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RESEARCH

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cxsAssociation study between single nucleotide polymorphism in thrombospondins *THBS1* gene and *THBS2* gene and coronary artery disease in Indian population

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Abstract

Background Coronary artery disease (CAD) is a complex medical condition characterized by atherosclerotic plaque accumulation in coronary arteries, leading to narrowed blood vessels and impaired blood flow. Endothelial dysfunction, smooth muscle cell proliferation, and various risk factors contribute to CAD development. Matricellular proteins, including thrombospondins (THBS), play crucial roles in vascular processes and cardiac function.

Methods A case–control study was conducted among 296 participants from Era's Lucknow Medical College and Hospital, India, to investigate genetic variations in THBS1 (N700S) and THBS2 (3′ UTR T → G) in relation to CAD. Genomic DNA was isolated, and PCR–RFLP was employed for genotyping. Clinical and biochemical parameters were assessed, and statistical analyses were performed using SPSS software.

Results The study revealed that age, serum cholesterol, HDL, VLDL, and LDL were significantly associated with CAD in the Indian population. However, no statistically significant associations were found between triglyceride and serum creatinine levels, as well as the studied THBS1 and THBS2 genetic polymorphisms, and CAD. The analysis of genotypic and allelic frequencies did not indicate significant associations with CAD risk.

Conclusions This study suggests that specific genetic variations in THBS1 and THBS2 may not be strongly linked to the development or risk of CAD in the studied Indian population. The associations observed between age, lipid profiles, and CAD highlight the multifactorial nature of CAD susceptibility. Further research with larger sample sizes and diverse populations is warranted to validate these findings and explore additional genetic factors contributing to CAD in specific populations.

Keywords Coronary artery disease (CAD), *Thrombospondin-1* (*THBS-1*), *Thrombospondin-2 THBS-2*, Polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP), Gene polymorphism

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Background

Coronary artery disease (CAD) is a medical condition characterized by the accumulation of atherosclerotic plaque within the blood vessels responsible for delivering oxygen to the heart. This process typically begins at an early stage in life and is thought to commence with the dysfunction of endothelial cells that line the coronary arteries. This dysfunction disrupts the regulation of vascular tone, specifically the constriction of blood vessels mediated by nitric oxide signaling. Subsequently, smooth muscle cells (SMCs) within the vessel walls undergo excessive proliferation, leading to structural changes in the vessels, ultimately resulting in narrowing and obstruction of blood flow [1].

Epidemiological investigations concerning coronary artery disease (CAD) have revealed that several factors significantly raise the risk of myocardial infarction (MI). These factors include advancing age, being male, obesity, elevated blood pressure, smoking, diabetes, and a sedentary lifestyle [2]. Additionally, CAD risk is linked to elevated levels of low-density lipoprotein (LDL) cholesterol, reduced high-density lipoprotein (HDL) cholesterol, and increased accumulation of fat (triglyceride-rich lipoproteins). In a clinical context, when these risk factors are present together, they can help identify specific subgroups within the population that are at a heightened risk of CAD. Identifying these high-risk groups is crucial for optimizing the application of preventive medical interventions [2].

Preventive strategies for coronary artery disease (CAD) primarily revolve around adopting a healthy lifestyle, which includes refraining from smoking, maintaining a non-obese weight, following a nutritious diet, and engaging in regular physical activity. Individuals at high risk for CAD can also benefit from additional medications aimed at reducing LDL cholesterol levels (such as statins), lowering blood pressure, and preventing blood clot formation (using aspirin) [2].

Significant alterations have frequently been observed in the composition of certain non-fibrillar matrix and matricellular proteins in patients with dilated cardiomyopathy. These matricellular proteins are non-structural components of the extracellular matrix (ECM) that regulate cell behavior and function. Examples of these proteins include tenascins, thrombospondins (THBS), periostin, osteopontin, osteonectin/secreted protein, and CCN proteins rich in cysteine (SPARC) [3]. THBS, in particular, are known to play a role in various processes within blood vessel walls, including the proliferation of smooth muscle cells (SMCs) and the migration and proliferation of endothelial cells. Additionally, THBS can bind to various other proteins in the extracellular matrix [3].

