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INVESTIGATION OF COMMON MITOCHONDRIAL MUTATIONS INCLUDING A7445G, A15555G AND A3243G IN INDIVIDUALS WITH NONSYNDROMIC DEAFNESS

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ABSTRACT

Although most nonsyndromic hereditary deafness caused by mutations in nuclear genes, significant contributions of mitochondrial DNA mutations (mtDNA) have become clearer in recent years. The aim of present study was to screen common mitochondrial mutations, including A7445G, A15555G and A3243G in individuals with nonsyndromic deafness.

In this study, 50 patients with nonsyndromic deafness and 50 healthy controls were studied. Samples were screened for common mutations in mtDNA using PCR-RFLP method. Sequencing was carried out to confirm observed mutations. None of A7445G and A3243G mutations were found in individuals with acquired and nonsyndromic deafness. But A15555G was observed in 2 patients with nonsyndromic deafness. There was no statistically significant correlation between the incidence of this mutation and the risk of deafness while comparing the control groups with patients (CI: 95%, OR: 1.41 and P: 0.24).

This study demonstrates that mitochondrial mutations mtDNA mutations play more prominent role in the origin of nonsyndromic deafness in the study population.

Key words: Mutation, mtDNA, nonsyndromic deafness, A7445G, A15555G and A3243G, PCR-RFLP

INTRODUCTION

Deafness is the most common disease in humans that affects 1 to 3 cases per 1000 births (Morton,1991 and Morton and Nance, 2006). Hearing loss can begin in the prelingual or postlingual stages (Woong *et al.*, 2012). Prelingual non-syndromic hearing loss almost includes 80% autosomal recessive (DFNB), 20% autosomal dominant (DFNA) and 1% X-linked and mitochondrial (Smith *et al.*, 2012). Deafness is a heterogeneous disease in terms of etiology, clinical and genetic aspects. Almost 70% of infants suffer from hereditary nonsyndromic deafness. A1555G mutation in 12s rRNA gene is a common cause for nonsyndromic deafness of maternal inheritance in some populations. This mutation can be associated with nonsyndromic deafness induced by aminoglycosides (Fischel-Ghodsian *et al*,1993 and Fischel-Ghodsian *et al.*, 1999). Although the majority of disease-causing mutations are heteroplasmy, A1555G often exists in individuals with or at risk of deafness (Kokotas, 2007).

A7445G in tRNA $^{Ser\ (UCN)}$ gene is another known mutation that has been reported previously as being associated with non-syndromic deafness (Bae *et al*., 2008; Reid *et al*., 1994).

Previous reviews on mitochondrial genes of 12s rRNA and tRNA $^{Ser\ (UCN)}$ led to the identification of A1555G and T1095C mutations in 12s rRNA gene that is almost seen in the homoplasmy form (Dai *et al.*, 2008). For the first time, Woong *et al*. studied mtDNA mutations in the population of korean patients with non-syndromic deafness in 2008 (Bae *et al.*, 2008). A3243G mutation in the tRNALeu (UUR) gene is also a common cause of non-syndromic deafness and diabetes (Van den Ouweland *et al.*, 1992). According t o a report by Dai *et al*. (2008) Maternally Inherited Diabetes and Deafness (MIDD), Progressive External Ophthalmoplegia (PEO) and Mitochondrial Encephalomyopaty Lactic Acidosis Syndrome (MELAS) is caused by A3243G variations.

The current study is aimed to determine the frequency of three known mutations of A1555G, A3243G and A7445G in individuals with acquired and non-syndromic deafness.

