Effect of angiotensin converting enzyme gene studies in nonalcoholic fatty liver disease subjects

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ABSTRACT

Nonalcoholic fatty liver disease (NAFLD) is associated with obesity, insulin resistance, and type 2 diabetes. ACE gene is the key to involve with transforming Angiotensin-I to the potent vasoconstrictor of angiotensin-II, well-known for the association with all the above-mentioned diseases. Limited studies have been documented with NAFLD and ACE gene I/D polymorphism in the global studies, and there are no studies that have been documented in the Saudi population. The aim of the current study was to investigate the genetic association between angiotensin converting enzyme (ACE) gene, insertion (I)-deletion (D) polymorphisms in NAFLD in the Saudi population. NAFLD is a clinic pathological syndrome produced due to the environment, genetic, and metabolic stress-correlated factors, which are demonstrated clinically as fat accumulation in hepatocytes. This is a hospital-based case-control study implemented in 95 NAFLD cases and 78 non-NAFLD subjects. Genomic DNA was extracted in all the subjects to perform the PCR with ACE gene I/D polymorphism, and the current study results revealed the negative association between the NAFLD cases and controls in the Saudi population (DD vs II; OR-0.17: 95% CI (0.04-0.64), p=0.04, DD+ID vs II: OR-0.19; 95% CI (0.05-0.70), p=0.006 and D vs I; OR-0.34: 95%CI (0.21-0.57), p=0.003. In conclusion, this study confirms NAFLD has no genetic role in the Saudi population with ACE gene I/D polymorphism analysis.

1. Introduction

Non-communicable diseases (NCD) accounts finished 70% of overall deaths as per the global burden of disease study (Cai et al., 2020). Nonalcoholic fatty liver disease (NAFLD) is one of the NCD and is documented as the most common liver disease throughout the world (Eslam et al., 2018). NAFLD is categorized as through excessive hepatic lipid accumulation connected with metabolic abnormalities in the absence of excessive alcohol consumption (Llovet et al., 2012). NAFLD burden is ripening swiftly with the ongoing epidemics of obesity, diabetes, and metabolic syndrome (MetS). However, NAFLD was not known as an autonomous disease distinctly from obesity and diabetes since the long era (Chalasani et al., 2018). The disease NAFLD spectrum spreads from modest hepatic steatosis to the concomitant presence of inflammation, and ballooning is defined as non-alcoholic steatohepatitis, also known as NASH (Chen et al., 2020). The prevalence of NAFLD is assessed to be 25.2% globally, and the prevalence of NAFLD with type 2 diabetes (T2D) is known to be two-fold higher in the normal subjects, and 55% was known to be the complete prevalence of NAFLD among T2D. Worldwide, NASH prevalence was found to be 37.3% among T2D subjects (Younossi et al., 2019). Inclusion of NASH in NAFLD is a foremost cause of liver correlated morbidity and mortality, and NASH patients have a latent risk of emerging hepatocellular carcinoma (Kanda et al., 2020).

The genetic connection between the variables in the pathogenesis of NAFLD and NASH developments has been concerned (Karrar et al., 2019). Earlier studies showed that NAFLD could exist in the non-obese subjects of 10-20%, most frequently associated with central adiposity, recent weight gain, and genetic risk factors (Namjou et al., 2019). Limited information is documented for understanding in developing the progress of NAFLD, and it's known to be a complex metabolite state in which genetic and pathogenetic factors will take place along with the lifestyle (Liu et al., 2019a).

NAFLD is known to be associated with numerous neurotransmitters, hormones, and proinflammatory cytokines (Diehl et al., 2005; Chaldakov et al., 2003).

