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Association of ACE2 Gene Polymorphisms (rs2285666 and rs4646142) with Dyslipidemia in Iraqi Patients with Type 2 Diabetes Mellitus: A Sex-Stratified Analysis

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Abstract: **Background: Growing evidence suggests that angiotensin-converting enzyme 2 (ACE2) is involved in lipid metabolism as well as cardiovascular homeostasis. Genetic variations in ACE2 have been linked to metabolic disorders including dyslipidemia which is one of the most common features of type 2 diabetes mellitus. However there has been very limited study on association between ACE2 polymorphisms with dyslipidemia in patients with type 2 diabetes mellitus specifically Iraqi population.**

Aim: This study aimed at investigating the association between ACE2 gene polymorphisms [rs2285666 and rs4646142] and dyslipidemia in Iraqi patients with T2DM by sex stratification.

Methods: Case control study was conducted on 463 patients with T2DM (222 women and 241 men). Participants were divided into case groups (dyslipidemia) and control groups (non-dyslipidemia) based on serum lipids levels. Genotyping for ACE2 polymorphisms using PCR-based methods and serum level of ACE2 were measured by ELISA.

Results: AA genotype of rs2285666 SNPs was significantly associated with dyslipidemia in female subjects (OR=3.19, 95%CI=1.31 – 7.78, p=0.01). A allele of rs2285666 SNPs was associated with dyslipidemia in male subjects (or=2.36, 95%CI=1.27 – 4.39, p=0.006). High ACE2 levels, elevated atherogenic indices, and adverse lipid profiles were observed in carriers of the A allele. No significant association was found for rs4646142 SNP.

Conclusion: The rs2285666 SNP was associated with dyslipidemia in Iraqi T2DM patients, with sex-specific differences in risk. The ACE2 variants may contribute to dyslipidemia pathogenesis in T2DM and highlight the potential for personalized risk stratification and therapeutic targets.

Keywords: **ACE2, rs2285666, rs4646142, Dyslipidemia, T2DM, Association.**

1. INTRODUCTION

ACE2 is a transmembrane protein with a single metalloproteinase active site and a transmembrane domain, making it tissue-bound or membrane-bound [1]. The ectodomain of ACE2 can be cleaved and released into the extracellular environment, resulting in the circulating or soluble form [2]. ACE2 adjusts blood pressure and sustains homeostasis due to its negative feedback on the renin-angiotensin system (RAS) [3]. ACE2 and its homolog ACE are central to the RAS component formation. It splits angiotensin I (Ang I) or Ang II into the inactive peptides Ang (1–9) and Ang (1–7). Ang (1–9) is also transformed into the vasodilator Ang (1–7). Thus, ACE2 mitigates the vaso-constrictive actions of the ACE-Ang II axis that raise blood pressure [4].

Research has shown that ACE2 activation improves lipid profiles by increasing HDL-C and decreasing LDL-C and triglyceride levels, thus lowering heart disorders. This occurs by transforming angiotensin II, a pro-inflammatory and prooxidant peptide, to angiotensin-(1-7), which is an anti-inflammatory and non-atherogenic substance [5]. In hyperlipidemia animal models, ACE2 overexpression reduces lipid deposition in the liver and improves insulin sensitivity, indicating protective effects against metabolic conditions [6]. ACE2 was shown to slow down the progression of atherosclerosis by minimizing oxidative stress and inflammation in the endothelium [7]. The lack of ACE2 exacerbated lipid-induced endothelial dysfunction, whereas activation was protective from lipid-mediated vascular injury [8].

