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Original Research Article

Prevalence of congenital non syndromic hearing loss among offspring of consanguineous marriage: a pilot study

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ABSTRACT

Background: Mutations in the gene encoding the gap-junction protein connexin-26, is understood to be the most important cause of non-syndromic hearing loss (NSHL). An attempt to identify the single nucleotide polymorphism (SNP) for W24X mutation was done. Consanguineous marriage was seen among the NSHL subjects.
Methods: SNP was identified using restriction fragment length polymorphism-polymerase chain reaction (RFLP-PCR). Forty-five subjects were screened for congenital hearing loss. Twenty subjects matched the inclusion criteria and were included in the study.
Results: 5 out of 20 subjects were found to have mutation i.e., 25%. Though consanguinity is known to cause autosomal recessive defect, the same could not be depicted in this study.
Conclusions: 25% of the study population had a mutation in their gene and the rest though had consanguineous marriage had not been affected genotypically.

Keywords: Hearing impairment, GJB2 mutation, W24X gene, RFLP

INTRODUCTION

An estimated prevalence of one in 1000 new born is seen among the hearing loss because of which normal communication, speech acquisition is hindered.[1,2] Palliative treatment with special education is needed as early as possible. Researchers have shown that the reason for hearing impairment among the children with prelingual hearing impairment is inherited and is autosomal recessive type. Though there are about 100 loci linked to congenital deafness, one among them is the gap junction beta 2 or the GJB2 gene, which is located at chromosome 13q12. This gene encodes for the protein connexin 26 which is mostly present in the cochlea and in the epidermis.[3]

Connexin 26 a small molecule which is actually a gap-junction protein, functioning in cell-to-cell diffusion of small molecules which is necessary for recycling of potassium in the cochlea is required for sensori neural hearing function.[4] This was the first gene to be associated with autosomal recessive form of hearing loss.[3,5] Connexin 26 mutations are responsible for at least 20% of all genetic hearing loss and 10% of all childhood hearing loss. In Indian population, more than 80 percent of cases of non-syndromic recessive deafness result from a mutated connexin 26 gene (pW24X).[6-8]

Studies have reported that the genetic and environmental issues are the causes of congenital deafness and early onset hearing impairment.

Consanguinity can affect the incidence of deafness in a population by expression of recessive genes. Hence genetic inheritance through consanguinity will increase the endurance and spread of defective hearing impairment

gene. Theoretically in consanguine marriage the initial phenotypic expression of genes is seen in these population hence the need of the study.

METHODS

After the approval of the study by institutional ethical clearance board of our institute, screening for non-syndromic congenital sensorineural hearing loss (SNHL) for GJB2 mutations was done at speech and hearing center of Mc Ganns teaching district hospital. This was a prospective study, where in subjects were selected as per the inclusion criteria. The study data represents information conducted over a period of one year (January to December 2019). We screened 45 unrelated Indian subjects with an age group within 21years. Subjects from Taranga school, Shimoga were included for this study. Most of the subjects were presented with severe-to-profound congenital hearing loss on pure-tone audiography, immittance audiometry, oto-acoustic emissions and evoked auditory brainstem response evaluation. These audiological tools were utilized for screening of all patients and to exclude those who do not match our criteria.

Patients who had conductive hearing loss, syndromic hearing loss, had taken ototoxic drugs, history of otorrhea, head trauma, meningitis, NICU admissions, kernicterus, any other perinatal pathology, maternal complications during pregnancy, or history of maternal consumption of ototoxic drugs during pregnancy; or any other known causes of hearing loss were not included in this study.

Two milliliters of blood samples were collected from each of the 20 patients, in EDTA vacutainers, after obtaining written informed consent from their respective parents (if minor). 25 subjects were excluded since they did not fulfill the inclusion criteria. DNA was isolated from these blood samples using Himedia blood genomic isolation kit. The DNA was aliquoted and stored at -80^{o} C.

Single nucleotide polymorphism was identified using RFLP after the polymerase chain reaction (PCR). Desalted primers were ordered from Metabion International Agency, Germany by Hysel India Pvt Ltd. The following primers were used for PCR (5'-TCT TTT CCA GAG CAA ACC GC-3' and 5'-GAC ACG AAG ATC AGC TGC AG-3').

PCR was performed using Himedia Taq mixture for a total reaction volume of 40 µl for 40 cycles (each cycle of 94° C for 30 seconds, 60° C for 30 seconds, and 72° C for 60 seconds). The PCR products were run on 1.5% agarose gel to visualize the bands. The products were then subjected to ALU I restriction digestion for overnight (ILS).

The subjects with no mutation in the gene of interest showed single band (286 bp) with two bands as homozygous (182,144 bp) and with three bands as heterozygous (286,182,144 bp).

For analysis, the data was entered in excel sheet and frequency in percentage was calculated based on the genotype.