Genome-wide association studies (GWAS) have been established numerous genetic variants connected with remarkable risk factors for NAFLD disease (Liu et al., 2019b). NAFLD is also connected with genetics through family history, aggregational, functional, experimental, twin studies, as well as single nucleotide polymorphisms in the GWAS and candidate genes (Sookoian and Pirola, 2017). Renin angiotensin system is connected with multiple human diseases such as nephropathy, liver, kidney, osteoarthritis, lungs, obesity, hypertension (HTN), chronic inflammation, fibrosis, and oxidative stress (Marshall et al., 2000; Poornima et al., 2015). Angiotensin converting Enzyme-I (ACE) is the key to involve with transforming Angiotensin-I to the potent vasoconstrictor of angiotensin-II (Bastard et al., 2006).

The ACE gene insertion/deletion (I/D) polymorphism denotes the appearance or disappearance of 287bp of Alu sequence present at the intron 16 region (Khan et al., 2014). Limited studies have been documented in the NAFLD with ACE gene I/D polymorphism (Tekatas et al., 2016; Sydorchuk et al., 2018; Güçlü et al., 2010). There are no documented studies have been stated in the Saudi population between I/D polymorphism in the ACE gene. So, the current study aims to explore the genetic association between angiotensin converting enzyme insertion and deletion polymorphism in the Saudi subjects diagnosed with NAFLD.

2. Materials and methods

2.1. Study enrolment

In this case-control study, 95 NAFLD cases and 78 non-NAFLD (controls) were involved. This study was performed in the Molecular Genetic pathology unit at King Saud University. The NAFLD patients and controls have opted from the surgery department in the KKUH premises. The inclusion criteria of the NAFLD subjects were based on ultrasound results confirming enlarged fatty liver after reconfirmation through comprehensive virology screening, subjects with obesity, T2D, and insulin resistant subjects. A histopathological NAFLD Assessment Score (NAS) of patients' liver biopsy in the final range of NAFLD. The histological findings of segment 4 liver biopsies fixed with formaldehyde and paraffin block sections stained with hematoxylin–eosin; Masson's trichrome; Prussian blue were evaluated by a single pathologist. The exclusion criteria of the NAFLD subjects are viral hepatitis, alcoholic hepatitis, drug-induced hepatitis, α1-antitrypsin deficiency, and Wilson's disease. The controls have opted for healthy subjects without any diseases. An ethical grant was received from IRB in the university premises, and informed consent was obtained from each patient involved in this study.

2.2. Molecular analysis

From each patient, 2ml of the peripheral blood was collected in ethylenediaminetetraacetic acid (EDTA) tubes, and simultaneously liver tissue was also collected for extraction of genomic DNA for the molecular analysis. Using the EDTA blood, genomic DNA will be extracted using the kit-based method, and DNA will be quantified using NanoDrop, spectrophotometer to visualize the genomic DNA (Al-Mutawa, 2019). Genotyping for 287bp Alu sequence was carried out with polymerase chain reaction (PCR), and primers were adapted from Khan et al. (2014). PCR profile comprises denaturation (95°C-30s), annealing (60°C-30s), extension (72°C-45s) with the involvement of initial denaturation, and final extension of 35 cycles. The PCR products were detected through 3% gel electrophoresis with ethidium bromide stained gel. Insertion is defined as 490bp, deletion as 190bp, and both the insertion and deletion as 490/190bp (Fig. 1).
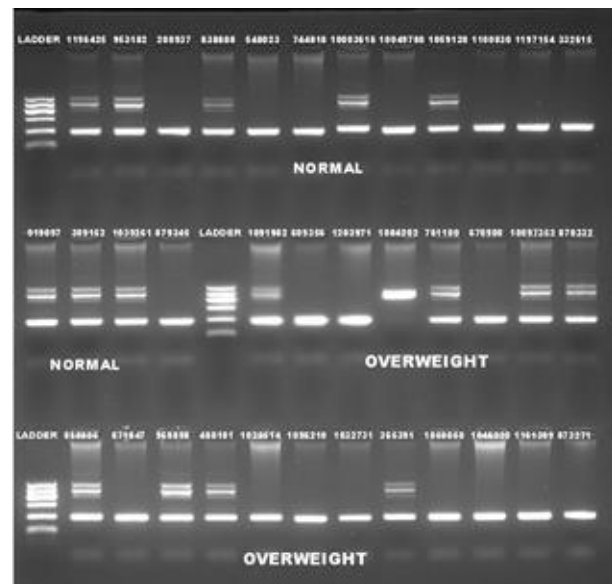

