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Coordinated suppression of lectin complement pathway effectors and upregulation of SERPING1 defines a thrombo-inflammatory regulatory signature in cardiovascular disease

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Abstract

Background

Cardiovascular disease (CVD), the leading global cause of death, is driven by thrombo-inflammatory processes. These involve oxidative stress, endothelial dysfunction, and innate immune dysregulation. The lectin complement pathway contributes to coagulation through thrombin-like activity and fibrin cross-linking, yet its transcriptional regulation under oxidative stress in human CVD remains unclear. We investigated whether CVD is associated with coordinated dysregulation of lectin pathway effector genes and the regulatory gene *SERPING1*.

Methods

In this cross-sectional study, 100 patients with clinically established CVD (acute coronary syndrome) and 50 healthy controls were enrolled. Systemic oxidative stress was assessed using serum superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA). The mRNA expression of *MBL2*, *MASP1*, *MASP2*, and *SERPING1* was quantified in peripheral blood mononuclear cells (PBMCs) by RT-qPCR. Associated statistical analyses were performed between the two groups.

Results

CVD patients showed significant oxidative imbalance, with reduced SOD ($p < 0.0001$) and elevated MDA ($p = 0.008$), while CAT remained unchanged ($p = 0.793$). Non-parametric analysis revealed significant downregulation of *MBL2* ($p = 0.017$), *MASP1* ($p = 0.028$), and *MASP2* ($p < 0.0001$), alongside upregulation of *SERPING1* ($p = 0.001$).

Conclusions

CVD is associated with systemic oxidative stress and a coherent four-gene lectin pathway regulatory signature, comprising downregulation of all three major *MBL2*, *MASP1*, and *MASP2*, concurrent with upregulation of *SERPING1*. This adaptive complement–coagulation modulation identifies the *MASP–SERPING1* axis as a potential biomarker and therapeutic target in cardiovascular disease. These findings support oxidative stress-linked transcriptional remodelling as a defining molecular feature of CVD, providing a mechanistic rationale for studies evaluating lectin pathway regulation in disease progression and intervention.

Introduction

Cardiovascular diseases (CVDs) comprise a broad spectrum of heart and vascular disorders, with coronary artery disease and acute coronary syndrome (ACS) being the most prevalent and clinically significant [1]. Collectively, CVDs remain the leading global cause of death, accounting for ~ 17.9 million deaths annually and nearly one-third of all mortality worldwide [2]. The burden is disproportionately borne by low- and middle-income countries, where urbanisation, dietary transition, and reduced access

to preventive care converge to drive epidemic levels of disease [3, 4]. In India specifically, CVD mortality has escalated sharply over the past three decades, driven by increasing metabolic risk factors alongside persistent exposure to tobacco and environmental pollutants [4]. Established modifiable risk factors, including diabetes, dyslipidaemia, hypertension, tobacco use, and alcohol consumption, contribute to disease progression through shared pathophysiological mechanisms centred on vascular inflammation, endothelial dysfunction, and oxidative injury [5].

Oxidative stress has emerged as a central and unifying mediator linking cardiometabolic risk factors to endothelial dysfunction and atherothrombotic progression. It arises when the generation of reactive oxygen and nitrogen species (ROS/RNS) exceeds the neutralising capacity of endogenous antioxidant defences, comprising enzymatic systems such as superoxide dismutase (SOD) and catalase, and non-enzymatic scavengers including glutathione and vitamin E [6]. Persistent elevation of ROS reduces nitric oxide (NO) bioavailability via peroxynitrite formation, promotes oxidative modification of low-density lipoproteins to generate pro-atherogenic oxLDL, and damage to endothelial membrane lipids, proteins, and nucleic acids [7, 8]. Malondialdehyde (MDA), a byproduct of lipid peroxidation, serves as a well-validated biomarker of this cumulative lipid oxidative injury and has been associated with adverse outcomes including mortality in ACS patients [9]. Oxidative stress further activates key inflammatory signalling cascades, including nuclear factor-κB (NF-κB) and the mitogen-activated protein kinase (MAPK) pathways that sustain endothelial activation, recruit monocytes to the vessel wall, and amplify pro-thrombotic cytokine release [10, 11].

The complement system, a major effector arm of innate immunity, has increasingly been recognised as a critical regulator of vascular inflammation and thrombosis [12, 13]. Complement activation proceeds through three convergent pathways: classical, alternative, and lectin, all converging on a shared cascade of C3 and C5 activation, anaphylatoxin generation, and membrane attack complex (MAC) formation [14]. The lectin pathway (LP) is initiated by soluble pattern recognition molecules, mannose-binding lectin (MBL), collectins (CL-10, CL-11), and ficolins that bind to carbohydrate motifs and oxidatively modified self-structures [15]. These molecules form complexes with MBL-associated serine proteases (MASP-1, MASP-2, and MASP-3) to propagate complement activation through sequential cleavage of C4, C2, and C3 [16]. While protective in host defence, dysregulated lectin pathway activation causes endothelial injury, platelet activation, and impaired fibrinolysis in atherosclerosis, ischaemic stroke, and thrombotic microangiopathy [17, 18].

A mechanistically important feature of the lectin pathway is its direct functional crosstalk with the coagulation cascade. MASP-1 and MASP-2 possess thrombin-like proteolytic activity, cleaving prothrombin, fibrinogen, and coagulation factor XIII, promoting thrombus stabilisation independently of the classical coagulation pathway and driving endothelial injury in thrombotic microangiopathy [18–20]. This complement–coagulation interface is tightly regulated by SERPING1 (encoding C1-inhibitor, C1-INH), a serine protease inhibitor that inactivates MASP-1, MASP-2, and C1r/C1s through covalent complex formation, and simultaneously inhibits contact pathway proteases including factor XIIa, factor XIa, and plasma kallikrein [21]. Elevated SERPING1 expression as a molecular feature of coronary artery

disease, reporting as a novel biomarker of impaired coronary collateral circulation in cardiovascular disease [22].

