Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

Original Research

The first successful application of preimplantation genetic diagnosis for hearing loss in Iran

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Received September 23, 2017; **Accepted** June 26, 2018; **Published** June 30, 2018

Doi: http://dx.doi.org/10.14715/cmb/2018.64.9.11

Abstract: Hearing impairment (HI) caused by mutations in the connexin-26 gene (*GJB2*) accounts for the majority of cases with inherited, nonsyndromic sensorineural hearing loss. Due to the illegality of the abortion of deaf fetuses in Islamic countries, preimplantation genetic diagnosis (PGD) is a possible solution for afflicted families to have a healthy offspring. This study describes the first use of PGD for *GJB2* associated non-syndromic deafness in Iran. *GJB2* donor splicing site IVS1+1G>A mutation analysis was performed using Sanger sequencing for a total of 71 Iranian families with at least 1 deaf child diagnosed with non-syndromic deafness. In Vitro Fertilization (IVF) was performed, followed by PGD for a cousin couple with a 50% chance of having an affected child. Bi-allelic pathogenic mutations were found in a total of 12 families (~17 %); of which a couple was a PGD volunteer. The deaf woman in this family was homozygous and her husband was a carrier of the IVS1+1G>A gene mutation. Among 8 biopsied embryos, two healthy embryos were implanted which resulted in a single pregnancy and subsequent birth of a healthy baby boy. This is the first report of a successful application of PGD for hearing loss in Iran. Having a baby with a severe hearing impairment often imposes families with long-term disease burden and heavy therapy costs. Today PGD has provided an opportunity for high-risk individuals to avoid the birth of a deaf child.

Key words: Sensorineural deafness; Connexin-26; Assisted reproductive technology; Preimplantation genetic diagnosis.

Introduction

Hearing loss is a common birth defect with an incident rate of 1 out of 2000 newborns (1-4). Near 70% of hearing impairments are of genetic origin and more than half of these cases are inherited (5-7). The most common type of heritable hearing impairment is autosomal recessive non-syndromic hearing loss (ARNSHL) which is more prevalent among populations with a high rate of consanguineous marriage (8). Over the last 2 decades, more than 40 different genes have been reported to be associated with non-syndromic hearing impairment. Despite this level of heterogeneity, connexin-26 gene (*GJB2*) mutations account for the majority of cases with inherited sensorineural deafness (1).

GJB2 encodes connexin 26 protein (Cx26), a member of connexin family that in an oligomeric form assemble gap junction channels between neighboring cells in the inner ear (7, 9, 10). These intercellular communication systems are responsible for K^+ circulation in cochlea where the conversion of sound vibration into electrobiochemical impulse takes place and further transfers into the auditory nerve in the brain (11). Accumulation of mutations in connexin genes has been linked to hearing impairment (7). Near 200 mutations in *GJB2* have been reported in different populations most of which were in coding regions (12). However, few studies have indicated the involvement of *GJB2* non-coding region mutations in hearing loss. So far, a few noncoding mutations, including c.-259C>T, c.-23G>T, and c.-23+1G>A have been identified in different populations including Czech Republic, Turkey, and Hungary (10). Splice mutation c.-23+1G>A known as IVS1+1G>A has also been reported as the second most common mutation in the Iranian population with ARNSHL (12).

Hearing loss imposes psychological distress and health issues, especially in the developing countries. High frequency of hearing loss with its heavy cost burden for the individual and society in addition to its personal and social problems reinforces the importance of developing proper rehabilitation interventions. One rehabilitation intervention for patients with hearing impairment is Cochlear Implantation (CI). Although some patients can be rehabilitated using hearing aids, the results are inconsistent due to the age of implantation, residual hearing, and the way of communication.

Moreover, surgery and post-operative care place a heavy cost burden on families and society (13-15).