Fig. 1: Agarose gel representation of ACE gene I/D polymorphism

2.3. Statistical analysis

Hardy Weinberg equilibrium (HWE) test was implemented to determine by the genotypes in NAFLD cases and the controls. The clinical analysis was performed with SPSS software (version 23.0). Continuous variables were measured with the mean±standard deviations and categorical variables with the percentages and their frequencies. Genotype and allele frequencies were carried out with Openepi software (version 3.01) (Khan et al., 2019). The P-values of <0.05 is considered as significant association.

3. Results

Genotype distribution between allele and genotypes for ACE I/D gene polymorphism is

regulated using the HWE. In this study, 95 NAFLD cases and 78 controls were involved in this study. The baseline characteristics of NAFLD cases and controls were shown in Table 1.

Table 1: Anthropometric features of NAFLD and non-NAFLD subjects

S. No	NAFLD (n=95)	Non-NAFLD (n=78)	pValue
Age (Years)	43.59±11.79	34.91±11.05	0.001
Gender (M: F)	30: 64	15: 63	0.55
Weight (kg)	83.9±15.37	78.1±15.79	0.01
Height (cms)	159.63±0.09	159.93±0.09	0.95
BMI (kg/m²)	32.9±5.93	30.5±5.79	0.008

The mean age of the NAFLD cases was 43.59±11.79, whereas in the controls, it was 34.91±11.05, and age was found to be a significant association (p=0.001). Gender was found to be almost similar and not associated (p=0.55). Weight (p=0.01) and height (p=0.95) showed the association with BMI (p=0.008) in the NAFLD cases when compared with the controls. Allele and genotype frequencies showed in Table 2.

The genotype frequencies in the NAFLD cases were 16.8% of II, 40% of ID, and 43.2% for DD, and allele frequencies were found to 63.2% in the D allele and 36.8% in I allele. The control groups showed 3.8% of II, 39.8% ID, and 56.4% of DD genotype association. 76.2% and 23.8% was the allele frequencies for D and I. None of the genotype model was found to be associated significantly (DD vs II; OR-0.17: 95% CI (0.04-0.64), p=0.04, DD+ID vs II: OR-0.19; 95% CI (0.05-0.70), p=0.006 and D vs I; OR-0.34: 95% CI (0.21-0.57), p=0.003.

Table 2: Genotype and allele frequency distributions in NAFLD and control subjects

ACE (ID)	NAFLD (n=95)	Non-NAFLD (n=78)	OR	95%CI	P-Value
II	16 (16.8%)	03 (3.8%)	-	Reference	-
ID	38 (40%)	31 (39.8%)	0.22	0.06-0.86	0.02
DD	41 (43.2%)	44 (56.4%)	0.17	0.04-0.64	0.04
ID+DD vs II	79 (83.2%)	75 (96.2%)	0.19	0.05-0.70	0.006
ID vs DD+II	38 (40%)	31 (39.8%)	1.01	0.54-1.86	0.97
DD vs II+ID	41 (43.2%)	44 (96.2%)	0.55	0.30-1.01	0.05
I	70(36.8%)	37 (23.8%)	-	Reference	-
D	120 (63.2%)	119 (76.2%)	0.34	0.21-0.57	0.003

4. Discussion

The aim of the present study was to investigate the genetic association with ACE gene polymorphism studies in NAFLD patients in the Saudi population. The current study will be documented as the initial study in the Saudi population. The HWE was shown to deviate between the NAFLD cases and controls. The current study results showed the non-significant association when compared between NAFLD cases and controls within the Saudi population. Allele and genotype frequencies were found to be a significant association. Genotype frequencies between deletion (56.4% in controls and 43.2% in cases) and heterozygous (40% in NAFLD cases and 39.8% in controls) were found high in non-NAFLD when compared with cases.