Despite substantial mechanistic evidence linking oxidative stress to lectin pathway engagement via cholesterol crystal deposition and endothelial neoantigen exposure [11, 23]. Direct transcriptional evidence of coordinated dysregulation of the lectin pathway in human CVD and its relationship to the systemic redox state remains limited. Prior studies have examined individual complement components or circulating protein levels in isolation, and have reported conflicting associations between MBL and cardiovascular risk depending on disease stage and population context [24, 25]. A concurrent transcriptional analysis of all major lectin pathway effector genes alongside their principal inhibitor, contextualised within the systemic oxidative stress profile, has not been reported in a single well-characterised CVD cohort. We therefore hypothesised that CVD is associated with dysregulation of components of the lectin complement pathway at the complement–coagulation interface, reflecting redox-driven modulation of thrombo-inflammatory signalling. To test this, we evaluated serum oxidative stress markers (SOD, catalase, MDA) alongside transcriptional profiles of *MBL2*, *MASP1*, *MASP2*, and *SERPING1* in peripheral blood mononuclear cells from patients with clinically diagnosed CVD and from healthy controls, and applied rigorous, distribution-appropriate statistical methods to ensure reproducible comparisons.

Materials and Methods

1. Study Design and Participants

This study was conducted as a cross-sectional observational investigation. Participants were stratified into two groups: patients with cardiovascular disease (CVD; n = 100) and healthy controls (n = 50). CVD patients were enrolled following a confirmed clinical diagnosis of acute coronary syndrome (ACS) at the Sri Jayadeva Institute of Cardiovascular Sciences and Research, Mysuru, India. Healthy controls were age-eligible volunteers with no known cardiovascular, metabolic, renal, hepatic, haematological, or autoimmune disease and not on any pharmacological treatment at the time of enrolment. Participant recruitment was not stratified by sex.

Inclusion criteria for CVD patients required a diagnosis of ACS confirmed on the basis of standard clinical, electrocardiographic, and biochemical criteria. Exclusion criteria included a prior history of hypertension or metabolic disease-associated CVD. The study was approved by the Institutional Human Ethics Committee, University of Mysore (Registration No. IHEC-UOM No. 184/Ph.D./2023–24) and by the Ethics Committee of the Sri Jayadeva Institute of Cardiovascular Sciences and Research, Mysuru. All procedures were conducted in accordance with the ethical principles for medical research involving human subjects as set out in the Declaration of Helsinki [26]. Written informed consent was obtained from all participants before enrolment.

2. Blood Sample Collection and Processing

Venous blood (5 mL) was collected from each participant. Samples were divided into clot activator tubes (centrifuged at 3000 rpm for 15 min at 4 °C to obtain serum) and EDTA tubes (stored at −80 °C for PBMC isolation and RNA extraction). Serum was used for biochemical assays, while PBMCs were processed for gene expression studies.

3. Evaluation of Oxidative Stress Parameters

All oxidative stress assays were performed on serum samples. Total protein concentration was determined before enzymatic assays to allow normalisation of all activity values to protein content. Spectrophotometric measurements were performed on a UV-Vis spectrophotometer.

3.1 Superoxide Dismutase Activity

SOD activity was measured using the modified method of Kakkar et al. (1984). The reaction mixture contained phosphate buffer (50 mM, pH 7.4), α-methionine (20 mM), hydroxylamine hydrochloride (10 mM), EDTA (50 µM), Triton-X (1%), and riboflavin (100 µM). Controls included mixtures without serum, and blanks lacked riboflavin. After incubation, Griess reagent was added, and absorbance was measured at 540 nm. Enzymatic activity was expressed as percentage activity per mg protein [27].

3.2 Catalase Activity

Catalase activity was determined by monitoring the decomposition of H_2O_2 (15 mM) in 0.1 M phosphate buffer (pH 7.4). Serum was added to the reaction mixture, and absorbance at 240 nm was recorded every 60 s for 180 s. Results were expressed as µmol H_2O_2 decomposed per mg protein [28].

3.3 Lipid Peroxidation

Malondialdehyde (MDA) levels were estimated using the thiobarbituric acid reactive substances (TBARS) assay. Serum was incubated with TBA (0.8%), acetic acid (20%, pH 3.5), and SDS (8.1%) at 95 °C for 30 min. The chromogen was measured at 535 nm, and the results were expressed as nmol MDA per mg protein [29].

3.4 Total Protein Estimation

Total serum protein was determined using the colourimetric Lowry method, with bovine serum albumin (BSA) as the standard. Protein concentration, expressed as milligrams per millilitre [30].

4. Gene expression Analysis

RNA was extracted from PBMCs using TRIzol reagent (Takara) [31], and quantified using NanoDrop™ 2000/2000c (ThermoFisher). Samples with A260/A280 ratios of 1.8–2.0 were accepted for downstream analysis, in accordance with MIQE guideline recommendations for RNA input quality [32]. One microgram of RNA was reverse-transcribed using PrimeScript™ RT Reagent Kit (Takara) on a SimpliAmp

Thermal Cycler (Applied Biosystems) under the following program: pre-incubation at 25 °C for 5 min, elongation at 50 °C for 60 min, and termination at 80 °C for 5 min.

