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IMPACT OF PON1 GENETIC DIVERSITY ON DIABETIC DYSLIPIDEMIA IN IRAQI PATIENTS

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ABSTRACT

Background: Diabetic dyslipidemia is a major contributor to cardiovascular complications in type 2 diabetes mellitus (T2DM). Paraoxonase-1 (PON1), an HDL-associated antioxidant enzyme, protects lipoproteins from oxidative modification, and genetic polymorphisms may influence its activity and susceptibility to dyslipidemia.

Aim: To investigate the association of PON1 polymorphisms (rs662, rs854571, and rs854572) with diabetic dyslipidemia in Iraqi patients with T2DM.

Methods: A case–control study comprising 150 type 2 diabetic patients with dyslipidemia and 150 without. Serum glucose, lipid profile, and PON1 concentrations were measured using standard biochemical methods and ELISA. Genotyping was performed by allele-specific polymerase chain reaction.

Results: Patients with dyslipidemia exhibited significantly elevated fasting blood glucose (FBG), triglycerides (TG), total cholesterol (TCH), and low-density lipoprotein (LDL-C), but lower high-density lipoprotein (HDL-C), PON1 concentrations, and HDL functional index than controls. The rs662 C/C genotype was associated with increased risk of dyslipidemia (OR = 2.63, p = 0.015), while the rs854571 C/C and rs854572 variant genotypes also conferred significant susceptibility. Linkage Disequilibrium (LD) analysis demonstrated weak correlations among the investigated loci. Haplotype analysis identified the CTC haplotype as significantly associated with dyslipidemia (OR = 2.49, p = 0.041).

Conclusions: PON1 genetic variation, particularly rs662, contributes to diabetic dyslipidemia in Iraqi patients. Combined haplotype analysis provides additional evidence that PON1 genetic diversity influences lipid homeostasis and may help identify individuals at increased cardiovascular risk.

KEYWORDS: PON1, Dyslipidemia, T2DM, Iraq, Gene polymorphism

1. INTRODUCTION

Type 2 diabetes mellitus (T2DM) is one of the fastest-growing chronic metabolic disorders worldwide. It remains a major cause of cardiovascular morbidity and mortality (Wong & Sattar, 2023). Persistent hyperglycemia promotes insulin resistance, oxidative stress, and chronic low-grade inflammation, resulting in profound disturbances of lipid metabolism. Diabetic dyslipidemia is typically characterized by elevated triglycerides, reduced HDL-C, and qualitative changes in LDL. Such alterations accelerate atherosclerosis and increase cardiovascular risk (Xourafa et al., 2024).

Beyond quantitative lipid abnormalities, oxidative modification of lipoproteins has emerged as a critical mechanism linking T2DM to vascular complications (Tsimikas & Witztum, 2024). PON1 is a calcium-dependent esterase synthesized primarily in the liver and transported in circulation bound to HDL particles (Mahrooz, 2024). By hydrolyzing oxidized phospholipids and lipid peroxides, PON1 preserves HDL antioxidant properties, inhibits LDL oxidation, attenuates vascular inflammation, and contributes to reverse cholesterol transport (Jakubowski, 2024). Experimental and clinical studies have consistently demonstrated reduced PON1 activity in patients with T2DM. Changes associated with diabetic dyslipidemia suggest an important role for PON1 in maintaining lipoprotein homeostasis (Durrington et al., 2023).

The human PON1 gene is located on chromosome 7q21–q22. It is highly polymorphic (Jakubowski, 2024). Both coding and promoter polymorphisms influence gene transcription, enzyme concentration, catalytic efficiency, and antioxidant capacity (Lewoń-Mrozek et al, 2024). Among the numerous variants identified, rs662 (Q192R), rs854571, and rs854572 have attracted considerable attention for their potential effects on lipid metabolism, which may confer high susceptibility to cardiovascular disease (Durrington, 2023). However, published findings remain inconsistent across different ethnic populations, indicating that the contribution of these variants may be population-specific and influenced by genetic background and environmental factors (Kunachowicz, 2023).

Despite the increasing prevalence of T2DM in Iraq, evidence regarding the relationship between PON1 polymorphisms and diabetic dyslipidemia in Iraqi patients remains scarce. Identifying genetic variants associated with dyslipidemia may improve understanding of disease pathogenesis, facilitate early risk stratification, and support the development of personalized preventive strategies. Therefore, the present study investigated the association of three common PON1 polymorphisms (rs662, rs854571, and rs854572) with diabetic dyslipidemia in Iraqi patients with type 2 diabetes mellitus.

