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Vascular Endothelial Growth Factor gene polymorphism (rs2010963) in ST- Segment Elevation Myocardial Infarction: An Egyptian Pilot Study

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Abstract

ST- segment elevation myocardial infarction (STEMI) is the most severe form of coronary artery disease. Vascular endothelial growth factor (VEGF-A) is critical in post- Myocardial Infarction (MI) angiogenesis. VEGF-A (rs2010963) gene polymorphism hasn't been explored in Egyptian ethnicity. This study aimed to explore the role of the VEGF-A gene (rs2010963) polymorphism in STEMI and its outcome. It was carried out on 50 STEMI patients and 50 controls who gave blood samples for VEGF-A level estimation by ELISA and VEGF-A gene(rs2010963) polymorphism by real-time PCR. We revealed that VEGF-A level was higher in STEMI cases vs. control at baseline and in STEMI cases at 2-weeks vs. baseline. At a cut off value > 20 pg/ml was a statistically significant discriminator of STEMI vs. control [AUC (95% CI) = 0.785 (0.692-0.861), sensitivity 82%, specificity 72%]. Participants with C/G-G/G genotypes of VEGF-A (rs2010963) had 2.8-times higher odds vs. those with C/C genotype to exhibit STEMI. C/G genotype was associated with highest VEGF-A level. STEMI participants with C/G genotype had 4.3-times higher odds vs. those with C/C-G/G genotype to exhibit poor outcome. In conclusions, VEGF-A level can discriminate STEMI cases from control. VEGF-A (rs2010963) gene polymorphism affects VEGF-A level, associated with risk of developing STEMI and affects clinical outcome.

Keywords: *STEMI, VEGF-A, gene polymorphism, ELISA*

1. Introduction

Acute myocardial infarction (AMI) is a leading cause of death associated with coronary artery disease (CAD) globally (1). AMI is caused by the rupture or erosion of a susceptible atherosclerotic plaque, which results in coronary artery blockage and progressive cell death (2). CAD accounts for 7.4 million deaths annually (3), with low- and middle-income countries contributing to more than 80% of mortality (4).

Smoking, hypertension, diabetes, dyslipidemia, stress, obesity, alcohol use, physical inactivity, and a diet poor in fruits and vegetables are all risk factors for the development of AMI (5). AMI is classified into two types based on electrocardiogram (ECG) results: ST segment elevation MI (STEMI) and non-ST segment elevation MI (NSTEMI) (6). STEMI is produced by transmural ischemia, or ischemia that affects the whole thickness of the myocardium, but NSTEMI does not (7).

STEMI is the most severe type of CAD, with high morbidity and death. STEMI must be diagnosed as quickly as possible, ideally within 10 minutes after the patient's first medical contact (8).

Angiogenesis contributes to the post-MI healing process by re-establishing microvascular circulation and supplying nutrient- and oxygen-rich blood to the cardiac tissue (9). Members of the VEGF family, notably VEGF-A, play an important role in post-MI angiogenesis. VEGF enhances endothelial cell (EC) survival, proliferation, and migration, as well as the formation of collaterals. Which improves heart function by increasing myocardial perfusion and decreasing infarct size (10).

Endogenous VEGF-A levels are significantly higher in STEMI patients. Pharmacologically modifying VEGF-A expression and activity would be a possible therapeutic approach to hasten angiogenesis after AMI, emphasizing its function post-AMI (11).

VEGF is located on chromosome 6p21.3 (12). VEGF possesses functional single nucleotide polymorphisms (SNPs) in its promoter region as well as 5′- and 3′-untranslated regions (UTRs) that may impact VEGF production (13). One of these functional VEGF gene polymorphisms is the -634 C/G polymorphism (rs2010963), which has been shown to affect blood VEGF levels (14).