Quantitative real-time PCR was performed using TB Green® Premix Ex Taq™ II (Takara) on a StepOnePlus Real-Time PCR System (Applied Biosystems). The cycling program consisted of an initial denaturation at 95 °C for 30 sec, followed by 40 cycles of denaturation at 94 °C for 8 sec and annealing/elongation at 60 °C for 45 sec. Primers were designed using Primer3 (v0.4.0), synthesised by Barcode BioSciences (Bangalore), and validated for specificity using NCBI BLAST to confirm target gene alignment with the human genome (GRCh38). Primer sequences are listed in Table1. Relative gene expression was normalised to *GAPDH* and calculated using the $2^{\wedge -\Delta\Delta Ct}$ method (Livak et al., 2001).

Tab1: Primer sequences used for gene expression analysis. Forward and reverse primer sequences were designed for target genes. All sequences are presented in the 5'–3' direction

Sl. No	Gene	Forward Primer (5' → 3')	Reverse Primer (5' → 3')
	GAPDH	CCAGAACATCATCCCTGCCT	CCTGCTTCACCACCTTCTTG
	MBL2	TCCCTCTCCTTCTCCTGAGT	TTTCTCCCTTGGTGCCATCA
	MASP1	CTCTGTGCTTCTCCCTGTCA	GAGGATTCCAAGTTGAAGTGCA
	MASP2	CTCCTGGGCCTTCTGTGTG	AAGTGGGTGAAGTAGAGGCG
	SERPING1	GTTTGCAAGACAGAGGCGAA	GGTTGTGTGGTGGGTTCATC

6. Statistical Analysis

Statistical testing, compared between groups using an unpaired Student's *t*-test, the distribution of all continuous variables was assessed using the Shapiro–Wilk test,Mann–Whitney *U* test, and Spearman test and data were presented as mean ± standard error of the mean (SEM). All statistical analyses and data visualisations were performed using Python.

Results

A total of 150 participants were enrolled, comprising 50 healthy controls and 100 patients with clinically diagnosed CVD. Baseline demographic, clinical, and biochemical characteristics are summarised in Table 2 (Supplementary Table S1). CVD patients were significantly older than controls (57.28 ± 1.02 vs. 30.62 ± 1.74 years; $p < 0.0001$). Gender distribution was comparable between groups (controls: 30 male, 20 female; CVD: 67 male, 33 female). Among CVD patients, 62% reported tobacco use and 53% reported regular alcohol consumption; both exposures were absent in the control group. These differences reflect the expected real-world profile of a chronic cardiovascular disease cohort relative to a healthy reference population.

Prior to statistical testing, all continuous variables were assessed for distributional normality using the Shapiro–Wilk test [34]. SOD activity was normally distributed in controls (Shapiro–Wilk $W = 0.983$, $p = 0.669$) and CAT activity was normally distributed in controls ($W = 0.952$, $p = 0.348$); these variables were therefore analysed using Student's *t*-test and reported as mean ± SEM. Lipid peroxidation (MDA) and all four lectin pathway gene expression variables exhibited significant deviation from normality in one or both groups (Shapiro–Wilk $p < 0.0001$ in all cases), consistent with the log-normal distribution characteristic of RT-qPCR relative quantification data [35]. These variables were analysed using the two-sided Mann–Whitney *U* test [36], and reported as median (interquartile range (IQR)). Rank-biserial *r* was calculated as the effect size measure for all Mann–Whitney comparisons [37] and reported in Supplementary Table S2.

Table 2

Baseline Characteristics and Molecular Profiles of Study Participants Normally distributed variables (SOD, CAT) are presented as mean ± SEM and compared by Student's t-test. Non-normally distributed variables (MDA and gene expression) are presented as median (IQR) and compared by two-sided Mann–Whitney U test with rank-biserial r as the effect size measure. Age difference between groups is an acknowledged study limitation; within-CVD age–gene expression independence is established by Spearman correlation. Abbreviations: SOD, superoxide dismutase; CAT, catalase; MDA, malondialdehyde; IQR, interquartile range; ns, not significant; –, not applicable. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

Sl. No.	Parameter	Controls Median (IQR) or Mean ± SEM	CVD Median (IQR) or Mean ± SEM	p-value	Effect size (r)
Demographic and Clinical Characteristics					
1	n	50	100	–	–
2	Age (years) †	30.62 ± 1.74	57.28 ± 1.02	< 0.0001	–
3	Gender (M/F)	30/20	67/33	–	–
4	Smoking (%)	0	62	–	–
5	Alcohol (%)	0	53	–	–
Oxidative Stress Parameters — Mean ± SEM; Student's t-test					
6	SOD (% inhibition/mg protein)	56.86 ± 1.09	49.12 ± 2.09	< 0.0001 ****	–
7	CAT (µmol H_2O_2/mg protein)	0.30 ± 0.001	0.29 ± 0.001	0.793 (ns)	–
Oxidative Stress Parameters — Median (IQR); Mann–Whitney U test					
8	Lipid Peroxidation (nmol MDA/mg protein)	0.73 (0.44–1.55)	1.48 (0.49–3.61)	0.008 **	0.267
Lectin Pathway Gene Expression — Median (IQR); Mann–Whitney U test with rank-biserial r					
9	*MBL2* (relative fold change)	0.99 (0.75–1.40)	0.81 (0.39–1.17)	0.017 *	−0.240
10	*MASP1* (relative fold change)	1.05 (0.54–1.39)	0.71 (0.47–1.10)	0.028 *	−0.221
11	*MASP2* (relative fold change)	1.01 (0.51–1.68)	0.63 (0.38–0.92)	< 0.0001 ****	−0.382
12	*SERPING1* (relative fold change)	1.20 (0.78–1.40)	1.96 (0.89–3.98)	0.001 ***	+ 0.330

