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Original Research Article

Association of Insertion-Deletion Polymorphism in the Angiotensin-Converting Enzyme Gene and Human Essential Hypertension among Sudanese in Al Nubba Village

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Abstract

This study was conducted to determine the association of Insertion-deletion Polymorphism in the Angiotensin-Converting Enzyme (ACE) Gene and Human Essential Hypertension among Sudanese in Al Nubba Village. A case-control community-based study was performed, Blood samples were collected from 54 subjects in a rural area around Khartoum **(**Al Nubba Village**).** The frequencies of the insertion (*I) and deletion (*D) alleles of the ACE gene were investigated, Polymerase chain reaction (PCR) was detected for the Insertion/Deletion polymorphism. Allele frequencies were calculated, our data indicated a preponderance of the *D allele among the Sudanese population. The percentage of Case/Control for Homozygous deletion DD, Homozygous insertion II and Heterozygous DI are almost the same. Further studies are recommended with a large sample size.

Keywords: Hypertension, ACE Gene, Insertion-deletion Polymorphism, case-control community-based, Sudanese.

INTRODUCTION AND LITERATURE REVIEW

Essential hypertension is a major public health problem in many countries due to its high prevalence and its association with coronary heart disease, stroke, renal disease, peripheral vascular disease and other disorders [1].

The Renin-Angiotensin System (RAS) has been under scientific scrutiny for the past two decades in efforts to understand it's physiology as well as to develop new pharmacological agents targeting the system. The ACE is a key regulator in the RAS which converts Angiotensin I into Angiotensin II. ACE encoding gene is located on the long arm of chromosome 17 in humans with an exon count of 26. The gene contains an insertion-deletion polymorphism in its intron 16 [2]; because of its involvement with the RAS, the insertion-deletion polymorphism of the ACE gene has been widely investigated in different populations and in case-control studies.

The present study was an attempt to determine the association of Insertion-deletion Polymorphism in the Angiotensin-Converting Enzyme (ACE) Gene and Human Essential Hypertension among Sudanese in Al Nubba Village.

An insertion/deletion polymorphism, commonly abbreviated “indel,” is a type of genetic variation in which a specific nucleotide sequence is present (insertion) or absent (deletion) [3].

According to Suliman A., [4] Sudan was the largest country in Africa and the ninth largest in the world, with an area of about one million square miles. It has a population of more than 39 million people. Hospital-based surveys in Sudan dating back from the middle of the last century have shown that hypertensive heart disease, particularly with its contribution to heart failure, is probably the commonest cause of cardiovascular disease. This can be explained by a number of factors, including high prevalence rates of hypertension. Also, the control of blood pressure in hypertensive patients seems to be poor in Sudan.

MATERIALS AND METHODS

The study was approved by the local ethical committee, and all participants gave written informed consent for all procedures.

The hypertensive cases and newly discovered were selected randomly. The control group from the same population. Exclusion criteria of any other chronic disease including diabetes. Control subjects are non-hypertensive, non-diabetic and in good health.

Blood samples were collected from 44 cases and 10 control individuals **(**Al Nubba Village**)** DNA was extracted using Quinidine Chloride protocol. PCR protocol and conditions Polymerase chain reactions were carried out using TECHNE Thermocycler UK for detection of the ACE Insertion/Deletion polymorphism: with a total volume of 25 ul containing 3ul of genomic DNA, 1ul of each primer, ready to use master mix use from iNtRON Biotechnology Company. The cycling parameters are presented in Table-1.

Primers Used:

Forward Primer: 5' CTGGAGAGCCACTCCCATCCTTTCT 3'
Reverse Primer: 5' GACGTGGCCATCACATTCGTCAGAT 3'

Table-1: Cycling parameters

Temperature (˚C)	Time	
94 ˚C initial denature	5 mins	
94 ˚C denature	1 mins	Repeat to 40 number of cycles.
65 ˚C annealing	1 mins	
72 ˚C extension	1 mins	
72 ˚C final extension	10 mins.	
4 ˚C	Forever	

The amplification products were subjected to electrophoresis on 1.5% TBE agarose gel, followed by staining using ethidium bromide and visualization under ultraviolet light. The DNA ladder was used for determination of band size, negative control also used (Figure-1).

The data was first coded in Excel sheet then analyzed using R, R Core Team [5]. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

RESULTS AND DISCUSSION

All cases completed the study protocol. The result of the PCR products, separated by gel electrophoresis showed a band corresponded to a 190-base pair fragment in the presence of the deletion and to a 490-base pair fragment in the presence of the insertion (Figure-1). D represents deletion polymorphism, I represents insertion polymorphism is shown in (Table-2).