RESULTS

We had 20 subjects with congenital sensorineural hearing loss. There were totally14 males and 6 females. Five out of 20 subjects were found to have mutation i.e., 25%. Among which 2 subjects were heterozygous and 3 were homozygous as shown in (Table 1).

Table 1: Genotype of the probands selected.

Gender	Total screened for SNP	Single band	Hetero-zygous	Homo-zygous
Male	14	11	1	2
Female	6	4	1	1
Total (%)	100	75	10	15

Table 2: Description of genotype for offsprings of consanguineous union.

	Not affected	Heterozygous	Homozygous	
First degree	8		1	9 (4 males, 4 females and 1 female)
Second degree		1	1	2 (1 male, 1 female)
Third degree	6	1		7 (7 male)
Non consanguineous	2		1	2 (2 males and 1 female)

As shown in the PCR gel figure, we observed that the subjects with single band are not affected by the mutation. Whereas the subjects with three bands are considered to be heterozygous (sample number 3) and subjects with two bands are homozygous (sample number 9 and 10). Since mutation in the gene creates a restriction site in the DNA, ALU I restriction enzyme cuts only those fragments of DNA where in a restriction site is generated. In the heterozygous subjects only one of the alleles are affected whereas in homozygous subjects both the alleles are affected (Figure 1).

The offspring of consanguineous unions are known to be at increased risk to genetic disorders. Since this research is about congenital sensorineural hearaling loss which is an autosomal recessive disorder, an insight into their presence or absence of consanguineous marriage is shown in (Table 2).

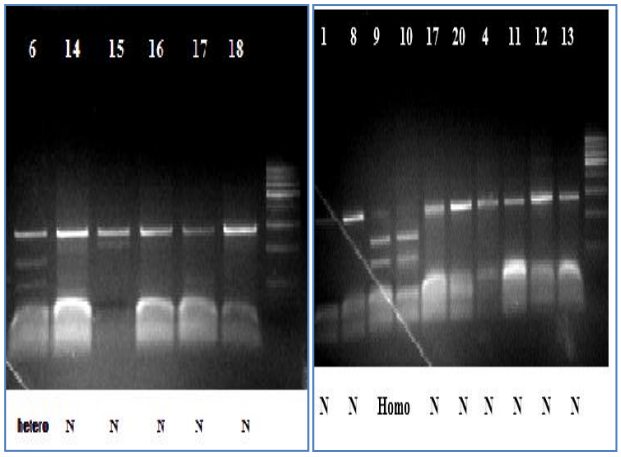

Figure 1: Agarose gel electrophoresis of restriction digestion of W24X gene polymorphism with ladder in the last lane.

Though 85% of the study population were a result of consanguineous marriage, their genotype was not affected by this W24X gene. Hence a Single band was observed and were grouped as not affected. Three subjects were homozygous among which one subject was not a result of consanguineous union hence with two bands. Two were heterozygous each second and third degree consanguineous and were with three bands.

DISCUSSION

This is a first study in Shimoga area presenting the prevalence of connexin 26 mutation (W24X) in the selected population. W24X is considered to be the most common single nucleotide polymorphism seen in the probands.

Though large diverseness is observed in hearing loss, most of the hearing impairment has been assigned to variants of GJB2 gene. A study done of Ashkenazi Jewish citizens have shown the 167 del T mutation to be more frequent in them, in Caucasians and in Mediterranean region 35delG was common and in Japanese subjects 235delC was more prevalent.[9-11]

A study by Shankar et al from southern and western parts of india has identified 17.7% of congenital deaf probands having biallelic mutation for connexin protein among which 95% were to W24X gene mutation.[7]

In our preliminary study 15% of probands were homozygous and 10% of probands were seen to be heterozygous for this mutation Since this is preliminary study, only a small number of samples has been analysed.

Though the subjects found (25%) to be mutated for W24X, in which 10% were heterozygous, the exact cause for deafness though had been ruled out, may be due to the result of mutation in another connexin gene.

Since the study population showed varied degree of consanguinity and since consanguinity is known to cause the spread of the autosomal recessive defect in our study 5% of the homozygous proband was a non consanguineous marriage. Hence the strength of consanguinity cannot be depicted.

CONCLUSION

Our results suggest that the frequency of mutation in W24X gene is considerably large and mutation in this gene is one of the major causes of congenital non-syndromic recessive deafness. The tendency towards the detectable progression in this fraction of children associated with this allele suggests that more awareness is needed. Detecting this mutation in early stages of life would facilitate in identifying the early diagnosis of deafness. This will help the subjects in implementing the intervention strategies like learning sign language, using hearing aids will help in acquiring formal education and betterment of life. Also, detection of deafness at an early stage will also help in identifying deserving candidates for cochlear implant surgery which is very essential in overcoming childhood hearing disability.

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