Oxidative Stress Parameters in CVD Patients and Controls

Oxidative stress biomarkers are presented in Fig. 1. CVD patients exhibited a significant reduction in serum SOD activity relative to healthy controls (mean 49.12 ± 2.09 vs. 56.86 ± 1.09% inhibition/mg protein; $t(148) = 4.596$, $p < 0.0001$; Fig. 1A), indicating substantially impaired superoxide scavenging capacity in the diseased state and in keeping with prior reports of depleted SOD in acute myocardial infarction and coronary artery disease [38, 39]. Catalase activity showed no significant difference between CVD patients and controls (mean 0.29 ± 0.001 vs. 0.30 ± 0.001 μmol H_2O_2 decomposed/mg protein; $t = 0.263$, $p = 0.793$; Fig. 1B), suggesting that catalase-dependent hydrogen peroxide clearance capacity is partially maintained at this stage of chronic disease. Lipid peroxidation, as reflected by MDA concentration, was significantly elevated in CVD patients (median 1.48 (IQR 0.49–3.61) vs. 0.73 (0.44–1.55) nmol MDA/mg protein; Mann–Whitney $U = 1832$, $p = 0.008$, rank-biserial $r = 0.267$; Fig. 1C), consistent with cumulative oxidative membrane injury in atherosclerotic disease and in line with previous findings linking elevated MDA to adverse cardiovascular outcomes [9].

Lectin Pathway Gene Expression in CVD Patients and Controls

All four-lectin pathway–associated genes showed statistically significant differences between CVD patients and healthy controls. Results are presented as median (IQR) and illustrated in Fig. 2. *MBL2* expression was significantly lower in CVD patients (median 0.81 (IQR 0.39–1.17)) compared with healthy controls (median 0.99 (0.75–1.40); Mann–Whitney $U = 3100$, $p = 0.017$, rank-biserial $r = -0.240$; Fig. 2A). Of note, this significant difference was not detected when the parametric *t*-test was applied to these non-normally distributed data ($p = 0.444$), highlighting the importance of appropriate statistical test selection for skewed gene expression data. *MASP1* expression was significantly downregulated in CVD patients (median 0.71 (0.47–1.10)) relative to controls (median 1.05 (0.54–1.39); Mann–Whitney $U = 3053$, $p = 0.028$, rank-biserial $r = -0.221$; Fig. 2B). MASP-1 contributes to thrombus biology through its thrombin-like capacity to cleave fibrinogen, activate factor XIII, and alter the fibrinolytic behaviour of blood clots [20, 40]. Its transcriptional suppression may therefore reflect adaptive attenuation of complement-driven coagulation amplification at the complement–coagulation interface. *MASP2* exhibited the most pronounced and significant downregulation among the effector genes in CVD patients (median .63 (0.38–0.92) vs. control median 1.01 (0.51–1.68); Mann–Whitney $U = 3454$, $p < 0.0001$, rank-biserial $r = -0.382$; Fig. 2C). The effect size for *MASP2* was the largest of the three effector genes, consistent with its central role as the primary C3-activating serine protease of the lectin pathway and its capacity for direct fibrin generation and thrombus stabilisation independent of the classical coagulation cascade [16, 41]. In contrast to the three effector genes, *SERPING1* was significantly upregulated in CVD patients (median 1.96 (0.89–3.98) vs. control median 1.20 (0.78–1.40); Mann–Whitney $U = 1674$, $p = 0.001$, rank-biserial $r = +0.330$; Fig. 2D), indicating heightened expression of the principal inhibitor of MASP-1 and MASP-2 activity. The concurrent downregulation of all three lectin pathway effector genes alongside significant upregulation of *SERPING1* constitutes a coherent four-gene regulatory signature, consistent with coordinated adaptive suppression of lectin pathway activity in the chronic cardiovascular disease state. The translational relevance of this finding is supported by prior identification of SERPING1 as a biomarker of impaired coronary collateral circulation in stable coronary disease and by evidence that

MASP-2 inhibition confers protection against complement-driven endothelial injury in thrombotic microangiopathy [18, 22].

Within-CVD and Control Age Independence of Gene Expression.

Given the substantial age difference between CVD patients (mean 57.28 years) and healthy controls (mean 30.62 years), Spearman rank correlations were computed between patient age and lectin pathway gene expression within the CVD group independently, to assess whether the between-group transcriptional differences could be attributable to age rather than disease status [42]. No significant correlations were identified between age and any of the four gene expression variables within the CVD cohort: *MASP1* (ρ = 0.021, *p* = 0.832), *MASP2* (ρ = 0.087, *p* = 0.390), *SERPING1* (ρ = −0.067, *p* = 0.505), and *MBL2* (ρ = −0.133, *p* = 0.188), Fig. 3A (Supplementary Table S3and S4).

These findings indicate that inter-individual variation in lectin pathway gene expression among CVD patients is not explained by chronological age, supporting the interpretation that the between-group transcriptional differences reflect disease-associated regulatory changes. Of note, within the healthy control group, both *MASP2* (ρ = −0.428, *p* = 0.002) and *SERPING1* (ρ = −0.438, *p* = 0.002); Fig. 3B showed significant negative correlations with age, suggesting that physiological age-related modulation of these complement genes occurs in healthy individuals a finding that underscores their biological relevance to vascular ageing and warrants further investigation in age-stratified prospective studies [15, 16].