Table-2: Codes given for the bands

Reading in the gel	code	
190	DD	Common homozygous
490/190	DI	Heterozygous
490	II	Rare homozygous

Allele frequencies were calculated from 44 hypertensive cases and 10 control subjects, Deletion allele (D): 0.77, Insertion allele (I): 0.23, (Figure-2). Our data indicated a preponderance of the *D allele among Sudanese population; Genotype Frequency: D/D (27) Proportion: 0.61, D/I (14) Proportion: 0.32, I/I (3) Proportion: 0.07. Hardy-Weinberg Equilibrium: Chi-square test for the genotypes with the status (case-controls), p-value = 0.6599, (Figure-3). Chi-square test for the gender with the status, p-value = 0.6542, (Figure-4).

This study revealed that there is no statistical association between insertion-deletion polymorphism in the ACE gene and human essential hypertension among Sudanese in Al Nubba Village. This goes in the same direction with the studies of Suliman A. and Alsatar H *et al*., [4, 6]. The percentage of case-control for homozygous deletion DD. Homozygous insertion II and heterozygous DI are almost the same.

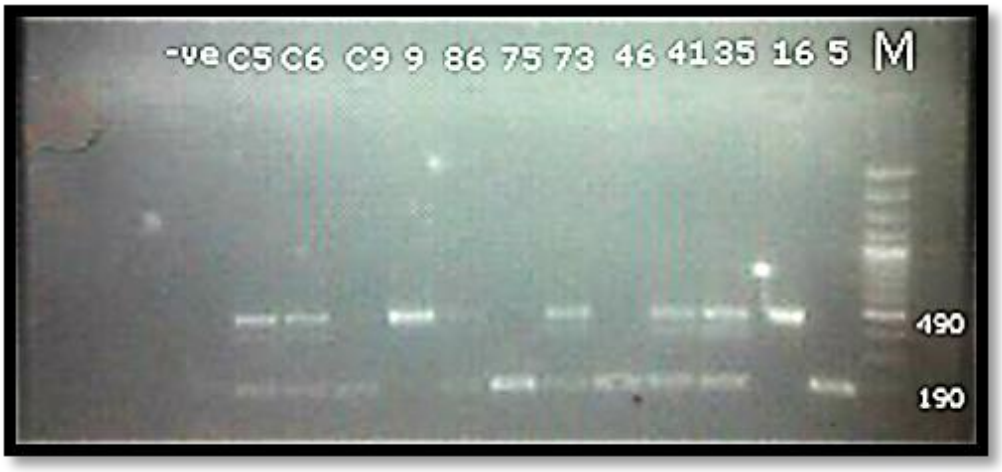

Fig-1: The genotyping results by 1.5% agarose (M represents DNA Ladder 100/BP. 5.46.75. control 9 represent a homozygous Deletion. 16 and 9 represent a Homozygous Insertion. 35, 41,73,86,control 6, control 5 represent a Heterozygous Insertion- Deletion)

