

Relationship of ACE Gene Insertion/Deletion (I/D) Polymorphisms with Hearing Loss (HL) in Patients from Bahawalpur, Pakistan

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Abstract. Hearing loss (HL) is an inability to hear completely or partially. It can be permanent or temporary depending upon the causative factor involved. One of the factors is high blood pressure that can be responsible for hearing loss at any age. A gene named Angiotensin-Converting Enzyme (ACE) gene that has nearly the size of 21kb is located on the 17q 23 chromosome in humans. The purpose of this study was to determine if any association is present between ACE insertion/deletion (I/D) polymorphisms in hypertensive patients suffering from hearing loss.

Keywords: hearing loss, ACE, polymorphism, hypertension

Introduction

Hearing impairment or Hearing loss (HL) is an inability to hear completely or partially. It can be permanent or temporary. The human hearing frequency is 20 to 20,000Hz. The three basic categories of hearing loss are conductive hearing loss sensorineural hearing loss, and mixed hearing loss. A number of factors like old age, hypertension (high blood pressure), exposure to noise, trauma to the ear, birth complications, infections, certain toxins or medications can be implicated in this regard. Hypertension can affect hearing in a number of ways, it can lead to a hemorrhage of the inner ear, the reason it is called “silent killer” it can also increase the viscosity of blood, causing reduced blood flow in the capillaries. Arterial hypertension can also cause ionic changes in the hair cells affecting their amplitude leading to hearing problems (Anjum *et al.*, 2014). Hearing impairment may be inherited but hypertension is one of the most common persistent conditions causing hearing impairment in older adults. An electrolyte regulator system called the Renin-angiotensin-aldosterone system (RAAS) mainly regulates the blood pressure. Genetic polymorphism related to this system has been under intensive study for many years to find out more about the genetic aspects of hypertension. There is a gene for the angiotensin-converting enzyme (ACE) that is an active vaso-constrictor and works by transforming angiotensin-I to angiotensin-II (Atik *et al.*, 2015). ACE gene of human is present on 17q 23

chromosomes and it is about 21kb in size. The most extensively studied insertion/deletion polymorphism is present on the intron 25 and 26 exons. It is considered due to the occurrence or else non-appearance of a 287 base pair repetitive sequence of Alu. There are three different types of genotypes including II representing homozygous insertion, DD representing homozygous deletion and ID representing heterozygous insertion/deletion (Bliznetz *et al.*, 2017). In this present study, we tried to find out the relation of ACE II, DD, and ID genotypes in people having the problem of hearing loss as ACE is involved in hypertension which is a key factor of hearing impairment.

Aim of the study. The main aim of this study is to find out how the ACE I/D polymorphisms are linked with hearing loss.

Materials and Method

This case control study of ACE I/D polymorphism was done in the Department of Microbiology and Molecular Genetics, University of Punjab. Samples were collected from hearing loss patients from Victoria Hospital Bahawalpur. IRB approval was obtained from both institutes. The study was done over a period of one year.

A sample size of 80 was calculated by Raosoft sample size calculator at the Confidence Interval (CI) of 90 %. Of the 80 subjects, 40 were cases and included hypertensive adults with average age of 31 years, of both sexes, diagnosed with hearing loss. Blood pressure

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categories followed as per American College of Cardiology (ACC) (Fang *et al.*, 2015) is

- Normal blood pressure is below 120/80 mm Hg; Elevated blood pressure is when systolic is between 120 to 129 mm Hg, while diastolic is below 80 mm Hg-mildly hypertensive; Stage 1 hypertension is when systolic is between 130 to 139, while diastolic is between 80 to 89 mm Hg-moderately hypertensive; Stage 2 hypertension is when systolic is at least 140 mm Hg *or* diastolic is at least 90 mm Hg-moderately hypertensive; Hypertensive crisis occurs when systolic pressure is over 180 mm Hg and/or diastolic is over 120 mm Hg-extremely hypertensive.