THBS are produced by a variety of cell types and play a central role in the composition of matricellular proteins. The THBS family can be classified into two subgroups based on their structural differences: subgroup A, which includes trimeric *THBS-1* and *THBS-2,* and subgroup B, consisting of pentameric *THBS-3, THBS-4,* and *THBS-5* [4]. All *THBS* genes have complex structures with multiple domains that allow them to interact with various ligands. Among these, *THBS-1* and *THBS-2* are notably involved in a wide range of physiological and pathological processes. Current research has generated numerous studies exploring the roles of *THBS-1* and *THBS-2* in different cardiovascular diseases (CVDs), indicating their potential as therapeutic targets [4].

THBS1 plays a crucial role in regulating angiogenesis and thrombosis, and it has the capability to increase the availability of TGF-β by releasing this cytokine from its inactive form [5]. Within the heart, *THBS2* is responsible for preserving the integrity of the cardiac matrix through its interaction with matrix metalloproteinases (MMPs) [5]. The N700S polymorphism of *THBS-1* has been linked to an elevated risk of myocardial infarction (MI), a condition where the blood supply to the heart muscle is obstructed, resulting in tissue damage. This suggests that individuals carrying specific genetic variations of the N700S polymorphism may be more prone to developing MI [6].

THBS-2 is closely linked to cardiac hypertrophy. Research has demonstrated that older mice lacking *THBS-2* (THBS-2 -/- mice) experience an exacerbated form of dilated cardiomyopathy, characterized by impaired systolic function, increased cardiac enlargement, and myocardial fibrosis. This suggests that *THBS-2* deficiency contributes to the development of age-related dilated cardiomyopathy [7].

In comparison to normal mice, mice with *THBS-2* knocked out exhibit higher mortality rates, along with a decline in cardiac function, increased cardiomyocyte apoptosis (cell death), and damage to the extracellular matrix (ECM) in a mouse model of doxorubicin-induced cardiomyopathy [8].

Elevated levels of *THBS-2* are predominantly associated with an unfavorable prognosis in heart failure patients. Among individuals with coronary heart disease and symptomatic congestive heart failure (CHF), there is an elevation in circulating *THBS-2* levels, which is linked to a heightened risk of CHF-related mortality, overall death, and repeated hospitalization over a three-year period [9].

In the context of blood vessels, *THBS1, THBS2,* and *THBS4* are closely linked to the control of both the structure and function of the vascular wall, as well as their interactions with blood-borne cells. A study in diabetic

rats, focusing on their clinical characteristics, revealed an increase in *THBS1* levels following vascular injury [10]. Additionally, in more advanced vascular lesions, *THBS2* plays a role in preventing atherosclerosis by enhancing the phagocytic activity of macrophages. This function is essential for clearing apoptotic, necrotic cells, and cellular debris within the lesions [10].

The study aimed to identify whether specific genetic variations in *THBS1* and *THBS2* genes are associated with an increased or decreased risk of CAD, which could provide valuable insights into the genetic factors contributing to the development of CAD in the Indian population.

Methods

Ethics and confidentiality

The current research adhered to the protocols established by the Institutional Ethical/Review Committee at Era's Lucknow Medical College and Hospital, Lucknow, as indicated by Reference number ELMC &H/R-Cell-/2019/24. All procedures conducted in this study were in compliance with the ethical standards outlined by ELMCH. Furthermore, informed written consent was acquired from each participant. The research was conducted in accordance with the principles of good clinical practice.

Study setting

Study participants were recruited from the Cardiology Unit of the Medicine Department at Era's Lucknow Medical College and Hospital in Lucknow, India. Diagnosis of CAD was determined by a cardiologist through a comprehensive assessment, including clinical examination, medical history review, and angiographic investigations that revealed more than 50% stenosis in one or more coronary arteries. Among the total of 296 study participants, 145 were individuals with CAD, while 151 were selected as controls based on age and gender matching, and they exhibited less than 50% luminal obstruction on coronary angiography. To ensure consistency, participants with recent cerebrovascular events within the past eight weeks, a history of acute coronary syndrome (ACS), chronic obstructive pulmonary disease (COPD), chronic liver and kidney insufficiency, cancer, or a history of repeated blood transfusions were included in the study. Additionally, CAD patients with comorbid diabetes and hypertension (HTN) were excluded from this research, as were patients whose CAD resulted as a secondary effect of other diseases. Furthermore, individuals with malignancies and other autoimmune disorders were not included in the study. The control group consisted of age and sex-matched individuals who did not have a history of peripheral vascular disease, CAD, or stroke.