VEGF-A gene polymorphism (rs2010963) has been studied in CAD patients of many populations (15), but up to our knowledge, it has not been explored in Egyptian ethnicity. In our study we aimed to assess the level of VEGF-A in acute STEMI and explore the relationship between the VEGF-A gene (rs2010963) polymorphism and the risk of STEMI and whether it has effect on clinical outcome within six months.

2. Materials and Methods:

This was a case control pilot study conducted on Egyptian population including 50 AMI patients, admitted to Intensive Care unit of Specialized Internal Medicine Hospital, Mansoura University who were diagnosed for STEMI by the clinical presentation: chest pain, dyspnoea and pallor, the 12-lead ECG showing elevation of ST-segment with elevated troponin and creatine kinase. Patients with acute STEMI confirmed by ECG, age > 18 years old with a lack of contraindications to PCI were included, while patients with known malignancies, pregnancy, contraindications to percutaneous coronary intervention (PCI) or established chronic heart failure were excluded. Control group 50 apparently healthy subjects who were nonsmokers, not diabetic, not hypertensive and were not obese. STEMI confirmed cases underwent primary PCI with successful stenting within 6-12 hours. The Gensini score (16) was calculated. Transthoracic echocardiography was performed at discharge. We assessed and recorded the left ventricular (LV) ejection fraction (LVEF), end-diastolic diameter (LVEDD), and end-systolic diameter (LVESD). The poor outcome, which we defined as the incidence of death or readmission with MI within six months, was established by patients' follow-up clinically for 6 months following PCI.

Blood sampling

5ml fasting venous blood samples were withdrawn from each control subject and from each patient immediately before PCI; 3 ml collected on plain tube; serum was separated after clotting and used for the biochemical investigations and for assessing VEGF-A level, and 2 ml blood were collected on EDTA containing tube stored at – 80°C until DNA extraction. Then 14 days after PCI another 3 ml venous blood samples were withdrawn from each patient and collected on plain tube; serum was separated after clotting for assessing VEGF-A level.

Biochemical investigations

Lipid profile, serum creatinine, creating kinase (CK) and creatine kinase-isoenzyme-MB (CK-MB) were done on COBAS C311 by Roche Diagnostics with SN 15807- 02, Chemiluminescent immunoassay was used to assess the amounts of troponin I (Tn I) on the Architect i1000SR, VEGF-A level was measured before PCI (basal) and after 14 days using Human VEGF-A ELISA Kit catalog No: E-TSEL-H0026.

VEGF-A gene determination of (rs2010963) polymorphism by real time PCR

Thermo Scientific's Gene JET PCR purification kit was used to extract DNA from peripheral blood leukocytes catalog Number K0701. Real-time polymerase chain reaction (PCR) DT-prime serial number A5D312 was used to genotype the G634C VEGF-A gene polymorphism positioned in the promoter region. The SNP ID is C_8311614_10 for VEGF-A RS2010963 with context sequence {CGCGCGGGCGTGCGAGCAGCGAAAG[C/G]GACAGGGGCAAAGTGAGTGACCTGC} from Thermo Fisher Scientific and the chromosomal location is Chr.6:43770613. TaqMan™ Genotyping Master Mix catalog number 4371353 from Thermo Fisher Scientific was used. Into a sterile microcentrifuge tube the reagents were pipetted according the following protocol (for each well): 10.0 µL(2X) of Universal PCR Master Mix, 0.5 µ(20X) SNP genotyping Assay,7.5 µL de ionized water, 2.0 µL DNA template. The following cycling stages were applied to develop reaction conditions: initial denaturation at 95°C for 10 minutes then 40 cycles of denaturation at 95 °C for 15 seconds, thereafter annealing/extension at 60 °C for 60 seconds, followed by final extension at 60°C for 30 seconds. Fluorescence intensity was measured at the endpoint in order to perform allelic discrimination. SDS software version 1.7 (Applied Biosystems, Foster, USA) was used to evaluate the measurement findings

and identify the genotype. This work was carried out at Clinical Pathology Department laboratories and molecular laboratory of oncology centre in Mansoura faculty of medicine.