Discussion

The present study investigated the relationship between systemic oxidative stress and transcriptional regulation of the lectin complement pathway in patients with cardiovascular disease (CVD) compared with healthy controls. Three principal findings emerged. First, CVD patients exhibited a state of pronounced oxidative imbalance characterised by significantly reduced superoxide dismutase (SOD) activity and elevated lipid peroxidation, while catalase activity decreased but was not significant. Second, transcriptional profiling revealed a coordinated pattern: *MBL2*, *MASP1*, and *MASP2* were significantly downregulated in CVD patients, while *SERPING1* was significantly upregulated. Third, within-group Spearman analysis confirmed that these transcriptional differences were not attributable to the age disparity between cohorts, supporting their association with disease status rather than chronological ageing. Together, these findings identify a coherent regulatory signature at the complement–coagulation interface that characterises the chronic cardiovascular disease state and has not been described across all four genes in a single human CVD cohort.

Oxidative stress in CVD

Oxidative stress plays a pivotal role in the pathophysiology of CVD, arising from an imbalance between the generation of free radicals and the capacity of endogenous antioxidant systems to neutralise them [8]. Superoxide dismutase catalyses the dismutation of the superoxide radical ($O2^{\bullet -}$) to hydrogen

peroxide; the depletion of enzyme activity allows superoxide to react with nitric oxide, generating peroxynitrite and reducing endothelial NO bioavailability, a hallmark of early atherogenesis [6, 7]. The significantly reduced SOD activity observed in CVD patients in the present study is consistent with a well-documented pattern of impaired superoxide scavenging in atherosclerotic and coronary artery disease [38, 39]. Catalase activity, however, did not differ significantly between groups. SOD and catalase trajectories have been documented in stable versus unstable cardiovascular phenotypes [39]. Consistent with deficient superoxide scavenging, lipid peroxidation, as assessed by malondialdehyde (MDA), was significantly elevated in CVD patients. Taken together, reduced SOD activity and elevated MDA levels, despite preserved catalase activity, signify a selective yet substantial oxidative imbalance in chronic cardiovascular disease, aligning with prior reports [8, 38].

Complement–Coagulation Crosstalk

The complement system provides a mechanistic yoke between oxidative vascular injury and thrombo-inflammatory exaggeration. Oxidative stress promotes the intracellular and extracellular deposition of cholesterol crystals within the arterial wall, directly activating the lectin pathway through ficolin-2 and MBL-dependent recognition of altered lipid structures on injured cell surfaces[11, 15, 23]. Once activated, the lectin pathway converges with the classical and alternative pathways, generating C3a and C5a, forming the membrane attack complex, and directly activating the coagulation cascade driving a self-amplifying cycle of complement-mediated endothelial injury and thrombosis [14, 17]. The lectin pathway is thus positioned at the convergence of innate immune surveillance and oxidative vascular damage, making it a particularly relevant molecular axis for investigation in human CVD [18, 19].

Transcriptional Downregulation of Effector Genes

The transcriptional downregulation of *MBL2*, *MASP1*, and *MASP2* in CVD patients may appear paradoxical, given oxidative stress–driven lectin pathway activation. However, in chronic disease states, sustained complement activation often induces adaptive transcriptional suppression to prevent uncontrolled consumption and collateral injury [17]. Evidence from other thrombotic and vascular conditions supports this interpretation: Lv et al. reported reduced complement gene expression in patients with symptomatic pulmonary embolism [43], and Orsini et al. demonstrated that targeting MBL provided long-lasting protection against endothelial injury in cerebral ischaemia [44], implying that lectin pathway activity is deleterious in chronic ischaemic settings and its suppression may be biologically protective. The transcriptional pattern observed in the present cohort is therefore best interpreted as a disease-stage–dependent regulatory shift, in which the chronic cardiovascular environment has driven the lectin pathway from acute effector activation towards an adaptive inhibitory state.

The significant downregulation of *MBL2* observed in this study (median fold change 0.81 vs. 0.99 in controls; $p = 0.017$, rank-biserial $r = -0.240$) warrants particular attention, as this association was not detected when the parametric *t*-test was applied to these markedly skewed data ($p = 0.444$). The association became statistically significant only upon application of the Mann–Whitney *U* test following

formal normality assessment a methodologically important demonstration of the sensitivity of test selection for gene expression data with a strongly non-parametric test. Its role in cardiovascular disease is nuanced in the existing literature: elevated circulating MBL predicted greater cardiovascular risk in haemodialysis patients [24], while lower MBL levels were associated with increased mortality in type 2 diabetes with established CVD [25]. This apparent contradiction likely reflects the context-dependent nature of lectin pathway activity protective in early pathogen clearance and acute-phase responses, but potentially injurious if excessively sustained in a pro-atherogenic or ischaemic vascular environment [15, 17, 45]. The transcriptional downregulation of *MBL2* in the present cohort of chronic CVD patients aligns with the adaptive suppression model and is consonant with the broader downregulation of all three effector pathway components described here.

The downregulation of *MASP1* and *MASP2* carries particular mechanistic significance due to their direct roles at the complement–coagulation interface. Both proteases thus represent molecular conduits through which lectin pathway activation translates directly into coagulation amplification [41]. Their transcriptional suppression in established CVD is a plausible endogenous mechanism for attenuating excessive complement-driven fibrin deposition in the context of chronic endothelial injury. The finding that *MASP2* exhibits the largest effect size of the three effector genes (rank-biserial $r = -0.382$) is consistent with its more prominent dual role in both complement propagation and direct coagulation crosstalk, and with the predominance of MASP-2–driven complement activity described in human thrombotic microangiopathy [18].

