DNA Marker Screening for High-Risk Non-syndromic Hearing Loss Associated to Gene Mutations

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Abstract

Background: Congenital hearing loss is a debilitating disease affecting 1–3 out of 1,000 live births. According to WHO's associated figures, in both ears, 278 million people globally suffer from moderate to extreme hearing loss. Most hearing-loss individuals live in developing countries. Many deafness and hearing problems cases were documented in our region. Those cases' exact cause is still unknown, so we performed this study and aimed to screen DNA in high-risk Non-syndromic hearing loss patients in Arbil city. **Methods:** This researchscreened 132 blood samples from (80 newborns and 52 individuals) **at** (Hiwa Institute for deaf and mutes); their ages patients from 14 to 22 years old**.***MTRNR1*genes were performed for molecular detection of mutant genes. The mutation gene wasamplified by multiplex tetra primer PCR. **Result:**G- mito-1555-F1, mito-1555-R1 (O), mito-1555-F2 (I), and mito-1555-R2 (I) hearing loss mutations were not observed in 132 blood samples from both classes and genotyped in MTRNR1. For mtDNA 12S rRNA mt.1555A>G, no mutant alleles were detected in all of the tests, and no false-positives were identified.Using all primers, fifty-two samples were easily separated on 2% agarose gel; two were outer primers, and others are inner primers. Two separate bands were observed with 52 molecular samples (wild type at 254bp and control at 341bp).Of 80 samples, 28 have control bands at 341 bp. We did not find any mutation in our 80 samples. **Conclusion**: MTRNR1 mutation genes were not present in collected samples in deafness-related mutation. Genetic tests for the deafness gene can better diagnose infant congenital NSHL cases than conventional screening procedures.

Keywords: *NSHL, Gene, Mutation, Tetra-Primer ARMS PCR*

Introduction

One of the most famous birth defects, the congenital birth defect deafness, is one of the most common sensory disorders of humans. The incidence before the age of 5 years is 2.7 / 1000, and in puberty, it rises to 3.5 per 1000.[1,2] Once a single gene mutation has occurred, it is unlikely that other genes are the primary culprit. Most likely, hybrid causes have one gene mutation and other environmental factors [3]. The role of both deafness's may be caused by biology and the climate. Fifty percent, in truth, all childhood deafness is known to be related to genetics.[4-6]

In the developed world, preinjury is the leading cause of death for babies younger than 37 weeks gestation.[7-8]Sensorineural infection is a widespread impairment in preterm infants.[9] Hearing deficiency or deafness is found in 7% of preterm babies.[8]A serious concern associated with the use of aminoglycoside

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is being present in infections, life support systems, and neonatal intensive care units.[10]As the perceptual, gestational age leads to hearing loss if an infant is born older, the mechanism remains underdeveloped. Extreme hyperbilirubinemia, which causes 80 percent of preterm hyperbilirubinemia, the risk of sensorineural hearing also increases for infants.[11] Hearing deficiency in premature babies may be progressive or progressive in the infant's hearing loss three years later; delayed-onset; at age.[12]The congenital hearing condition where there is no evidence of disease, such as with or without congenital deafness (NSHL). In certain circumstances, NSHL is caused by a single gene with the remaining 30 percent expressed in various signs.[8]Monogenic hearing loss can be hereditary. At 80 percent, autosomal recessive hearing loss (ARNSHL) occurs in usually pre-lingual. However, in about 20% of the cases, hearing deficits are caused by autosomal dominant non-syndromic and syndromic conditions. As part of a complicated succession mechanism of inheritance, 1% of cases (one in a thousand) are attributed to either the X-chromosome or the mitochondria.[11]Heteroplasmas Deferred extension, also known as the MTR1 12S mitochondrial ribosomal-s gene, is a hot spot for sensory hearing deficiency mutations.In total, it has been reported that over 30 mutations cause in MTRNR1[13], a condition such as m.1555A, non-syndromic hearing loss mutations of m.1494C>T and m.1095T>C.[14,15]Bilateral, incremental, and sensorineural hearing loss also occurs with mitochondrial mutations. A few additional neurological symptoms are observed. The severity of hearing loss can range from moderate to absolute lack of hearing.It has been estimated that m.1555A> G's penetrance is between 28-75 percent, with an average of about 60 percent. The date of birth ranges from early birth to adulthood.To date, it has been found that 46 genes are causally linked to NSHL. There are different frequencies of mutations in these genes across multiple ethnicities.[2,16]It appears to differ widely in the degree of mitochondrial hearing loss prevalence among populations. In Asian countries, the frequency of m.1555A>G has been estimated to be higher than in the rest of the world[6,17]however, one may also say that differences between countries and regions also exist.[18]Based on large-scale epidemiological studies, in a study in China were done in 28 provinces and municipalities, over 90% of those with a hereditary hearing impairment" (>80%).[13,15,19]

