ORIGINAL ARTICLE
Iran J Allergy Asthma Immunol
October 2020; 19(5):478-483.
Doi: 10.18502/ijaai.v19i5.4463

The Critical Role of Prenatal Genetic Study in Prevention of Primary Immunodeficiency in High-risk Families: The Largest Report of 107 Cases

Seyedeh Zalfa Modarresi[1,2], Nastaran Sabetkish[1], Mohsen Badalzadeh[1], Shaghayegh Tajik[1], Behnaz Esmaeili[1], Mohammad Reza Fazlollahi[1], Massoud Houshmand[3], Jaber Gharehdaghi[4], Shirin Niroomanesh[5], Fatemeh Rahimi Sherbaf[5], Zahra Alizadeh[1], Nazanin Khodayari Namini[1], Marzieh Maddah[1], Zahra Pourpak[1], and Mostafa Moin[1,6]

[1] Immunology, Asthma and Allergy Research Institute, Children's Medical Center Hospital, Tehran University of Medical Sciences, Tehran, Iran
[2] Department of Pediatrics, Shahid Sadoughi University of Medical Sciences, Tehran, Iran
[3] National Institute for Genetic Engineering and Biotechnology, Tehran, Iran
[4] Legal Medicine Research Center, Legal Medicine Organization, Tehran, Iran
[5] Division of Perinatalogy, Department of Obstetrics and Gynecology, Yas Hospital, Tehran University of Medical Sciences, Tehran, Iran
[6] Department of Immunology and Allergy, Children's Medical Center Hospital, Tehran University of Medical Sciences, Tehran, Iran

Received: 1 February 2020; Received in revised form: 19 August 2020; Accepted: 23 August 2020

ABSTRACT

This study aims to investigate the role of prenatal diagnosis (PND) in Iranian couples with a previous history of primary immunodeficiency disorders (PIDD) in their family.

All referred couples with a family history of PIDD and a tendency for PND were included in this project. Based on gestational age, chorionic villus sampling (CVS) was performed to analyze the molecular defect of the fetus according to the previous gene defect of the affected case in the family. Postnatal confirmation was performed by immunological screening tests.

In a total of 100 cases, CVS was not evaluated in 19 patients due to unwillingness (n=5), late prenatal referral (n=7), miscarriage before CVS (n=3), and female fetus with x-linked diseases in previous children (n=4). In the remaining 81 patients, heterozygous and homozygous mutations were found in 33 and 23 cases, respectively. The hemizygous mutation was obtained in 6 and no pathogenic mutations were found in 19 individuals. Postnatal evaluations revealed that a total of 65 babies were healthy, 32 fetuses were aborted (3 cases before CVS, 2 spontaneous abortions of a healthy and as affected fetus in the CVS subgroup, and 27 cases were aborted due to therapeutic causes). One fetus from the heterozygous subgroup was spontaneously aborted with severe combined immunodeficiency (SCID) and one fetus from the homozygous subgroup that was supposed to be healthy was affected by the autosomal dominant-chronic granulomatous disease (AR-CGD). The diagnostic error was 1.2%.

PND is highly recommended in families with a history of PID in their previous child to prevent an affected baby being born and to reduce the government, family, and personal burden of these diseases.

Keywords: Aborted fetus; Consanguinity; Genetic counseling; Prenatal diagnosis; Primary immunodeficiency disorders

Corresponding Author: Zahra Pourpak, MD, PhD; Immunology, Asthma, and Allergy Research Institute, Children's Medical Center Hospital, Tehran University of Medical Sciences, Tehran, Iran. Tel: (+98 21) 6691 9587, Fax: (+98 21) 6642 8995, E-mail: pourpakz@sina.tums.ac.ir

Published by Tehran University of Medical Sciences (http://ijaai.tums.ac.ir)

INTRODUCTION

Primary Immunodeficiency Diseases (PIDDs) compromise a heterogeneous group of hereditary disorders; causing defects in different components of the immune system.[1] Patients affected with severe forms of PIDD are highly susceptible to severe and recurrent or unusual infections. Moreover, other disorders including malignancy, autoimmunity, and allergy are common in these patients.[2,3]