Statistical analysis of data:

IBM-SPSS software (IBM Corp., 2019) was used to enter and evaluate data. IBM SPSS Statistics for Windows, Version 26.0 (IBM Corp, Armonk, NY). Statistical study of SNPs Data was input and analysed using the SNP Stats software: https://www.snpstats.net/start.htm, which included allele and genotype frequencies.

3. Results and Discussion:

STEMI is the most severe form of CAD, with significant morbidity and death as a result of transmural ischaemia (17).

VEGF family members, especially VEGF-A, play an important role in post-MI angiogenesis. VEGF promotes endothelial cell survival, proliferation, and migration through attaching to its receptor, VEGF receptor 2 (VEGFR2). VEGF's angiogenic capabilities during vascular development or tumour angiogenesis have quickly led to an assessment of its function in post-MI angiogenesis. In the ischemic heart, VEGF is promptly induced (18).

Multiple common SNPs in the VEGF gene have been linked to VEGF level. Different SNPS in the VEGF gene may alter influence the angiogenesis process, VEGF protein concentrations, and may be linked to inter-individual susceptibility to various disorders and cardiac ones on top (19).

So, we aimed to assess the VEGF-A serum level in patients with acute STEMI, explore the relationship between the VEGF-A gene (rs2010963) polymorphism and the risk of STEMI and whether it has effect on clinical outcome.

A total of 50 confirmed STEMI cases and 50 controls were enrolled in our study, with their mean age 56.2 ± 10.7 and 52.5 ± 9.5y respectively. Males represent the greater proportion about 75% of the whole number of participants. There was no significant difference between cases and control regarding sex and age (P value 0.488 and 0.068 respectively. Regarding the characteristics of the STEMI cases 58% current smokers, 52 % hypertensive, 30% diabetic, 18% obese, 12 % with history of cardiovascular disease, 40% inferior STEMI, 60% anterior STEMI and 11% faced poor outcome (poor outcome = death or readmission with MI within six months). Data not shown

On comparing laboratory tests in STEMI cases vs. control, a statistically significantly higher serum creatinine, CK-Total, and CK-MB and a statistically significantly lower HDL-C in STEMI cases vs. control were detected (table 1).

Our finding went in hand with some researchers like Mehran et al.(20) who additionally determined that CK-MB elevation corresponds with calcification and coronary artery plaque load ,both of which might be the main factors for myocardial ischemic lesions.

Rao et al. (21) also noted that Serum TC, TG, LDL, VLDL levels were significantly higher in the STEMI group compared to age-matched controls, and the levels of these lipid profile parameters in STEMI patients and severity of the disease was assessed by the number of vessels involved, and only LDL levels were found to be significantly correlating with severity of the disease.

And our study also revealed a statistically significantly higher VEGF-A level in STEMI cases vs. control at baseline, and a statistically significantly higher VEGF-A level in STEMI cases at 2-weeks vs. baseline (table 1). While VEGF-A at a cut off value > 20 pg/ml is a statistically significant discriminator of STEMI cases vs. control with a good diagnostic accuracy [AUC (95% CI) = 0.785 (0.692-0.861), sensitivity 82%, specificity 72%, SE = 0.046] (figure 1).

Table (1): Comparisons of laboratory tests in STEMI cases vs. control

Characteristic	STEMI cases	Control	HLE	P1-value
Serum creatinine (mg/dl)	1.0 (0.9 – 1.2)	0.9 (0.8 – 1.1)	-0.100	0.020
CK-Total (IU/L)	976.5 (513.7 – 1780.7)	57.5 (40.2 – 85.5)	-915.0	<0.001
CK-MB (IU/L)	104.5 (59.7 – 194.2)	7.4 (5 – 9)	-98.50	<0.001
Total cholesterol (mg/dl)	205 (182.2 – 250.5)	204 (184 – 237)	-4.500	0.667
Triglycerides (mg/dl)	147.5 (115.2 – 198)	129 (96.5 – 194.2)	-16.50	0.155
LDL-C (mg/dl)	153 (125.2 – 190.2)	134.7 (117.3 – 159.6)	-16.90	0.052
HDL-C (mg/dl)	26.5 (19.7 – 33)	40 (35 – 45)	13.00	<0.001