The advance of pre-implantation genetic diagnosis (PGD) technology brought about the opportunity to avoid the birth of a deaf child. Pre-implantation genetic testing includes genetic analysis of 1 or 2 biopsied cells from 6-8 cell embryos. Those embryos result from fertilizing retrieved eggs with sperm *in vitro*. Biopsied cells are then used to identify unaffected embryos before implantation (16, 17).

With regards to the low interest in abortion of a deaf fetus in different populations (18), it seems that the use of PGD can be welcomed more than prenatal diagnosis (PND) as it certifies that only unaffected embryos are transferred to the uterus. Moreover, by using polymorphic informative markers linked to the specific mutation, the chance of missing recombination events is decreased (2).

Iran with a consanguineous marriage rate of about 38.6% shows an incidence of approximately one in 166 newborns with hearing loss (19). Due to the illegality of aborting a deaf fetus in Iran, PGD is considered as the only solution available for Iranian parents with a medical history of deafness who do not want to have a deaf offspring.

The present study reports the results of a genetic testing for *GJB2* gene mutation performed on a group of deaf individuals of whom only one led to the PGD. As far as the authors are concerned, this is the first successful application of PGD for ARNSHL in Iran. This process resulted in a singleton pregnancy and subsequent birth of a healthy baby boy.

Materials and Methods

Subjects

A total of 71 families with at least one deaf member were investigated in this study. All of the participants were referred to Otolaryngology Department, Valieasr Hospital, between March 2012 to December 2015 (Imam Khomeini Hospital Complex, Tehran, Iran). Auditory brainstem responses (ABR) as well as otoacoustic emissions (*OAEs*) were measured to determine the sensorineural hearing loss. Genetic counseling sessions were arranged for all the families to get a proper understanding of the Hearing loss genetics. A questionnaire was filled in by all adult deaf individuals or parents with a deaf child. An informed consent form was signed by all participants. This study was approved by the Otorhinolaryngology Research Center of Tehran University of Medical Sciences, with the consideration of ethical issues.

Mutation Analysis

Peripheral blood samples were collected from volunteers for genomic DNA extraction using salting out method (20). Both coding and noncoding sequences of the *GJB2* gene including the exon–intron boundaries were investigated in one affected member of each family by direct sequencing method. This was performed using the BigDye Terminator v3.1 kit (Life Technologies, USA). Primer sequences are available upon request.

Determination of disease-associated haplotype

PGD quality assessment was performed on a cousin couple (a deaf woman and her hearing husband) who volunteered to do PGD. Single lymphocytes were isolated from fresh peripheral blood of the woman as previously described (21). Twelve short tandem repeat (STR) markers linked to the *GJB2* gene were designed and tested. Finally, 5 informative markers were selected to establish the disease-associated haplotype within the family (Table 1).

Five STR markers, D13GJB2SU40.7, D13GJB2SU16.5, D13GJB2SD9.5, D13GJB2SD10.6, and D13GJB2SD12, were located on chromosome 13 regions flanking the *GJB2* gene.

"D" stands for DNA. The following number is the chromosome where the STR marker is located. "U" and "D" stands for upstream and downstream, respectively and refer to the location of the STR marker compared to the gene of interest. The distances of D13GJB2SU40.7 and D13GJB2SU16.5 were estimated to be 4070 and 1650 kb upstream of the *GJB2* gene, respectively. STR markers D13GJB2SD9.5, D13GJB2SD10.6, and D13GJB2SD12 were 950, 1060 and 1200 kb downstream of *GJB2* gene, respectively.

In-vitro fertilization (IVF)

The selected couple had normal fertility criteria, thus a fresh IVF cycle was planned for them. Intracytoplasmic sperm injection (ICSI) was used to fertilize the ova and obtain embryos. Embryos were biopsied on day three. All the steps were carried out in Erfan Hospital (Tehran) and all biopsied blastomeres were sent to Kawsar human genetics research center (Tehran, Iran) for preimplantation genetic diagnosis.

