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ACE GENE AND ITS ASSOCIATIONS WITH INFLAMMATION OR INFLAMMATORY DISORDERS

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ABSTRACT

Angiotensin I-converting enzyme (ACE) gene as part of the renin-angiotensin system (RAS) is involved in the maintenance of body fluids, salts and majorly in regulation of blood pressure levels. ACE is a potent vasoconstrictor of the RAS and is a vasodilator of a kallikrein-kinin system that has impact on the process of inflammation. Inflammation is generally body's defence mechanism however if inflammation is slow and long lasting for prolonged periods of several months to years it is than called as chronic inflammation and is a disease condition. ACE I/D polymorphism is seen to be associated with many inflammatory diseases. This study aims to determine the frequency and association of I/D Polymorphism in the Pakistani population and its distribution among various ethnicities. The objectives were to determine the frequency of I/D in various ethnicities and to determine the association of I/D polymorphism with inflammatory diseases. The cross-sectional study was carried comprising 400 subjects. Anthropometric measurements were taken; body mass index was calculated. Diabetes was assessed by measuring blood sugar levels. Cardiovascular disease condition was assessed by measuring blood pressure and lipid profiling. Psychiatric disorder of subjects was assessed by asking questions related to standard indicators of anxiety and depression. The biochemical analyzer was used for complete reactive protein and lipid profiling. I/D polymorphism was identified through genetic screening via PCR. Statistical analysis was done to observe associations and chi-square values and odds ratios were calculated. For ACE gene I/D polymorphism in the population 67.2% was insertion while 32.75% was a deletion. The prevalence of diabetes was observed at 14% no significant association was observed with ACE gene Polymorphism. Prevalence of obesity was observed 54% no significant association was observed with ACE gene Polymorphism. The prevalence of Cardiovascular disorder was observed at 35% in our selected population and there was no significant association observed with ACE gene Polymorphism. The prevalence of the psychiatric disorders that were observed 55%, association with ACE gene polymorphism was not significant. We found that deletion increases genetic susceptibility towards obesity, cardiovascular disorders, diabetes, and psychiatric disorders. However, this is a preliminary study and the results need to be confirmed in a larger cohort.

Keywords: Ace gene, Inflammation, Diabetes, Obesity, CVD, Polymorphism

INTRODUCTION

Angiotensin-I converting enzyme (ACE) gene is known to be first genetic element that has shown impact on human physical performance (Bilchick *et al*., 2019)**.** This enzyme was first discovered by Leonard T. Skeggs Jr. in 1956 (Dorer *et al*., 1970)**.** The formation of ACE enzyme is controlled by ACE gene. The ACE enzyme is significant part of renin-angiotensin system (RAS) that is involved in maintenance of body fluids, salts and majorly regulate blood pressure of human body (Savoia *et al*., 2011)**.** This enzyme can cleaves off angiotensin I at specific locations and transform it into another proteins called as angiotensin II. Angiotensin II is physiologically active peptide and is effective vasopressin, it also stimulates aldosterone. It controls fluid electrolyte balance and also causes vasoconstriction that increases blood pressure. The ACE gene is located on chromosome number 17. It has 26 exons and 25 introns. The size of the ACE gene is 27546bp (Gribouval *et al*., 2005). If more than one allele occupies in gene's locus within population, the condition is said to be polymorphism in gene. Along with having more than one allele at a specific locus, the occurrence of this allele has a rate of at least 1% in a population. The most important polymorphism in the ACE gene is insertion/deletion polymorphism which occurred at intron 16 of the gene on chromosome 17q23 (Okamoto *et al*., 2002). A**s** ACE act as a potent vasoconstrictor of RAS, it also inactivates Bradykinin and also act as vasodilator of Kallikrein-kinin system, it is found to be involved majorly in process of inflammation. According to some studies, ACE can modulate the cutaneous neurogenic inflammations (Hyman *et al*., 2006)**.** Reports have also shown the Associations between the I/D polymorphism of the ACE gene in intron 16 and autoimmune diseases (Lehmann *et al*., 2005).