VEGF-A level (pg/ml)	Baseline	65 (30 – 160)	20 (10 – 42.5)	Z 4.985	P2 <0.001
	After 2 weeks	220 (80 – 250)			
		Z -4.884 P3 <0.001			

Notes: Data is median (Q1-Q3). The test of significance is Mann-Whitney U-test (P1). HLE = Hodges-Lehman Median Difference.

Test of significance is Mann-Whitney U-test (P2) and Wilcoxon's signed ranks test (P3).

ErZen et al. (22) reported that VEGF levels were significantly elevated in post-MI patients compared to controls. The effect of VEGF-A on cellular and physiological characteristics of the myocardium, followed by adaptive or dysadaptive remodelling, oxygen transport, increasing blood vessel development, perfusion of the myocardium, cardiac circulation, and the entry of energy substrates into cardiac cells, may explain VEGF-A level elevation. Moreover, the presence of VEGF-A and its receptors in cardiac fibroblasts and non-endothelial cells having fibroblast-like characteristics that perform tissue regeneration and development suggested that mediators had a significant role in the process of myocardial remodelling in ischemia.

Additionally, Hueso et al. (11) reported that STEMI patients had considerably greater circulating VEGF-A levels than controls. Also, Palmer et al. (23) showed that compared to levels seen in healthy controls, the mean levels of VEGF-A they assessed in their sample were very comparable or slightly increased.

Likewise, Hojo et al. (24) reported serum VEGF levels in STEMI patients reached a peak on day 14 and were considerably higher than in control participants. Also, Harada et al.(25) demonstrated that overall VEGF-A levels rose following PCI and peaked on day 7.

Up to our knowledge no one before had used VEGF-A cut off value for diagnosis, however it has been used for prediction only of poor outcome.

However, Petyunina et al. (26) emerged with a result that a cut-off VEGF-A level of 172.4 pg/ml on the 7th day (area under curve (AUC) 0.697, with specificity 50.9% and sensitivity 88.9%; 95% C 0.567-0.807, P = 0.0515) became a predictor for further infarctions within six months. And they also added a questionnaire for anxiety that appeared to be one of the factors that decreased the VEGF-A level affecting the neovascularization leading to angina development post infarction.

