Original Article

Impact of Fibrinogen-β Chain Polymorphism (rs4220), Plasma Fibrinogen and Thrombin-Antithrombin Complex in Iraqi Patients with Coronary Artery Disease

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ABSTRACT

Objective: To measure the concentration of plasma thrombin-antithrombin complex and fibrinogen, and to analyze the single-nucleotide polymorphism of the fibrinogen B gene (rs4220) in Iraqi coronary artery disease patients and healthy controls

Study Design: Case-control study

Place and Duration of Study: This study was conducted at the Ibn Al-Nafees Hospital, Baghdad Governorate, Iraq, from 1st December 2025 to 28th February 2026.

Methodology: One hundred and twenty participants were enrolled, 40 patients with both diabetes mellitus and coronary artery disease (GI), 40 patients with coronary artery disease only (GII), and 40 apparently healthy controls (GIII). An enzyme-linked immunosorbent assay is used to quantify P. fibrinogen and thrombin-antithrombin complex concentrations. The deoxyribonucleic acid was isolated from blood, followed by polymerase chain reaction amplification using a thermocycler and a newly designed pair of primers targeting the fibrinogen B gene. The Sanger sequencing technique detected the rs4220 single-nucleotide polymorphism.

Results: The highly significant difference in plasma fibrinogen and thrombin-antithrombin complex concentrations compared with controls ($P< 0.01$). The genotypes ($P<0.01$) of the rs4220 single-nucleotide polymorphism in the fibrinogen B gene differed significantly between coronary artery disease patients with diabetes mellitus and controls; no significant difference was observed between the coronary artery disease patients without diabetes mellitus and controls ($P=0.061$). The allele frequencies ($P<0.01$) differed significantly between the G1 and GIII.

Conclusion: The rs4220 polymorphism in the fibrinogen B gene is associated with an increased risk of coronary artery disease and help to assess the genetic risk and inform effective prevention strategies in Iraq.

Key Words: Coronary artery disease, Plasma fibrinogen, Single nucleotide polymorphism, Thrombin-antithrombin complex.

doi:http://doi.org/10.60110/medforum.37.10.06

Citation of article: Salih ZS, Hassan AS, Humaish HH. Impact of Fibrinogen-β Chain Polymorphism (rs4220), Plasma Fibrinogen and Thrombin-Antithrombin Complex in Iraqi Patients with Coronary Artery Disease. Med Forum 2026;37(10):31-36. doi:http://doi.org/10.60110/medforum.37.10.06.

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INTRODUCTION

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Received: June, 2026
Reviewed: July-August, 2026
Accepted: September, 2026

The primary cause of infirmity and death in developed countries is coronary artery disease (CAD), and it is associated with approximately 17.8 million deaths yearly worldwide[1]. The pathogenesis of the various complications of CAD, including recurrent myocardial infarction (MI), is attributable to dysfunction of hemostatic function[2]. For preserving blood flow within the circulation, Hemostasis represents the homeostatic system, which seeks to balance between pro-coagulant and anti-coagulant forces[3].

The thrombin-antithrombin complex (TAT) indicates activation of the coagulation cascade and has been related to thrombotic conditions in both venous and arterial systems[4]. TAT complexes are more easily detected than thrombin. Elevated TAT levels have been associated with coronary slow flow and proposed as a diagnostic tool[5].

Plasma fibrinogen is a glycoprotein that is involved in coagulation. The most significant factor contributing to the pathophysiology of atherosclerosis[6]. Elevated levels have been widely linked to multiple CVD-related phenotypes, involving thrombosis, rising blood viscosity, atherosclerosis, and the subsequent development of CAD[7].

A 65-kilobase region on the human chromosome 4 (4q23-q32) contains three genes (FGB, FGG, and FGA) that encode plasma fibrinogen. The greatest focus has been on variations in fibrinogen structural genes,which are explicitly linked to the disruption of fibrinogen[8]. Variations of the fibrinogen β chain (FGB) gene, like rs4220, are associated with increased levels of fibrinogen[9].