Inflammation is part of the body's defense mechanism to recognize and remove harmful stimuli and begin the healing process however if inflammation is slow and long lasting for prolonged periods of several months to years it is than called as chronic inflammation and is a disease condition. In general, the extent and effects of chronic inflammation may vary with the cause of the injury and the capacity of the body to repair and heel the damage of the body (Viitanen *et al*., 2001a)**.** The causes of chronic inflammation include failure of elimination of the agent causing acute inflammation, prolonged exposure to particular irritant or foreign material, autoimmune disorder, or frequent recurrent episodes of acute inflammation. It is observed that 3 to 5 people die due to chronic inflammatory disorders such as stroke, chronic respiratory diseases, cardiovascular disorders, cancer, obesity and diabetes, that makes chronic inflammation the most significant cause of death in the world (Porcelli, 2018).

Clear evidence is present that shows that there is an association between cardiovascular diseases and chronic renal diseases with coagulation disorders, inflammation, endothelial dysfunctions, and fibrosis. There are different markers of the inflammation and fibrinolytic system that includes D-dimmers and C-reactive proteins (CRP). They play an important role in the pathogenesis of renal and cardiovascular disorders. In atherosclerosis and coronary heart diseases, elevated levels of CRP are considered an important risk factor. The smallest fibrin degradation product is D-dimmer. Increased blood coagulation activation and fibrinolytic action pathway are reflected by the plasma levels of D-dimers. By the consideration of the fibrogenic markers that include the transforming growth factors β1 (TGF-β1) that is a multifunctional cytokine and is considered as a key driver of fibrosis. It works as the regulator of cell proliferation and the formation of collagen in renal and cardiovascular diseases. Another potent regulator of fibrinolysis known as plasminogen activator inhibitor 1(PAI-1) is also being involved in some physio-pathological processes. Its functions or expressions can cause deleterious outcomes depending upon the disease (Chábová, 2018).

Some of the previous studies also show the interactions between ACE I/D polymorphic, fibrinolytic cascade, and RAS (Bilchick *et al*., 2019)**.** From this, it can be hypothesized that there may be any associations between the elevated levels of plasma and inflammation. During the RAS system when Angiotensin I is being converted into Angiotensin II, ACE is formed that is also involved in the formation of the Bradykinin that plays important role in the degeneration and also in the inhibitory pathways of Angiotensin II. Angiotensin II also forms the AT1 Receptor and the AT2 Receptor. AT1 receptor plays an important role in growth inhibition, salt re-absorption, oxidative stress, and apoptosis. Apart from these, it is also involved in the vasoconstriction that leads towards CVD, Lipogenesis that leads towards obesity, reduced insulin sensitivity that leads towards diabetes and the most important is cytokines (chemokines) that are involved in the inflammation. The AT2 receptor is also produced through Angiotensin II that involved in vasodilation and increased insulin sensitivity. (Lines ending with a perpendicular segment represent inhibitory pathways). All the major mechanisms in which the Ace gene is involved and how it led towards the inflammatory disorders is given below in Fig. 1.

ACE I/D polymorphism is seen to be associated with many inflammatory diseases in which mostly researches have been done on vitiligo, aggressive periodontitis, asthma, pancreatitis, metabolic syndrome in elderly Slovaks, myocardial infarction, and obesity (Bilchick *et al*., 2019; Gribouval *et al*., 2005; Lehmann *et al*., 2005; Scholzen *et al*., 2003; Viitanen *et al*., 2001a, 2001b)**.** Insertion/deletion polymorphism of the ACE gene does not influence the gene structure but it affects the function of the gene (Gribouval *et al*., 2005; Manning *et al*., 2003; Schmidt *et al*., 2007)**.** I/D polymorphism is reported to be associated with various diseases and disorders. This study aims to determine the frequency and association of I/D Polymorphism in the Pakistani population and its distribution among various ethnicities to determine the frequency of I/D in various ethnicities and to determine the association of I/D polymorphism with inflammatory diseases (Sun *et al*., 2009).