It is estimated that between 10 and 30% of Finnish people have non-syndromic hearing loss, the frequency of me.1555A>G,it is reported to be 2.6% Some research studies show that in Japan suggests that the percentage of deaf and hard of hearing individuals in Japan is about 5%. Among Spanish individuals with hearing loss, the prevalence is higher than 23%.[18] The mutation frequencies of M.1494C>T and m.1095T>C were similarly stated to remain hidden in Asian populations and go unnoticed by other populations.[8]

In some instances, aminoglycoside antibiotics can bind to the human mitochondrial DNA. It has been suggested that a mutation could be used to transform the human mitochondrial ribosome into a bacterial ribosome, improving the binding affinity of the drug molecules. A single dose of aminoglycosides can cause deafness at any age for the mutation carrier MTRNR1.[5]However, premature and newborns and infants are more sensitive. Often premature babies are born and develop viruses and infections, and sepsis, with aminoglycoside treatment being given to up to 90% of preterm infants.[7]The most important factor affecting a child's ability to acquire language is when hearing impairment is found.[7]It is also hampered by early identification, early diagnosis, and intervention may help to improve listening voice, and communication in children.[15]

In satisfying the needs of neonates with hearing damage, early diagnosis is of critical significance. Universal screening costs for the diagnosis of newborns with hearing damage are comparatively low[9]and can be economically advantageous even in developed countries.[20]Also, the discovery of mutations in aminoglycoside-induced mitochondrial

genes. Deafness may prevent these medications from being misused. Studies have demonstrated that concomitant gene screening for newborn hearing is an essential supplement to traditional hearing tests for better infant care[21] and can recognize infants whose hearing loss exists.[10]

For a small number of different versions and samples, PCR is exceptionally cost-effective, readily suitable at different health care distribution sites, and tests directly aimed at NSHL-associated variants have been licensed for use in clinical diagnosis.[2]However, standard PCR had difficulties in scaling to vast numbers of samples, with various approaches in play.[22]The most accurate and efficient way to recognize hereditary variations linked to hearing loss in public health efforts remains unanswered. [8,23]Therefore, this study aimed to investigate the same genetically mutant gene among newborns with Nonsyndromic Hearing Losscompared to healthy individuals.

Materials and Methods

Patients and sample collection

This research has been carried out throughout the time between November 2019 till October 2020. **The study enrolled a total of** 80 newborns**with non-**syndromic hearing loss **and 52**healthy individuals**at** (Hiwa Institute for deaf and mutes) **their** ages patients from 14 to 22 years old**.** After gaining the Medical Research Center/ Hawler Medical University's ethics committee's approval, the study was undertaken. Samples were collected inpatients whom specialized physicians diagnosed. Three ml of peripheral blood samples wereperformed and aseptically collected by syringe and transferred intoan EDTA tube, then transferred to the laboratory for further tests.

Preparing the Primers

Lyophilized forward and reverse primers of *MTRNR1*gene:

mt.1555A>G, mt.1555A>G-mito-1555-F1 (O) (TGTAGCCCATGAGGTGGCAAGAAATG), mito-1555-R1(O) (TTAGCTCAGAGCGGTCAAGTTAAGTTGAAA) mito-1555-F2(I) (TTTATATAGAGTAGG), mito-1555-R2 (I) (CACTTTCCAGTACACT TACCATGTTACGACGTGT).

This procedure was performed depending on the manufacturer's instruction by dissolving the lyophilized sample with nuclease-free water to give the final concentration (100 pM/µl) (as a stock solution) then rotating down briefly. To prepare 10µM of working primer (working aliquot), re-suspended tenpM/µl of stock primer in 90µl of deionized water to reach a final concentration of 10µM.These primers were synthesized by GeNet Bio Company (Korea).