2. MATERIALS AND METHODS

2.1. Study design and participants

A case-control study was conducted between September 2025 and April 2026 in the Postgraduate Laboratory, Department of Biochemistry, College of Medicine, University of Kufa, Iraq. The study included 300 unrelated Iraqi adults with previously diagnosed T2DM. They were allocated into two equal groups. The case group comprised 150 patients with diabetic dyslipidemia. The control group consisted of 150 T2DM patients without dyslipidemia. Participants were recruited from the Specialized Centre for Diabetes and Endocrinology at Al-Sadr Medical City, Al-Najaf, Iraq. Patients with diabetic dyslipidemia fulfilled the American Diabetes Association (ADA) diagnostic criteria for T2DM. Their HbA1c ≥6.5% or fasting plasma glucose (FBG) ≥126 mg/dL, together with dyslipidemia defined as HDL-C ≤40 mg/dL in men or ≤50 mg/dL in women, triglycerides ≥150 mg/dL and/or LDL-C ≥130 mg/dL. Eligible participants were older than 30 years. Patients with type 1 diabetes mellitus, thyroid disorders, malignancy, liver disease, renal failure, pregnancy, or other severe systemic diseases were excluded. The diabetic control group consisted of patients with T2DM but without dyslipidemia. Their ages, sex, and geographical origin were matched as closely as possible. They were subjected to the same exclusion criteria.

2.2. Ethical approval

The study protocol was reviewed and approved by the Review Board of the College of Medicine, University of Kufa (Approval No. MEC-31). Written informed consent was obtained from all participants before enrollment. All procedures were conducted in accordance with the principles of the Declaration of Helsinki.

2.3. Sample collection

Following an overnight fast of 10–12 hours, approximately 5 mL of peripheral venous blood was collected from each participant. Three millilitres were transferred into plain gel tubes, allowed to clot, and centrifuged at 4,000 × g for 10 minutes to obtain serum. Serum aliquots were stored at −20°C until biochemical analyses. The remaining 2 mL were collected into EDTA-containing tubes for genomic DNA extraction and subsequent genotyping. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters (kg/m²). Clinical and demographic information was recorded using a standardized questionnaire.

2.4. Biochemical measurements

FBG, TCH, TG, and HDL-C concentrations were determined using enzymatic colourimetric assay kits according to the manufacturers' instructions. LDL-C was calculated using the Friedewald equation whenever triglyceride concentrations were below 400 mg/dL. Serum PON1 concentration was quantified using a commercially available sandwich enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's protocol. Absorbance was measured at 450 nm using a microplate reader, and concentrations were calculated from the standard calibration curve generated for each assay. The HDL-Functionality index (HDL-FI) was calculated by dividing the levels of PON1on HDL.

2.5. DNA extraction and genotyping

Genomic DNA was isolated from whole blood anticoagulated with EDTA using a commercial genomic DNA extraction kit according to the manufacturer's instructions. Three common PON1 polymorphisms were investigated: rs662, rs854571, and rs854572. Genotyping was performed using allele-specific polymerase chain reaction (AS-PCR). Specific primer sets were designed for each polymorphism. The characteristics of the primers are described in Table 2.1.

Table 2.1: Characteristics of the primers

Primer	Sequence (5′→3′)	Template strand	Length (bp)
rs662 (T>C)			
Allele T (Normal)	CCAAATACATCTCCCAGGATT	Plus	271
Allele C (Mutant)	CCAAATACATCTCCCAGGATC	Plus	
Reverse primer	AAGGATTGTATCGGCAGGAC	Minus	
rs854571 (T>C)			
Allele T (Normal)	GGAATACCCTCTGCTGAAGAAT	Plus	175
Allele C (Mutant)	GGAATACCCTCTGCTGAAGAAC	Plus	
Reverse primer	CTGGGACCAAAAGCCTTGA	Minus	
rs854572 (C>G)			
Forward primer	GGTCGGGGATAGACAAAGGG	Plus	929
Allele C (Normal)	AGCAGACAGCAGAGAAGAGAG	Minus	
Allele G (Mutant)	AGCAGACAGCAGAGAAGAGAC	Minus	

Amplification reactions were carried out in a thermal cycler under optimized conditions, as described previously. The amplification was performed using two separate PCR tubes for each DNA sample. One tube was specific for the wild-type allele, and the other for the variant allele. Each reaction was prepared in a final volume of 20 µL. The reaction mixture in each tube consisted of 5 µL FIREPol® Master Mix (Solis BioDyne, Tartu, Estonia), 1 µL of the common reverse primer, 1 µL of the allele-specific primer, 8 µL of nuclease-free water, and 5 µL of genomic DNA template. The amplification reaction was carried out using an initial denaturation step at 95°C for 5 min, followed by 32 cycles of denaturation at 95°C for 30 s, annealing at 57°C for 30 s, and extension at 72°C for 1 min, with a final extension at 72°C for 7 min.

