians, hematologists, geneticists, pediatricians and molecular biologists have also facilitated this trend. The importance of the teamwork among all program components and the fundamental contribution of each of them, the timely exchange of data and expertise must be particularly underlied.

The possibility of carrying out diagnosis by sampling of fetal cells in circulation in maternal peripheral blood, will still lead to still greater acceptability by the couples at high risk for β-thalassemia but, as reported by the experience of our centre, the use of this technique is not yet diagnostic and, at present, it is not applicable in the routine clinical practice [30]. Using next generation sequencing on cell-free DNA will be a promising method for new approaches for non-invasive antenatal assesment of β-thalassemia [31].

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