Non-Invasive Prenatal Diagnosis (NIPD): Clinical applications in the early detection of fetal diseases. The case of thalassemia

Introduction

Non-Invasive Prenatal Diagnosis (NIPD) is a low-risk genetic test carried out on a sample of maternal blood that screens for birth defects and inherited diseases. However, fetal cells are usually scarce in the maternal circulation. To achieve an effective diagnosis, they need to be enriched in order to isolate a sufficient amount of them. Fetal DNA is then analyzed by the means of various techniques, the more frequent being PCR and MPS. NIPD is being increasingly used in the early and effective detection of monogenic diseases.

One of the most common monogenic diseases is **thalassemia**: a hemoglobinopathy characterized by abnormal formations of hemoglobin. These include α-globin and β-globin mutations that result in the underproduction or even the absence of normal globin cells. These anomalies cause a variety of symptoms that may differ from one patient to another.

Objectives

- To develop, deepen and enhance specific scientific knowledge on NIPD: its evolution, its clinical applications, how it is and should be performed and the particular techniques and medical equipment usually involved in the testing process.
- To compose an accurate definition and a structured, research-based report on thalassemia, including a precise account of its types, world presence, and observable symptoms in order to determine the most suitable ways to detect and diagnose it.

Methodology

This paper is a literature review based on a guided online research in accordance with selected keywords: 'NIPD', 'Non-Invasive Prenatal Diagnosis' and 'thalassemia'.
Renowned medical databases were consulted (PubMed, ScienceDirect, Google Scholar) and papers were chosen according to their journal impact.

Results

Non-Invasive Prenatal Diagnosis

An accurate analysis of **fetal material** is crucial in order to perform an efficient prenatal diagnosis:

- **Intact fetal cells** that can be found in the maternal plasma:

  - **Trophoblasts** can be detected via the use of specific antibodies that are able to detect placental antigens. They usually are present in the maternal blood (and can therefore be isolated from it) during the first trimester of pregnancy only.

  - **Leukocytes** usually continue to circulate in the maternal blood long after pregnancy.

  - **Nucleated red blood cells (NRBC)** have a short half-life. They present an uncommon, distinctive morphology.

- **Cell-free fetal DNA (cffDNA)** originates in the placenta. It derives from the genetic material that is released after fetal cells undergo apoptosis or lysis, the latter being triggered by the mother's immune system.
- **Cell free fetal RNA (cffRNA)** originates in active genes present in the placenta. It tends to appear in a smaller amount than cffDNA.

	cffDNA	Fetal cells
Earliest detection (weeks)	4	7
Proportion (%)	5-10	0.0001-0.01
Persistence in maternal blood	< 24 hours	> 27 years
Important physical properties	Short fragments	Dense nucleus
Advantages	High proportion in plasma Existence of assays for trisomies Automated plataform available	Complete fetal DNA Whole genomic DNA amplification Confirm fetal identity in 1st trimester
Disadvantages	Fragmented nature of DNA High cost Specific assay	Low proportion in plasma No automatic process Placental mosaicism within trophoblastic fetal cells

Table 1. Differences between fetal cells and cffDNA.

Universal fetal markers				
	SNP or mutated points	Polymorphic segments	Epigenetic modifications	Some mRNA or proteins derived of those
Allow to	Identify origin of the mutated allele (maternal/ paternal)	Identify segment's origin (maternal/ paternal)	Perform imprinting	Determine activity levels of fetal and placental genes

Table 2. Most important and useful fetal markers.

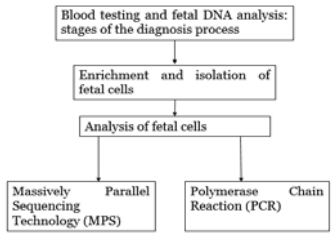

Table 3. Blood testing and fetal DNA analysis: stages of the diagnosis process.

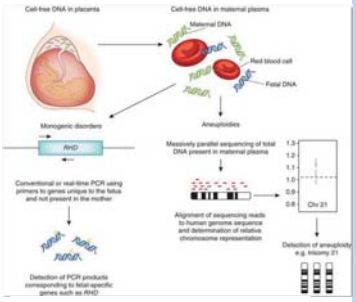

Figure 1. Process of analyzed DNA fetal in maternal blood.

Invasive prenatal diagnosis techniques:

Amniocentesis is a process in which amniotic fluid is sampled using a hollow needle inserted into the uterus.

Chorionic villus sampling (CVS) is a testing procedure which involves sampling the developing placenta during the first trimester of pregnancy in order to examine the fetal karyotype and/or genotype.