Pre-implantation Genetic Diagnosis (PGD)

PCR Amplification

Single cell lysis was done according to Cui et al. (22). DNA was extracted by salting out method using KBC extraction kit (KBC co., Tehran, Iran). The first round of the nested PCR was performed using 5 pairs of outer primers to amplify DFNB1-linked microsatellites. a Mastercycler® pro (Eppendorf, Germany) was utilized and the following program was performed: 6 min denaturation at 96°C, followed by 15 cycles of 1 min at 96°C, 45s at 60°C, 1 min at 72°C, and 5 min at 72°C.

Table 1. STR markers designed for *GJB2* gene.

Kit Name	STR maker	Chromosome position	Distance from the gene (kb)
Deafness	D13GJB2SU40.7	chr13:24843253-24843465	4070 kb from upstream of gene
	D13GJB2SU16.5	chr13:22422656-22423020	1650 kb from upstream of gene
	D13GJB2SD9.5	chr13:19806578-19807031	950 kb from downstream of gene
	D13GJB2S10.6	chr13:19697590-19698109	1060 kb from downstream of gene
	D13GJB2SD12	chr13:19554432-19555232	1200 kb from downstream of gene

The quality of the PCR products was tested by running samples on 2% agarose gels.

For the second round of PCR, 1 µl of the primary PCR products was added to another tube containing 2 µl of 10X PCR Buffer (500 mMKCl, 100 mM Tris-HCl, pH 8.3), 200 mM of each dNTPs (Invitrogen, USA), 2 IU Taq DNA Polymerase (Invitrogen, USA) and 10 pmol of each inner primer. The targeted region was amplified in the second round PCR using the following conditions: 3 min denaturation at 93°C, followed by 10 cycles of 1 min at 93°C, 1 min at 64°C, 2 min at 72°C and finally 5 min at 72°C. Second PCR was carried out on Mastercycler® pro (Eppendorf, Germany).

Multiplex fluorescent PCR

Multiplex amplified products from the second-round PCR were tagged with different fluorochromes using labeled primers. Multiplex fluorescent PCR was performed as previously described by Piyamongkol et al. (23). The PCR conditions were as follows: initial activation of the Taq DNA Polymerase for 4 min at 94°C, followed by 30 cycles of 1 min denaturation at 94°C, annealing for 45 s at the 55 °C, and extension for 2 min at 72°C.

Results

Among the 71 investigated cases, a total of 12 families (~17 %) were identified with a pathogenic c.-23+1G>A mutation in both alleles. Of 12 candidate families, only one family was willing to do PGD. The woman in this family was homozygous for the c.-23+1G>A mutation and her husband was a carrier of this mutation. Therefore, the chance of having an affected child was 50 percent with each conception for this couple. Genotyping of the informative STR markers showed a homozygous haplotype in the woman and a heterozygous genotype for her husband. At the first IVF/PGD fresh cycle, eight embryos were biopsied (Figure1).

Among the biopsied embryos, three were diagnosed as healthy carriers of the c.-23+1G>A mutation that were suitable for embryo transfer. Three other embryos had a homozygous haplotype associated with the aforementioned mutation and two other embryos remained undiagnosed as they failed to amplify. Allele dropout (ADO) was observed only for one marker.

Two healthy embryos were transferred and one embryo was preserved. A singleton pregnancy was achieved. Prenatal diagnosis confirmed the PGD analysis and ultimately a healthy baby boy was delivered. For documentation, afterbirth, the hearing of the neonate was tested with ABR and OAEs; both determined a normal hearing.

Discussion

This study was developed to describe the first successful use of PGD for non-syndromic deafness caused by a mutation in *GJB2* gene which resulted in the birth of a healthy unaffected baby boy.