METHODOLOGY

Subjects and Clinical data

The study design for this research was cross-sectional for that sampling was carried out from 2018 to 2019 at several regions of Rawalpindi, Pakistan. An inclusion criterion was subjects with age range from 18 to 80 years, with no any physical and mental disability and non-pregnant women. An exclusion criterion was the subjects having age below 18 or above 80 years, subjects with physical or mental disability and pregnant women. Socio-demographic information of subjects was judged by work type and level of education that was obtained on properly designed data acquisition form. Informed consent was also obtained from all participants of the study.

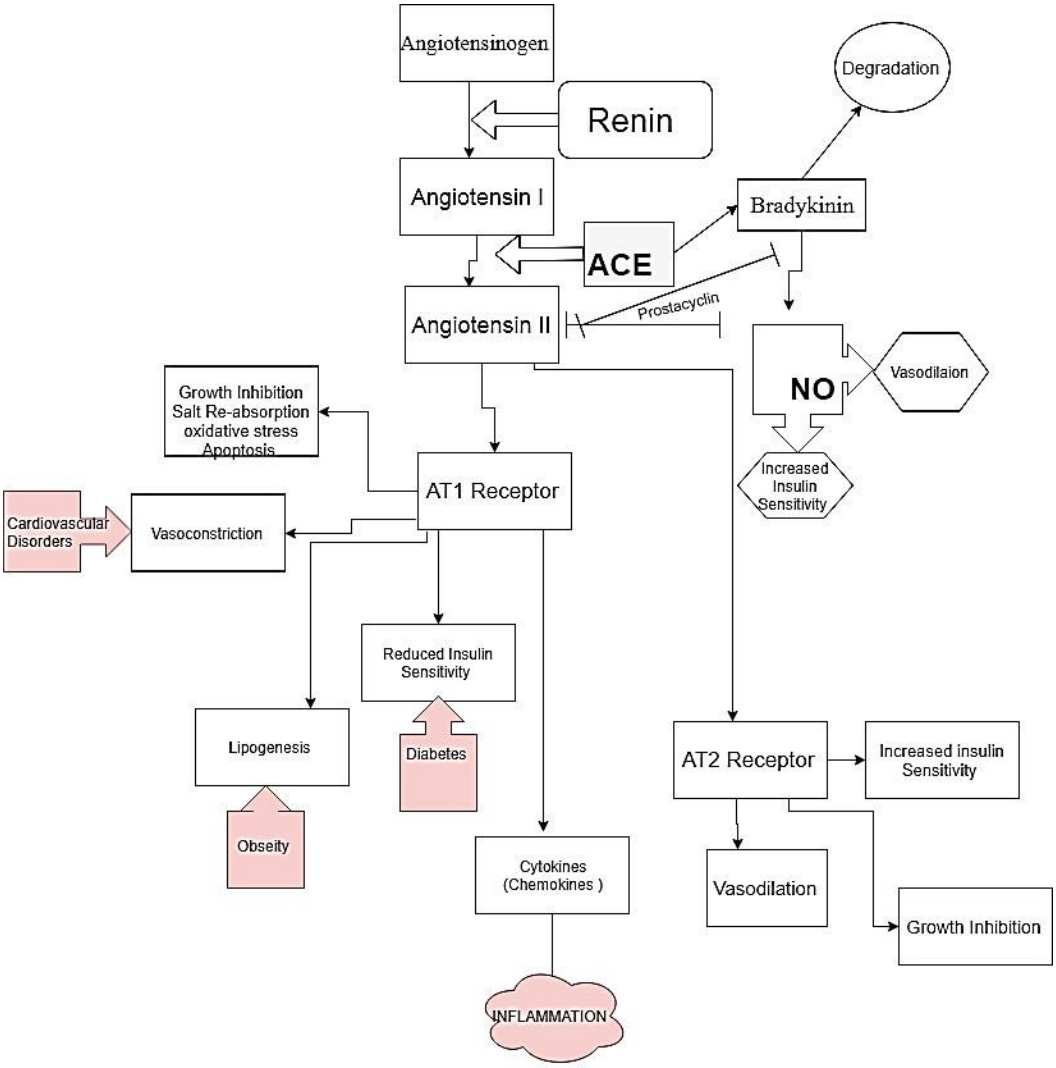

Fig. 1. The process of the RAS system and its action.