T2DM is a recognized risk factor for heart disease, stroke, hypertension, and atherosclerosis [9]. The influence of insulin resistance on the vascular system and kidneys is involved in the development of hypertension in individuals with T2DM [10]. A global population-based study reportedly showed that increased plasma levels of ACE2 were associated with a greater risk of cardiovascular diseases [11]. Increased ACE2 levels are also demonstrated to be associated with a risk of cardiovascular diseases (CVD) in T2DM [12]. Serum ACE2 levels were found to be correlated with total cholesterol (TC) and triglyceride levels (13). Thus, the ACE2 role may differ in T2DM from that in healthy individuals. The **ACE2** gene is on the X chromosome at the cytogenetic location Xp22.2**.** Its expression and genetic variation are gender-dependent and, thus, differ between males and females [14]. It has been found that genetic background is involved in 67% of circulating ACE2 level variation [15]. ACE2 polymorphisms, rs879922, rs233575, rs2158083 and rs2074192, and the haplotype C-G-C of three of these SNPs were explored to induce vulnerability to obesity and hyperlipidemia in women [16]. ACE2 SNP, rs879922, may also be a susceptibility genetic locus marker for T2DM and its related cardiovascular risks [17]. ACE2 can direct changes in both hyperlipidemia and T2DM.

Studies concerning the involvement of ACE2 gene polymorphism in T2DM dyslipidemia are limited, particularly in Iraq. The present study was conducted to assess the impact of the ACE2 gene polymorphism (rs2285666 & rs46461420 SNPs) on dyslipidemia in type 2 Iraqi diabetic patients.

2. METHODS

2.1. Study Participants

A case-control study was conducted involving two groups of participants. The women group comprised 222 type diabetic women, 104 with dyslipidemia (cases) and 118 without (control). The women's age range was 52.4± 8.4 y and 53.1± 8.8 y, respectively. The men group contained 241 type 2 Iraqi diabetic men, 116 with dyslipidemia (cases) and 125 without (control). The men's age range was 53.97 ± 9.77 y and 53.33 ± 9.15 y, respectively. Participants were recruited from the Al-Sadder Teaching Hospital in Najaf, Iraq. The cases and control groups were diagnosed as type 2 diabetic patients according to the WHO guidelines for type 2 diabetes. Dyslipidemia was confirmed from the assessment of serum lipid profile measurement. Cases with diseases other than T2DM and dyslipidemia were excluded, while individuals with diseases other than T2DM were excluded from the control group. All participants were provided informed consent, and the Scientific Committee of the Department of Biochemistry, College of Medicine, University of Kufa, Iraq, approved the study. Data collection, biochemical analyses, and genetic testing were conducted from March to October 2024 in the Department of Biochemistry, College of Medicine, University of Kufa, Iraq. A detailed questionnaire was administered to collect demographic information, medical history, family history, and other relevant data. The body mass index (BMI) was calculated by dividing weight in Kg on square height in meter.

2.2. Biochemical Measurements

ACE2 levels in serum were determined by ELISA (sandwich enzyme-linked immunosorbent assay) from Sunlong Biotech (China) according to manufacturer's guidelines. Serum ACE2 levels are represented in units of ng/mL. The serum lipid profiles at fasting conditions of total cholesterol (TC), triglycerides (TG), and HDL-C were determined with commercial kits as standard. Very low-density lipoprotein cholesterol (VLDL-C) and low-density lipoprotein cholesterol (LDL-C) were determined indirectly [18]. The atherogenic index of plasma (AIP) and the cholesterol ratio (cholesterol/HDL-C) were calculated indirectly using established formulas.

2.3. Genotypic Analysis of ACE2 Polymorphisms

Genomic DNA was extracted from whole blood samples collected in EDTA-anticoagulant tubes using a G-spin™ Total DNA Extraction Mini Kit. Genotyping of ACE2 gene polymorphisms (rs2285666 G>A and rs4646142 G>C) was performed using restriction fragment length polymorphism polymerase chain reaction (RFLP-PCR) for the rs2285666 SNP and allele specific-PCR (AS-PCR), for the rs4646142 SNP. Primers for the rs2285666 SNP were selected based on previously

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published sequences [19]: F; 5-CATGTGGTCAAAAGGATATCT-3 and R; 5-AAAGTAAGGTTGGCAGACAT-3. Primers for rs4646142 SNP were designed using Primer3 software. They were:

F-Wild: 5-GCTGGTTGCTTTGTAGTCTCG-3

F-Mutant: 5-GCTGGT TGCTTTGTAGTCTCC-3

R: 5-GCAATG GTGGACCAGGTAGG-3

The PCR amplification reactions of the two SNPs were performed in a total volume of 25 µL. The RFLP-PCR reaction mixture of the rs2285666 SNP contained 10 µL of Taq Plus Master Mix, 1.5 µL of each primer (F and R), 100 ng (5 µL) of genomic DNA template, and 7 µL nuclease-free water. PCR products were visualized by agarose gel electrophoresis. The product was digested with the restriction enzyme AluI (2 U) in a 20 µL reaction volume containing 10× buffer and 5 µL of PCR product. Digestion was conducted at 37°C for 10 hours, followed by electrophoresis on a 2% agarose gel to identify genotypes. For the allele-specific PCR of the rs4646142 SNP, the reactions were set up in two separate tubes: the first tube contained 1 µL each of the forward primer specific to the wild-type allele (F-Wild) and the reverse primer (R), while the second tube contained 1 µL each of the forward primer specific to the mutant allele (F-Mutant) and the same reverse primer (R). For each tube, 13 µL of Taq Plus Master Mix, 100 ng (4 µL) of genomic DNA template, and 6 µL nuclease-free water were added. Products were analyzed by 2% agarose gel electrophoresis.

2.4. Statistical Analysis

Continuous variables were expressed as mean ± standard deviation (SD). Quantitative data analyses were carried out using the student's t-test for variables of two groups or by one-way analysis of variance (ANOVA) for more than two groups. Genotype and allele frequency distribution of ACE2 were calculated using the chi-square test. Qualitative women participant data were evaluated using logistic regression analysis under co-dominant and dominant genetic models. Associations between SNPs and dyslipidemia were analyzed using multivariate logistic regression, adjusted for conventional risk factors, and expressed as odds ratios (ORs) with 95% confidence intervals (CIs). A two-tailed p-value <0.05 was considered statistically significant.

3. RESULTS

3.1 Demographic and Biochemical Profiles of Cases and Controls

The demographic and biochemical characteristics of the study participants are presented in Table 1. Among men, those with dyslipidemia exhibited significantly higher BMI values ($p < 0.0001$), ACE2 ($p< 0.0001$), TG ($p= 0.030$), TC ($p = 0.0004$), and LDL-C levels ($p< 0.0001$), and atherogenic indices (AIP: $p< 0.0001$; TC/HDL-C: $p < 0.0001$) compared to controls. Conversely, HDL-C levels were significantly lower in cases ($p < 0.0001$). Similarly, type 2 diabetic women with dyslipidemia had higher BMI values ($p = 0.01$), ACE2 ($p < 0.0001$), TG ($p = 0.022$), TC ($p = 0.0003$), and LDL-C levels ($p < 0.0001$), and atherogenic indices (AIP: $p < 0.0001$; TC/HDL-C: $p < 0.0001$) compared to controls. HDL-C levels were significantly lower in cases ($p < 0.0001$).

Table 1: Demographic and Biochemical Profiles of Cases and Controls: A Sex-Stratified Analysis

	Women			Men		
	Mean ± SD			Mean ± SD		
	Cases	Control	P-value	Cases	Control	P-value
No	104	118		116	125	
Age (y)	52.4± 8.4	53.1± 8.8		53.97 ± 9.77	53.33 ± 9.15	
BMI (kg/m^2)	26.81 ± 4.49	25.36 ± 3.55	0.01	28.10 ± 4.69	25.77 ± 3.15	<0.0001
ACE2 (ng/mL)	0.269 ± 0.05	0.180± 0.05	<0.0001	0.217 ± 0.05	0.168± 0.06	<0.0001
TG (mmol/L)	1.54 ± 0.89	1.25 ± 0.74	0.022	1.15 ± 0.58	0.96 ± 0.55	0.030
TC (mmol/L)	4.10 ± 0.86	3.55 ± 1.05	0.0003	4.06 ± 0.95	3.44 ± 0.89	0.0004
HDL-C (mmol/L)	1.08 ± 0.43	1.37 ± 0.38	<0.0001	1.15 ± 0.48	1.34 ± 0.36	<0.0001
LDL-C (mmol/L)	2.70 ± 0.95	1.62 ± 0.94	<0.0001	2.56 ± 0.98	1.65 ± 0.98	<0.0001
VLDL-C (mmol/L)	0.61 ± 0.41	0.58 ± 0.34	0.07	0.71 ± 0.41	0.66 ± 0.38	0.06
AIP (Log TG/HDL)	0.17 ± 0.06	0.08 ± 0.03	<0.0001	0.18 ± 0.06	0.09 ± 0.03	<0.0001
TC/HDL-C	3.05 ± 1.63	2.05 ± 1.26	<0.0001	3.11 ± 1.86	2.15 ± 1.32	<0.0001