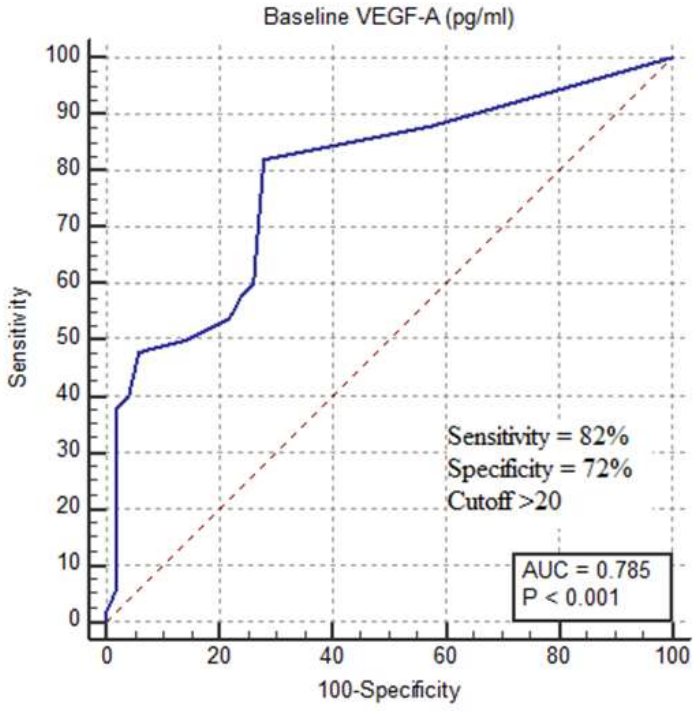

Figure (1): ROC curve for VEGF-A (pg/ml) in discriminating cases vs. control

Applying Hardy Weinberg Equilibrium revealed that VEGF-A gene (rs2010963) genotyping in both patients and controls were independent (i.e., they were in HWE equilibrium), p>0.05 (table 2)

In terms of VEGF-A allele frequencies (rs2010963) among STEMI patients, the observed frequencies of variations of G634C VEGF-A (rs2010963) revealed that CG was the most frequent genotype, while in control participants CC was the most frequent genotype (table 3). In line with this Kutia et al. (27) showed that the observed frequencies of variations of the G634C VEGF-A (rs2010963) CG and GG were the most frequent genotype 'G' allele is higher in STEMI cases. Lin et al. (28) interpreted that when the VEGF-A (rs2010963) 'C' allele was compared to the 'G' allele, there were excellent coronary collaterals.

Regarding VEGF-A (rs2010963) genotyping this study showed that 'C' allele is higher in control (70/129=54.3%) vs. STEMI (59/129= 45.7%), while 'G' allele is higher in STEMI cases (41/70= 58.5%) vs. control (30/70= 42.5%). However, this difference did not achieve statistical significance, and that the dominant model is the best inheritance model (with the lowest p-value, AIC, and BIC). Participants with C/G-G/G genotypes have 2.8-times higher odds (adjusted for age and sex) vs. those with C/C genotype to exhibit STEMI (table 3)

Table (2): Hardy-Weinberg Equilibrium (HWE) Test

Group	$\chi 2$	Pearson's p	Fisher's p
Case	1.976	0.372	0.668
Control	0.113	0.944	1.000
All participants	0.489	0.782	0.899

Notes: The test of significance is Pearson's chi-square and Fisher's exact tests.

Table (3): Allele frequencies of VEGF-A (rs2010963) in cases vs. control subjects, SNP association with STEMI (n=100, adjusted for age and sex)

Allele	STEMI cases	Control	All	Chi-square ($\chi 2$) test			Logistic regression		
	N=50	N=50	N=100	$\chi 2$	ϕ	p-value	COR	95% CI	p-value
C	59 (59%)	70 (70%)	129 (64.5%)	2.642	0.115	0.104	r(1) 1.62	r(1) 0.90-2.91	0.105
G	41 (41%)	30 (30%)	71 (35.5%)						

Model	Genotype	Group		AOR	96% CI	p-value	AIC	BIC
		Control	STEMI cases					
Co-dominant	C/C C/G G/G	25 (50%) 20 (40%) 5 (10%)	15 (30%) 29 (58%) 6 (12)	r(1) 2.97 2.03	r(1) 1.19 – 7.44 0.48 – 8.54	0.058	136.2	149.3
Dominant	C/C C/G-G/G	25 (50%) 25 (50%)	15 (30%) 35 (70%)	r(1) 2.77	r(1) 1.15 – 6.67	0.020	134.5	144.9
Recessive	C/C-C/G G/G	45 (90%) 10 (10%)	44 (88%) 6 (12%)	r(1) 1.11	r(1) 0.30 – 4.14	0.880	139.9	150.3
Over-dominant	C/C-G/G C/G	30 (60%) 20 (40%)	21 (42%) 29 (58%)	r(1) 2.54	r(1) 1.08 – 5.97	0.030	135.2	145.6
Log-additive	-	-	-	1.83	0.94 – 3.55	0.068	136.6	147.0

Notes: Data is N (%). Phi (ϕ) is a measure of the strength of association. COR = crude odds ratio. CI = confidence interval. AOR = adjusted odds ratio. CI = confidence interval. AIC = Akaiki information criterion. BIC = Bayesian information criterion.