Blood sample collection, processing, storage and genotyping

Three milliliters of venous blood samples were collected from all participants with their informed consent through a single-time venipuncture from the antecubital vein. The collected samples were stored in EDTA-coated vials. Genomic DNA was subsequently isolated from the white blood cells (WBCs) using the conventional phenol–chloroform isolation method, and these DNA samples were stored at -20 °C until they were needed for further analysis. The quality and quantity of the high molecular weight genomic DNA was assessed using spectrophotometer quantification and analyzed through gel electrophoresis. Additionally, the extracted DNA's quality was verified using a Nanodrop spectrophotometer (NANODROP 2000), and its purity was determined by calculating the 260/280 ratio. For PCR amplification, a ratio between 1.5 and 1.8 was considered acceptable. The genotyping process was carried out using the PCR–RFLP method.

Genotyping

For THBS-1 (N700S), the following primers were used:

Forward: GCATGGTGTACCCTCAGGTG

Reverse: TGTTTTGATAAGGTGATGGGC.

PCR amplification of the *THBS1* gene was performed in a 20 µl reaction mixture, which included 2 µl of DNA template, 1 µl each of forward and reverse primers, 10 µl of Master mix, 0.1 µl of MgCl2, 0.1 µl of Taq Polymerase, and 6 µl of nuclease-free water. Subsequently, the PCR tubes were placed in a thermal cycler for amplification. The PCR conditions consisted of an initial denaturation step at 94 °C for 5 min, followed by 33 cycles of denaturation at 94 °C for 30 s, annealing at 56 °C for 30 s, extension at 72 °C for 30 s, and a final extension at 72 °C for 5 min. The resulting PCR products were then analyzed on a 2% agarose gel containing ethidium bromide (EtBr) and visualized using a gel documentation system (Biorad). Restriction fragment length polymorphism (RFLP) analysis of the THBS1 gene was carried out in a 20 µl reaction mixture, comprising 6 µl of PCR product and 14 µl of the restriction enzyme (RE) reaction mixture. The digested PCR products were subsequently analyzed on a 3% agarose gel containing EtBr and visualized using a gel documentation system (Biorad [11]). PCR products was 293 bp size, and digestion with *BsrI* (3 h, 65 °C) generated two additional fragments of 191 bp and 102 bp in the presence of the G allele (Fig. 1).

For THBS-2, (3′ UTR T → G) the following primers were used

Forward: CTGTGCATGCCATGGTCCCTAGA;

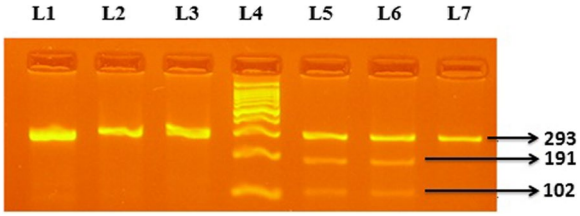


Fig. 1 PCR products was 293 bp size, and digestion with BsrI restriction enzyme (3 h, 65 °C) generated 2 additional fragments of 191 bp and 102 bp in the presence of the G allele

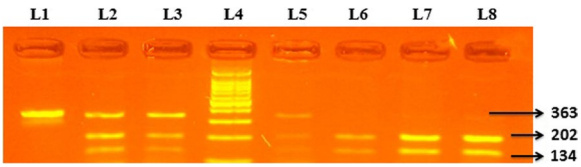


Fig. 2 PCR products was 363 bp size, and digestion with DdeI restriction enzyme (12 h, 37 °C) generated 3 fragments of 27 bp, 134 bp, and 202 bp in the presence of the T allele and an additional 336-bp band in the presence of the G allele

Reverse: TATCATAATGGCTTATGCACAGTATTC CCTTCA.