In this study, *GJB2* mutation screening in 71 unrelated deaf individuals from different families led to determining the cause of hearing loss in 12 families (~17 %). The detection rate in this study was similar to the findings of a recent study on genetic causes of hearing loss in Iran (12). In the aforementioned study, Zeinali et al., investigated *GJB2* c.−23+1G>A mutation in a total of 418 Iranian individuals with an autosomal recessive non-syndromic sensorineural hearing loss. Bi-allelic pathogenic mutations in the *GJB2* gene were found in 81 patients (~19.4 %). Besides, 13 cases were diagnosed with a monogenic mutation. Previously, Hashemzade et al., showed a frequency rate of 26.5% (9 out of 34 families) for *GJB2* mutations in the deaf families; however, the prevalence of this specific mutation was very low where only one among 34 studied families was determined as a carrier of this gene mutation (24). This amount of variation in the frequency of *GJB2* c.−23+1G>A mutation might be due to differences in the number and the ethnicity of the studied populations. A similar prevalence of this gene mutation (20%) has been reported

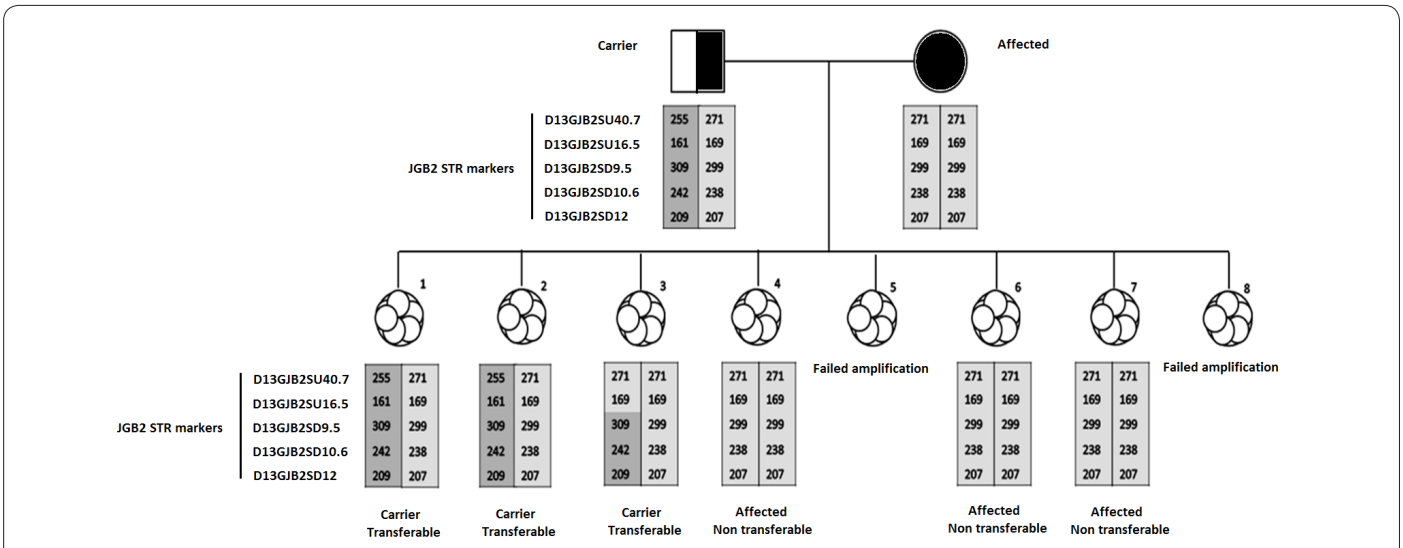

Figure 1. Preimplantation genetic haplotyping for hearing loss by genomic DNA analysis of *GJB2* gene linked markers. Haplotypes of father (carrier), mother (ARNSHL-affected) and 6 embryos are shown. The mutated and the wild-type alleles were depicted in light-shaded and dark-shaded columns, respectively. The homozygous haplotype (affected) linked to *GJB2* c.-23+1G>A mutation was presented in embryos No. 4, 6 & 7 while heterozygous haplotype (carrier) was observed in the embryos No. 1 & 2. A recombination was observed in the embryo No. 3. Since the crossing over was occurred between U16.5 and D9.5 of the gene, sequencing was done confirming that it was not affected. The embryos number 5 and 8 failed to amplify.

in Syrian population with a non-syndromic sensorineural hearing loss (25). The Yacut, a subarctic region of Russia, population has the highest prevalence of this gene mutation among studied Asian populations (26). A cohort study on patients with autosomal recessive deafness in Yacut showed a frequency rate of 51.82% for c.-23+1G>A mutation.