Molecular Mutation Detection in MTRNR1 mt.1555A>G gene by Tetra-Primer ARMS PCR

DNAwas collected from blood samples and genotyped using the genomic extraction kits (Addbio/ Korea), depending on the manufacturer's instructions. A Nanodrop spectrophotometer performed forthe extracted genomic DNA was tested to estimate the extracted DNA's concentration and purity of the absorbance reading at (260 /280 nm).

The *MTRNR1* mt.1555A>G mutation was identified by multiplex tetra primer amplification mutation system PCR. The total of 25 µl PCR master mix reaction volume was performed containing 3µl of genomic DNA, 12.5 µl of 2X GoTaqGreen Master Mix(Promega, USA), and 1.5µl was added for each of the forward and reversed primers of MTRNR1 mt.1555A>G-mito-1555-F1,mito-1555-R1(O),mito-1555-F2 (I),mito-1555-R2 (I)[24]then the mixture was completed by adding 3.5 µl of nuclease-free water.Amplification was initiated according to the manufacturers' instructionsan initial denaturation at 94°C for 5 minutes, 35 intervals accompany it at 94°C for 30 sec, 59°C for 30 sec, 72°C for 1 minute, and the last extension at 72°C for 5 minutes.The components of the PCR are lined with 2% agarose. The 254bp, 156bp, and 341bp signify wild form, mutation, and regulation presence, respectively.

Result

A total of 132 (80 newborns and 52 individuals) were genotyped for mt.1555A>G, G-mito-1555-F1,mito-1555-R1 (O),mito-1555-F2 (I),andmito-1555-R2 (I) hearing loss mutations in MTRNR1. No mutant alleles were observed among all samplesfor mtDNA 12S rRNA mt.1555A>G,and no false-positive were found. Allowing just 52 samples to be run on a 2% agarose gel gives an almost transparent cut-and-and-dried result; two of them are outer primers, and others are inner primers. Two different bands were observed with 52 samples with different molecular sizes (wild type at 254bp and control at 341bp). 28 out of 80 samples have only control bands at 341 bp. In this study, we could not detect any mutation in our 80 samples.

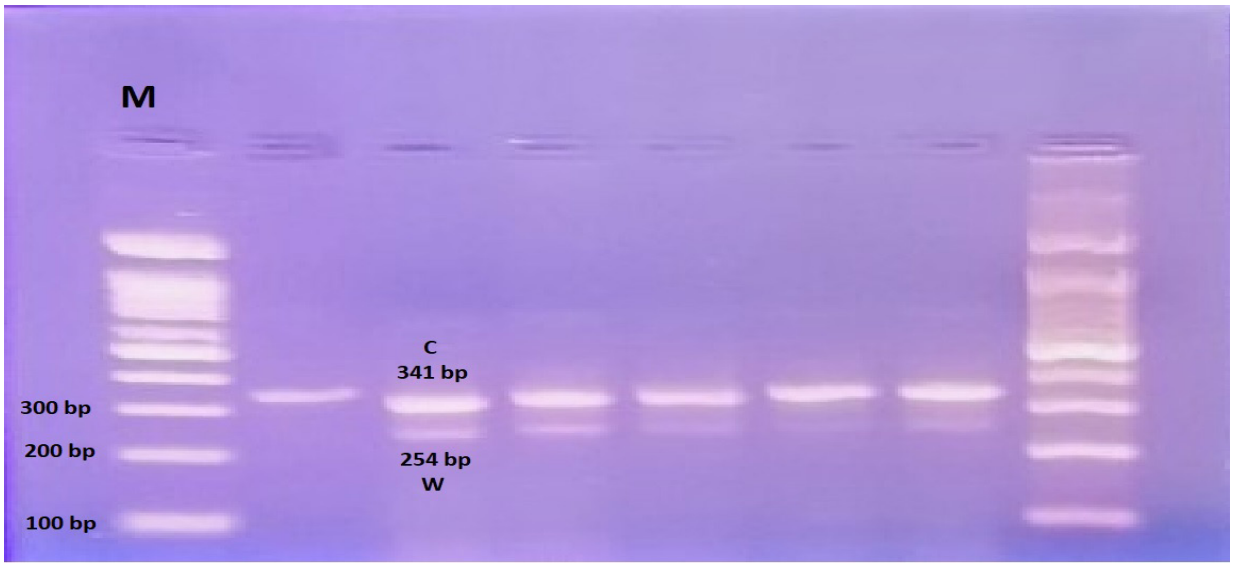

Figure 1: PCR for gene MTRNR1 mt.1555A>G100bp M(marker) , 254 bp W(wild type), 341bp C(control)