List of abbreviations

ACE	Angiotensin Converting Enzyme
RAS	Renin-angiotensin system
I/D	Insertion/Deletion
CVD	Cardiovascular Disorders
PCR	Polymerase chain reaction
BMI	Body mass index

Sample Size

To calculate sample size for study

$$Sample\ Size = \frac{Z^2 * P(1-p)/e^2}{Z^2 * P(1-p)/e^2 N}$$

formula was used (Reshetnikov *et al*., 2015). N represents the size of population, e is the margin error and Z represents the z-score of the number of standard deviation (SD). Using above formula for sample size, 381 subjects were required for Rawalpindi population with 5% margin, 95% level of confidence and 1.96 z-score. For this study we included 400 subjects for samples.

Evaluation of Obesity

Obesity was evaluated by measuring height and body weight that were recorded without shoes and with light clothing for all participants selected for the study. BMI was calculated and with respect to calculated BMI subjects were categorized as Normal weight, overweight and obese as per WHO classification of obesity for Asian people (Schmidt *et al*., 2009).

Evaluation of Diabetes

Diabetes was assessed by measuring blood sugar levels using Microlab 400 ELI Tech Group Reagents. Diabetes type II was defined as if a subject has random plasma glucose of 200 mg/dL or greater than this threshold value (Yang *et al*., 2020)**.**

Evaluation of Cardiovascular disorders

Cardiovascular disease conditions were defined as if the subjects have systolic blood pressure (SBP) greater than 140 mmHg and diastolic blood pressure (DBP) greater than 90 mmHg. Questions were asked via questionnaire from subjects to assess information about the CVD condition of the subject (Bilchick *et al*., 2019).

Evaluation of Psychiatric Disorders

Psychiatric disorder of subjects was assessed by asking questions related to standard indicators of anxiety and depression. A psychiatric disorder was defined as if the subject complains about having a feeling of nervousness, restless or tense, feeling sense of danger become panic or doom, or complains about increased heart rate, fast breathing, sweating, trembling, weakness, tiredness. A person having trouble in concentrating about anything about the present worry, couldn't be able to sleep properly or complains about gastrointestinal problems, couldn't be able to control worry or having the urge to avoid things that trigger anxiety (Qu *et al*., 2020).

Clinical Examination

Anthropometric measurements were taken that include personal details of subjects, which include age, gender, ethnicity, family history, disease history, lifestyle with a special focus on dietary habits and use of medicinal procedures.

Biochemical Analysis

The biochemical analysis was performed to identify any discrepancy or deviations from standard level indicating the presence of disease. The biochemical analyzer was used for CRP (complete reactive protein) and lipid profiling. Prepared samples were analyzed to measure total cholesterol and blood glucose levels by Microlab 400 with ELI Tech Group reagents. For the detection of the CRP in blood reagents, C-reactive protein detection was done. The appearance of visible agglutination under artificial light indicated the presence of CRP in serum samples.

Genetic Screening

Genetic screening was used to identify the presence of Polymorphism at the DNA level which was or could be associated with the disease. I/D polymorphism was identified through genetic screening. During the genetic screening, simple PCR was run on the extracted DNA from the collected blood samples by the simple organic method. The size of the products of PCR was 190bp for the deletion and it was 390bp for the insertion polymorphism (Sun *et al*., 2009). Primers were designed by using a tool called Primer3. The primers for the insertion/deletion polymorphism were

FORWARD 5-CTGGAGACCACTCCCATCCTTTCT-3
REVERSE 5-GATGTGGCCATCACATTCGTCAGAT-3.

Statistical Analysis

Statistical analysis through the SPSS v 25.0 was performed to find out and verify associations and chi-square ratios and their odds ratios were found out. For the odd ratios, the confidence level was 95% and lower and upper values were found out from which we find out the average values of the odds ratios. Results were presented in the form of a percentage, *p-value,* and odds ratio. The p-value of < 0.05 was marked as significant for association studies.