The remaining 40 were non-hypertensive without hearing impairment, age and sex matched healthy individuals.

Informed consent forms were signed by all the participants of this study. Participants' complete the medical information was recorded with the help of a proforma. The BMI of all the participants were calculated. The blood samples were collected observing the sterile technique according to the standard protocol. 5 mL of blood was collected from the antecubital fossa of each subject and transferred to the EDTA vials which were stored at -20 °C in the refrigerator. The samples were transported from Bahawalpur to the Department of Microbiology and Molecular Genetics in the University of the Punjab, Lahore in dry ice containers. In the laboratory at the Department of Microbiology and Molecular Genetics, DNA isolation was performed from the blood samples following the protocol of (Miller *et al.*, 1988). After that, the nested polymerase chain reaction (PCR) was performed to obtain the ACE gene banding pattern with a particular focus on ACE I/D polymorphism. Nested PCR was used because it reduces non specific binding of primers and thus decreases contamination. Annealing was done at 58 °C. Genotypes of ACE were identified by observing the banding pattern on the agarose gel. The 210 bp showed the DD genotype, 498bp showed II genotype, while 264 bp showed the ID genotype. Polymorphism of ACE I/D was observed within the 16th intron belonging to the ACE gene as a functional polymorphism marker (Fig. 2).

Statistical data analysis was done by using SPSS ver 13. In this study, the application of the chi-square test helped in finding out the connection of ACEI/D polymorphism with hypertensive subjects suffering from hearing loss.

Results and Discussion

A greater number of men presented with hearing loss as compared to women, the ratio seems to be 5:3. Most of the men presenting with the complaint were above the age of 35 years as compared to women who presented at a much earlier age of 20 years (Ali and Hussain, 2021a and b). An average age of hearing loss patients was 31 years regardless of the gender (Fig. 1). Subjects having Body Mass Index (BMI) between 18 and 24.9 were considered normal, while those having a value of ≥ 25 were considered as overweight (Table 1). It is clear that most of the male hearing loss (HL) patients (n=13) lie within the overweight group while the majority of female hearing loss (HL) patients (n=8) were in the normal BMI range. Most of the patients suffering from hearing loss belong to the severely hypertensive group irrespective of gender. The affection status of the HL patients showed that among 40 patients, 30% were mild hypertensive (8 were males and 4 females), 32% moderately hypertensive (8 were males and 5 females) and 38% were severely hypertensive (9 were males and 6 females).

Table 1. Demographic data of cases (n=40) and controls (n=40)

Study groups	BMI	P-value
Cases males (n=25)	Mean	25.20
females (n=15)	standard deviation	1.13
Control males (n=25)	Mean	21.08
females (n=15)	standard deviation	1.66

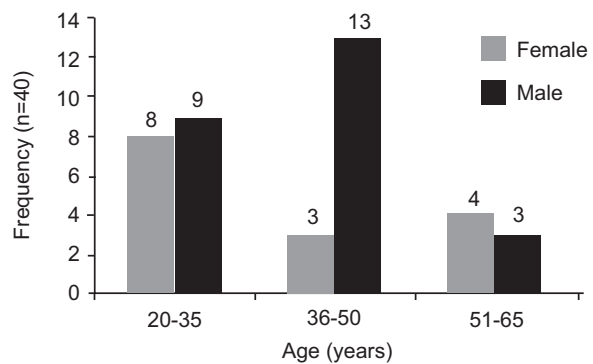


Fig. 1. Age distribution among HL patients (n=40).

All the healthy controls showed the DD genotype, while the HL patients showed both the ID and DD genotypes. The genotype DD association was more common in both controls (n=40) and patients (n=40). A substantial association was not present between ACE I/D polymorphisms and HL significant at $P > 0.05$. In the present study, the frequency of ACE DD genotype in the mildly hypertensive HL patients was 22% and in the case of moderately hypertensive HL patients, it was 31%, while it was 45.7% frequent in the severely hypertensive patients. ID genotype was found in 5 of the hypertensive HL patients, whereas no II genotype was found in controls as well as in the patients (Table 2).