2.6. Agarose gel electrophoresis

PCR amplification products were resolved by agarose gel electrophoresis (1.5%) using Goldview stain, and an appropriate DNA ladder as a molecular size marker. Electrophoresis was performed under standard conditions, and amplified fragments were visualized using a UV transilluminator.

2.7. Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics software. Continuous variables were expressed as mean ± standard deviation (SD), whereas categorical variables were summarized as frequencies and percentages. Comparisons between the two study groups were performed using Student's t-test for continuous variables and the χ^2 test for categorical variables. The genotype distribution of each polymorphism was evaluated for compliance with Hardy–Weinberg equilibrium (HWE). Associations between PON1 polymorphisms and diabetic dyslipidemia were estimated by multinomial logistic regression under codominant, dominant, and recessive genetic models after adjustment for age, sex, BMI, and HbA1c. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. LD and haplotype analyses were performed to evaluate the combined effects of the investigated SNPs. Statistical significance was defined as a two-tailed P value <0.05.

3. RESULTS

3.1. Anthropometric and Biochemical Characteristics

The comparison of anthropometric and biochemical parameters between patients and the control group is presented in Table 3.1. FBS, TG, TCH, and LDL-C levels were significantly ($p < 0.001$) elevated in patients compared with controls. In contrast, HDL-C levels were significantly ($p < 0.001$) lower in patients than in controls. Regarding oxidative and functional parameters, PON1 levels were significantly ($p < 0.001$) lower in patients than in controls. Furthermore, the HDL-FI was significantly ($p < 0.001$) lower in patients compared to the control group ($p < 0.001$).

Table 3.1: Anthropometric and biochemical characteristics of patients and the control group

	Mean ± SD		P-value
	Patients	Control	
Age (years)	53.57 ± 9.08	54.65 ± 8.41	0.374
BMI (kg/m²)	30.22 ± 4.71	30.70 ± 4.24	0.447
FBG (mg/dL)	187.25 ± 61.90	230.30 ± 77.26	<0.001
HbA1c (%)	8.56 ± 2.23	9.24 ± 1.90	0.154
TG (mg/dL)	120.81 ± 17.25	244.09 ± 68.91	<0.001
TCH (mg/dL)	181.52 ± 26.54	228.67 ± 40.66	<0.001
LDL-c (mg/dL)	94.12 ± 23.26	132.28 ± 39.96	<0.001
HDL-c (mg/dL)	63.16 ± 9.48	48.01 ± 13.27	<0.001
PON1 (ng/mL)	34.37 ± 7.80	14.98 ± 5.02	<0.001
HDL-FI (HDL/PON1)	0.555 ± 0.142	0.330 ± 0.132	< 0.001

3.2. Genotyping of PON1

PCR-AS products were collected from150 T2DM patients with dyslipidemia and 150 T2DM patients without dyslipidemia. For rs662, participants with the wild-type homozygous TT showed a single 271 bp band. Those with the homozygous CC mutant showed a single 271 bp band. Whereas carriers of heterozygous TC genotypes showed two bands, one band for each of the T and C alleles (Fig 3.1). Results for rs854571 showed TT and CC genotypes at 175 bp, while the heterozygous TC showed two 175 bp bands (Figure 3.2). Results for rs854572 showed a 929 bp band for those with the CC and GG genotypes. The heterozygous genotype (CG) demonstrated two 929 bp bands (Figure 3.3).

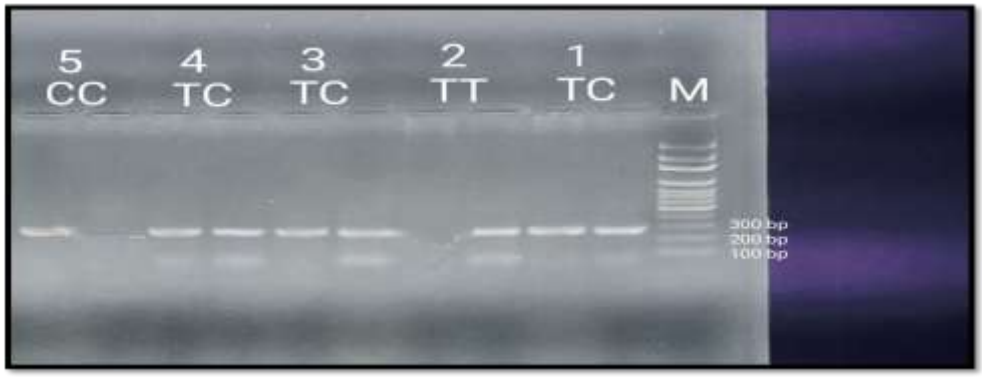

Figure 3.1: AS-PCR product of PON1 rs662 on 1.5% agarose gel electrophoresis. M: Ladder. Lanes 1, 3, and 4 show the 271 bp TC genotype. Lane 2 indicates genotype TT (271 bp). Lane 5 indicates genotype CC at 271 bp.