Timely diagnosis and treatment of these diseases lead to reduced morbidity and mortality.[4] Although our knowledge of PIDDs is expanding, some aspects of their diagnosis, treatment, and prevention are still in the dark. Prophylactic antibiotics, replacement therapy, and hematopoietic stem cell transplantation (HSCT) are done for many PIDDs. However, high risks of graft rejection and drug adverse effects, as well as high treatment costs, are among the disadvantages of these methods. Moreover, the quality of life of both patients and their families is reduced.[5]

There are more than 300 forms of PIDDs associated with unique genetic defects in the world.[6] Thus, genetic counseling and prenatal diagnosis (PND) has become an important part of the management in families with an affected child suffering from PIDD. The ideal PND strategy is the identification of the disease-causing mutation in the fetus and parents and performing chorionic villus sampling (CVS) between 10-14th weeks of gestational age.[6]

In some countries, PND is now possible for many PIDDs through CVS, amniotic fluid cell culture, fetal blood sampling, ultrasound-guided cordocentesis, or amniocentesis in the second trimester of pregnancy.[1,7-9] In Iran, PND of PIDDs has been performed in the Immunology, Asthma, and Allergy Research Institute (IAARI) since 2008 to help the families with affected children and their relatives who wish to have a healthy child or in cases of unintended pregnancies.

This study aims to report the role of PND in families who are at risk of giving birth to a child with PIDD during 12 years in a tertiary center and try to prevent the birth of affected children according to abortion authorization in Iranian Legal Medicine Organization (ILMO).

PATIENTS AND METHODS

All pregnant women who had a child diagnosed with a PIDD or had a confirmed positive family history of PIDDs and were referred to IAARI for PND between April 2008 and July 2020, were included in the study. The Ethics Committee of IAARI approved the study protocol (No: 412/P/86/327).

Informed consent forms were filled out by the parents for CVS and the genetic study of the fetus. They were also informed about the risks of PND including incorrect results and potential risks of the procedure[10] as well as treatment options that were available for the families if the fetus was affected (Table 1).

If the fetus was at 10-14 weeks of gestation, ultrasound-guided CVS was done during the first trimester[11] in the In Vitro Fertilization Research Center (IVFRC), Yas hospital, Tehran University of Medical Sciences, Iran. According to strict rules of legal abortion in Iran that are only permitted before 19 weeks of gestation, we decided to perform CVS at this time to have adequate time for performing genetic analysis and further decision making. CVS samples were collected in EDTA tubes. Genomic DNA of each CVS sample was isolated by a specific DNA extraction kit (Qiagen, USA) and the genomic study was performed in IAARI. Contamination with maternal DNA was ruled out with DNA extracted from the fibroblasts obtained during CVS.[12] PCR was performed according to the standard protocol.[13] The sequence of primers is available upon request. Mothers with a confirmed diagnosis of PIDD in their fetuses were referred to the ILMO to obtain permission for legal abortion.

Table 1. Characteristics of pregnant mothers undergoing chorionic villus sampling *(*CVS)

Mothers undergoing CVS	81 (81%)
Mean±SD age of mothers	32 ± 4.2 (years)
Mean±SD gestational age	10.07 ± 4.5 (weeks)
Parents consanguinity	83 (83%)

Published by Tehran University of Medical Sciences (http://ijaai.tums.ac.ir)

In all confirmed or unconfirmed cases of PND, a vaccine exemption letter was given to the pregnant women so that the side effects of vaccination would not be added to the newborn babies that may be affected. If the pregnancy was terminated successfully, the newborn baby was followed up with three months intervals for 12 months period for immunological and advanced screening tests[14] and receiving killed vaccines.

Genetic testing was not performed in parents with a previous child suffering from an X-linked disorder and a present female fetus confirmed by chromosomal evaluations (PCR) of maternal blood samples.

RESULTS

During a period of 12 years; from April 2008 to July 2020, 114 women were referred to our center during pregnancy. These families or their relatives had diagnostic or treatment records in the Institute. Of 114 pregnant mothers, 100 had regular visits that were included in the study, and 14 were excluded due to incomplete information or lack of follow-up. The mean ± SD age of the mothers was 32±4.2 years (20-40 years) and the mean±SD gestational age was 10.07±4.5 weeks (1-37 weeks) in the first visit.