High blood pressure along with the problems of hearing loss is quite prevalent in the populations of elderly people. It is also an established fact that noise can drastically enhance the probability of hypertension (Koohiyan *et al.*, 2018). This study has established here forth that arterial hypertension is an individual risk factor for the loss of hearing. On the other hand, there is an effect of overweight on the hearing loss because obesity restricts overall blood flow in the body, thus our inner ear is working on a reduce blood flow in such

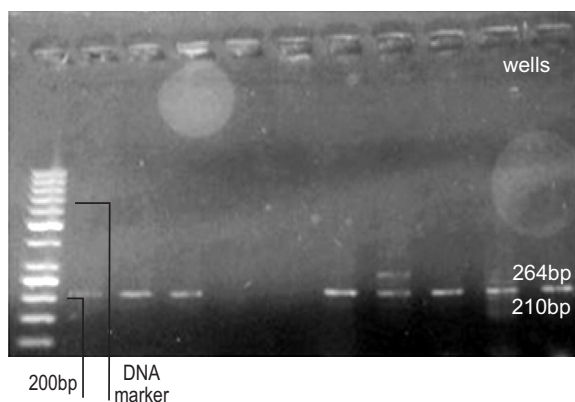


Fig. 2. Nested PCR results showing 210bp:DD genotype, 264bp ID genotype.

conditions. In relation to ACE I/D and G2350A gene polymorphisms, people who are the carriers of genotypes AG, GG and DD are comparatively more susceptible to hypertension when exposed to higher levels of noise for a long time. However, no relationship was found between hypertension and noise induced hearing loss (NIHL) in the Egyptian population (Zawilla *et al.*, 2014). According to the National Health Survey of Pakistan, a study was done according to which 18% of people in Pakistan are patients of hypertension and every third such person who is above 40 is increasingly susceptible to a variety of other diseases. Also, it showed that gender and age are indeed important factors associated with the disorder of hearing impairment even in the people who show no previous history of this disorder. It has also been described that males show a substantial drop in their ability to hear as they age, while women did not show such patterns (Naseri *et al.*, 2018). The current study showed that men are more prone to develop hearing impairment due to high blood pressure as compared to females. A number of factors are involved like environmental, genetic factors and also because of physiological low HDL level in males.

The prevalence of high blood pressure and hearing loss is a very important area of research in elderly populations. And this study has demonstrated that arterial hypertension is definitely a very important and independent risk factor for the occurrence of hearing loss. In one study, including the northern Indian subjects it was reported that secondary hypertension in the population is because of the I alleles of the ACE I/D polymorphisms (Naz *et al.*, 2017). In the present study, a fine comparison was made between the controls and HL patients to detect polymorphism in ACE I/D and the results found were significant as the P-value is less than 0.05. However, the frequency DD genotype of ACE I/D polymorphism was higher in both the controls and the cases. Unlike this study, genotype II was more common in the Malaysian HL population (Jayapalan *et al.*, 2008).

Table 2. Frequency of ACE polymorphism in cases (HL patients) and controls according to ACC guidelines

Type of polymorphism	Control (n=40)	Mild hypertension	Moderate hypertension	Severe hypertension	p-value	OR
ID	0	0	0	5	0.516	OR could not be calculated as one of the cells contain "0"
DD	40	9	13	18		
II	0	0	0	0		

Conclusion

So, the null hypothesis of independence was rejected and concluded that there exists a significant relationship between the levels of hypertension and ACEI/D polymorphism in hearing loss patients. So, preemptive actions must be done to keep the levels of blood pressure normal and hence decrease the risk of hearing impairment.

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Conflict of Interest. The authors declare no conflict of interest.

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