	IPD		NIPD
	Amniocentesis	CVS	
Early detection (weeks)	15	11-14	4-7
Risk of miscarriage (%)	1	1-2	0
Test type	Diagnostic	Diagnostic	Screening

Table 4. Invasive and non-invasive prenatal diagnosis: comparative data

Clinical Applications				
	Fetal sex	Single-gene diseases	Fetal chromosomal abnormalities	Pregnancy problems
Diagnosis of	X-linked diseases Several endocrinal disorders	Autosomal dominant or recessive monogenic diseases	Trisomies Monosomies	Hypersensitivity reaction between the mother and her unborn fetus
Via the analysis of	SRY gene	One or two mutated genes	Variations in the karyotype Gain or loss of genetic material in chromosomal regions	Fetal Rh antigen
Most frequent technique/s	Conventional PCR	PCR or MSP	PCR or MSP	PCR

Table 5. Principal clinical applications of NIPD.

Thalassemia

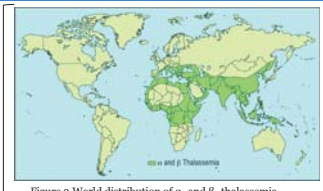

Figure 2.World distribution of α- and β- thalassemia

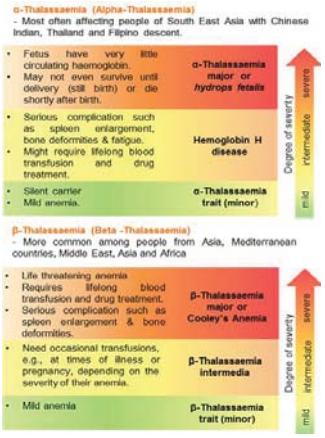

Table 7. Types of thalassemia and their characteristics.

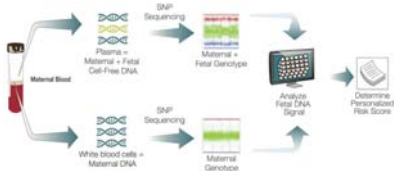

Figure 3. Stages of the analysis of fetal cells in maternal blood using MPS.

Table 6. Thalassemia diagnosis: different methods.

Most relevant diagnosis techniques:

- **Fluorescent Immunohistochemistry (IHC)** is a method for demonstrating the distribution of proteins in tissue sections. It is performed via specific antibodies that recognize the target protein.
- **Real-time PCR combined with fluorescence** testing is performed in order to determine whether a fetal globin chain is an ordinary or a mutated one.
- **PCR in two steps** is used when a paternal allele is concerned.
- **MPS** involves identifying single nucleotide differences in fractionated DNA in order to discriminate potential mutation points in the fetal genome.
- **Real-time quantitative PCR** is used to amplify, detect and/or quantify a targeted allele, be it a normal or a mutated one. Markers from paternal and maternal DNA are employed in the sample analysis.

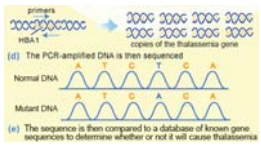

Figure 4. Analysis of fetal cells in maternal blood by PCR technique.

Bioethics

It is essential to have in consideration the legal framework of the country where the diagnosis is to be performed: in countries where abortion is an illegal practice, NPID results could put pregnant women in a vulnerable, even dangerous position.

It is extremely important to provide the couples with detailed information on these procedures, their possibilities, and their limitations. A well-trained genetic counselor who would be able to thoroughly explain the techniques, advice the couples and answer their questions stands out as a highly-recommended option.

In order to respect the principle of autonomy, the women to be potentially tested need to be adequately informed before giving their consent.

In order to respect the principle of justice, women should receive good medical care regardless of social, economic or ethnic reasons.

The Spanish Context

In Spain, NPID is being increasingly used in the private sector in order to detect Down's syndrome and Edward's syndrome.

As for the future of non-invasive prenatal testing, statistics show a tendency to implement the use of NPID in the public health sector as well.

A widespread practice of NPID is thought to be followed by a decrease of invasive testing, resulting in a decline of miscarriage risks and improved safety for pregnant women.

Conclusions

- NIPD stands out as a good screening prenatal test. If the results turn to be positive, it can then be followed by invasive procedures aiming to confirm the pathology.
- Since it entails but a simple blood test, NIPD emerges as a socially welcomed test.
- Thalassemia is confirmed to be one of the most common blood disorders. There exist two different types of this disease, each one of them entailing different levels of risk.
- Usage of NIPD in the early detection of thalassemia is still going through an experimental phase. However, numerous research projects aiming to promote and spread NIPD procedures in clinical applications are currently being conducted.

References

Figure 1. D. W. Bianchi. From prenatal genomic diagnosis to fetal personalized medicine: progress and challenges. Nature Medicine. 2012; (18): 1041-1051.
Figure 2. En línea: phm.utoronto.ca/~jeffh/PPT/phm14thal.ppt
Figure 3. En línea: www.panoramatest.com/sites/default/files/images/how%20it%20works.jpg
Figure 4. En línea:www.ironhealthalliance.com/disease-states/thalassemia/diagnosis/genetics-studies.jsp (17/01/2014)
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Table 7. En línea: http://dna-laboratories.com/?p=289 (05/11/2011)