Among 12 families with the known cause of deafness, there was only one family who decided to do PGD. Fortunately, their fresh IVF/PGD ended up with the birth of a healthy baby boy. To the best of our knowledge, this is the first successful application of PGD for hearing loss in Iran. The first report of PGD for non-syndromic deafness was in 2009 by Altarescu et al., who demonstrated that blastomere biopsy in PGD analysis is a reliable method for non-syndromic deafness. They implemented 12 polymorphic biomarkers related to GJB2/GJB6 mutations in 14 couples. Their developed protocol resulted in the delivery of 3 healthy children (2).

A recent study by Xiong Wen Ping in 2015 is also another example of a successful application of PGD done for a family with a *GJB2*-associated hearing impairment to prevent transmission of the gene mutation causing hearing impairment to the newborn. They reported a successful delivery of a baby boy (27).

There is a growing interest in different populations for using assisted reproductive technologies as a possible solution for families with a high probability of having deaf children. It has been determined that cultural and religious backgrounds affect people's attitudes toward prenatal diagnosis (PND), and termination of pregnancy (TOP) for hearing loss or deafness. An example of such investigation is Alsulaiman et al.'s study in which 70 parents' attitudes towards (PND) and (TOP) were investigated. Concerning attitudes toward PND, there were an 81.4% average acceptance rate and a 25.2% average TOP acceptance rate among the participants (28). Another example is Middleton et al.'s study done in 2001. They studied individuals of whom 644 were deaf, 143 had a severe hearing loss, and 527 had normal hearing with either a deaf parent or a child. Their results showed that 21% of deaf individuals, 39% of individuals with severe hearing loss and 49% of cases with normal hearing had a tendency to use PND for preventing birth of a deaf child. Six percent of the first group, 11% of the second group, and 16% of the third group said that they would prefer a TOP if the deafness of the unborn baby was definite. It was interesting that two percent of the deaf participants said they would prefer to have a deaf child and would consider a TOP if the fetus was identified with a normal hearing (29).

Overall, knowing that *GJB2*-associated gene mutations are the most common cause of hearing impairment imposes significant considerations on conducting more studies to examine this gene to prevent the birth of infants with hearing problem. In Iran, aborting a fetus with hearing loss is prohibited. On top of that, religious beliefs, and cultural and emotional factors prevent parents from terminating a pregnancy. As a solution, preimplantation genetic testing provides an opportunity for high-risk individuals to avoid birth of a deaf child. As the number of *GJB2* positive families in the present study was not enough to conclude the tendency of Iranian deaf families to do PGD, further studies are required to investigate Iranian attitudes toward PGD and provide proper conditions for such families who like to have normal hearing children. Since financial issues may affect interest rate and may stop some parents from performing pre-implantation genetic testing, governments should provide financial support to deal with this issue.

Acknowledgement

We would like to thank our nursing team including Nayereh Seyedmovaghar, Zhaleh Mohammadi, Sedigheh Sharifi and Yeganeh Ferassati for their kind assistance in collecting blood samples. We would also like to thank the audiology team of Valiasr Hospital for their sincere collaboration. This study was supported by the Kawsar Human Genetics Research Center.

Interest Conflict

All authors declared no conflict of interest.

Authors Contribution

Alireza Karimi Yazdi, Dr.Fatemeh Ramezanzadeh, Dr. Saeid Amanpour and Dr.

Sirous Zeinali were involved in initial study design. Sample and data collection was performed by Mahtab Rabbani Anari, Ali Eftekharian and and Ardavan Tajdini. Solmaz Sabeghi, Kiyana Fatemi, and Zohre Sharifi did participate in pre-PGD and PGD process. Elham Davoudi-Dehaghani, Paanti Fouladi, and Elmira Ebrahimi wrote the preliminary draft of the manuscript.