RESULTS

Population Characteristics and Metabolic Measures

A total of 381 samples were collected for the study, out of which the percentage of both genders is almost equal (50%). All the respondents were divided into 3 age groups that include teens age (18, 19), young adults that include (20 to 35), and then adults all above (35 above). Table 1 is showing the percentages of all the respondents according to which out of 381 7% were teenagers, 16% of subjects were young adults and 77% were young adults.

Table 1. Percentages of Gender and Ages of all the respondents included in this research project.

Variables Distribution	Category	frequency or	Insertion	Deletion
Gender	Male	50%	74%	26%
	Female	50%	74%	26%
Age	Young	7%	10%	30%
	Young adults	16%	24%	49%
	Adults	77%	67%	33%
Data collected through questionnaire **BMI is classified according to the WHO's recommendation of BMI classification for the Asian population**				

Table 2. Various variables with their prevalence and their associations with the I/D polymorphism.

Variables	Category	Frequency distribution	Insertion	Deletion	P-value	Odd ratio
Obesity * (obese 54%)	Obesity class I	30%	63.5%	36.5%	1.251 (for insertion) 0.801 (for deletion)	0.020 (for insertion) 0.167 (for deletion)
	Obesity class II	15%	62.5%	37.5%	0.852 (for insertion) 1.261(for deletion)	0.164 (for insertion) 0.212 (for deletion)
	Obesity class III	9%	71.4%	28.6%	1.933 (for insertion) 1.140 (for deletion)	0.236 (for insertion) 0.156 (for deletion)
Diabetes **	Diabetic	14%	20%	80%	0.875 (for insertion) 0.877 (for deletion)	0.979 (for insertion) 1.046 (for deletion)
Cardiovascular disorders ***	CVD	35%	68.75%	31.25%	0.808 (for insertion) 0.718 (for deletion)	1.022 (for insertion) 0.955 (for deletion)
Psychiatric disorders ****	Anxiety	55%	49%	51%	0.285 (for insertion) 0.286 (for deletion)	0.911 (for insertion) 1.210 (for deletion)

Worldwide frequencies of I/D with obesity are 67.25% and 32.75% (Abraham *et al*., 2015)**,** diabetes has 48.6% and 39.6% (Abraham *et al*., 2015; Flegal *et al*., 2016; Ward *et al*., 2016)**,** CVD has 40% and 77% (Savoia *et al*., 2011),

and psychiatric disorders have 40% and 41.0% respectively (Reshetnikov *et al*., 2015)**.** The results of our research are presented in Fig. 2.

