SCREENING OF THE CONNEXIN 26 (35DELG) MUTATION IN EGYPTIAN PATIENTS WITH AUTOSOMAL RECESSIVE NON-SYNDROMIC DEAFNESS AND ITS RELATION TO THE PATIENTS' IQ

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Hearing is one of the five senses which have effect on important faculties i.e., speech, communication, education, social and intelligence development. Hearing loss or deafness is one of the most common prevalent congenital sensory disorder. Its incidence worldwide is 1:500 in newborn and 1:300 in children by the age of 4 (Chan & Chang, 2014). Sixty percent of all the cases refer to genetic causes and the rest occur due to environmental factor. More than 100 genes are involved in hearing loss i.e, GJB2, GJB3, GJB6.... etc (Frei et al., 2002; Kaskalan et al., 2014; Banjara et al., 2016).

Connexins are large family of gap junction proteins, which are involved in direct cell to cell transfer of small molecules and ions. Connexin 26 (Cx26) is an important protein, which is encoded by GJB2 gene. GJB2 gene is located on the chromosomal location 13q11-12 (Cordeiro-Silva et al., 2010; Yoshikawa et al., 2011). The Cx26 (35delG) mutation is a deletion of six guanine bases that extend from 30-35 on the GJB2 gene, resulting in a stop codon. This deletion resulted in truncated polypeptide chain consisting of 12 amino acids instead of 226 amino acids (Petersen and Willems, 2006; Serrão de Castro et al., 2013; Kaskalan et al., 2014).

Cx26 protein plays an important role in the recycling and movement of potassium ions (K+), which have a key role in hearing mechanism as a part of a signal transduction in the inner ear (Cordeiro-Silva et al., 2010). Therefore, the mutation in the Cx26 protein cause autosomal non-syndromic deafness (ANSD) (Masindova et al., 2012; Kaskalan et al., 2014).

In many populations, the alteration in the connexin 26 is considered the most frequent cause of deafness, as it is responsible for approximately 50% of the Mediterranean region, North American and North and South European patients with
Although several studies worldwide have proved that mutation in \( GjB2 \) gene is associated with hearing loss and its frequency in different population, there are few studies reported on the frequency of this mutation in the Egyptian population. Therefore, our study aims to screen the Cx26 (35delG) in the Egyptian population, in addition to correlate between the Cx26 (35delG) mutation and the IQ of the studied patients.

**MATERIALS AND METHODS**

**Subjects**

The present study was carried out with the consent of all patients' and controls' parents to share in the study as well as acceptance of the ethics committee of the University. In the present study, 120 patients were diagnosed with congenital non-syndromic hearing loss. Their ages were between 1.5 to 13 years with a mean of 6.19 years (SD = 2.758). In addition, 120 healthy children were examined as control.

**Clinical diagnosis and IQ measurement**

Clinical evaluation of children was carried out including general and systemic examination to exclude the presence of any other congenital abnormality, and include local examination of throat, ear and nose. Assessment of mental age was done using the intelligence scale according to Stanford-Binet (2003) to calculate the intelligence quotient (IQ). Subnormal intellectual functions are diagnosed when IQ is below 70.

**DNA extraction**

DNA was extracted from whole blood for both patient and control using spin column method of Gene-JETTM Genomic DNA purification kit (Fermentas Life Sciences, Finland). The eluted DNA was stored at -20°C until further use.

Genotyping for the Cx26 (35delG) mutation was performed by amplification refractory mutation system (ARMS) analysis using a thermal cycler (Biometra, Germany) according to Mustafa (2004). A total of 20 ng of genomic DNA was amplified in a PCR containing 0.2 Mm of forward primer and 0.2 Mm of reverse primer, 10xPCR buffer, 1.5 mM MgCl\(_2\), 200 mM dNTPs, and one unit of Taq DNA Polymerase (Promega, UK) in a 25 mL volume. Amplification conditions were 95°C for 4 min, 95°C for 30 s, 60°C for 30 s, 72°C for 30 s, Steps 2-4 were repeated for 35 cycles followed by 72°C for 8 min.