The purpose was to measure the plasma levels of TAT Complex and fibrinogen in CAD patients, and to assess the rs4220 polymorphism in the FGB gene in CAD patients and controls to explore the possible effect of this polymorphism of the FGB geneon fibrinogen as early markers of ischemia and improve the management and treatment strategies in Iraqi CAD patients.

METHODOLOGY

The case-control study was conducted at Ibn Al-Nafees Hospital, Baghdad Governorate, Iraq from 1st December 2025 to 28th February 2026 vide letter No. 166 dated 11th July 2025 and included 80 patients with CAD (19 females and 61 males) aged 23 to 86 years and 40 apparently healthy controls with the same age range. Forty samples were collected for each group after ensuring that all the required characteristics within the research plan were 100% matched.

About 5 mL of blood was collected from each subject. The specimens were divided into two aliquots: the first tubes were EDTA tubes containing 2.5 ml of blood, used in the Polymorphism (rs4220) of FGB Gene study. The second tubes are sodium citrate tubes containing 2.5 ml of blood in sodium citrate tubes, and then centrifuged at 1,100 x g for 10 minutes to extract plasma that is used for the measurement study biomarkers by the sandwich ELISA technology based on the procedure protocol of Elabscience® (China) (Catalog No: E-EL-H6106, Catalog No: E-EL-H1788, respectively) by ELISA-Human Reader HS (Human, Germany).

Deoxyribonucleic acid was isolated from 200 µL of blood using the protocols provided by the TransGen DNA extraction kit (China). After extraction, the DNA was used in a PCR assay to amplify the FGB gene at a specific region using a newly designed set of primers from Macrogen, Korea. The forward primer was 5`ACGGTGGTGGATGGTGGTAT3` with 64.1 Tm C and 55% GC content, and the reverse primer was 5'CGTCTGCTTGAGAGTTTTAGAGGAA3` with 60.7 Tm C and 44% GC content. The PCR product was 303 bp. The reaction mixture volume was 27.5 µL, comprising 12.5 µL[2]×EasyTaq® PCR SuperMix, 10 µL of extracted DNA template, 0.5 µL of reverse primer, 0.5 µL of the forward primer, and 4 µL of nuclease-free water. DNA sample amplification was performed using a Thermal cycler device based on the PCR steps, including the initial denaturation step of 5 minutes at 94°C, for only one cycle. The denaturation, annealing, and extension steps take 30 seconds for the 35 cycles at (94°C, 63°C, and 72°C), respectively. The final step is called the final extension step of 8 min at (72°C) for one cycle. The PCR product was visualized using gel electrophoresis (Fig. 1). The PCR product delivered to Macrogen Company in South Korea for Sanger sequencing to detect any sequence variation in a specific region. Chromas Pro (Technelysium Pty. Ltd.) and Jal view are used to analyze and sequence alignments for SNP detection[10].

The data was entered and analyzed through SPSS-26. Chi-square is used to compare percentages. One-way analysis of variance (ANOVA) was used to assess differences among the groups under the study, and post hoc tests were then used for multiple comparisons. Cut-off values, specificity, and sensitivity were estimated by ROC analysis. The Hardy-Weinberg was calculated to examine genotypes and alleles in the gene used.

RESULTS

The mean ages were 56.15±8.145 years in GI, 56.17±14.04 years in GII, and 55.5±8.51 years in GIII, with no statistically significant difference among groups (P=0.877).

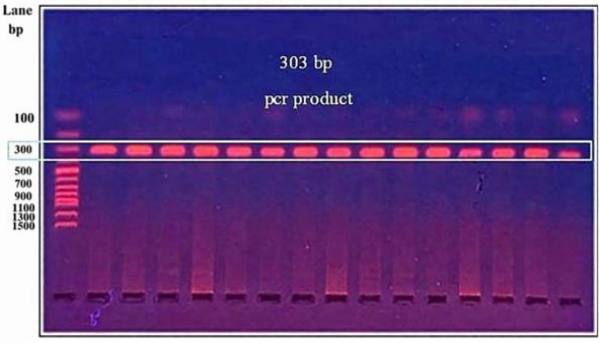


Figure No. 1: Amplification of the FGB gene using 1.5% agarose gel electrophoresis with Gel Stain, 100-1500 bp DNA ladder marks 1-15 resembling a 303 bp PCR product.