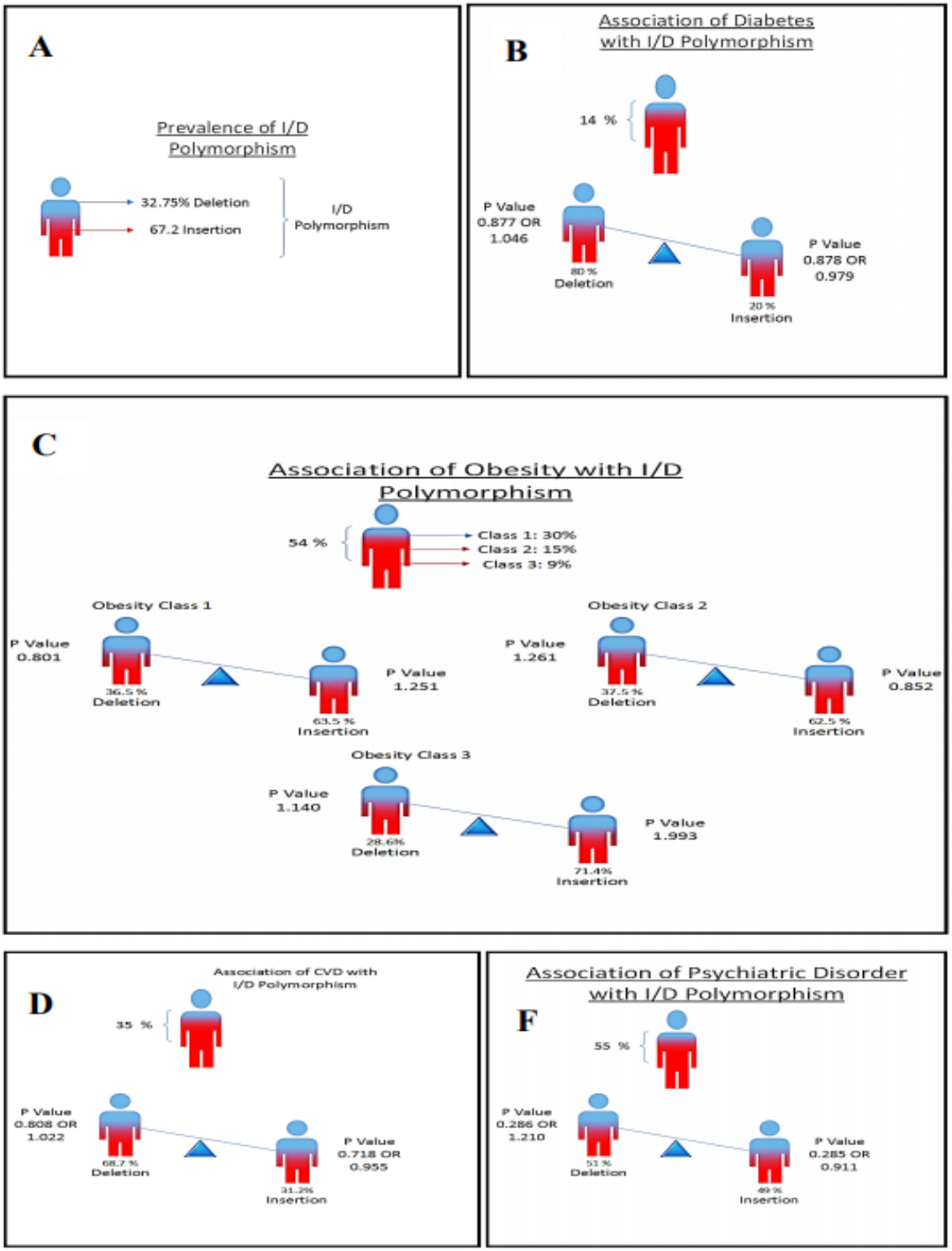

Fig 2. (A) is showing the percentage of I/D polymorphism in the population. (B) is showing the prevalence of diabetes. Prevalence of obesity is shown in (C), (D) is showing the prevalence of Cardiovascular disorder. (E) is showing the prevalence of psychiatric disorders.

Status of I/D Polymorphism

The percentage of I/D polymorphism of the ACE gene in the population of 381 samples was 67.2% is insertion while they remain 32.75% is the deletion based on PCR results (Fig. 2. (A)).

Diabetes association with I/D Polymorphism

The prevalence of diabetes was observed at 14% and its association with the insertion is noted as 20% with 0.878 p-values and OR of 0.979. It shows more association with deletion as of 80% with a p-value of 0.877 and 1.046 OR. But their p-values do not show any significance because they are greater than 0.05 (Fig. 2. (B)).

Obesity association with I/D Polymorphism

Prevalence of obesity as shown in Fig. 2. (C) according to which 54% people are obese and they are classified into 3 classes, obese class1, obese class 2 and obese class 3 according to the WHO's classification of BMI in Asian people. According to this 30%, people are categorized into obese class 1 who shows more association with insertion with the 63.5 % and p-value of 1.251 while with the deletion it has 36.5% with a p-value of 0.081. 15% of people categorized into obese class 1 have 62.5% association with insertion having a p-value of 0.852 and 37% association with deletion with a p-value of 1.261. Obese Class 3 has 9% of people who also show more association with the insertion having 71.4% with a p-value of 1.933 and 28.6% with deletion having 1.140 p-values. All these p-values present no significant association between the I/D polymorphism and the obese people (Table 2).

CVD association with I/D Polymorphism

The prevalence of cardiovascular disorder was observed at 35% in our selected population. Its association with insertion is 68.7% with 0-808 p-values (OR 1.022) and with the deletion it has 31.25% with p-values of 0.718 (0.955). These p-values also have no significant value Fig. 2. (D).