Of 100 families who were included in the study, 17 (17%) families had non-consanguineous marriage; while 83 cases (83%) had consanguineous marriage. The prevalence of different diseases that were diagnosed in their families was as follows: Bruton disease (n=4), autosomal dominant-chronic granulomatous disease (AR-CGD) (n=11), X-Linked Chronic Granulomatous Disease (XL-CGD) (n=5), Hemophagocytic lymphohistiocytosis (HLH) (n=5), Leukocyte adhesions deficiency syndrome (LADS) (n=18), congenital neutropenia (n=7), Severe combined immunodeficiency (SCID) (n=15), wiskott aldrich syndrome (WAS) (n=6), Griscelli syndrome (n=9), and combined immunodeficiency (CID) (n=1).

Figure 1 depicts the schematic diagram of all included patients. Of 100 included pregnant women, 81 individuals underwent CVS. CVS was not performed in 19 cases due to late prenatal visits (7 mothers), miscarriage before CVS (3 mothers), female sex of the fetus, and diagnosis of an X-linked disease in the previous child (one XL-CGD and three Brouton disease in previous children of mothers with a present female fetus), and unwillingness (5 mother). From these participants, 14 babies were healthy, and 2 cases were affected due to being referred so late or unwillingness to undergo the CVS and were born with SCID without undergoing CVS.

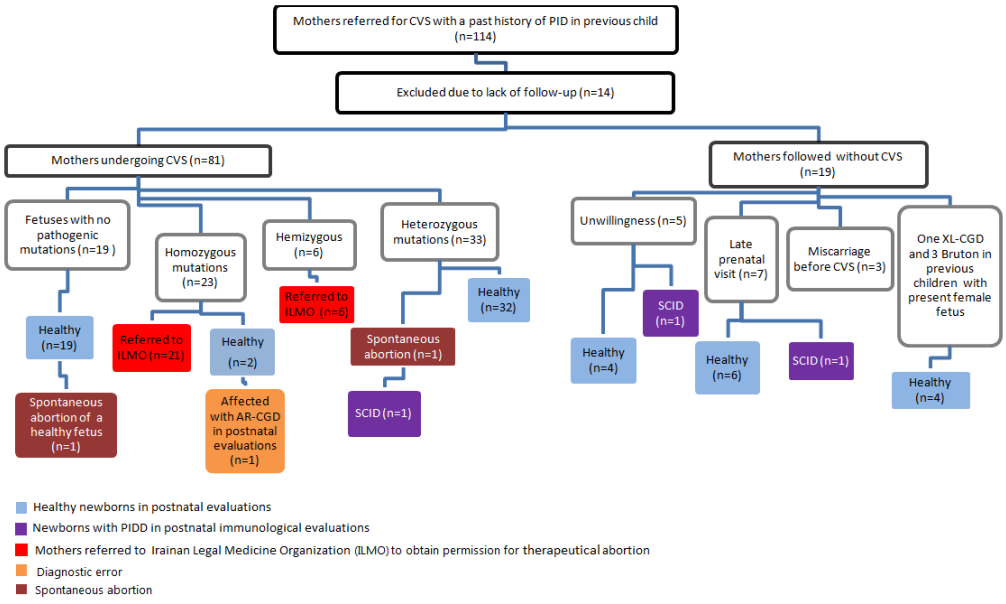

Figure 1. The schematic diagram of included patients

From the total included cases in the study (n=100), 52 babies were healthy according to the PND that was confirmed in postnatal evaluations. Twenty-nine cases were aborted from which 2 abortions were spontaneous; while 27 abortions occurred due to therapeutic causes. Additionally, one baby was diagnosed with a PIDD in postnatal evaluations (AR-CGD). This case with AR-CGD was born because of an incorrect PND.

