Connexin 26 (GJB2) Gene Mutations Linked With Autosomal Recessive Nonsyndromic Sensorineural Hearing Loss in Iraqi Population

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Abstract

Background:

Deafness is a total or partial hearing loss that may appear at any ages with different degrees of severity. Approximately 50% of hearing loss have a genetic origin, among them, the nonsyndromic sensorineural deafness represents about 70% of the cases. From them 80% corresponding to autosomal recessive inheritance deafness.

Objective: Autosomal recessive deafness was not been studied enough at molecular level in Iraq, so this study aimed to detect the prevalence of the three most common mutations of Connexin 26 (GJB2) gene in nonsyndromic sensorineural deafness for Iraqi population.

Method: The current case-control study was conducted from January 2018 to January 2020 at molecular laboratory in Anatomy and Histology Department/ faculty of Medicine/ Kufa University/Najaf/ Iraq. The study was included 95 deaf patients (55 males and 40 females) their age range between 11-40 years old and 21.5 ± 6.3 year (mean ± SD) and 110 healthy control group, their ages range between 10-40 years old and 20.1 ± 5.9 year (mean ± SD), these two groups were matched in age and gender. In order to detect c.35delG, 235delC and 167delT mutations in GJB2 gene, we were employed the polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) technique.

Results: From 95 deaf patients with ARNSHL who were participated in this study, the c.35delG was the main frequent mutation encountered with GJB2 gene, among them 35(36.8%) were homozygous, 40(42.1%) were heterozygous and 20 (21.1%) were wild genotypes. The second degree mutation in GJB2 gene was c.235delC mutation, which from the 95 deaf patients, there were 21 (22.1%) carried out homozygous, 33 (34.7%) heterozygous and 42(44.2%) wild genotypes. None of the 95 deaf patients were showed the c.167delT mutation, on the other hand these variants were not detected in healthy control group which was studied parallel with patients group.

Conclusion: Our data conclude that the GJB2 c.35delG and c.235delC gene mutations were the main cause of ARNSHL in Iraqi deaf population.

Introduction

Congenital Hearing loss is the most common form of sensorineural deafness, which occurs in 1/350–1/1000 newborns and 70% of these cases are genetic source [1, 2]. Inherited hearing loss was divided to syndromic, which was accounted 30% of inherited congenital hearing loss that associated with other clinical characteristics as well as hearing loss and non-syndromic, which was accounted the other 70% who were phenotypically appeared with hearing loss only[3]. Regarding to the non-syndromic hearing loss, autosomal recessive is the major common form that ranged from 75 to 85% of deaf cases[4, 5].

The gap junctions between neighbouring cells formed by collection of transmembrane proteins that called connexins. It was two decades ago that connexins were found to be involved in genetic deafness. There were two types of connexins: Gap Junction α (GJA) and Gap Junction β (GJB) with variable distribution in tissues. Most of the nonsyndromic inherited deafness 60–80% of cases is associated with mutation of the genes encoding connexins 26, 30, 31, 32, 43 but the higher risk of mutations were found in connexin 26 (GJB2) that located on chromosome 13q11-12 and has 4804 bp in length[6]. The GJB2 gene which encodes connexins 26, these proteins are dependent in both terms of expression and localization, within the cochlea, connexins 26 form heterometric gap junctions which obviously contribute to the up keeping homeostasis of cochlea. The mutation of this gene as heterozygous or homozygous form influenced on the actions of the gap junctions and therefore prevent the conversion of the mechanical signal to the electrical signal. Hence deafness was installed as symmetrical and bilateral most often severe or profound[7].

In 1997, Kelsell et al[8] found that the GJB2 gene is related to the pathogenesis of autosomal recessive nonsyndromic hearing loss (ARNSHL). Though further studies have reported that GJB3 and GJB6 mutations also linked to hereditary deafness, while GJB2 consider as the main heritable factor in this condition. It was reported to have up to 50% mutations in all patients from various populations[9, 10].