On applying dominant model, there was no statistically significant difference between C/C vs. C/G-G/G genotypes except for STEMI which was statistically significantly higher in C/G-G/G genotypes vs. C/C genotype (table 4)

In the present study, the VEGF-A baseline level was compared between the three genotypes and the highest level was associated with C/G genotype then C/C, and the lowest with G/G (p-value= 0.048). Although VEGF-A rs2010963 G > C polymorphism is a functional polymorphism linked to an increase in VEGF-A level and is known to impact VEGF transcription and expression. However, the reason for this could be that, in a highly polymorphic gene like VEGF, it is more likely that the influence of multiple SNPs, or haplotypes, is responsible for VEGF production rather than a single SNP (figure 2)

Table (4): Comparisons of C/C genotype vs. C/G-G/G genotypes

Characteristic	Genotype C/C N=40	Genotype C/G-G/G N=60	Test of significance	
	Categorical		$\chi 2$	p-value
STEMI	15 (37.5%)	35 (58.3%)	4.167	0.041
			z-value	p-value
Serum creatinine (mg/dl)	0.9 (0.9 – 1.1)	1 (0.9 – 1.2)	-1.290	0.197
CK-Total (IU/L)	117.5 (55 – 513)	445 (61.3 – 1272.2)	-1.414	0.157
CK-MB (IU/L)	10 (6.13 – 52.5)	59 (7.5 – 129.7)	-1.721	0.085
Total cholesterol (mg/dl)	218 (187 – 254)	204 (180 – 245.7)	-1.066	0.286
Triglycerides (mg/dl)	158 (110 – 199.5)	132 (100 – 197)	-1.094	0.274
LDL-C (mg/dl)	146.5 (126 – 176.6)	134.7 (119 – 184)	-0.486	0.627
HDL-C (mg/dl)	35 (27.2 – 44)	32 (25 – 40)	-1.090	0.276
Baseline VEGF-A (pg/ml)	20 (10 – 76.5)	30 (20 – 80)	-1.873	0.061

Notes: Data is N (%) for categorical variables (test of significance is chi-square test), mean ± SD for age (test of significance is independent-samples t-test), and median (Q1-Q3) for laboratory tests (test of significance is Mann-Whitney U-test).

Patients were followed up for six months for detecting poor outcome. As for SNP association with poor STEMI outcome, over-dominant model was the best inheritance model (with the lowest p-value, AIC, and BIC). STEMI participants with C/G genotype had 4.3-times higher odds vs. those with C/C-G/G genotype to exhibit poor outcome (table 5)

LCX-RCA culprit vessel, Age > 59 years, serum creatinine > 1.3 mg/dl, total CK > 1150 IU/L, CK-MB > 175 IU/L, and multiple stents were statistically significant predictor variables on the likelihood of poor outcome within 6-months of PCI (table 6)

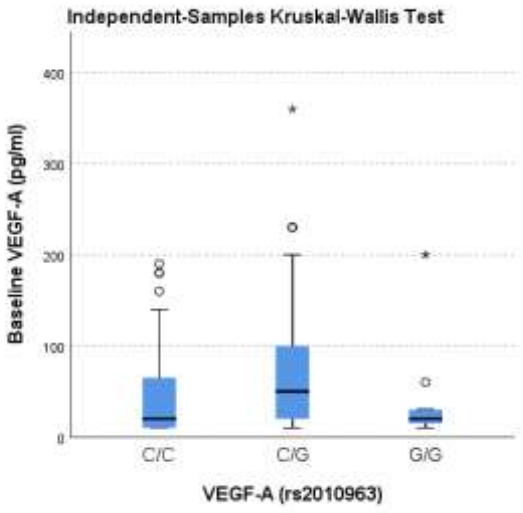

figure (2) Boxplot shows VEGF-A baseline (pg/ml) level in 3 genotypes C/C, C/G and G/G