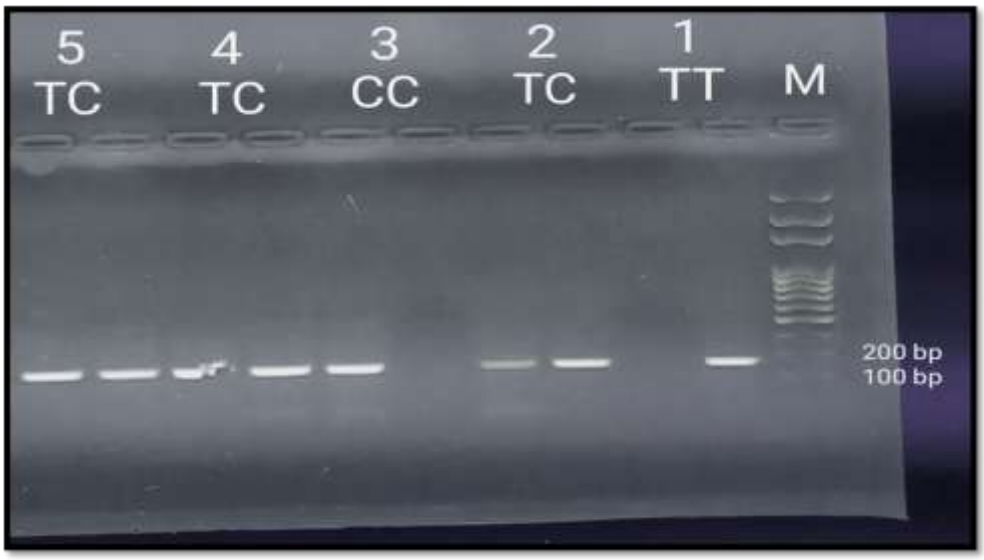

Figure 3.2: AS-PCR product of PON1 rs854571 on a 1.5% agarose gel electrophoresis. M: Ladder. Lane 1 indicates the 175 bp (TT) genotype. Lanes 2, 4, and 5 indicate the 175 bp (TC) genotype. Lane 3 indicates the 175 bp (CC) genotype.

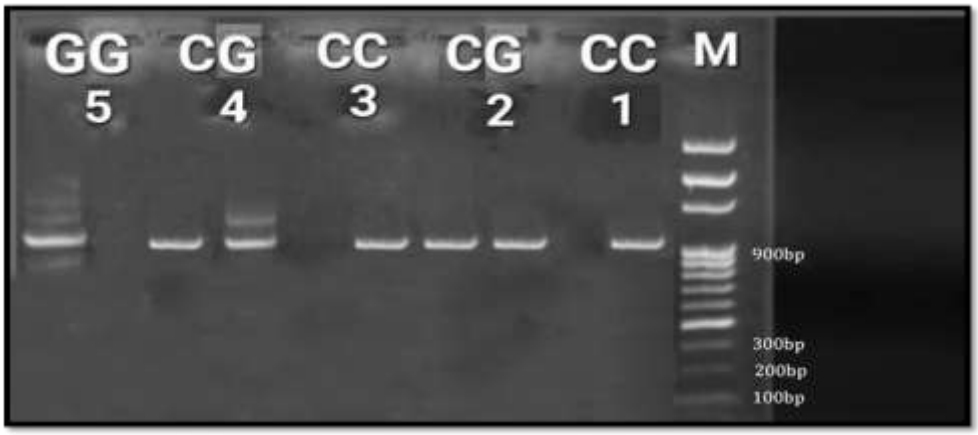

Figure 3.3: AS-PCR product of PON1 rs854572 on 1% agarose gel electrophoresis. M: Ladder. Lanes 1and 3 indicate genotype (CC) 929bp. Lanes 2 and 4 indicate the 929 bp genotype (CG). Lane 5 indicates genotype (GG) at 929 bp.