In spite of numerous mutations were recognized (35delG, 235delC, 167delT, V37I, 109G-A, etc), but 235delC was found to be the most common GJB2 mutation in East Asians populations, c.35delG in Caucasians and 167delT predominant in Ashkenazi Jews [7– 12].

There is a few knowledge about GJB2 gene was recorded in Iraqi population with autosomal recessive non syndromic sensorineural hearing loss. for that reason, this study was aimed to investigate the common c.35delG, c.235delC and c.167delT mutations in the gene of connexin 26 (GJB2) in Iraqi deaf patients.

Patients And Methods

The current case-control study was conducted from January 2018 to January 2020 at molecular laboratory of Anatomy and Histology Department/ faculty of Medicine/ Kufa University/Najaf/ Iraq. The study was included the consent forms approved by medical ethics committee in Faculty of Medicine, Kufa University.

All the patients were remitted to our laboratory by otolaryngology Department of Al-Sadder medical city in Al-Najaf/ Iraq. The sample size was calculated by using the online software OSSE (online sample size estimator)[13]. Ninety five patients were diagnosed with bilateral ARNSHL according to family history of patients and audiometric parameters, they were (55 males and 40 females) their ages between 11-40 years old and 21.5 ± 6.3 year (mean ± SD). Among them, 75 were sporadic cases of hearing loss, compatible with recessive inheritance while the other 20 probands were from families with more than one sib of non-syndromic hearing loss, age and gender were matched with control group which were

consisted of 110 voluntary individuals unrelated to patients, they were selected from general population and apparently healthy, their ages ranged between 10-40 years old and 20.1 ± 5.9 year (mean ± SD). Only individuals free from signs and symptoms of any chronic diseases such as DM, cardiac diseases, hypertension, renal diseases or others were selected to participate in this study.

Consent information was obtained from all participant relatives and patients younger than 18 years from their parents. Assessment was included a complete case history, using Pure Tone Audiometry and physical examination. The inclusion criteria was ultimately finding of examinations with ARNSHL, while the exclusion criteria were including: acquired hearing loss related to environmental causes and patients who diagnosed with syndromic hearing loss.

Audiology

All study participants underwent pure-tone audiometry by using diagnostic audiometer instrument (Siemens Danplex DA 74 clinical diagnostic audiometer, USA) in a soundproof room. Which averages pure-tone of more than 25 dBHL (dBHL at 500, 1000, and 2000 Hz) were definite as hearing loss according to the hearing loss classification [(14)]. The hearing impairment degrees were ranged from moderate to profound in current study.

Degree of Hearing Loss Tone Average	Hearing Loss range(dB HL)
Normal	-10 to 15
Slight	16 to 25
Mild	26 to 40
Moderate	41 to 55
Moderately severe	56 to 70
Severe	71 to 90
Profound	91 to equipment limits[(14)]

Mutation Analysis

One ml of venous blood was collected from all individuals participated in this study. Genomic DNA was isolated and purified from peripheral blood lymphocytes by using ReliaPrep™ Blood gDNA Miniprexp System (Promega) according to the standard protocol of the manufacturer.

The extracted DNA was used in the screening of selected mutations. Polymerase Chain Reaction-Restriction fragment length polymorphism (PCR-RFLP) analyses were applied to detect the three GJB2 common mutations that described previously and as appeared in Table (1). Restriction enzymes and PCR primers were purchased from Biolabs (USA). The amplification of GJB2 genes was performed by using genomic DNAs (100-200 ng) in 25 µl master mix containing 50mM KCl, 1.5 mM MgCl2, 10 mM Tris HCl

(pH 8,3), 200 M dNTPs and 1.25 U tag DNA Polymerase (Promega, USA) and 10 pmol of each primer. The PCR procedure was done by incubation at 94°C for 10 min, followed by 35 cycles of 94 °C for 30 sec, 55°C for 30 sec. and 72 °C for 60 sec, 72 °C for 7 min for final extension . Then the products of PCR were digested by using BstI, ApaI and MwoI enzymes to search of the connexin 26 (GJB2) variations c.35delG, c.235delC and c.167delT respectively.