Table (5): SNP association with poor STEMI outcome (n=50)

Model	Genotype	STEMI outcome		COR	96% CI	p-value	AIC	BIC
		Not poor	**Poor**					
Co-dominant	C/C	14 (35.9%)	1 (9.1%)	r(1)	r(1)	0.13	54.7	60.4
	C/G	20 (51.3%)	9 (81.8%)	6.3	0.72 – 55.51			
	G/G	5 (12.8%)	1 (9.1)	2.8	0.15 – 53.71			
Dominant	C/C	14 (35.9%)	1 (9.1%)	r(1)	r(1)	0.063	53.2	57.1
	C/G-G/G	25 (64.1%)	10 (90.9%)	5.6	0.65 – 48.43			
Recessive	C/C-C/G	34(87.2%)	10 (90.9%)	r(1)	r(1)	0.73	56.6	60.4
	G/G	5 (12.8%)	1 (9.1%)	0.68	0.07 – 6.52			
Over-dominant	C/C-G/G	19 (48.7%)	2 (18.2%)	r(1)	r(1)	**0.059**	53.1	57
	C/G	20 (51.3%)	9 (81.8%)	4.3	0.82 – 22.39			
Log-additive	-	-	-	1.82	0.61 – 5.48	0.28	55.5	59.3

Notes: COR = Crude odds ratio. CI = confidence interval. AIC = Akaiki information criterion. BIC = Bayesian information criterion.

Multivariable binary logistic regression analysis was run to ascertain the effects of 4 predictor variables (three in each of the two models) on the likelihood of occurrence of poor outcome within 6-months; in the first model, of the 3 predictor variables, older age was the only statistically significant independent predictor of the likelihood of occurrence of poor outcome within 6-months, the model correctly classified 86% of cases at 72.7% sensitivity and 89.7% specificity. The model is statistically significant at $\chi 2$ (3) of 14.910 and p-value 0.001. Participants with age > 59 years have 14.1-times higher odds to exhibit poor outcome within 6-months (table 7)

While in the second model, of the 3 predictor variables, older age and CK-MB > 175 IU/L but not VEGF-A SNP were statistically significant independent predictors of the likelihood of occurrence of poor outcome within 6-months, the model correctly classified 88% of cases at 45.5% sensitivity and 100% specificity. The model is statistically significant at $\chi 2$ (3) of 22.019 and p-value <0.001. Participants with age > 59 years and CK-MB > 175 IU/L have 28.8- and 15.1-times higher odds to exhibit poor outcome within 6-months (table 7)

In a similar context, Fach et al. (29) indicated that death rates in elderly patients with STEMI were significantly higher, as was the risk of strokes during follow-up, and that the one-year major accidental cardiac and cerebrovascular events (MACCEs) rate was over five times higher in older patients.

In our study, Sex didn't achieve statistical significance being a risk factor for poor outcome within six months. However, Cenko et al. (30) looked at the sex-age interaction as a risk factor for mortality, they discovered that women under the age of 60 had a greater probability of dying young than males in the same age group. Differences in 30-day mortality between men and women were not significant for individuals aged 60 to 74 years or for those aged 75 and up. However, they concluded that all-cause mortality rose with age.

In the study conducted by Kutia et al. (27), predictors for the 6-month clinical outcome were determined abdominal obesity, anterior location of STEMI, atrial fibrillation, Peak TnI at admission, TIMI score, GC/CC variations of the VEGF-A gene, and dynamic changes in VEGF-A levels in serial measures were all found to be predictors of the whole clinical outcome in the univariate linear regression analysis.