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3.2 Genotype and Allele Frequencies of ACE2 Gene Polymorphism in Cases and Controls

Table 2 presents the genotype and allele frequencies of the ACE2 gene polymorphisms in cases and controls. In women, the codominant model revealed a significant association between the AA genotype of the rs2285666 SNP and dyslipidemia (OR = 3.19, 95% CI = 1.31–7.78, p = 0.01). The minor allele frequency (MAF) of the A allele was higher in cases (36.5%) compared to controls (32.7%). In men, the A allele was significantly associated with dyslipidemia (OR = 2.36, 95% CI = 1.27–4.39, p = 0.006), with a higher MAF in cases (31%) compared to controls (16%). Regarding rs4646142 SNP, the results of neither women nor men exhibited a significant association with dyslipidemia.

3.3 Demographic Characteristics, Lipid Profiles, and ACE2 Levels Across Genotypes of Genotypes of rs2285666 SNP

Table 3 presents the demographic, biochemical, and ACE2 level differences across the rs2285666 genotype carriers. ACE2 levels increased significantly (p= 0.001) among women who carried GG to GA and AA genotypes. HDL-C levels were significantly ($p < 0.0001$) lower in those of the AA genotype compared to the GG and GA genotypes. Atherogenic indices were significantly (AIP $p < 0.0001$; TC/HDL-C $p < 0.0001$) higher in carriers of the AA genotype compared to the GG and GA genotypes. ACE2 levels were significantly (p= 0.02) higher in A allele carriers compared to G allele carriers. HDL-C levels decreased significantly ($p < 0.0001$) in the A allele than in the G allele carriers. Similarly, AIP and TC/HDL-C were significantly (AIP: $p < 0.0001$; TC/HDL-C: $p < 0.0001$) higher in the A allele than in the G allele carriers.

4. DISCUSSION

4.1 Demographic and Biochemical Profiles

In the current study, significant differences in demographic and biochemical profiles were obtained between type 2 diabetic individuals with and without dyslipidemia stratified by sex.

The observed differences in BMI, lipid profiles and ACE2 (angiotensin-converting enzyme 2) among case and control subjects are likely the result of the interaction between genetic predispositions for these conditions and metabolic dysregulations [20]. The X-linked gene encoding ACE2 plays an important role in both the renin-angiotensin system (RAS), and lipid metabolism. It has also recently been identified to play significant roles in insulin sensitivity. Thus, elevated ACE2 activity in dyslipidemic subjects could indicate adaptive or compensatory mechanisms to counteract metabolic disturbances since ACE2 has been demonstrated to have effects on lipid homeostasis and inflammation [21]. Further, the increased BMI found in the study groups is strongly related to obesity and its comorbidities including insulin resistance and dyslipidemia that require attention. Adipose tissue dysfunction will worsen lipid abnormalities as well as cause systemic inflammatory responses [22] . These results therefore highlight the pathophysiologic significance of ACE2 in the relationship between dyslipidemia in type 2 diabetes mellitus, particularly regarding its ability to regulate lipid profile and atherogenic indices. Furthermore, the sex-specific variations call for individualized approaches to managing dyslipidemia in T2DM since women and men may respond differently regarding genetics and metabolism [23].