PCR amplification of *THBS 2* was evaluated in a 20 µl reaction mixture containing 2 µl of DNA template, 1 µl of each forward and reverse primer, 10 µl of Master mix, 0.1 µl $MgCl_2$, 0.1 µl Taq Polymerase & 6 µl of nuclease free water. PCR conditions were 94 °C for 5 min 1 cycle followed by 33 cycles of 94 °C for 30 s, 56 °C for 30 min, 72 °C for 30 s followed by final extension at 72 °C for 5 min. The amplified PCR products were analyzed on 2% agarose gel containing ethidium bromide (EtBr) and visualized using gel documentation system (Biorad). RFLP of *THBS2* gene was evaluated in 20 µl of reaction mixture containing PCR Product 6 µl and RE reaction Mixture 14 µl. The digested PCR products were analyzed on 3% agarose gel containing ethidium bromide using and visualized gel documentation system (Biorad). The undigested PCR product was represented by a 363 bp band and it was digested with *DdeI* restriction endonucleases [12]. The restriction digestion generated two alleles, where the presence of T allele was confirmed by a single band of 363 bp while G allele was visualized by two bands of 202 bp and 134 bp (Fig. 2).

Data collection and statistical analysis

Statistical analysis was conducted using SPSS software version 21. χ^2 analysis and Fisher's exact test were employed to compare clinical and demographic information among the study participants. To compare genotype and allele frequencies between mild and severe patients, a 2×2 contingency table was created, and Fisher's exact test was applied. Statistical significance was considered for *p*-values less than 0.05. Haplotype analysis was carried out using the Shesis Software online version (Fig. 3).

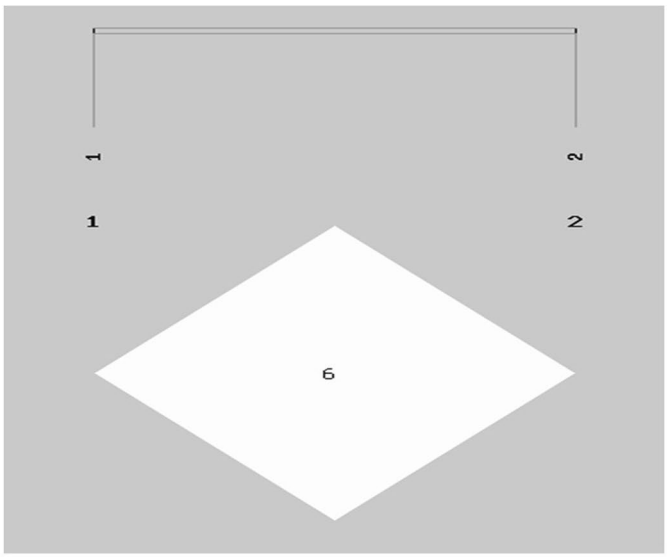
Results

Patients' characteristics and clinical manifestations

The table presents the comparison of various parameters between a control group and CAD group. The table includes the mean and standard deviation (SD) for each parameter and indicates the *p*-values for statistical significance. Mean age of 49.21 ± 8.46 years was found in the control group while the CAD group has a higher mean age of 55.10 ± 9.32 years. Mean serum cholesterol level was 146.23 ± 22.74 mg/dL in the control group whereas the CAD group has a higher mean of 157.29 ± 25.6 mg/dL. The mean TG level was 130.36 mg/dl ± 26.65 in the control group, while the CAD group has a slightly higher mean of 133.3 mg/dl ± 34.67. No significant difference was found in TG levels between the two groups ($p = 0.413$). The mean HDL (High-Density Lipoprotein) level was 26.97 mg/dl ± 5.45 in the control group, whereas the CAD group has a slightly higher mean of 29.08 mg/dl ± 9.54. A significant difference was found in HDL levels between the two groups ($p = 0.02$). Mean VLDL (Very Low-Density Lipoprotein) level was 23.07 mg/dl ± 4.14 in the control group, whereas significantly higher mean of 32.48 mg/dl ± 11.05 was found in the CAD. The *p*-value (< 0.001) suggests a significant difference in VLDL levels between the two groups. The mean LDL (Low-Density Lipoprotein) level was found to be 79.39 mg/dl ± 10.67in the control groups, while the CAD group has a higher mean of 86.79 mg/dl ± 17.54. The *p*-value (< 0.001) indicates a significant difference in LDL levels between the two groups. The mean serum creatinine level was 1.33 mg/dl ± 1.82 in the control group, whereas the CAD group has a slightly lower mean of 1.18 mg/dl ± 0.53. The *p*-value (0.33) suggests no significant difference in serum creatinine levels between the two groups. In summary, the table shows that individuals with CAD have significantly higher mean values for age, serum cholesterol, HDL, VLDL, and LDL compared to the control group. However, there is no significant difference in triglyceride and serum creatinine levels between the two groups (Table 1).