ACE (EC 3.4. 15.1) is a central component of RAS that controls HTN through regulating the volume of fluids in the body, which converts Angiotensin I to Angiotensin II by vasoconstrictor and also inactivates the bradykinin (Shen et al., 2012). ACE gene is a zinc metallopeptidase that appears on chromosome17q23. It is one of the utmost genetic variables responsible for the variability of ACE serum activity (Khan et al., 2014; Poornima et al., 2015). II, ID, and DD are the three types of genotypes that exist in the human population. Till now, the complete pathogenesis is not confirmed (Zhang et al., 2019). There are limited studies have been recorded with NAFLD in the global population and showed both significant and non-significant association (Güçlü et al., 2010; Tekatas et al., 2016; Sydorchuk et al., 2018). Our study is also in agreement with the previous studies, which showed a negative association (P>0.05).

Meta-analysis studies are defined as the statistical analysis of a large collection of analysis results from specific studies for the purpose of integrating the findings. The concept of performing meta-analysis studies is to confirm the statistical procedure of the combined data of the global studies and also based on single study results, the utility of an intervention of a hypothesis cannot be done (Haidich, 2010), and it has been proven by an enormous number of meta-analysis studies. ACE gene polymorphism has been implemented the meta-analysis studies in almost all the human diseases and showed both the effects of association (Ahmad et al., 2019; Aslbahar et al., 2018; Han et al., 2017; Luo et al., 2016; Mengesha et al., 2019; Xu et al., 2018; Yuan et al., 2017; Zheng and Huang, 2020). However, there are no meta-analysis studies have been documented due to the limited number of global studies have been documented.

NAFLD is known to be chronic liver disease and affects up to 25% globally. NASH cirrhosis is connected with NAFLD through the growth with a 170% growth in cirrhosis. NASH cirrhosis is known to be the leading indication for liver transplantation. The major risk factors for NAFLD are associated with Insulin resistance, obesity, and T2D (Danford et al., 2018). The sub-studies in the Danford et al. (2018) studies have documented the heritability of NAFLD disease within the affected families. Family aggregational studies confirmed NAFLD affected overweight children have major chances to inherit the disease when compared with overweight children without the disease. In our study, the BMI for opted NAFLD patients (n=95) were found to be as; 8 of them were normal weight, overweight was documented to be 28 patients, obesity 1 and obesity

2&3 were enrolled as 32 and 27 NAFLD patients. However, the BMI data for the enrolled patients were not recorded.

A mutation in rs738409C>G in the *PNPLA3* gene is confirmed using the genome-wide association studies. The rs738409C>G variant is known to be I148M mutation is the initial allele associated with intrahepatic fat content, which appears to be a major genetic determinant of hepatic steatosis in the growth of NAFLD (Romeo et al., 2008). The rs58542926C>T, rs780094C>T, and rs641738C>T variants in the TM6SF2, GCKR, and MBOAT7 genes are connected with hepatic steatosis and NAFLD (Danford et al., 2018). In this study, single ACE gene polymorphism was studied was implemented, and the current study data showed the negative association. In the Saudi population, ID polymorphism studies in the ACE gene has been implemented with different diseases and showed positive and negative associations (Alharbi et al., 2013a; 2013b; Al-Mutawa, 2018; Fawwaz et al., 2017; Sabir et al., 2019).

The strength of the current study was to involve Saudi subjects, and this is the first molecular-based study in the Saudi population. One of the limitations of this study is the low sample size, implemented the single ID polymorphism, and missed out on the serum analysis. In conclusion, the present study in the Saudi population in NAFLD subjects showed a negative association with ACE I/D gene polymorphism studies. Future studies should be performed in the global population to implement the meta-analysis studies between ACE gene I/D polymorphism and NAFLD-NASH disease.

Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

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