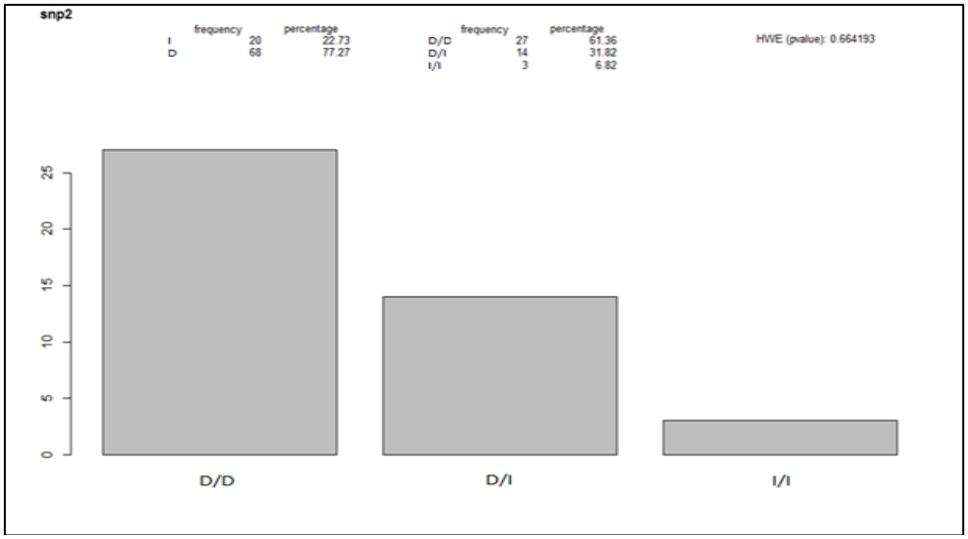

Fig-2: Represents Allele frequencies

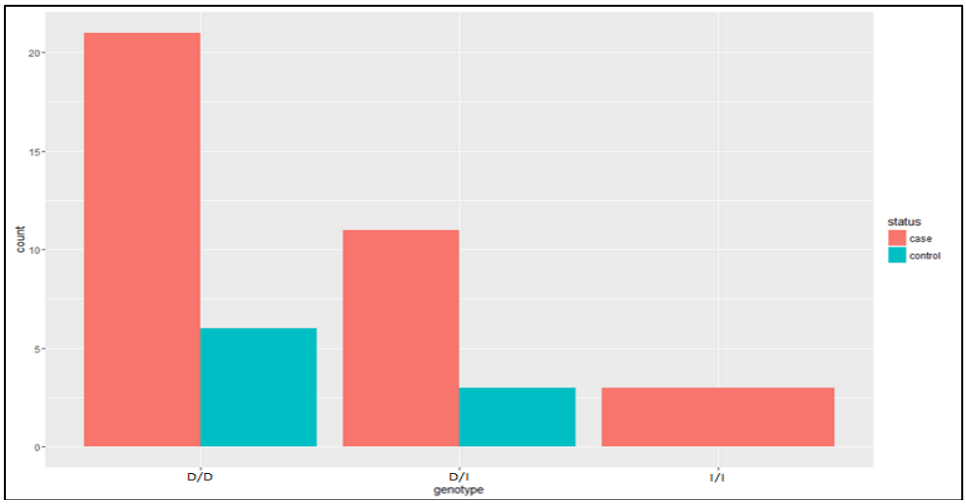

Fig-3: Comparison of insertion-Deletion polymorphism in the Angiotensin-Converting Enzyme (ACE) Gene among cases and control

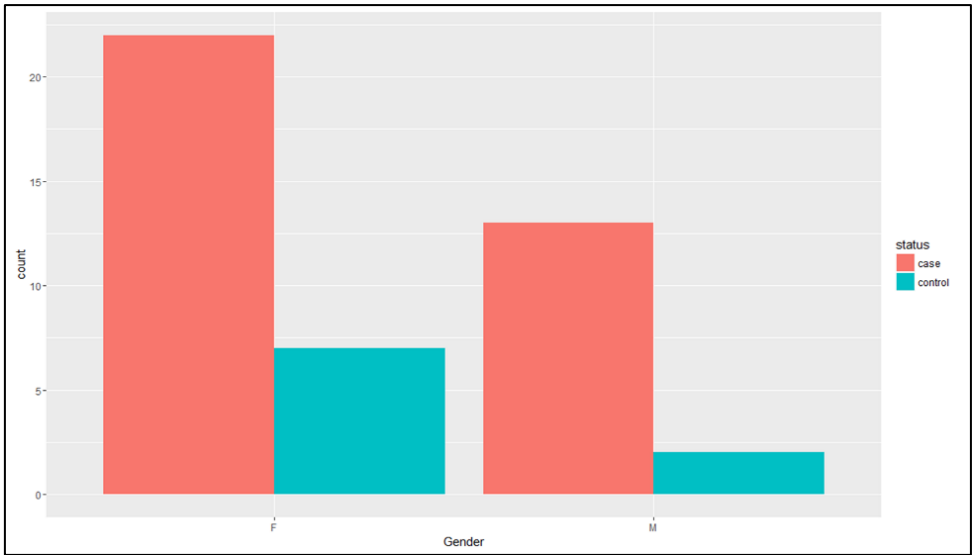

Fig-4: Male and Female Distribution among cases and control

CONCLUSION

The percentage of Case/Control for Homozygous deletion DD, Homozygous insertion II and Heterozygous DI are almost the same. Cases and control were not significantly different in the three genotypes respectively, were in Hardy-Weinberg equilibrium, this study showed that there is no Association of Insertion-deletion Polymorphism in the Angiotensin-Converting Enzyme (ACE) Gene and **Human Essential Hypertension among Sudanese in Al Nubba Village.** Further studies are recommended with a larger sample size.

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