Wild type PCR products were cut by the enzymes in c.35delG and c.235delC genes and showed two bands in gel electrophoresis, whereas c.167delT gene was uncut and produced single band when applied by electrophoresis in 2% agarose gel stained with ethidium bromide, and the results were recognized by gel documentation instrument.

Results

The current study was involved three mutations in connexin 26 (GJB2) gene related with non syndromic autosomal recessive deafmute in 95 patients. Among them, 55 (57.9%) were males and 40 (42.1%) were females. The results of questionnaire were appeared that endogamy marriage between parents of deaf patients is more common which accounts (80%), and there is a family history in the mother's family (39%) or the father's family (42%). As well as, 89/95 (94%) of the patients' parents have normal hearing. The presented data for five of remaining sex patients was appeared that their paternal and maternal grandparents have normal hearing. hence, from the interview outcomes were considered all patients as having autosomal recessive non syndromic hearing loss.

A total of 95 deaf patients with ARNSHL who recruited in this study, were included 75 simplex proband which refers to sporadic patients whose families have no one suffering from hearing loss and 20 multiplex proband which means that there is at least one first or second degree deaf relative in their families. Clinical characteristics of deaf patients and hearing tests confirmed that the level of hearing loss was severe to profound in 91 patients, the remaining 4 patients showed moderate hearing loss as appeared in Table 2.

Table 1
Mutations and parameters for PCR-RFLP.

Primer sequence	Mutation	PCR Product (bp)	Restriction Enzyme	Allele size (bp)	Ref.
F:TCTTTTCCAGAGCAAACCGC R:GCTGGTGGAGTGTTTGTTCACA	c.35delG	89	*BstI*	wild: 69 + 20 Hetero: 89 + 69 Homo: 89	Freitas et al. 2010
F: TGTGTGCATTCGTCTTTTCCAG R:GGTTGCCTCATCCCTCTCAT	c.235delC	722	*ApaI*	wild: 451 + 277 Hetero:722 + 451 + 277 Homo: 722	Masyita et al. 2009
F: GATTGGGGCACGCTGCA R: CCCTTGATGAACTTCCTCTTCT	c.167delT	322	*MwoI*	wild: 322 Hetero: 322 + 161 Homo:161	Shahin et al. 2002
bp: base pair, F: Foreword, R: Reverse, Ref: Reference					

Table 2
Clinical characteristics of deafness patients

Parameters	Simplex proband n = 75	Multiplex proband n = 20
Sex	42	13
Male 55	33	7
Female 40		
Age at the test	40	9
6–18 years	37	11
19–40 years		
Severity of hearing loss	0	0
Mild	4	0
Moderate	11	3
Severe	60	17
Profound		
n: number		

The present study revealed that c.35delG was the most frequent encountered mutation in GJB2 gene. from 95 deaf patients, there were 35 (36.8%) appeared with homozygous genotype, 40 (42.1%) with heterozygous genotype while the remaining 20 (21.1%) were revealed normal genotype.

On the other hand, c235delC mutation was represented as the second degree mutation in GJB2 gene, from deaf patients, there were 20 (21.1%) were carried out homozygous, 33 (34.7%) heterozygous genotype, and 42(44.2) wild genotypes for the tested mutation.

None of the 95 deaf patients showed the c.167delT mutation. As well as there were no mutations appeared in control group. The frequency of the detected mutations was summarized in Table 3.

Table 3
Frequency of the GJB2 mutations in deafness patients.