C1-INH is a serine protease inhibitor (serpin) and the principal physiological brake on both the classical and lectin complement pathways: it inactivates MASP-1, MASP-2, and C1r/C1s through the formation of stable covalent complexes with their active sites, thereby arresting complement activation at the level of the pattern recognition molecule–associated proteases [21]. Importantly, C1-INH also inhibits plasma kallikrein, factor XIIa, and factor XIa, positioning it as a multi-functional regulator of innate immune–coagulation crosstalk well beyond the complement system [21]. The significant upregulation of *SERPING1* has been reported in the coronary disease and thrombotic contexts [22]. The simultaneous downregulation of *MBL2*, *MASP1*, and *MASP2*, and upregulation of *SERPING1*, constitutes a coherent four-gene regulatory signature: lectin pathway effectors are transcriptionally suppressed while their primary inhibitor is concomitantly induced.

Age and Confounding Factors

A potential confound in the present study is the substantial age difference between CVD patients (mean 57.28 years) and healthy controls (mean 30.62 years). To address this directly, Spearman rank correlations were computed between patient age and each lectin pathway gene within the CVD group independently. None of the four genes showed a significant age-dependent variation within the CVD cohort: *MASP1* ($\rho = 0.021$, $p = 0.832$), *MASP2* ($\rho = 0.087$, $p = 0.390$), *SERPING1* ($\rho = -0.067$, $p = 0.505$), and *MBL2* ($\rho = -0.133$, $p = 0.188$). The absence of any significant age–expression association within the disease group indicates that the inter-individual variation in lectin pathway gene expression among CVD

patients is not driven by chronological age, and supports the conclusion that the between-group differences reflect a disease-associated transcriptional state. Notably, within the control group, both *MASP2* (ρ = −0.428, $p = 0.002$) and *SERPING1* (ρ = −0.438, $p = 0.002$) showed significant negative correlations with age, suggesting that these lectin pathway genes do undergo age-related transcriptional modulation in the healthy vascular state a finding that may reflect physiological changes in complement regulation with healthy ageing and that merits further investigation in prospective, age-stratified cohorts [15, 16].

The cross-sectional observational design precludes causal inference: the associations reported between CVD status and lectin pathway gene expression are correlational, and prospective or longitudinal studies will be required to establish temporal directionality and to determine whether transcriptional dysregulation precedes, accompanies, or follows disease progression. The absence of an age-matched control group remains a structural limitation of the study design; while the within-CVD Spearman analysis addresses this concern at the gene expression level, it does not exclude age-related contributions to the oxidative stress parameters, for which analogous within-group age analyses were not performed. Future work should confirm with additional protein-level quantification of MASP-1, MASP-2, and SERPING1 by ELISA or Western blot. The study did not include direct measures of coagulation pathway activation, such as thrombin–antithrombin complexes, D-dimer, or fibrin degradation products or of platelet activation, which would have directly linked the observed transcriptional changes to downstream thrombo-inflammatory outcomes. Residual confounding by tobacco use (62% of CVD patients) and alcohol consumption (53%) cannot be excluded, as both exposures independently modulate oxidative stress and innate immune gene expression, and their near-complete absence in the control group prevents statistical adjustment. The relatively modest sample size, particularly for the control group ($n = 50$), limits statistical power for subgroup analyses and warrants replication in larger, independently recruited cohorts.

Notwithstanding these limitations, the present study offers several contributions. The use of appropriate non-parametric testing, guided by formal Shapiro–Wilk normality assessment, revealed a significant downregulation of *MBL2* that was undetectable by the parametric approach, a methodological finding with broader implications for the reporting of RT-qPCR data from clinically heterogeneous cohorts. The coherent four-gene regulatory signature described here, encompassing all three major lectin pathway effector components and their principal inhibitor, provides a more complete transcriptional picture of lectin pathway dysregulation in CVD than has been reported from a single human cohort in the existing literature. From a translational perspective, the complement–coagulation interface defined by the MASP–SERPING1 axis is an area of active clinical development: recombinant C1-INH preparations are already in clinical use for hereditary angioedema [21], and MASP-2 inhibitory monoclonal antibodies are under clinical investigation for thrombotic microangiopathy and ischaemia-reperfusion injury [18].

Conclusion

The present findings support the relevance of this axis in atherothrombotic disease and provide a transcriptional rationale for future studies investigating lectin pathway components as biomarkers of disease burden and as therapeutic targets in chronic cardiovascular disease. Future investigations employing age-matched, longitudinal designs with concurrent measurement of circulating complement proteins, coagulation activation markers, endothelial dysfunction indices, and multi-gene normalisation will be required to build upon and fully contextualise the transcriptional observations reported here.

Statements and Declaration

CRediT authorship contribution statement:

Managalli G Manohara: Writing – review & editing, Writing – Original draft, Visualisation, Validation, Methodology, Investigation, Formal analysis, Data curation, and Conceptualisation. **Jahnavi R:** Methodology and Investigation. **Dr. Veena Nanjappa:** Investigation, Formal analysis, and Sample Provision. **Dr. Devaraju Chandagal Javaregowda:** Investigation, Formal analysis, and Sample Provided. **Dr. Manjunath M:** Investigation, Formal analysis, Data curation. **Suttur Malini:** Writing – review & editing, Writing – Original draft, Visualisation, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, and Conceptualisation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data Availability:

No Data availability.