Genotyping of THBS1 and THBS2 polymorphisms

The table provides information about genotypes and their association with controls and cases in a study. In THBS1 G > A; AA genotype is 88.97% in the cases and 88.74% in controls with no significant difference ($p = 0.811$). GA genotype was found in 11.03% of the cases and in the control group, 11.26% had this



Loci	Case (freq)	Control (freq)	Chi2	Fisher's	Pearson's p
A G*	248.62 (0.857)	254.16 (0.842)	0.04	0.840804	0.840784
A T*	28.38 (0.098)	29.84 (0.099)	0.007	0.931398	0.931374
G G*	12.38 (0.043)	13.84 (0.046)	0.047	0.828465	0.828446
G T	0.62 (0.002)	4.16 (0.014)	--	--	--

Fig. 3 Haplotype analysis

Table 1 Biochemical parameters in Cases and control subjects

Parameters	Controls (n = 151) Mean ± SD	CAD Cases (n = 145) Mean ± SD	P Value
Age (years)	49.21 ± 8.46	55.10 ± 9.32	**< 0.001**
Serum cholesterol (mg/dl)	146.23 ± 22.74	157.29 ± 25.6	**< 0.001**
Triglyceride (mg/dl)	130.36 ± 26.65	133.3 ± 34.67	0.413
HDL (mg/dl)	26.97 ± 5.45	29.08 ± 9.54	**0.02**
VLDL (mg/dl)	23.07 ± 4.14	32.48 ± 11.05	**< 0.001**
LDL (mg/dl)	79.39 ± 10.67	86.79 ± 17.54	**< 0.001**
Serum creatinine (mg/dl)	1.33 ± 1.82	1.18 ± 0.53	0.33

Values ≤ 0.05 are considered significant

genotype. The *p*-value (0.948) and unadjusted OR was 0.916 (95% CI 0.448–1.875), indicates no significant association with the disease. The adjusted OR after controlling for confounding factors was 1.04 (95% CI 0.337–3.197). A* allele was found in 94.48% of the cases group and 94.37% in the control group. G* allele was found in 5.52% of the cases group and 5.63% in the control group. The *p*-value (0.817) indicates no significant association with the disease. The unadjusted OR was 0.921 (95% CI 0.532–1.736), suggesting no significant difference in the odds of the disease between the two alleles.

In THBS2 G > T; GG genotype was found in 80% of the cases group and 79.47% in the control group. No significant difference ($p = 0.894$) was found in the distribution of this genotype between the two groups. GT genotype was 17.93% in the cases group and 18.54% in the control group. The *p*-value (0.670) and unadjusted OR 0.961 (95% CI 0.532–1.706), indicates no significant association with the disease. The adjusted OR after controlling for confounding factors was 1.24 (95% CI 0.457–10.881). TT genotype was found in 2.07% of the cases group and 1.99% in the control group. The *p*-value (0.967) and unadjusted OR was 1.034 (95% CI 0.205–5.230) indicates no significant association with the disease. The adjusted OR after controlling for confounding factors was 0.84 (95% CI 0.065–10.881). G* allele was 88.97% in the cases group and 88.74% in the control group. T* allele was 11.03% in the cases group and 11.26% in the control group. The *p*-value (0.59) and the unadjusted OR was 0.868 (95% CI 0.519–1.451) suggest no significant association with the disease (Table 2).