The following primers were used to amplify the target sequence which contained the Cx26 (35delG):

Common forward primer: 5'- GAA GTA GTG ATC GTA GCA CAC GTT CTT GCA -3'.

Reverse 1 (R1) primer for the normal allele: 5'-TTG GGG CAC GCT GCA GAC GAT CCT GGG GAG -3';
Reverse 2 (R2) primer for the mutant allele: 5'- TTG GGG CAC GCT GCA GAC GAT CCT GGG GAT -3', and the PCR products were visualized via 2% agarose gel electrophoresis.

**Statistical analysis**

Statistical analyses were carried out using statistical package of social sciences (SPSS) software version 14 and P < 0.01 was considered significant. Quantitative data were presented as mean ± SD for normally distributed data and as medians and percentiles for skewed data. Quantitative data were presented in the form of frequencies and percentages. Comparisons between genotype frequencies between groups were calculated using non-parametric chi square test.

**RESULTS AND DISCUSSION**

Connexin 26 (35delG) mutation ranks among the most frequent causes of the autosomal recessive non-syndromic deafness. Beside the presence of the mutation in homozygous form, it can be found as compound heterozygous; coupled with other mutations in the same gene or other one.

One hundred twenty patients diagnosed by congenital non-syndromic hearing loss and 120 healthy children were examined to screen for the existence of the Cx26 (35delG) in the GJB2 gene in the Egyptian population. 68 (56.7%) out of the patients were males and 52 (43.3%) were females. The patients' ages ranged from 1.5 to 13 years with mean of (6.19 ± 2.758). There were no significant differences in age and gender between deafness patients and their control. The average of the patients’ IQ was 72.6 ± 12.418. Genotyping for the Cx26 (35delG) mutation was carried out using ARMS analysis and Fig. (1) shows the differences between the three genotypes visualized on agarose gel.

Among all the patients, there were 35 individuals (29.2%) with homozygous mutation (35delG/35delG). Moreover, 60 individuals (50%) with heterozygous mutation (35delG/unknown). Whereas, 25 individuals (20.8%) with normal genotype for this mutation (Table 1). The allelic frequency of the 35delG mutation in the patient (54.2%) was significantly very high in comparison with the control (12%).

To our knowledge, many studies demonstrated the association between Cx26 (35delG) mutation and deafness also, its distribution in different countries, but in Egypt only few studies were carried out. Our results disagreed with Mustafa (2004) who showed that only 8 (9.6%) patients were homozygous and 6 (7.2%) patients were heterozygous and the 35delG mutation is much less common in Egypt (~10%). Moreover, Mohamed et al. (2010) found that the allelic frequency of the Cx26 (35delG) mutation in Upper Egypt was 8.7% (27 out of 310 investigated alleles in 155 patients), in which the homozygous patients were 11 and the carrier patients were 5. In addition, El-Barbary et al. (2015) reported that four
patients were homozygous and four patients were heterozygous out of 51 patients, with allelic frequency of 10.8% for the Cx26 (35delG) in Alexandria, Egypt. Social and environmental variations could have an impact on the expression of this character.

On the other hand, our findings partially agreed with Cordeiro-Silva et al. (2010), who reported that the Cx26 (35delG) mutation was considered the most frequent mutation (60-85%) of all the Caucasian population, while Mahasneh and Battah (2006) recorded 85% of all the GJB2 mutant allele in the Mediterranean populations.

Chan and Chang (2014) reviewed the occurrence among most of the worldwide studies, and reported that the frequency of the Cx26(35delG) was 57%, which is ranging from 0% in South and East Asian countries and 100% in the European, North African and the middle Eastern. About 82.4% of these alleles are homozygous for the Cx26 (35delG) and the rest (17.6%) are compound heterozygous with other disease causing.