Table 2: Adjusted genotype and allele frequencies of ACE2 Gene Polymorphism in cases and control individuals

		Cases	Control	OR (95%CI)	P-value
rs2285666	**Women**				
	No	104	118		
	Codominant				
	GG	50 (48%)	58 (49.2%)		
	GA	32 (30.8%)	52 (44%)	0.71 (0.40–1.28)	0.25
	AA	22 (21.2%)	8 (6.8%)	3.19 (1.31–7.78)	0.01
	Dominant				
	GG	50 (48%)	58 (49.1%)		
	GA+AA	54 (52%)	60 (50.9%)	1.04 (0.62–1.77)	0.87
	MAF%	36.5	32.7		
	Men				
	No	116	125		
	G	80 (69%)	105 (84%)		
	A	36 (31%)	20 (16%)	2.36 (1.27–4.39)	0.006
	MAF%	31	16		

rs4646142	**Women**				
	No	104	118		
	Codominant				
	GG	55 (52.9%)	62 (52.5%)		
	GC	38 (36.5%)	40 (33.9%)	1.07 (0.62–1.85)	0.80
	CC	11 (10.6%)	16 (13.6%)	0.78 (0.34–1.76)	0.54
	Dominant				
	GG	55 (52.9%)	62 (52.5%)		
	GC+CC	49 (47.1%)	56 (47.5%)	0.99 (0.58–1.67)	0.96
	MAF%	28.8	30.5		
	Men				
	No	116	125		
	G	72 (62.1%)	80 (64%)		
	C	44 (37.9%)	45 (36%)	1.09 (0.64–1.83)	0.76
	MAF%	37.9	36		

The combination of enhanced ACE2 along with unfavorable lipid patterns arising in individuals with dyslipidemia may speed up the progression of T2DM or its complications. The dysfunction of the endothelium induced by hyperlipidemia contributes to insulin resistance, chronic inflammation, and the development of diabetic complications, cardiomyopathy, and death [24]. The lower HDL-C and higher LDL-C levels observed in cases would worsen cardiovascular risks as HDL-C protects against atherosclerosis, whereas LDL-C promotes plaque formation [25]. The variations in atherogenic indices (AIP and TC/HDL-C) significantly point to an enhanced cardiovascular risk in dyslipidemic individuals, with these indices being excellent indicators of atherosclerosis [26].

4.2 Genotype and Allele Frequencies of ACE2 Gene Polymorphism

Genotyping of the ACE2 gene sequence showed that the AA genotype was associated with dyslipidemia among type 2 diabetic women, the OR of 3.19 depicted how risk factor AA carriers are to develop such metabolic alteration. However, the association seemed to be with sex-related differences. In men, the OR was found to be 2.16, showing it as a 2.16 risk factor for developing dyslipidemia in T2DM. Such results brought about critical insights into the genetic underpinnings of dyslipidemia with the ACE2 gene and could lead to potential implications in personalized medicine and risk stratification for T2DM.

The interaction between polymorphisms of the ACE2 gene and dyslipidemia might help understand how the ACE2 gene impacts lipid metabolism and works with gender-specific genetics within both men and women. Therefore, it was found that there are an increase of the risk of dyslipidemia for the AA genotype in women and the A allele in men, therefore it can say that the ACE2 gene could be altered by the A allele and contribute to the disruption of lipid homeostasis. The RAS system is a regulatory system for lipid metabolism and insulin sensitivity and ACE2 regulates the RAS system [21]. Since ACE2 is X-linked; there exists the potential for a gender specific difference in the risk of dyslipidemia due to women having two x-chromosomes compared to men who have only one [27]; which may indicate some form of dosage compensation effect(s) or inactivation. The lack of an identifiable relationship between the rs4646142 SNP and dyslipidemia suggests that not all variations of the ACE2 gene equally affect the risk of developing dyslipidemia. This highlights the complexity of the genetics behind metabolic traits.

The association of ACE2 polymorphisms with dyslipidemia may have significant implications for the progression of T2DM and its complications. Dyslipidemia is a major risk factor for CVD, which is the leading cause of morbidity and mortality in T2DM patients [28]. The rs2285666 SNP, by potentially altering ACE2 function, may exacerbate lipid abnormalities, insulin resistance, and endothelial dysfunction, thereby accelerating the development of atherosclerosis and other diabetic complications [24]. The higher frequency of the A allele in dyslipidemic individuals suggests that this variant may contribute to a more aggressive disease phenotype, warranting closer monitoring and early intervention in carriers [25].