3.3. Association of PON1 Polymorphisms with diabetic dyslipidemia

The HWE analysis of the PON1 polymorphisms studied in patients with diabetic dyslipidemia and the control group was performed. Results for the genotype distributions of rs662, rs854571, and rs854572 in the control group showed no deviation from HWE. The association between PON1 polymorphisms and dyslipidemia in type 2 diabetic patients, after adjustment for age, sex, BMI, and HbA1c, is presented in Table 3.3. The codominant model of rs662 showed that individuals with the C/C genotype had a significantly higher risk of dyslipidemia than those with the T/T reference genotype (OR = 2.63, 95% CI: 1.20–5.56, p = 0.015). Moreover, under the dominant model, carriers of the T/C + C/C genotypes exhibited a significantly increased risk of dyslipidemia (OR = 1.74, 95% CI: 1.09–2.80, p = 0.021). The recessive model also revealed significant effects of the C allele. Results are supported by a significant trend test of allelic dose (p = 0.008). For the rs854571 SNP, only the codominant model showed a significant association (OR = 1.98, 95% CI: 1.01–3.90, p = 0.046) between the C/C genotype and the risk of dyslipidemia. For the rs854572 SNP, the codominant model revealed that both C/G and G/G genotypes were significantly associated with increased risk of dyslipidemia (OR = 1.98, 95% CI: 1.15–3.40, p = 0.014 and OR = 2.62, 95% CI: 1.02–6.72, p = 0.045, respectively). The dominant model further confirmed the association with the risk of dyslipidemia in carriers of C/G + G/G (OR = 2.05, 95% CI: 1.25–3.36, p = 0.004). Results are also supported by a significant allele-dose trend (p = 0.008; Table 3.2).

Table 3.2: Association of PON1 polymorphism with dyslipidemia in type 2 diabetics after adjustment for age, sex, BMI, and HbA1c

	Control	Cases	OR (95%CI)	P-value
rs662				
Codominant				
T/T	73 (49%)	54 (36%)	1.00	
T/C	63 (42.3%)	72 (48%)	1.58 (0.80–3.10)	0.18
C/C	13 (8.7%)	24 (16%)	2.63 (1.20–5.56)	0.015

Dominant				
T/T	73 (49%)	54 (36%)	1.00	0.021
T/C+C/C	76 (51%)	96 (64%)	1.74 (1.09–2.80)	
Recessive				
T/T+T/C	136 (91.3%)	126 (84%)	1.00	0.051
C/C	13 (8.7%)	24 (16%)	2.06 (1.00–4.27)	
TTAD (C allele)				0.008
rs854571				
Codominant				
T/T	54 (36.2%)	40 (26.7%)	1.00	—
T/C	71 (47.6%)	72 (48%)	1.32 (0.70–2.47)	0.39
C/C	24 (16.1%)	38 (25.3%)	1.98 (1.01–3.90)	0.046
rs854572				
Codominant				
C/C	84 (56.4%)	55 (36.7%)	1.00	—
C/G	56 (37.6%)	77 (51.3%)	1.98 (1.15–3.40)	0.014
G/G	9 (6%)	18 (12%)	2.62 (1.02–6.72)	0.045
Dominant				
C/C	84 (56.4%)	55 (36.7%)	1.00	—
C/G+G/G	65 (43.6%)	95 (63.3%)	2.05 (1.25–3.36)	0.004
Recessive				
C/C+C/G	140 (94%)	132 (88%)	1.00	—
G/G	9 (6%)	18 (12%)	1.78 (0.75–4.25)	0.19
TTAD (G alleles)	-----	---	---	0.008

TTAD: Trend Test of Allelic Dose (0 → 1 → 2)

3.4. Haplotype association assessment

The LD analysis among the studied PON1 polymorphisms is presented in Table 3.3. In patients with diabetic dyslipidemia, weak LD was observed among most SNP pairs. Significant weak LD was identified between rs854571 and rs854572 ($D' = 0.1939$, $r = -0.1487$, $p = 0.010$), indicating a modest non-random association between these loci in patients. Other pairs did not reveal significant LD. In the control group, all SNP pairs exhibited weak LD with no statistically significant associations.

Table 3.3: Linkage disequilibrium metrics of PON1 polymorphism in type 2 diabetics with dyslipidemia and control groups

	SNP Pair	D	D'	r	P-value
Patients	rs662- rs854571	0.0238	0.1177	0.0974	0.0918
	rs662- rs854572	-0.0092	0.0608	-0.0386	0.5038
	rs854571- rs854572	-0.036	0.1939	-0.1487	0.010
Control	rs662- rs854571	0.0189	0.1052	0.0842	0.1461
	rs662- rs854572	0.0031	0.018	0.0159	0.7843
	rs854571- rs854572	-0.0121	0.1215	-0.057	0.3255