Meanwhile, Table (2) showed that, there were 20 (16.7%), 17 (14.2%), 33 (27.5%), 48 (40%) and 2 (1.7%) patients with their IQ being average, low average, borderline impaired, mildly impaired and moderately impaired, respectively.

Among all the patients, the average IQ (90-109), none (0%) was mutant homozygote, 12 (60%) of them was heterozygote and 8 (40%) of them was normal in this mutation. In addition, for the low average IQ (80-89), 4 (23.5%), 9 (52.9%) and 4 (23.5%) of them were mutant homozygote, heterozygote and normal, respectively. However, in the borderline impaired group (70-79), 11 (33.3%) of them were mutant homozygote, 13 (39.4%) were heterozygote and 9 (8.3%) were normal. Although for the mildly impaired (55-69) the mutant homozygote patients were 20 (41.7%), the heterozygote patients were 24 (50%) and the normal patients were 4 (8.3%). Finally, all 2 (100%) of the moderately impaired IQ (45-54) were compound heterozygote, this could be due to the presence of other mutation beside the Cx26 (35delG) mutation.

It is noteworthy that 20 (57.1%) out of 35 mutant homozygous patients and 24 out of 60 (40%) heterozygous patients were mildly impaired. These may be referred to the presence of the Cx26 (35delG) mutation (Table 2).

Although, it is reported that the IQ scores of hearing impaired children are often less than those of normal hearing (Brauer et al., 1998), our results showed significant association between the Cx26 (35delG) mutation and the patient’s IQ. No previous studies were reported on the relationship between the Cx26 (35delG) mutation and the patient’s IQ. Therefore, further studies are needed on larger population and different ages and region.

**CONCLUSION**

This article briefly indicates that the prevalence of the Cx26 (35delG) mu-
SCREENING OF THE CONNEXIN 26 (35DELG) MUTATION IN EGYPTIAN PATIENTS

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tation is 54.2% in the Egyptian population, and there is a good probability of association between the presence of this mutation and the patient’s IQ. It is noteworthy that further studies of considerable number of samples and varying age groups are needed to substantiate our findings. Also, we can conclude that the genetics diagnosis of the ANSD is very important in the early treatment of the patient.

SUMMARY

Deafness is one of the most common and widespread congenital sensory disorder. Mutation in the connexin 26 (35delG) is considered the most frequent cause of the autosomal recessive non-syndrome deafness (ARNSD). This study aimed to determine the prevalence of the Cx26 (35delG) mutation in the Egyptian population. To achieve this goal 120 patients were evaluated for this mutation. The Cx26 (35delG) was screened using amplified refractory mutation system analysis (ARMS) analysis. The Cx26 (35delG) mutation was found in the 29.2% and 50% in the patients as homozygous and compound heterozygous, respectively. These results were significantly very high in comparison with the control. The frequency of the mutant allele was 54.2% in this population. These findings revealed the presence of the studied mutation in the Egyptian population.

REFERENCES

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Yoshikawa, S., A. Kawano, C. Hayashi, N. Nishiyama, S. Kawaguch, H.

Table (1): Genotype and allele frequencies and percentage of the Cx26 (35delG) mutation in the connexin 26 gene.

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<th>Genotypes</th>
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<th>Control</th>
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<td>No.</td>
<td>Percent</td>
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<td>35delG allele frequency</td>
<td>54.2%</td>
<td>12%</td>
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* P < 0.05 is considered statistically significant.
Table (2): Correlation between IQ and genotype frequencies and percentage.

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Fig. (1): PCR Products of a fragment from the GJB2 gene for the detection of the Cx26 (35delG) using amplification refractory mutation system (ARMS) analysis.
Lane 1 and 2: show the normal homozygous genotype
Lane 3 and 4: show the mutant homozygous genotype
Lane 5 and 6: show the heterozygous genotype