Likewise, no significant difference was noted in sex distribution of the patients and controls (χ^2 = 0.80, P=0.670). The mean TAT complex levels differed significantly across GI, GII, and GIII, with the highest values observed in GI, followed by GII and GIII (P<0.01). Similarly, plasma fibrinogen levels were significantly elevated in GI and GII compared to GIII (P<0.01) [Table 1].

Receiver operating characteristic (ROC) analysis was performed to evaluate plasma TAT complex and fibrinogen in GI and GII. In GI, the TAT complex

showed excellent diagnostic performance (AUC = 0.998) with 100% sensitivity and 97.5% specificity at a cut-off value of 359.18 pg/mL, while fibrinogen demonstrated an AUC of 0.966 with 95% sensitivity and specificity at 332.88 ng/mL. In GII, the TAT complex had an AUC of 0.907 with 92.5% sensitivity and 80% specificity at 310.868 pg/mL, whereas fibrinogen showed lower performance (AUC = 0.822), with 90% sensitivity and 75% specificity at 258.55 ng/mL. All results were statistically significant (P = 0.000) [Table 2, Fig. 2].

Table 3 showed the genotype frequencies of the rs4220 polymorphism in the FGB gene among the studied groups. In GI, the genotype distribution was GG (35%), GA (50%), and AA (15%), whereas in GIII it was GG (75%), GA (25%), and AA (0%). A statistically significant difference was observed between GI and GIII (χ^2 = 15.152, P < 0.01). The odds ratio (OR = 3.000) indicated that individuals with the heterozygous genotype (GA) in GI have a threefold higher risk of developing CAD compared to those with homozygous genotypes (GG and AA). All genotype distributions were consistent with Hardy–Weinberg equilibrium. In addition, allele frequencies showed a highly significant difference between GI and GIII (χ^2 = 15.626, P<0.01). For GII, the genotype frequencies were GG (55%), GA (45%), and AA (0%), compared to GIII [GG (75%), GA (25%), AA (0%)]. No statistically significant difference was found between GII and GIII (χ^2 = 3.516, P=0.061). However, the odds ratio (OR = 2.454) suggested that individuals with the GA genotype in GII have a higher risk of CAD compared to those with GG and AA genotypes. Similarly, no significant difference in allele frequency was observed between GII and GIII (χ^2 = 2.771, P=0.072). When comparing GI and GII, the genotype distributions were significantly different (χ^2 = 7.883, P<0.01), with GI showing GG (35%), GA (50%), and AA (15%), and GII showing GG (55%), GA (45%), and AA (0%). The elevated odds ratios (>1) indicated that both heterozygous (GA) and homozygous mutant (AA) genotypes in GI are associated with an increased risk of CAD. Furthermore, allele frequencies differed significantly between GI and GII (χ^2 = 5.072, P=0.013). In this study, GG was considered the wild-type homozygous genotype, GA the heterozygous genotype, and AA the mutant homozygous genotype.