Table 2 Genotypic and allelic frequencies of THBS1 and THBS2 polymorphisms in controls (n = 151) and CAD cases (n = 145)

Genotypes	Cases (n, %)	Controls (n, %)	Cases vs. Control Unadjusted OR (95% CI)	p value
THBS1 genotypes				
A	129 (88.97)	134 (88.74)	(Ref.)	
GA	16(11.03)	17 (11.26)	0.98(0.47–2.02)	0.95
A*	274 (94.48)	285 (94.37)	Ref	
G*	16(5.52)	17(5.63)	0.98(0.48–1.98)	0.95
THBS2 genotypes				
GG	116 (80)	120(79.47)	(Ref.)	
GT	26(17.93)	28(18.54)	0.96(0.53–1.74)	0.89
TT	3(2.07)	3 (1.99)	1.03 (0.20–5.23)	0.96
G*	258 (88.97)	268 (88.74)	(Ref.)	
T*	32 (11.03)	34(11.26)	0.98(0.59–1.63)	0.93

Discussion

In our comparative analysis of age-related parameters between Controls (n = 151) and CAD Cases (n = 145), our study revealed significant differences, indicating that CAD cases were, on average, older (55.10 ± 9.32 years) than the control group (49.21 ± 8.46 years) with a statistical significance of $P < 0.001$. This contrasts with findings from prior studies, including Boekholdt et al. (2002), where cases and controls had average ages of 40 ± 6 and 39 ± 7 years, respectively [12]. Koch et al. (2008) reported an older age in both cases (64.0 ± 12.0 years) and controls (60.3 ± 11.9 years), and Loon DEV et al. (2011) presented the highest mean age of participants at 68.7 ± 8.7 years, with 60.2% being female [13, 14]. These variations underscore the importance of considering demographic differences across studies, potentially influenced by factors like geographic location, study design, and inclusion criteria. The observed disparities highlight the diverse age distributions in populations studied for coronary artery disease, emphasizing the need for nuanced interpretation and exploration of contributing factors.

In our study, individuals with CAD exhibited significant disparities in lipid profiles. Serum cholesterol (TC), high-density lipoprotein (HDL), very-low-density lipoprotein (VLDL), and low-density lipoprotein (LDL) levels were notably higher in CAD cases. Specifically, serum cholesterol showed a *p*-value less than 0.001, HDL exhibited a *p*-value of 0.02, VLDL had a *p*-value less than 0.001, and LDL also had a *p*-value less than 0.001, all indicating statistical significance with CAD cases displaying elevated values. However, triglyceride levels and serum creatinine showed no significant difference ($P = 0.413$ and $P = 0.33$, respectively). On the other hand, Bansal SK et al. (2015) also reported significantly elevated total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and triglyceride (TG) levels in CAD cases compared to controls. Interestingly, the HDL-C levels were significantly lower in CAD cases in their study, contrary to our study's finding of higher HDL levels in CAD cases [15].

In our study focusing on THBS1, AA genotype showed no significant difference between cases (88.97%) and controls (88.74%) ($p = 0.811$). The GA genotype also exhibited no significant association with the disease (11.03% in cases vs. 11.26% in controls, unadjusted OR = 0.916, $p = 0.948$). A* and G* alleles also showed no significant differences between cases and controls (A*: 94.48% vs. 94.37%, G*: 5.52% vs. 5.63%, $p = 0.817$). The unadjusted OR for allele comparison was 0.921 (95% CI 0.532–1.736), suggesting no significant difference in disease odds. In Zhang XJ et al.'s meta-analysis, associations were observed between the THBS1 N700S polymorphism and CAD risk in the general population (heterozygote model: OR = 1.14, 95% CI 1.03–1.26; dominant model: OR = 1.13, 95% CI 1.00–1.29), European population (heterozygote model: OR = 1.13, 95% CI 1.00–1.27), and Asian population (heterozygote model: OR = 1.57, 95% CI 1.01–2.44; dominant model: OR = 1.56, 95% CI 1.00–2.43) [16]. The THBS2 3' untranslated region (UTR) polymorphism and THBS4 A387P polymorphism were not associated with overall CAD risk. However, an association was observed between the THBS4 A387P polymorphism and CAD risk in the American population (allele model: OR = 1.09, 95% CI 1.00–1.18; homozygote model: OR = 1.29, 95% CI 1.04–1.61; recessive model: OR = 1.27, 95% CI 1.02–1.58). Boekholdt SM et al. study examined THBS-1 genotypic distributions, revealing no significant associations between THBS-1 genotypes and the risk of premature CAD or MI, with genotypic frequencies for GG, GA, and AA across controls, CAD patients, and MI patients [12].