Genetic testing on 81 CVS samples showed 23 homozygous and 33 heterozygous mutations in fetuses. Nineteen fetuses were healthy without any mutations and 6 fetuses were diagnosed with hemizygous mutations. Of 33 heterozygous fetuses, 32 were born healthy, one fetus was spontaneously aborted. Of 23 cases with homozygous mutations, 21 were referred for legal abortion, and from the 2 remaining fetuses that were supposed to be healthy, one case was born with AR-CGD due to diagnostic error. Of 19 cases with no pathogenic mutations, one fetus was spontaneously aborted.

The prevalence of different PIDDs among fetuses that were legally aborted (n=27) is as follows: AR-CGD (n=2), XL-CGD (n=2), HLH (n=3), LADS (n=7), congenital neutropenia (n=1), SCID (n=5), WAS (n=3), and Griscelli syndrome (n=4). Healthy cases revealed normal immunological tests and were vaccinated during the one-year follow-up without any complication.

Finally, of 81 cases that underwent CVS for further clinical and genetic evaluations, 80 correct PND were made; while diagnostic errors occurred in one case (1.2%).

DISCUSSION

From a total of 100 pregnant cases that were referred for prenatal evaluations, CVS was done for 81 cases with a family history of PIDD. From these patients, twenty-seven fetuses were referred to ILMO to obtain permission for legal abortion due to detected mutation after CVS, 2 cases were spontaneously aborted, 51 cases were born healthy, and one case was born with PIDDs. From these 2 affected cases, one case was spontaneously aborted with SCID, while diagnostic error (1.2%) occurred in one case that was born with AR-CGD. The reason for the incorrect diagnosis may be due to insufficient CV samples that hindered reverse PCR. Among 19 individuals not referred for CVS due to different causes, spontaneous abortion occurred in 3 cases, 14 fetuses were born healthy, and 2 were affected with SCID because of late referral or unwillingness of undergoing the PND process.

Despite advances in our understanding of the pathogenesis and diagnostic methods of PIDDs, their management is still a major challenge. Considering the genetic and hereditary nature of these diseases and the high rate of consanguineous marriages in some countries like Iran,[15] it is predicted that the incidence of giving birth to babies with different types of PIDDs is high. Genetic counseling and prenatal diagnostic evaluations are important components in the management of PIDDs and may have a great role in decreasing the mortality and morbidity rate of every country. There are some limited case studies on PND of PIDDs but all of these diseases have not been addressed in a comprehensive study.[7-9,16,17]

Different sampling methods like CVS, amniocentesis, and cordocentesis are used for prenatal evaluation depending on the gestational age, among which CVS has been known as the most common and safest method in the first trimester. To avoid an incorrect diagnosis, it should be borne in mind that the rate of contamination with maternal cells is estimated to be as high as 16.7% in cytogenetic studies.[18,19] In a recent study, 4 pregnant women with 5 fetuses and definite diagnoses of LAD-1 in their previous children underwent CVS the result of which revealed 2 new mutations in 2 fetuses that were legally aborted.[20]

Some studies have used molecular and genetic methods and flow cytometry to evaluate the samples. In the study of Mishra et al., flow cytometry was used to evaluate 13 cases with a positive family history of PIDD.[21] The results showed that 9 cases were healthy, 3 cases were affected, and one sample could not be assessed due to contamination with maternal cells. They concluded that the results of prenatal and postnatal assessments were consistent and flow cytometry was introduced as a sensitive and rapid method in families with a definite diagnosis when molecular methods are not available.[21] However, flow cytometry was performed at 18 weeks of gestation and this time-point is not appropriate for Iranian families as there will be no time for legal abortion in case of diagnosing any kind of PIDD. Flow cytometry was also

used for PND of a series of immunodeficiency and hematologic diseases in the study of Curtis et al. The results showed that flow cytometry; especially protein-based and functional flow cytometry assays, can be used to confirm the diagnosis of PIDS.[22] The negative points about flow cytometry are that required reagents may not be available everywhere and that it is dependent on the operator's expertise. Moreover, this method is not useful in all immunodeficiency diseases and although it is less costly and less invasive for PND, no study has compared its sensitivity and specificity with genetic tests.[4]