Table No. 1: Mean levels of studied biomarkers among the study groups

Biomarker	GI	GII	GIII	P value
P. TAT (pg/mL)	533.04±31.45	390.34±68.87	284.68±48.7	<0.01(HS)
P. fib. (ng/mL)	397.15±59.36	287.61±31.15	229.23±48.7	<0.01(HS)

HS: Highly significant (P<0.01)

Table No. 2: Receiver operating characteristic curve of the biomarkers in the study groups

Group	Biomarker	Cut-off	AUC	P value	95% CI		Specificity	Sensitivity	PPV	NPV	Youden index
					LB	UB					
GI	P. TAT (pg/mL)	359.18	0.998	0.000**	0.951	1.000	97.5	100	0.97	1	0.925
	P. fib. (ng/mL)	332.88	0.966	0.000**	0.925	1.000	95	95	0.95	0.95	0.9
GII	P. TAT(pg/mL)	310.868	0.907	0.000**	0.822	0.961	80	92.5	0.82	0.91	0.725
	P. fib. (ng/mL)	258.55	0.822	0.000**	0.680	0.896	75	90	0.78	0.88	0.65

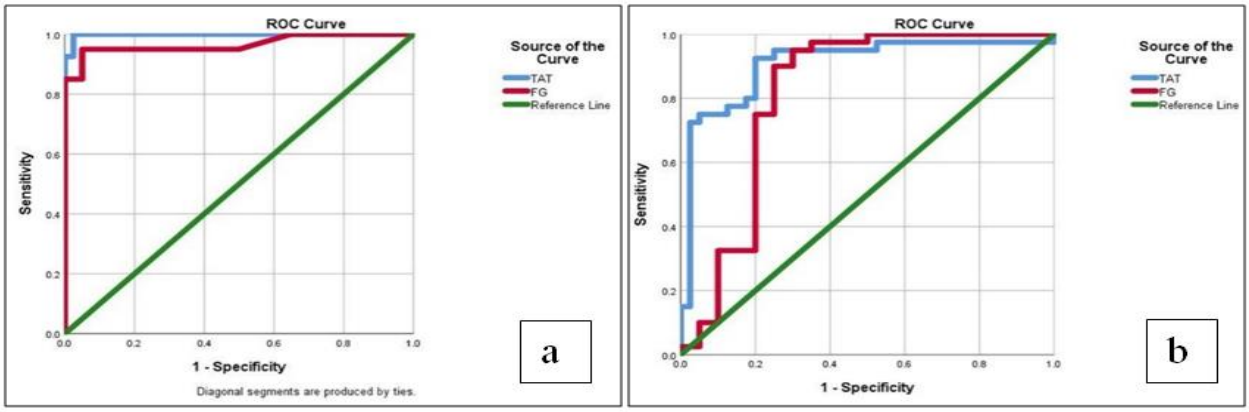


Figure No. 2: Receiver operating characteristic curve for Plasma levels of fibrinogen and TAT complex in the (a) GI group and (b) GII group

Table No. 3: Genotypes and Allele Frequency of rs4220 in the FGB gene in the study groups

Genotype	**GI (n= 40)**	**GIII (n= 40)**	**OR**	**C.I.**	**χ2**	**DF**	**P value**
GG (Homozygous)	14 (35%)	30 (75%)	0.179	0.068 to 0.471	15.152	2	<0.01** (HS)
GA (Heterozygous)	20 (50%)	10 (25%)	3.000	1.164 to 7.732			
AA (Mutant)	6 (15%)	-	15.26	0.829 to 280.73			
Allele	**Frequency**		**Total**				
G	48 (60%)	70 (87.5%)	118 (73.75%)		15.626	1	<0.01**(HS)
A	32 (40%)	10 (12.5%)	42 (26.25%)				
Genotype	**GII (n= 40)**	**GIII (n= 40)**	**OR**	**C.I.**	**χ2**	**DF**	**P value**
GG (Homozygous)	22 (55%)	30 (75%)	0.407	0.157 to 1.052	3.516	1	0.061 (NS)
GA (Heterozygous)	18 (45%)	10 (25%)	2.454	0.95 to 6.339			
AA (Mutant)	-	-	1.000	0.019 to 51.63			
Allele	**Frequency**		**Total**				
G	62(77.5)	70 (87.5)	132 (82.5)		2.771	1	0.072 (NS)
A	18(22.5)	10 (12.5)	18 (17.5)				
Genotype	**GI (n= 40)**	**GII (n= 40)**	**OR**	**C.I.**	**χ2**	**DF**	**P value**
GG (Homozygous)	14 (35%)	22 (55%)	0.440	0.179 to1.083	7.883	2	<0.01** (HS)
GA (Heterozygous)	20 (50%)	18 (45%)	1.222	0.5076 to 2.943			
AA (Mutant)	6 (15%)	-	21.48	1.159 to 398.41			
Allele	**Frequency**		**Total**				
G	48 (60%)	62 (77.5%)	110 (68.75%)		5.072	1	0.013*(S)
A	32 (40%)	18 (22.5%)	(31.25%)				