3.5. Haplotype Association of PON1 polymorphisms with Dyslipidemia

The association between PON1 haplotypes and dyslipidemia in type 2 diabetic patients, after adjustment for age, sex, BMI, and HbA1c, is presented in Table 3.4. Using the TTC haplotype as the reference, several haplotypes demonstrated varying degrees of association with dyslipidemia. The CTC haplotype showed a significant association with an increased risk of dyslipidemia (OR = 2.49, 95% CI: 1.04–5.99, $p = 0.041$), indicating that carriers of this haplotype have approximately 2.5-fold higher odds of dyslipidemia compared with the reference. Other haplotypes did not show significant associations with dyslipidemia in T2DM. The global haplotype association test was significant ($p = 0.035$), indicating that the distribution of PON1 haplotypes differs significantly between patients with dyslipidemia and controls.

Table 3.4: Fully adjusted haplotype association of PON1 gene with Dyslipidemia in type2 diabetics

Haplotype	Frequency	OR (95% CI)	P-value
TTC (Ref)	0.2628	1.00	—
CCC	0.1341	2.95 (0.73–11.93)	0.129
CTC	0.1012	2.49 (1.04–5.99)	0.041
CTG	0.0671	1.19 (0.07–19.72)	0.905
TCC	0.1891	1.71 (0.90–3.26)	0.099
TCG	0.0762	----------	0.999
TTG	0.1223	2.31 (0.92–5.80)	0.075
Global haplotype association			0.035

4. DISCUSSION

4.1. Anthropometric and Biochemical Characteristics

The results indicated a significant disruption in all aspects of glucose regulation, lipid metabolism, oxidative balance, and HDL function among Iraqi diabetic dyslipidemic patients. These data support the idea that T2DM has a profound impact on metabolic functions, resulting in an elevated cardiovascular risk and rapid progression of atherosclerotic disease. The latter is the leading cause of both mortality and morbidity among diabetic patients.

FBS levels were significantly elevated in diabetic patients, indicating poor glycemic control and persistent hyperglycemia. Chronic hyperglycemia is a hallmark of type 2 diabetes mellitus. It is strongly associated with insulin resistance, impaired glucose utilization, and enhanced hepatic glucose production (Lu et al, 2024). Persistent elevation of glucose levels promotes oxidative stress by promoting reactive oxygen species production, protein glycation, and activation of inflammatory pathways. Significantly increased levels of TG, TCH, and LDL-c were observed in diabetic patients compared with controls. These findings are characteristic of diabetic dyslipidemia; they may be explained by insulin resistance–mediated disturbances in lipid metabolism (Xourafa et al, 2024).

In contrast, HDL cholesterol levels were significantly lower in patients than in healthy controls. Reduced HDL levels are commonly observed in diabetic dyslipidemia. They may result from increased HDL catabolism and impaired HDL maturation secondary to hypertriglyceridemia and oxidative stress (Hussain et al, 2019). HDL particles exert anti-inflammatory, antioxidant, and reverse cholesterol transport functions, protecting against cardiovascular disease (Denimal et al, 2023). Thus, reduced HDL concentration may contribute to diminished cardiovascular protection in diabetic patients.

One of the most important findings of the present study was the marked reduction in serum PON1 levels in diabetic patients. PON1 is an HDL-associated antioxidant enzyme involved in protecting both HDL and LDL particles against oxidative modification. Reduced PON1 levels suggest impaired antioxidant defence mechanisms and increased oxidative stress in diabetic dyslipidemia. Hyperglycemia-induced oxidative stress may directly inactivate PON1 activity via oxidative modification and glycation of the enzyme or its HDL carrier particles (Jakubowski, 2024). Previous studies have similarly reported reduced PON1 activity and expression in patients with T2DM and dyslipidemia. Klen et al. (2023) demonstrated that PON1 activity was associated with atherogenic lipid indices in diabetic patients. Thus, emphasising the role of PON1 in modulating oxidative stress and lipid oxidation. Similarly, the importance of PON1 in protecting against lipid oxidation and atherosclerosis has been highlighted (Durrington et al., 2023).

The significantly reduced HDL functional index observed in the current study further supports the concept of dysfunctional HDL in diabetic dyslipidemia. It has emerged that HDL functionality is a more important indicator of cardiovascular protection than HDL concentration alone (Denimal et al., 2023). The HDL/PON1 ratio used in the present study reflects the antioxidant and antiatherogenic capacity of HDL particles. Lower HDL-FI values in diabetic patients indicate impaired HDL functionality and reduced antioxidant efficiency (Mahrooz, 2024). Dysfunctional HDL could diminish its ability to suppress LDL oxidation, promote cholesterol export and reduce vascular inflammation. It strongly contributes to accelerated atherosclerosis in diabetic individuals.