Ethical Approval:

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Consent to Participant:

All participants gave written informed consent as per the Huma Ethical Committee Guidelines.

Consent to Publication:

Not Applicable.

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Figures

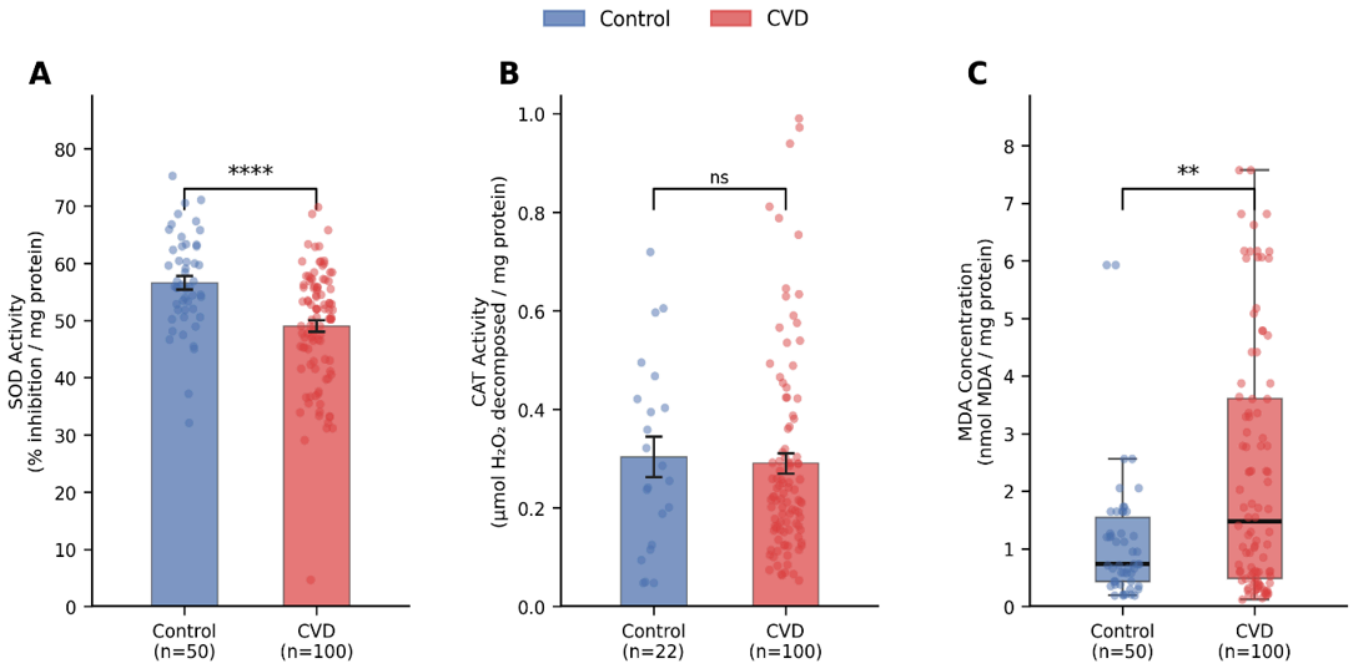


Figure 1

Oxidative stress parameters in CVD patients and healthy controls. (A) Superoxide dismutase (SOD) activity. (B) Catalase (CAT) activity. (C) Malondialdehyde (MDA) concentration reflects lipid peroxidation. SOD and CAT are presented as mean ± SEM and compared by unpaired Student's *t*-test. MDA is shown as a box-and-whisker plot (line = median; box = IQR; whiskers = 10th–90th percentile) with individual values overlaid, compared by two-sided Mann–Whitney *U* test, *n* = 50 (Control), *n* = 100 (CVD). **$p < 0.01$, ****$p < 0.0001$; ns, not significant.

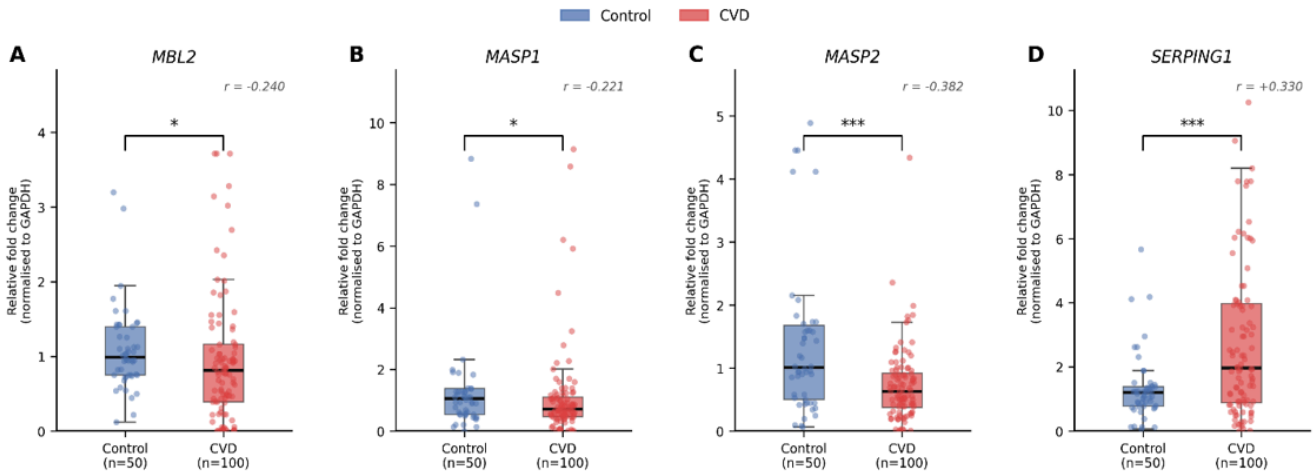


Figure 2

Lectin pathway gene expression in CVD patients and healthy controls. (A) *MBL2*; (B) *MASP1*; (C) *MASP2*; (D) *SERPING1*. All panels show median (IQR) as box-and-whisker plots (line = median; box = IQR; whiskers = 10th–90th percentile) with individual data values overlaid as scatter points. Comparisons by two-sided Mann–Whitney *U* test, rank-biserial *r* shown as effect size in the upper right of each panel. Expression normalised to *GAPDH* by the $2^{-\Delta\Delta Ct}$ method. *n* = 50 (Control), *n* = 100 (CVD). **p* < 0.05, ****p* < 0.001, *****p* < 0.0001.