Psychiatric Disorders association with I/D Polymorphism

The prevalence of psychiatric disorders was 55 %. Their association with the insertion is 49% and 51% with the deletion. Their p-values and OR with the insertion are 0.285 and 0.911 while with the deletion it is 0.286 and 1.210. All these p-values show no significant importance (Fig. 2. (E)).

DISCUSSION

In the Rawalpindi region prevalence of obesity is 54%. As per WHO classification for obesity 6% of subjects were observed underweight (BMI >18), 40% of individuals were normal weight (BMI >18 to 24.9) 28% were overweight (BMI >25 to 29.9), 15% of subjects were identified as Obese Class I, 8 % subjects from participants were identified as Obese Class II, 3 % subjects were categorized under Obese Class III and 54% subjects whose BMI was greater than 30 were considered as Obese. The overall prevalence was observed 54% in Rawalpindi region.

The prevalence of obesity according to research 39% of adults were overweight and 13% were obese (Hyman *et al*., 2006)**.** The prevalence that was found out from our research is 28% are overweight and 26% are obese. Females have more obese because of their lifestyle and daily habits. Pregnancy is one of the major causes of obesity is seen in Pakistani women because during that time their eating habits change completely and rest becomes the major part of their routine and they didn't go for exercise that causes more obesity in females. Cardiovascular diseases overall make 32.5% of the population worldwide (Flegal *et al*., 2013) and our research results make 35% of the population that have CVD. The overall prevalence of diabetes was 9.9% in the US population in 2015 (Abraham *et al*., 2015; Flegal *et al*., 2016; Prina *et al*., 2015; Reshetnikov *et al*., 2015) but in our research, it is found to be 14%. This situation can be alarming because by comparing with the US population our prevalence of the disease is about 5% more so precautionary measures are needed to be taken to control this situation.

The overall prevalence of psychiatric disorders in the world is 32.9% (Abraham *et al*., 2015; Flegal *et al*., 2016) and our research shows that anxiety has 55% in the Rawalpindi population. The prevalence of psychiatric disorders is also very high that means that we have to check which are causing these problems in our population before it goes out of control. ACE gene and its associations with the inflammatory disorders remain controversial because in some regions it shows high associations while in the other ethnicities, it can be seen that the ACE gene and its any polymorphism (ID, II, DD) does not show any association with any inflammatory disorders. But in most of the cases, it is seen that it is present in the conditions. So, it can be concluded that these associations can be dependent on the ethnicities because their association ratios vary from one ethnicity to the other ethnicity.

All these diseases show associations with the I/D polymorphism of the ACE gene but their p-values are non-significant which means that there is no link between the inflammatory disorders and the I/D polymorphism. There are some studies according to which there is no reason through which we can predict that there is no association between the inflammation and the ACE genotype. Some studies like polycystic ovary disease (Marushchak1 *et al*., 2020) and spontaneous miscarriages (Rasha *et al*., 2020) do not find an association between them.

CONCLUSION

We conducted a study to find out the prevalence and associations of some inflammatory diseases that majorly include obesity, diabetes, CVD, and psychiatric disorders. Three hundred eighty one (381) subjects were enrolled in this study as per calculated using sample size formula with 5% margin and 95% level of confidence and 1.96 z-score. Samples were collected by the random sampling methods with some exclusion criteria that were people with some major physical abnormalities or injuries and pregnant women. For this study genetic screening and data analysis were performed. The percentage for the insertion was 67.3% and for the deletion, it was 32.6%. From this data, we find out the associations of this polymorphism with all the diseases like obesity, diabetes, CVD, and psychiatric disorders.

According to the odds ratios, diabetes and psychiatric disorders show high exposure association with deletion while cardiovascular disorders show higher exposure associated with the insertion. All the other values are indicating the low exposure association. As the rate of all the inflammatory disorders is notably high in this population which means that there is something else that is causing these inflammatory disorders that can be genetic, environmental, or epigenetic. In conclusion, we find that Deletion increases genetic susceptibility towards obesity, cardiovascular disorders, diabetes, and psychiatric disorders. However, this is a preliminary study and the results need to be confirmed in a larger cohort.

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Author's Contribution

All Authors contributed equally.

Conflict of Interest

There is no conflict of interest exists.

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