The results are consistent with previous studies showing that polymorphisms in PON1 may influence lipid levels but do not necessarily indicate a direct effect on disease susceptibility. Malinowski et al. (2024) found correlations between PON1 variants and changes in total cholesterol and LDL levels, but no direct association with the risk of unstable angina. Similarly, Durrington et al. (2023) demonstrated that PON1 variants may be associated with cardiovascular disease through changes in lipid metabolism and oxidative stress, rather than directly conferring susceptibility to genetic factors.

4.2. Association of PON1 Polymorphisms with Diabetic Dyslipidemia

The HWE analysis showed that the three SNPs, rs662, rs854571, and rs854572, did not deviate from HWE. This finding indicates that genotype distributions were consistent with expected frequencies and supports the genetic stability of the study population. Conformity to HWE also suggests accurate genotyping, minimal population stratification, and the absence of major selection bias. These results strengthen the validity and reliability of the subsequent genetic association analyses involving PON1 variants (Salanti et al., 2024).

PON1 polymorphisms were found to be associated with an increased risk of developing dyslipidemia among individuals with T2DM. The rs662 SNP showed the strongest and most consistent associations with the risk of dyslipidemia across multiple inheritance models. The rs662 SNP results in an amino acid substitution that alters the enzyme's substrate specificity and antioxidant activity (Lewoń-Mrozek, 2024). It has been found to influence PON1 activity. In parallel, LDL oxidation and HDL's protective function are also modulated. Malinowski et al. (2024) demonstrated a role of the variant allele in increasing the oxidised LDL, TG, TCH, and LDL-c levels. Similar findings were reported by Luo et al. (2018) in Asian populations and patients with metabolic disorders.

The current findings are consistent with those of El-Lebedy et al. (2014), who demonstrated significant associations between the PON1 r662 polymorphism and cardiovascular risk in Egyptian diabetic patients. Also, Yari et al. (2023) identified the rs662 G allele as a risk factor among Iranian individuals. Suneja et al. (2025) found an association between the GG genotype at rs662 and increased cardiovascular disease risk among diabetic patients, accompanied by lower serum PON1 levels. These observations support the concept that the rs662 polymorphism may impair the antioxidant and antiatherogenic functions of PON1, thus contributing to dyslipidemia.

Several mechanisms may explain the observed association between rs662 and dyslipidemia in type 2 diabetics. PON1 is an HDL-associated antioxidant enzyme that protects lipoproteins against oxidative modification (Durrington et al., 2023). Functional alterations resulting from the rs662 polymorphism may reduce PON1's ability to hydrolyze oxidised lipids. This thereby increases oxidative stress and promotes the accumulation of atherogenic lipoproteins. Impaired PON1 activity may contribute to HDL dysfunction, reduced cholesterol efflux capacity, and increased LDL oxidation. Oniki et

al. (2024) reported significant effects of PON1 variants on cholesterol efflux capacity, independent of HDL concentration, emphasizing the importance of HDL functionality over HDL quantity alone.
The rs854571 SNP indicated a significant association with dyslipidemia. Carriers of the C/C genotype showed increased susceptibility compared with those with the wild T/T genotype. The rs854571 SNP is located in the promoter region of the PON1 gene (Vavlukis et al., 2022). It may influence transcriptional activity and enzyme expression. Leviev and James (2000) reported that promoter-region polymorphisms strongly affect PON1 expression and antioxidant activity. Reduced transcriptional activity associated with promoter variants may consequently decrease serum PON1 concentration and weaken HDL-associated antioxidant defence mechanisms (Mahrooz, 2024).
Both heterozygous and homozygous variant genotypes at the rs854572 SNP were found to be associated with increased risk of dyslipidemia. Such findings suggest that rs854572 may also contribute to metabolic dysregulation and impaired lipid homeostasis in diabetic individuals. The explanation of the observed association between rs854572 and dyslipidemia may involve altered regulation of PON1 expression and activity. It may impair protection against lipid oxidation (Mahrooz, 2024).
Previous reviews have emphasized that both coding and promoter SNPs influence HDL-associated antioxidant capacity and cardiovascular susceptibility. Shunmoogam et al. (2018) highlighted that genetic variability in PON1 contributes to interindividual differences in oxidative stress response and lipid metabolism. Small changes in either the structure or the regulation of the PON1 gene could significantly affect its stability and antioxidant activity (Lewoń-Mrozek et al., 2024). It has been indicated that not just the level of HDL is important for cardioprotective mechanisms in individuals with diabetes, but also the functional ability of HDL (Lui & Tan, 2024).