Discussion

The investigation of hereditary deafness and other genetic conditions has increased significantly with the advent of genomics. Population-specific hearing loss mutations have been found by innovative genetic technology, which continues to change and become more widespread.[23,25]Many researchers have shown that the approximate figures range from 60 to 80% of pre-lingual (HL) and (NSHL) is caused by genetic causes, with empirical evidence from developing countries indicating that 60% of deaf people have inherited deafness.[8, 11, 14, 22, 24, 26, 27]Deaf disease gene translation and cloning have made significant strides since the human genome project was completed. The fundamental hereditary susceptibility to NSHL has been increasingly revealed by molecular genetics and its molecular epidemiology.[28]The positive screening figure has been 1% in China since the UNHS was broadly adopted in 2000, 2.5–3% were seen for the trials that had included 5-year-olds and juvenile respondents, and there has been other research that has seen prevalence estimates of 3.4–4% for 14-year-olds a sharp rise in the incidence of neonatal hearing loss and drug-induced hearing loss.[21-29]This scenario highlights the severe drawbacks of relying entirely on a newborn hearing screening to diagnose NSHL. This method does not diagnose the drug-induced deafness hearing gene carrier or those of delayed-type deafness. The MTRNR1 mt.1555A>G gene was performed for Nonsyndromic Hearing Lossgenotyping purposes; 132 samples (80 newborns and 52 individuals) were enrolled. For the mtDNA 12S rRNA mt.1555A>G, no

mutant alleles were found in any of the samples, and no false-positive were found. Two distinct bands were discovered with 52 samples of different molecular sizes (wild type at 254bp and control at 341bp). However, only control bands at 341 bp are found in 28 of the 80 samples. In a study by [2]in China, among 27573 newborn samples, 1810 carried pathogenic mutations. Furthermore,[2] recorded (0.298%) NSHL mutant MTRNR1gene, which does not agree with our findings. Our research findings for the infant and stable adults were distinct, most likely due to genetic trends in the two groups. The sample size may have impacted these effects.Since it's commonly correlated with non-syndromic hearing loss, the m.1555A>G entails a middle non-syndromic hearing loss in the mother and brother of the patient.[26]Furthermore, the m.1555A>G mutation results in a wide range of family members' clinical phenotypes. In reality, the penetrance of this mutation varies between families. Moreover,[26]detected MTRNR1gene in patients using PCR-RFLP using the restriction enzymeHaeIIIin Tunisia family members.

On a 2.5 percent agarose gel, all samples were quickly separated (Fig. 1).[24]screened 1181 newborns for NSHL mutant genes, they found 29 newborns had one or two mutant alleles; for MTRNR1 mt.1555A>G mutation, just one exception was found.[24]

As has been associated with genetic and acquired hearing loss, as well as an aminoglycoside-induced mutation.[9,30,31]Tetra-primer ARMS-R has been widely reported. Our study used point mutation genotyping methods have been the most commonly used.[24,30]The flow system used in the Tetra ARMS-R package, which includes running DNA through an agarose gel and examining it under a microscope for three separate lanes, has been significantly improved. We also found that a genotype usually takes about three hours.It has few necessary mutations and insertions, so it is an efficient and straightforward molecular classification technique that only requires a small quantity of material for typing. While the kit for large-scale population genotyping detection is successful, the Tetra-primer ARMS-PCR findings are less accurate and applicable. They have a hazy picture of the nucleotide they're looking for; additional recheck procedures are expected to better clarify the Tetra-primer ARMS-PCR kit's performance. This technique is a crucial factor in avoiding false positives and negatives.

Finally, we show that the Tetra-primer ARMS-PCR kit mentioned here is a suitable method for use in a wide range of smaller settings, especially in Iraq's underdeveloped rural areas. Larger-scale epidemiological research on inherited hearing loss in Iraq is required in the future to incorporate more diagnostic targets and develop molecular diagnosis and genetic therapy.

Conflict of Interests: None.

Source of Funding: Self.

Ethical clearance: The study was undertaken after gaining the Medical Research Center/ Hawler Medical University's ethics committee's approval.

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