MATERIALS AND METHODS

In this experimental descriptive study, a total of 50 blood samples were collected from non-syndromic hearing loss with autosomal recessive pattern under the auspices of Welfare Organization of Lorestan Province using easy sampling method. After obtaining informed consent from all individuals, their clinical and demographic information (non-syndromic sensorineural hearing loss with autosomal recessive pattern) were obtained through 5 ml of blood in tubes containing EDTA (0.5 molar). Then DNA was extracted using known phenol-chloroform method and the concentration of extracted DNA was measured using a spectrophotometer (Unico Model 2100 –USA) (Grimberg *et al*., 1989; Kleihues *et al*., 1997). R and F primer sequences were designed and made to identify 3 mutations of mtDNA molecules using mitochondrial genome sequence with access password of NC-012920 as well as Primer 3 Software (Table 1). PCR was performed on DNA samples using a thermocycler to investigate mitochondrial mutations of A7445G, A15555G and A3243G (TECHNE TC-512 UK) according to the following reaction and thermal procedure: Initial denaturation of 94° C for 5 minutes, then 30 cycles of: denaturation at 94° C for 30s, 63° C for binding primers to the target DNA for 30s, 72° C for 50s to develop complementary strands and eventually the final development at 72° C for 6 min (Table 1). PCR reaction conditions were the same for the 3 mutations and thus each PCR micro-tube for each mutation contained 1µL (1µL) of Primer R (10PM), 1µL of Primer F (10PM), 1µL of enzyme Taq DNA polymerase (5U / µL), 0.5µL of mixed NTP(10Mm), 2 / 5µL of PCR buffer (10X), 1 / 5µL of $MgCL_2$ (50mM) and 1µL DNA (100ng) that was reached to a final volume of 25µL using dH_2O. For final approval, all PCR products were electrophoresized on 2% agarose (1: 6) for 1 hour under 120V voltage for final confirmation. The resulting gel was stained by fluorescent colors and PCR-RFLP method was used to study mutations (Table 1). In each microtube, 10µL of PCR product was mixed with 1µL of restriction enzyme (10U/µL) and 2µL of buffer and 7µL distilled water and was placed at 37° C for 16 hours. The products were electrophoresized on 2% agarose gel with 90V voltage for 2 h (Table 1).

Table 1. Mitochondrial mutations, primer sequences, PCR products size, restriction enzymes, normal and mutant PCR-RFLP products.

Name of mitochondrial mutations	Primers	PCR product size	Restriction enzymes	Normal PCR-RFLP products	Mutant PCR-RFLP products
A1555G	F- 5' CAC AAA ATA GAC TAC GAA AGT GGC 3' R- 5' ACT TAC CAT GTT ACG ACT GTG 3'	566bp	HaeIII	455bp 111bp	91bp 20bp 455bp
A3243G	F-5' CCT CCC TGT ACG AAA GGA C 3' R-5' GCG ATT AGA ATG GGT ACA ATG 3'	238bp	HaeIII	169bp 37bp 32bp	97bp 72bp 37bp 32bp
A7445G	F- 5' GAG AAG CCT TCG CTT CGA AG 3' R-5' GAG GGC GTGATC ATG AAA GGT 3'	348bp	XbaI	119bp, 229bp	348bp

RESULTS

A total of 50 deaf men and women were studied in the present experimental-descriptive study. Patients included 29 men (58%) and 21 women (42%) and the control group consisted of 27 men (54%) and 23 women (46%). There was no significant difference in terms of gender (P=0/74) between the two groups of patients and controls. None of A7445G, A15555G and A3243G mutations was found in the PCR-RFLP conducted on the control samples. Only, A15555G mutation was observed in the homoplasmy form in 2 out of 50 probands of patients with non-syndromic deafness (Fig. 1). A15555G mutation causes the 111bp fragment to form an additional position for the enzymes in

12s rRNA gene 1555 position by converting A to G. This mutation converts 111bp fragment into two 20bp and 91bp fragments (Fig. 1).

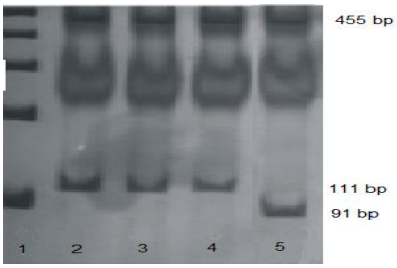

Fig. 1. PCR-RFLP products on 2% agarose gel for analysis of A1555G mutation
Band 1(100 bp DNA Ladder) band 2 – 5(PCR-RFLP products).

The phenotype of A1555G mutation carriers was highly variable and A1555G mutation was observed in two mothers, all healthy and deaf sister and brothers except the father (Table 2).

Table 2. Clinical characteristics of patients carrying known pathological mutation A1555G.

Patient Number	Right ear hearing (db)	Left ear hearing (db)	Family members with A1555G mutation	Severity of hearing loss of affected family members
1	96	104	Yes (all except the Father)	All healthy
2	91	101	Yes (all except the Father)	Hearing-impaired sister