4.3. Linkage Disequilibrium Analysis of PON1 Polymorphisms

Weak LD was identified between rs854571 and rs854572 in diabetic patients, suggesting a modest non-random association between these two promoter-region polymorphisms. However, the D′ and r values remained relatively low. This observation is biologically plausible because both SNPs are located within regulatory regions of the PON1 gene. They may be inherited together more frequently than chance would predict (Vavlukis et al., 2022). Promoter-region polymorphisms frequently affect PON1 transcription and antioxidant function. Leviev and James (2000) demonstrated that regulatory polymorphisms within the PON1 promoter region significantly affect serum PON1 levels. Therefore, the modest LD observed between rs854571 and rs854572 may reflect coordinated regulatory effects on PON1 transcription and HDL-associated antioxidant activity.
The observed weak LD pattern in the current study is consistent with previous investigations that have demonstrated variable LD among PON1 SNPs across ethnic populations. Genetic recombination rates, population history, migration, and ethnic diversity may seriously influence LD structure within candidate genes. Shunmoogam et al. (2018) emphasized that PON1 polymorphisms exhibit considerable interpopulation variability regarding allele frequencies and linkage relationships. Vavlukis et al. (2022) reported heterogeneous LD patterns among PON1 variants in cardiovascular and metabolic diseases in different ethnic groups.
In the control group, no significant LD pattern was observed for any of the analysed SNPs. Hence, such a result may suggest relatively independent segregation of the studied loci in the general Iraqi population. The lower LD observed among controls compared with diabetic patients may indicate that disease-related metabolic processes or population-specific genetic architecture contribute to differences in haplotype distribution (Wysocka & Zwolak, 2021).

4.4. PON1 Haplotype Association with Diabetic Dyslipidaemia

The current study shows that certain haplotypes formed by PON1 polymorphisms have a greater effect on the risk of developing dyslipidemia than a single polymorphism. A significant association was found between the CTC haplotype and an increased risk of dyslipidemia, with an odds ratio of 2.5. The evidence presented here indicates that when individuals carry multiple alleles, there are additive and possibly synergistic effects on PON1 and lipid metabolism (Batista-Herrera et al., 2026). PON1 is known to be involved in HDL particles and has significant antioxidant properties. Thus, defects that occur at various sites within the PON1 gene and can affect the structure or regulation of the PON1 enzyme will diminish HDL's ability to protect against oxidative damage to lipids (Durrington et al., 2023).
The significant relationship identified for the CTC haplotype is biologically plausible; it involves variations in the coding and promoter regions of the PON1 gene. Variations at the coding site could influence the enzyme activity and substrate specificity (Al-Barqaawi et al, 2022). Therefore, haplotype combinations may better reflect the impact of PON1 genetic variation than analyses of individual polymorphisms. Leviev and James (2000) demonstrated strong effects of promoter polymorphism on PON1 expression. Durrington et al. (2023) emphasised that rs662 was associated with oxidative lipid metabolism and cardiovascular risk.
The Global Haplotype Association Test revealed a substantial difference in total haplotype distributions between diabetic dyslipidemic patients and the control group. Therefore, the overall genetic arrangement of PON1 haplotypes significantly contributes to the likelihood of developing dyslipidemia in type-2 diabetic patients. This finding indicates that studying multiple genes, rather than a single SNP, is biologically important (Batista-Herrera et al., 2026).
The observed associations of haplotypes with dyslipidemia were generally weaker than those detected for individual polymorphisms. This finding suggests that susceptibility to dyslipidemia among the enrolled individuals is driven predominantly by individual functional variants rather than by synergistic interactions of multiple loci (Kunachowicz et al., 2023). Numerous interpretations may be recognised to explain such an observation. The relatively weak LD observed among the studied SNPs may have limited the ability of haplotypes to capture additional genetic information. The inclusion of variants with modest or negligible functional effects within the haplotypes may attenuate the effect of the susceptibility allele. Other factors, such as effect sizes and statistical power, are not excluded (Cinar & Viechtbauer, 2022).

The present findings are in agreement with those reported previously. Shunmoogam et al. (2018) highlighted that the combined interaction among several polymorphisms in the PON1 gene can significantly alter antioxidant potential and increase susceptibility to metabolic diseases. Vavlukis et al. (2022) reported that PON1 haplotypes may influence lipid oxidation and atherosclerosis progression through cumulative effects on enzyme activity and HDL-associated antioxidant defence.

In conclusion, PON1 genetic variation, particularly rs662, contributes to diabetic dyslipidemia in Iraqi patients. Combined haplotype analysis provides additional evidence that PON1 genetic diversity influences lipid homeostasis and may help identify individuals at increased cardiovascular risk.

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