GJB2 mutation	Effect	Genotype / Frequency (%)				Allele frequency
		Wild	Heterozygous	homozygous	Total affected	
c.35delG	frameshift	20 (21.1%)	40 (42.1%)	35 (36.8%)	75(78.9%)	0.42 %
c.235delC	frameshift	42(44.2%)	33 (34.7%)	21 (22.1%)	54(56.8%)	0.61 %
c.167delT	frameshift	Not detected				-

Discussion

The GJB2 gene encoding the gap-junction protein connexin 26, was revealed to cause distinct forms of hearing impairment, in particular autosomal recessive non syndromic hearing impairment. Mutations in GJB2 are the most common cause of moderate-to-profound congenital inherited hearing impairment in numerous populations[10].

In Iraq, there was no more details about this type of mutations except Jarada et al[15] who was studied other variant mutations in GJB2 gene of Iraqi people who were resident in Jordan kingdom. Therefore, we performed this case-control study to investigate the role of c.235delC, c.35delG and c.167delT mutations in the GJB2 and their interaction with other environmental factors to susceptibility of this condition.

In fact, the literature review was showed that the distribution of connexin 26 (GJB2) mutations widely differs among ethnicities, GJB2 c.35delG variant is found in 60% of Caucasians, Northern Europeans and Turkish suffering from hereditary hearing loss[16, 17].

The results were revealed that the c. 35delG was evident in 75 (78.9%) consisting of 35 (36.8%) homozygous and 40 (42.1%) heterozygous genotypes of mutant alleles, which was agreed with (70%) prevalence of the c.35delG mutation recorded in Cuba and (74%) in Spain for studying patients by Yenitse et al from Cuba and Spain[18]. As well as, The GJB2 c.35delG mutation was recorded in (60%) of North European and Turkish who suffer from hereditary deafness[16, 17]. It was arranged among the highest mutation in GJB2 when compared with its frequency in other Arabic populations rates. The GJB2 mutations account in Algeria 40%, in Lebanon 33.3%, in Palestine 23%, in Tunisia 17% and in Jordanian 16.9%. However, this mutation very rare in Asian patients and it is frequently encountered GJB2 mutation in Caucasians[19]. Accordingly, the high level of this mutation is result of exposure to depleted uranium and chemical radiation from wars that happened in the last decades.

Another less frequent frameshift mutation, c.235delC was reported in this study. Which detected in 54(56.8%) from all ARNSHL patients including 21 (22.1%) homozygous and 33 (34.7%) heterozygous genotypes of mutant alleles. Our results consistent with other studies that found the GJB2 235delC

genetic mutation was presented in 12.2–33% of individuals with hereditary deafness[9, 10, 20]. The c.235delC mutation was found as the most common mutation cause premature protein termination in patients suffering from hearing loss in East and Southeast Asia, while lower frequencies was recorded in Oceania and Europe [21–23]. It was also presented in Japanese, Korean, and Mongolian populations also mutation in GJB2 was a significantly contributed to the recessive inheritance NSHL in the Chinese population and also appeared in other ethnic groups [13, 24–27].

The third mutation c.167delT in GJB2 gene, which was not appeared in our study subjects, was mainly presented in Ashkenazi Jews[7, 10, 28]. Also, this mutation was detected in a Palestinians group from Bethlehem[2], which representing that the variant of GJB2 mutant allele frequency also may be marked in the groups of the similar population.

The variation between various studies in type of GJB2 mutations and terms of frequency associated with ARNSHL may be due to several causes that include: sample size (large sample size increase the probability for detecting rare mutations), selection criteria of the patients, accuracy of genotyping method that employed and consanguineous marriage rate.

Conclusion

We concluded from current study that Connexin 26 (GJB2) c.35delG and c.235delC gene mutations was the causative agent for congenital autosomal recessive hearing loss in Iraqi population. These data can support the screening programs of audiometric recognizing neonates with congenital inherited hearing loss in Iraqi endogamy parents.

Declarations

due to technical limitations, Declaration section is not available for this version.

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