NS = Not significant *S = Significant **HS = Highly significant

DISCUSSION

In the present study, the mean age of patients was 56.15 years in GI and 56.17 years in GII. CAD is widely recognized as a manifestation of advanced atherosclerosis, predominantly affecting older individuals[11]. The severity and incidence of CAD increase as individuals grow older, with multi-vessel CAD being more common in people over 75 years[12].

This study identified highly significant differences between genders, with males more prevalent than females in the CAD groups. Higher oscillatory conditions in males have been reported in previous studies and are considered a predictor of neointimal thickening, an early stage of atherosclerosis development. This finding suggests that males may be hemodynamically more prone to obstructive vascular lesions than females[13].

Our study results are in line with the results by Su et al[14], which showed an increase in P. fibrinogen levels in CAD patients with or without DM. Fibrinogen encourages atherogenesis by causing low-density lipoprotein (LDL) cholesterol to accumulate in arterial walls[15]. Plasma fibrinogen is known to play a major role in inflammatory response, smooth muscle cell migration, and endothelial dysfunction. Therefore, the relationship of P. fibrinogen levels with CV events and atherosclerosis has long been relevant[16].

Our results are compatible with Valente-Acosta et al[17],

Our results are compatible with Valente-Acosta et al[17], CAD patients have elevated TAT complex levels. TAT complex, a verified indicator of thrombin production, is created when it is rendered inactive by irreversibly attaching to antithrombin. These multifaceted actions underscore its critical role in vascular pathophysiology.[18] Borissoff et al[19] also showed initial stage of atherosclerotic damage presented both significantly higher TAT complex levels and enhanced thrombin production capabilities. Clinical research indicates that increased levels of P. TAT complex are strongly related to the existence and degree of CAD.[20].

In CAD patients who have diabetes, our findings of the rs4220 polymorphisms are consistent with Koch et al[21]. However, our study in CAD patients without DM is in line with the studies by Mallik and Majumder[22]. Standeven et al[23] reported that platelet-rich arterial thrombi, which can induce myocardial infarction, have a heterogeneous fibrin structure affected by fibrinogen levels and FGB gene polymorphisms, the BβArg448Lys fibrinogen variant, along with diabetes, alters fibrin network structure, increasing CV risk. Carriers of the Bβ448Lys variant were younger than Bβ448Arg carriers, indicating a possible association with early mortality in this population[24]. By altering the charge of the molecule, Arg448Lys in FGB may impairthe C-terminal of the B chain in D-domains of the fibrinogen and slightly worsen the condition. It's currently unknown how the Arg448Lys polymorphism affects fibrinogen. According to some earlier research, it is related to P. fibrinogen level and the risk of atherothrombotic disease[25].

CONCLUSION

The individuals with the rs4220 polymorphism in the FGB gene can develop heart diseases with coagulation

defects compared to individuals without this defect; these defects are associated with elevated P. fibrinogen levels, especially in diabetic patients. To date, the variation in this SNP (rs4220) and the level of the thrombin-anti-thrombin complex have not been examined in the Iraqi population.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Zainab Saad Salih, Ahmed Saadi Hassan
Drafting or Revising Critically:	Zainab Saad Salih, Haider Hafudh Humaish
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

Conflict of Interest: The study has no conflict of interest to declare by any author.

Source of Funding: None

Ethical Approval: No.166 Dated 11.07.2025

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