DISCUSSION

MtRNA gene mutations, along with hereditary deafness usually occur in the genes coding the protein synthesis machinery, including rRNA and tRNA genes. MtRNA various mutations causing nonsyndromic deafness with maternal inheritance pattern were identified (Kokotas, 2007). Mutations in 12s rRNA and tRNA $^{Ser\ (UCN)}$ genes are the main cause of most nonsyndromic deafness cases with maternal inheritance pattern (Kokotas, 2007; Van den Ouweland *et al*., 1992). This study was conducted on 50 deaf individuals to investigate the relationship between nonsyndromic sensorineural deafness and common mutations in human mitochondrial genes. According to previous studies, less than 1% of sensorineural deafness is caused due to mitochondrial mutations. A3243G and A7445G mutations were not observed in this study. The small sample size in this study is probably the reason for not getting more mutations. However, the results showed a different pattern of A1555G mutation in the population with acquired and nonsyndromic deafness, although there was no statistically significant correlation between the incidence of this mutation and the risk of deafness while comparing the control with the patient groups. The results also showed that none of the control samples have mutations in mentioned genes. These results suggest that A1555G mutation, to exert it to the pathogenesis effect, depends on the effect of other factors, including mitochondrial haplotypes, modifier genes and environmental factors. The most common 12s rRNA mutation is A1555G mutation identified in many families with different ethnic backgrounds. In fact, A1555G was the first mutation that was shown to cause nonsyndromic deafness in humans and is found in hundreds of families around the world and is considered as one of the most common genetic causes of deafness. A1555G mutation was first described in an Israeli Arab family tree, in which the majority of family members suffered from sensorineural deafness and the incidence of severe or very severe form during childhood .But a minority of family members developed the disease during childhood or early adulthood (Prezant *et al.*, 1993). A1555G mutation can be found in 0/6 to 2/5% of clinical population with nonsyndromic deafness in Caucasus, although its frequency is high, particularly in the Spanish population (Estivill *et al.*, 1998). The incidence of A1555G mutation is also high in Asian populations, including 2/9% in China (Li *et al*., 2005), 3% in Japan (Usami *et al.*, 2000) and 5/3% in Indonesia (Malik *et al*., 2003). A1555G mutation usually occurs in the homoplasmy form, but it also has been identified in the heteroplasmy form

in a number of families (El-Schahawi *et al.*, 1997 and Del Castillo *et al*., 2003). However, there is no clear relationship between the mutation load and deafness severity. It seems that the prevalence of these mutations in the eastern population is lower than in western society. Also, the frequency of A1555G in inherited nonsyndromic hearing impairment in a Spanish family is about 27/1%, but the same frequency is about 55/9% progressive deafness family (Kokotas, 2007; Torroni *et al.*, 1999). Woong investigated mitochondrial mutations in 227 unrelated patients with nonsyndromic deafness in Korea for the first time in 2012 (Woong *et al.*, 2012). Two patients with A1555G mutation was detected among these patients. In addition, two new types of C895T and 12s rRNA gene may be a hot place for mitochondrial mutations leading to deafness in the Korean population (Woong *et al.*, 2012). These findings suggest a possible increase in biochemical defect in people who suffer from the synthesis of mitochondrial proteins caused by A1555G mutation. So, changing the age and type of attack will lead to hearing damages (Dai *et al.*, 2008). In another study patients with A1555G mutation in the mitochondrial 12s rRNA gene suffered from increased risk of developing hearing loss after being treated with aminoglycosides. But carriers showed deafness without exposed to the drug (Prezant *et al.*, 1993; El-Schahawi *et al.*, 1997).

In another study, A1555G mutation was reported in 2%of Japanese patients with prelingual deafness that was consistent with our study (Jacobs *et al.*, 2003). The incidence of A1555G mutation phenotype is highly variable that include carriers with normal hearing to carriers with absolute deafness (Ballana *et al.*, 2006). Age of onset and varying severity in patients show that some people are deaf at birth while others show a slowly progressive hearing loss at the age of puberty. Variable nature of phenotype of patients with A1555G mutation implies other factors affecting the phenotypic emergence of mtDNA mutations, which affects the age at onset and progressive deafness (Ballana *et al.*, 2006). A1555G mutation produces a clinical phenotype that is significantly changed among family members and varies from severe congenital hearing loss to moderate progressive hearing loss to the age of puberty to a completely normal hearing. The remarkable point is that some pharmacological and environmental factors can affect phenotypes of individuals with mutations. The results of previous studies indicate that the amino glycoside antibiotic can cause disease progression in patients with mitochondrial mutations. In a retrospective study, it was shown that a group of patients with A1555G mutation who have healthy to impaired phenotype suffered from hearing loss after exposure to aminoglycoside drugs (Estivill *et al.*, 1998).

Conclusion

Since, similar to the rest of the country, the rate of consanguineous marriage in the Lorestan province is high, this population is an invaluable resource for research on Autosomal recessive genetic diseases such as deafness. Certainly, the results of these studies can be helpful in screening for deafness in our population and consequently systematic genetic counseling and pre implantation genetic diagnosis (PGD).

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