Dr. Amanpour & Dr. Karimi were involved in the revisions of the manuscript.

All authors approved the final version of the manuscript.

References

1. Mani RS, Ganapathy A, Jalvi R, Srisailapathy CS, Malhotra V, Chadha S, et al. Functional consequences of novel connexin 26 mutations associated with hereditary hearing loss. European Journal of Human Genetics. 2009;17(4):502-9.

2. Altarescu G, Eldar-Geva T, Brooks B, Zylber-Haran E, Varshaver I, Margalioth EJ, et al. Preimplantation genetic diagnosis (PGD) for nonsyndromic deafness by polar body and blastomere biopsy. Journal of assisted reproduction and genetics. 2009;26(7):391-7.

3. Rabionet R, Gasparini P, Estivill X. Molecular genetics of hearing impairment due to mutations in gap junction genes encoding beta connexins. Human mutation. 2000;16(3):190.

4. Bakhchane A, Bousfiha A, Charoute H, Salime S, Detsouli M, Snoussi K, et al. Update of the spectrum of GJB2 gene mutations in 152 Moroccan families with autosomal recessive nonsyndromic hearing loss. European journal of medical genetics. 2016;59(6):325-9.

5. Lazăr C, Popp R, Trifa A, Mocanu C, Mihut G, Al-Khzouz C, et al. Prevalence of the c. 35delG and p. W24X mutations in the GJB2 gene in patients with nonsyndromic hearing loss from North-West Romania. International journal of pediatric otorhinolaryngology. 2010;74(4):351-5.

6. Tóth T, Kupka S, Haack B, Riemann K, Braun S, Fazakas F, et al. GJB2 mutations in patients with non-syndromic hearing loss from Northeastern Hungary. Human mutation. 2004;23(6):631-2.

7. Bundey S, Alam H. A five-year prospective study of the health of children in different ethnic groups, with particular reference to the effect of inbreeding. European journal of human genetics: EJHG. 1992;1(3):206-19.