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Table 3: Demographic Characteristics, Lipid Profiles, and ACE2 Levels Across Genotypes of ACE2 Gene Polymorphism (rs2285666) Stratified by Gender

	Mean ± SD			
Women				
Subgroup	**GG**	**GA**	**AA**	**P-value**
No	50	32	22	
Age (y)	51.83 ± 8.15	51.88 ± 8.32	52.45 ± 8.57	0.07
BMI (kg/m^2)	26.01 ± 4.36	26.54 ± 4.45	26.81 ± 4.49	0.11
ACE2 (ng/mL)	0.261 ± 0.05	0.276 ± 0.05	0.289 ± 0.05	0.001
TG (mmol/L)	1.49 ± 0.86	1.52 ± 0.88	1.54 ± 0.89	0.072
TC (mmol/L)	3.98 ± 0.83	4.06 ± 0.85	4.10 ± 0.86	0.081
HDL-C (mmol/L)	1.25 ± 0.42	1.11 ± 0.43	0.95 ± 0.43	<0.0001
LDL-C (mmol/L)	2.62 ± 0.92	2.67 ± 0.94	2.70 ± 0.95	0.45
VLDL-C (mmol/L)	0.59 ± 0.40	0.60 ± 0.41	0.61 ± 0.41	0.75
AIP (Log TG/HDL)	0.165 ± 0.06	0.186 ± 0.06	0.223 ± 0.06	<0.0001
TC/HDL-C	2.96 ± 1.38	3.31 ± 1.51	3.70 ± 1.63	<0.0001
Men				
Subgroup	**G**	**A**		
No	80	36		
Age (y)	52.89 ± 9.57	54.51 ± 9.87		0.06
BMI (kg/m^2)	27.54 ± 4.60	28.38 ± 4.74		0.08
ACE2 (ng/mL)	0.213 ± 0.05	0.233 ± 0.05		0.02
TG (mmol/L)	1.13 ± 0.57	1.16 ± 0.59		0.22
TC (mmol/L)	3.98 ± 0.93	4.10 ± 0.96		0.67
HDL-C (mmol/L)	1.13 ± 0.47	0.94 ± 0.48		<0.0001
LDL-C (mmol/L)	2.51 ± 0.96	2.59 ± 0.99		0.35
VLDL-C (mmol/L)	0.70 ± 0.40	0.72 ± 0.41		0.96
AIP (Log TG/HDL)	0.176 ± 0.06	0.220 ± 0.06		<0.0001
TC/HDL-C	3.05 ± 1.82	3.54 ± 1.88		<0.0001

4.3 Phenotype-ACE2 Gene Polymorphism Relationship

An analysis examining the relationship between phenotype and ACE2 gene polymorphism identified pertinent links between possession of the A allele from rs2285666 SNP with levels of HDL-C and atherogenic indices (AIP and TC/HDL-C) in patients diagnosed with both dyslipidemia and type 2 diabetes. Such findings offer crucial insights into how ACE2 is involved in lipid metabolism.

The variations in ACE2, lipid profiles, and associated atherogenic indexes found in subjects differing genetically at rs2285666 appear to arise from the metabolic regulatory functions of ACE2. The presence of specific genes at the locus rs2285666 influences how the variation in ACE2 influences lipid metabolism. An alteration in one part of the ACE2 gene can affect ACE2's ability to express itself or it's catalytic efficiency. In this case, this would result in disruption of lipid balance. Importantly, there was significantly greater levels of ACE2 observed in female individuals who carried two alleles for the A genotype when compared to male individuals who also had an A allele but less than two; which indicated that this variant is capable of enhancing the function of the enzyme. Enhanced ACE2 function can cause imbalance within the RAS pathway which contributes to the development of dyslipidemia [21]. Given that ACE2 plays significant roles in modulating both endothelial function and inflammation as they relate to lipid regulation, lower HDL-C values along with higher atherogenic indices (AIP and TC/HDL-C) in AA carriers further support the involvement of ACE2 in lipid metabolism [29].