Several studies have evaluated PND of CGD, as one of the most common PIDDs with definite genetic diagnostic methods, and have reported that the X-linked form is the most common,[11,23] which is in contrast to our findings. In our study, only 5 out of 16 cases of CGD were X-linked; this finding has been already presented in a comprehensive report from Iran.[15] In a study by Yavuz Köker et al in Turkey, a pregnant woman who was a CGD carrier underwent CVS at 12 weeks' gestational age and genetic testing was done on fetal DNA. The results indicated a healthy fetus. After birth (first postpartum week), dihydrorhodamine (DHR) flow cytometry assay confirmed the baby's health. The authors stated that a mutation study with direct sequencing is a proper method for PND of CGD.[7]

Since legal abortion of genetically approved affected fetuses is not allowed after 18 weeks' gestational age in Iran and DHR can be done on the cord blood after 18 weeks' gestational age,[24] this test is not recommended because legal abortion is not possible if the test is positive. A recent study has suggested an analysis of NADPH activity of fetal blood (FB) neutrophils for PND of CGD in families approaching late in pregnancy.[25] However, legal abortion may still not be applicable if positive results are obtained after 18 weeks of gestational age.

In all religions and all countries, there are cultural and religious limitations for abortion. In a study conducted in Israel in 2004, 3 out of 7 families with a history of SCID refused to undergo any PND procedure. In their study, prenatal assessments were done on 5 fetuses from 4 families; one of the fetuses was aborted spontaneously due to a homozygous mutation and four fetuses were diagnosed to have heterozygous mutations and their health was confirmed with molecular methods after birth.[12] Since many couples are counseled in our center and are provided with adequate information about the disease and its outcomes, the choice of abortion by families is not unexpected. Fortunately, abortion acceptance was very high in our study, and all the families undergoing CVS accepted to abort their fetus.

Although in our country, abortion is performed according to religious and legal rules, some incorrect cultural beliefs may result in denial of abortion in some families which can be considered as a limitation of this investigation. Additionally, the diagnostic error; although being compatible with previous studies (1.2%), is another limitation of the current investigation which could be resolved by the application of more novel modalities.

In conclusion, considering the high rate of consanguineous marriages in Iran and the high likelihood of PIDDs in children, especially in families with a positive history of PIDDs, the prenatal diagnosis will aid in preventing the birth of affected children, maintain the quality of life of the family, and reduce the financial burden of immunodeficiency diseases. We recommend performing the PND in susceptible families with a positive history of PIDDs in the center with substantial experience in the diagnosis of PIDDs to reduce the birth of affected children and decrease the related burdens.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGEMENTS

None

REFERENCES

1. Picard C, Al-Herz W, Bousfiha A, Casanova JL, Chatila T, Conley ME, et al. Primary Immunodeficiency Diseases: an Update on the Classification from the International Union of Immunological Societies Expert Committee for Primary Immunodeficiency 2015. J Clin Immunol. 2015;35(8):696-726.
2. Sponzilli I, Notarangelo LD. Severe Combined Immunodeficiency (SCID): from molecular basisto clinical management. Acta Biomed. 2010;82(1):5-13.
3. Ameratunga R, Woon S-T, Neas K, Love DR. The clinical utility of molecular diagnostic testing for primary