8. Snoeckx RL, Huygen PL, Feldmann D, Marlin S, Denoyelle

F, Waligora J, et al. GJB2 mutations and degree of hearing loss: a multicenter study. The American Journal of Human Genetics. 2005;77(6):945-57.
9. Kelley PM, Harris DJ, Comer BC, Askew JW, Fowler T, Smith SD, et al. Novel mutations in the connexin 26 gene (GJB2) that cause autosomal recessive (DFNB1) hearing loss. The American Journal of Human Genetics. 1998;62(4):792-9.
10. Matos T, Simões-Teixeira H, Caria H, Cascao R, Rosa H, O'Neill A, et al. Assessing noncoding sequence variants of GJB2 for hearing loss association. Genetics research international. 2011;2011.
11. Mahdieh N, Rabbani B, Wiley S, Akbari MT, Zeinali S. Genetic causes of nonsyndromic hearing loss in Iran in comparison with other populations. Journal of human genetics. 2010;55(10):639-48.
12. Zeinali S, Davoudi-Dehaghani E, Azadmehr S, DabbaghBagheri S, Bagherian H, Jamali M, et al. GJB2 c.− 23+ 1G> A mutation is second most common mutation among Iranian individuals with autosomal recessive hearing loss. European Archives of Oto-Rhino-Laryngology. 2015;272(9):2255-9.
13. Ryu N-G, Moon IJ, Chang YS, Kim BK, Chung W-H, Cho Y-S, et al. Cochlear implantation for profound hearing loss after multimodal treatment for neuroblastoma in children. Clinical and experimental otorhinolaryngology. 2015;8(4):329.
14. Iizuka T, Kamiya K, Gotoh S, Sugitani Y, Suzuki M, Noda T, et al. Perinatal Gjb2 gene transfer rescues hearing in a mouse model of hereditary deafness. Human molecular genetics. 2015;24(13):3651-61.
15. Hao Y, Chen D, Zhang Z, Zhou P, Cao Y, Wei Z, et al. Successful preimplantation genetic diagnosis by targeted next-generation sequencing on an ion torrent personal genome machine platform. Oncology letters. 2018;15(4):4296-302.
16. Verlinsky Y, Cohen J, Munne S, Gianaroli L, Simpson JL, Ferraretti AP, et al. Over a decade of experience with preimplantation genetic diagnosis: a multicenter report. Fertility and sterility. 2004;82(2):292-4.
17. Middleton A, Hewison J, Mueller RF. Attitudes of deaf adults toward genetic testing for hereditary deafness. The American Journal of Human Genetics. 1998;63(4):1175-80.
18. Sloan-Heggen CM, Babanejad M, Beheshtian M, Simpson AC, Booth KT, Ardalani F, et al. Characterising the spectrum of autosomal recessive hereditary hearing loss in Iran. Journal of medical genetics. 2015:jmedgenet-2015-103389.
19. Saadat M, Ansari-Lari M, Farhud D. Short report consanguineous marriage in Iran. Annals of human biology. 2004;31(2):263-9.
20. Miller S, Dykes D, Polesky H. A simple salting out procedure for extracting DNA from human nucleated cells Nucleic Acids Res 16: 1215. Find this article online. 1988.
21. Hussey ND, Donggui H, Froiland DA, Hussey DJ, Haan EA, Matthews CD, et al. Analysis of five Duchenne muscular dystrophy exons and gender determination using conventional duplex polymerase chain reaction on single cells. Molecular human reproduction. 1999;5(11):1089-94.
22. Cui X, Li H, Goradia TM, Lange K, Kazazian HH, Galas D, et al. Single-sperm typing: determination of genetic distance between the G gamma-globin and parathyroid hormone loci by using the polymerase chain reaction and allele-specific oligomers. Proceedings of the National Academy of Sciences. 1989;86(23):9389-93.
23. Piyamongkol W, Harper JC, Delhanty JD, Wells D. Preimplantation genetic diagnostic protocols for α-and β-thalassaemias using multiplex fluorescent PCR. Prenatal diagnosis. 2001;21(9):753-9.
24. Chaleshtori MH, Farhud D, Taylor R, Hadavi V, Patton M, Afzal A. Deafness–associated connexin 26 gene (GJB2) mutations in Iranian population. Iranian Journal of Public Health. 2002;31(3-4):75-9.
25. Al-Achkar W, Al-Halabi B, Ali B, Moassass F. First report of prevalence c. IVS1+ 1G> A and del (GJB6-13S1854) mutations in Syrian families with non-syndromic sensorineural hearing loss. International journal of pediatric otorhinolaryngology. 2017;92:82.
26. Barashkov NA, Dzhemileva LU, Fedorova SA, Teryutin FM, Posukh OL, Fedotova EE, et al. Autosomal recessive deafness 1A (DFNB1A) in Yakut population isolate in Eastern Siberia: extensive accumulation of the splice site mutation IVS1+ 1G> A in GJB2 gene as a result of founder effect. Journal of human genetics. 2011;56(9):631-9.
27. Xiong W, Wang D, Gao Y, Gao Y, Wang H, Guan J, et al. Reproductive management through integration of PGD and MPS-based noninvasive prenatal screening/diagnosis for a family with GJB2-associated hearing impairment. Science China Life Sciences. 2015;58(9):829.
28. Alsulaiman A, Mousa A, Kondkar AA, Abu-Amero KK. Attitudes of Saudi parents with a deaf child towards prenatal diagnosis and termination of pregnancy. Prenatal diagnosis. 2014;34(2):153-8.
29. Middleton A, Hewison J, Mueller R. Prenatal diagnosis for inherited deafness—what is the potential demand? Journal of genetic counseling. 2001;10(2):121-31.24.