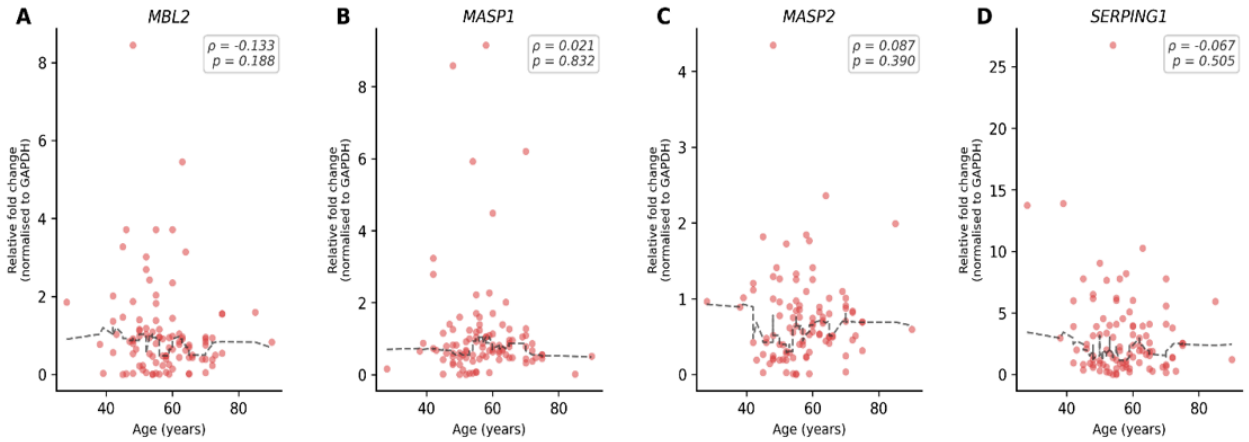


Figure 3A

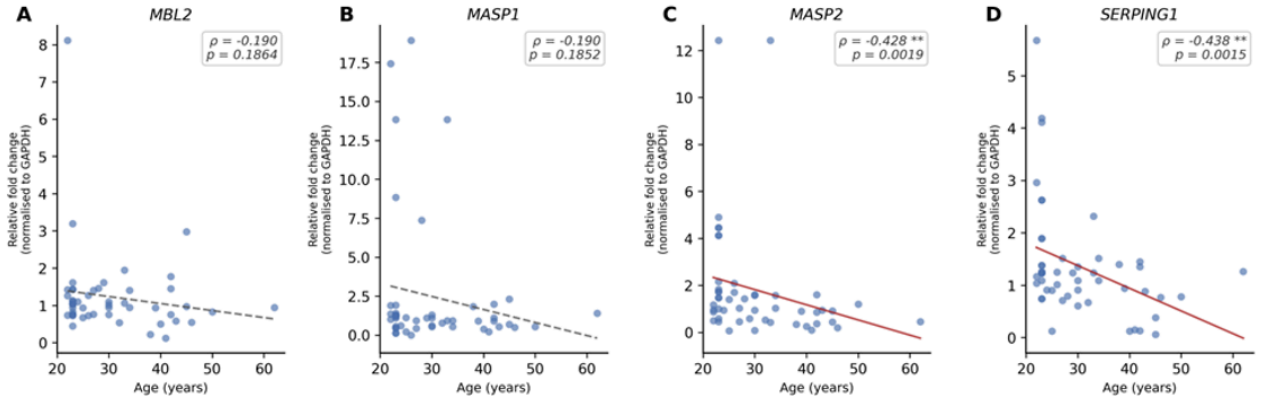


Figure 3B

Figure 3

A. Scatter plots showing individual data points for age (x-axis) versus relative fold change (y-axis) for (A) *MBL2*, (B) *MASP1*, (C) *MASP2*, (D) *SERPING1* within the CVD group (n = 100). Dashed lines indicate rolling median trend. Spearman ρ and p-values are shown in the upper right of each panel. None of the four genes showed a significant association with age within the CVD cohort (all p > 0.18), supporting the interpretation that between-group transcriptional differences reflect disease-associated rather than age-driven regulatory changes.

B Scatter plots showing individual data points for age (x-axis) versus relative fold change (y-axis) for (A) *MBL2*, (B) *MASP1*, (C) *MASP2*, (D) *SERPING1* within the healthy control group (n = 50). Solid regression lines indicate significant correlations ($p < 0.05$); dashed lines indicate non-significant trends. *MASP2*($\rho = -0.428$, $p = 0.002$) and *SERPING1* ($\rho = -0.438$, $p = 0.002$) showed significant negative correlations with age in healthy controls, suggesting physiological age-related downregulation of these complement genes in the healthy vascular state.

Supplementary Files

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- SupplementaryMaterials.docx