Additionally, GC/CC polymorphisms in the VEGF-A gene, Peak TnI at admission for 6 months, and VEGF-A levels remained the merged independent endpoint predictors in the unadjusted multivariate regressive logistic analysis. After 6 months of adjusting for dynamic variations in VEGF-A levels, GC/CC polymorphism gene was a reliable predictor of poor clinical outcomes Kutia et al. (27).

Table (6): Predictors of the likelihood of occurrence of poor outcome (univariate analysis):

Predictor	Univariate analysis		
	COR	95% CI	p-value
Culprit vessel			
LAD	r(1)	r(1)	0.027
LCX-RCA	5.333	1.2-23.5	
VEGF-A (rs2010963)			
C/G	r(1)	r(1)	0.086
C/C-G/G	4.3	0.82-22.4	
Obesity			
No	r(1)	r(1)	0.085
Yes	3.88	0.82-18.23	
History of CVD			

No	r(1)	r(1)	0.097
Yes	4.5	0.76-26.5	
STEMI localization			
Anterior	r(1)	r(1)	0.079
Inferior	3.5	0.86-14.1	
Age			
<59	r(1)	r(1)	0.003
>59	13.05	2.4-70.8	
Serum creatinine (mg/dl)			
<1.2	r(1)	r(1)	0.007
>1.3	10	1.8-53.2	
CK-Total (IU/L)			
<1150	r(1)	r(1)	0.027
>1150	5.33	1.2-23.5	
LVEDD			
<5	r(1)	r(1)	0.077
>5	6.96	0.8-59.8	
CK-MB (IU/L)			
<175	r(1)	r(1)	0.021
>175	5.48	1.2-23.1	
Multiple stents			
0-1	r(1)	r(1)	0.025
>1	5.667	1.2-25.7	

Notes: COR = crude odds ratio. CI = confidence interval. r(1) = reference category. The test of significance is binary logistic regression.

Table (7): Predictors of the likelihood of occurrence of poor outcome (multivariate analysis):

Predictor	Multivariate analysis					
	Model 1			Model 2		
	AOR	95% CI	p-value	AOR	95% CI	p-value
Age						
≤59	r(1)	r(1)	0.006	r(1)	r(1)	0.005
>59	13.5	2.1-86		28.8	2.7-305.5	
VEGF-A (rs2010963)						
C/G	r(1)	r(1)	0.110	r(1)	r(1)	0.123
C/C-G/G	4.8	0.7-34		4.9	0.65-37.3	
Culprit vessel				-	-	-
LAD	r(1)	r(1)	0.067			
LCX-RCA	5.2	0.9-30				
CK-MB (IU/L)						
≤175	-	-	-	r(1)	r(1)	
>175				15.1	1.4-156.9	0.023

Notes: COR = crude odds ratio. CI = confidence interval. r(1) = reference category. The test of significance is binary logistic

4. Conclusions

Variations in plasma concentrations of VEGF-A from baseline in STEMI may give idea about disease progression and outcome. VEGF-A (rs2010963) gene polymorphism is a functional SNP that affect VEGF-A expression and function, so can be used as a heriditery indicator linked to the outcome and prognosis.

limitations:

Small sample size, VEGF-A level was measured only at baseline and on 14th days of PCI, and VEGF-A (rs2010963) was the only assessed gene polymorphism

Recommendations:

Repeated measuring of VEGF-A levels and comparing its levels may be of value in predicting angiogenesis and reperfusion in STEMI cases, detection of 'G' allele and VEGF-A (rs2010963) genotypes is recommended as prognostic and screening genetic biomarker in patients and their relatives respectively. Further large population – based wide scale studies are required to understand the genetic contribution of VEGF-A (rs2010963) as a risk factor for developing STEMI,

Declarations

Participant consent and ethics approval

To participate in this study, all participants provided informed consent, and the research was authorized by the institutional review board (IRB) under the number MD.21.05.447. Date 30/6/2021

Potential conflict of interest

The authors assert that were no competing objectives.

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