immune deficiency disorders: a case based review. Allergy Asthma Clin Immunol. 2010;6(1):12.
4. Abraham RS. Relevance of laboratory testing for the diagnosis of primary immunodeficiencies: a review of case-based examples of selected immunodeficiencies. Clin Mol Allergy. 2011;9(1):6.
5. Modell V, Gee B, Lewis DB, Orange JS, Roifman CM, Routes JM, et al. Global study of primary immunodeficiency diseases (PI)—diagnosis, treatment, and economic impact: an updated report from the Jeffrey Modell Foundation. Immunol Res. 2011;51(1):61.
6. Derpoorter C, Bordon V, Laureys G, Haerynck F, Lammens T. Genes at the Crossroad of Primary Immunodeficiencies and Cancer. Front Immunol. 2018;9:2544.
7. Ayşe M, Dirk R. Prenatal diagnosis of chronic granulomatous disease in a male fetus. Iranian J Allergy Asthma Immunol. 2009;8(1):57-61.
8. Ariga T, Iwamura M, Miyakawa T, Takahashi H, Mikami A, Sakiyama Y. Prenatal diagnosis of the Wiskott–Aldrich syndrome by PCR-based methods. Pediatr Int. 2001;43(6):716-9.
9. Madkaikar MR, Gupta M, Rao M, Ghosh K. Prenatal diagnosis of LAD-I on cord blood by flowcytometry. Indian J Pediatr. 2012;79(12):1605-9.
10. Wah YM, Leung TY, Cheng YKY, Sahota DS. Procedure-Related Fetal Loss following Chorionic Villus Sampling after First-Trimester Aneuploidy Screening. Fetal Diagn Ther. 2017;41(3):184-90.
11. Wessels T-M, Krause A. Genetic counselling in primary immunodeficiency disorders. Current Allergy & Clinical Immunology. 2012;25(4):199-203.
12. Tabori U, Mark Z, Amariglio N, Etzioni A, Golan H, Biloray B, et al. Detection of RAG mutations and prenatal diagnosis in families presenting with either T–B–severe combined immunodeficiency or Omenn's syndrome. Clini Genet. 2004;65(4):322-6.
13. Nadkarni A, Hariharan P. Molecular Techniques for Prenatal Diagnosis. Hematopathology: Advances in Understanding. 2019:469.
14. E. Richard Stiehm HDO, Jerry A. Winklestein. Part II (Primary Immunodeficiencies). Immunologic Disorders in Infants and Children2004. p. 289-355.
15. Saadat M, Ansari-Lari M, Farhud D. Short report consanguineous marriage in Iran. Ann Hum Biol. 2004;31(2):263-9.
16. DiSanto JP, Markiewicz S, Gauchat J-F, Bonnefoy J-Y, Fischer A, de Saint Basile G. Prenatal diagnosis of X-linked hyper-IgM syndrome. N Engl J Med. 1994;330(14):969-73.
17. Weening R, Bredius R, Wolf H, Van der Schoot C. Prenatal diagnostic procedure for leukocyte adhesion deficiency. Prenat Diagn. 1991;11(3):193-7.
18. Akolekar R, Beta J, Picciarelli G, Ogilvie C, d'Antonio F. Procedure-related risk of miscarriage following amniocentesis and chorionic villus sampling: a systematic review and meta-analysis. Ultrasound Obstet Gynecol. 2015;45(1):16-26.
19. Alfirevic Z, Navaratnam K, Mujezinovic F. Amniocentesis and chorionic villus sampling for prenatal diagnosis. The Cochrane Library. 2017.
20. Esmaeili B, Ghadami M, Fazlollahi MR, Niroomanesh S, Atarod L, Chavoshzadeh Z, et al. Prenatal diagnosis of leukocyte adhesion deficiency type-1 (five cases from iran with two new mutations). Iranian J Allergy Asthma Immunol. 2014;13(1):61-5.
21. Mishra A, Gupta M, Dalvi A, Ghosh K, Madkaikar M. Rapid Flow cytometric prenatal diagnosis of primary immunodeficiency (PID) disorders. J Clin Immunol. 2014;34(3):316-22.
22. Curtis MG, Walker B, Denny TN. Flow cytometric methods for prenatal and neonatal diagnosis. J Immunol Methods. 2011;363(2):198-209.
23. Winkelstein JA, Marino MC, Johnston Jr RB, Boyle J, Curnutte J, Gallin JI, et al. Chronic granulomatous disease: report on a national registry of 368 patients. Medicine. 2000;79(3):155-69.
24. Richardson M, Ayliffe M, Helbert M, Davies E. A simple flow cytometry assay using dihydrorhodamine for the measurement of the neutrophil respiratory burst in whole blood: comparison with the quantitative nitrobluetetrazolium test. J Immunol Methods. 1998;219(1):187-93.
25. Kulkarni M, Gupta M, Madkaikar M. Phenotypic Prenatal Diagnosis of Chronic Granulomatous Disease: A Useful Tool in The Absence Of Molecular Diagnosis. Scand J Immunol. 2017;86(6):486-90.