Current findings emphasize the importance of ACE2 gene polymorphisms in influencing lipid profiles and atherogenic risk in T2DM patients. The rises in ACE2 and atherogenic indices among AA genotype carriers point to the genetic contributor, rs2285666 SNP, as a predisposition marker for dyslipidemia risk. These are relevant to individualized medication, i.e. identification of subjects genetically more predisposed to dyslipidemia risk who can be managed accordingly, which would

reduce the ensuing cardiovascular risk [25]. The difference between the sexes in ACE2 levels and lipid levels reinforces the need for sex-specific approaches to dyslipidemia treatment in T2DM patients.

The associations of rs2285666 genotypes with deficit lipid profiles and increased ACE2 levels may meaningfully relate to T2DM progress, as well as ensuing complications. The reduction in HDL-C levels among carriers of the A allele exacerbates the pre-existing risk, as HDL-C plays a protective role against atherosclerosis by promoting cholesterol efflux and anti-inflammatory functions [24]. These findings stress the need for early identification and management of dyslipidemia in at-risk genotypes as a means of averting or postponing diabetic complications [30].

4.4 Comparison of the present findings with those reported in the literature

Several previous reports demonstrated consistent results with the current findings. Li et al. illustrated an association of rs2285666 SNP in the ACE2 gene with dyslipidemia and insulin resistance in a Chinese population. They found that carriers of the A allele had higher triglyceride levels and lower HDL-C levels [31]. Pinheiro et al. reported sex-specific differences in ACE2 expression associated with cardiovascular risk factors in T2DM patients [32]. Soro-Paavonen et al. explored the involvement of ACE2 in lipid metabolism associated with cardiovascular risk [33]. Tukiainen et al. highlighted the implication of ACE2's role in directing sex-specific genetic effects on dyslipidemia [27]. Patel et al. discussed the role of ACE2 in modulating lipid homeostasis and inflammation [21]. Rader et al. reported that dyslipidemia, particularly low HDL-C and high LDL-C levels, contribute to CVD risk in T2DM patients [25]. Ginsberg et al. emphasized the role of dyslipidemia in endothelial dysfunction and insulin resistance [24].

Some investigations explored research results that were inconsistent with our findings. Niu et al. highlighted an insignificant association of ACE2 polymorphisms, including rs2285666, with lipid profiles in a European population [34]. South et al. indicated a higher ACE2 expression associated with improved lipid profiles in women [35].

The current study found the same contradictions as prior studies. Patel et al. were able to show that ACE2 is involved in lipid metabolism [21], but similar to other studies (Soro-Paavonen) they could not establish an association between dyslipidemia and ACE2 genetic variation in every population group studied [33]. Differences in genetics, environment, ethnicity, or research methodology may have produced the difference between their data and the data of this study.

5. CONCLUSION

ACE2 polymorphism rs2285666 is associated with dyslipidemia in Iraqi T2DM patients, with sex-specific differences in risk. The ACE2 variants may contribute to dyslipidemia pathogenesis in T2DM and highlight the potential for personalized risk stratification and therapeutic targets.

6. LIMITATIONS

The current study has some important limitations. Firstly, this study was carried out in one hospital centre in Iraq. Therefore, although the number of participants (which is acceptable) does not reflect all the genetic variability that exists in the wider Iraqi population. It will therefore require extensive, multi-centre studies to confirm these results in other populations with a variety of ethnic backgrounds. Secondly, this study did not account for possible confounders (e.g., diet, physical exercise, drugs) that may affect both lipids and ACE2. Thirdly, whilst it has used sex stratification in the analysis, it has not examined how sex hormone variation and/or x chromosome inactivity might influence the association seen here. Finally, this study has no data on whether the rs2285666 and rs4646142 polymorphisms influence ACE2 function/ expression. This means that it limits the biological interpretation of what was found.

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