In our study, THBS2 G > T, revealed no significant difference in the distribution of GG genotype between cases (80%) and controls (79.47%) ($p = 0.894$). Similarly, the GT

genotype does not show a significant association with the disease (17.93% in cases vs. 18.54% in controls, unadjusted OR = 0.961, $p = 0.670$). Adjusting for confounding factors resulted in a non-significant adjusted OR of 1.24 (95% CI 0.457–10.881). The TT genotype also displayed no significant association (2.07% in cases vs. 1.99% in controls, unadjusted OR = 1.034, $p = 0.967$), with an adjusted OR of 0.84 (95% CI 0.065–10.881). Allele comparison for G* and T* showed no significant differences between cases and controls (G*: 88.97% vs. 88.74%, T*: 11.03% vs. 11.26%, $p = 0.59$). The unadjusted OR for allele comparison was 0.868 (95% CI 0.519–1.451), indicating no significant association with the disease. Contrastingly, Boekholdt SM et al. study on the THBS-2 gene revealed a different pattern. Among 1028 individuals, they observed a GG genotype prevalence of 6.7%, GT genotype prevalence of 35.7%, and TT genotype prevalence of 57.2%. Statistical analyses indicated a significant association between GG and TT genotypes (0.58, $p = 0.048$), suggesting distinct outcomes for individuals with these genotypes. The GT genotype did not show significant associations with the outcome compared to TT (0.89, $p = 0.33$). While the combined effect of GG and GT genotypes versus TT showed a trend towards association (0.84, $p = 0.15$), statistical significance was not achieved at the conventional threshold [12]. Additionally, study by Koch et al. investigated the THBS2 gene variant rs8089, showing a prevalence of the TT genotype in 56.7%, TG in 37.3%, and GG in 6.0%. Notably, the study reported statistically significant differences in allele frequencies between groups ($p = 0.054$ for TT genotype and $p = 0.027$ for T allele), suggesting a potential association between the rs8089 variant and the studied populations [13].

It is essential to note that the roles of *THBS-1* and THBS-2 in CAD are still under investigation, and the precise mechanisms through which they contribute to the disease process remain incompletely understood. Further research is required to fully elucidate their functions and explore potential therapeutic implications in the context of CAD.

Conclusion

In conclusion, the study suggests that age, serum cholesterol, HDL, VLDL, and LDL are associated with CAD in the Indian population. However, triglyceride and serum creatinine levels, as well as the studied THBS1 and THBS2 genetic polymorphisms, do not show significant associations with CAD in this specific group. Further research and larger sample sizes may be needed to validate these findings and explore other potential genetic factors related to CAD in this population.

Abbreviations

THBS1	Thrombospondin 1
BMI	Body mass index
CAD	Coronary artery disease
CI	Confidence interval
DBP	Diastolic blood pressure
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
THBS2	Thrombospondin 2
HDL	High-density lipoprotein
LDL	Low-density lipoprotein
OR	Odd ratio
P	*P*-Value
PCR	Polymerase chain reaction
RFLP	Restriction fragment length polymorphism
SBP	Systolic blood pressure
SNP	Single nucleotide triphosphate
SPSS	Statistical package for the social sciences
χ^2	Chi-square test

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Author contributions

STR has done overall supervision. AE has done overall search and compilation of data. MA has done statistical analysis. IAW and ZS helped in sample collection. SS has helped in the manuscript compiling.FM did final reviewing of the manuscript.

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None.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical approval and consent to participate

Protocol and procedures in the study complied with the declaration of Institutional Ethical/ Review Committee (Ref no. ELMC &H/R-Cell-/2019/24) of Era's Lucknow Medical College and Hospital, Lucknow.

Consent for publication

Written informed consent for publication was obtained from all the participants before sample collection.

Competing interests

The authors declare that they have no competing interests.

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