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Lectin-Like OLR1 3′UTR Rs1050286 Gene Polymorphism and Plasma Oxidized-LDL in Coronary Artery Disease and Their Relation to Cardiovascular Risk and Outcomes

Hanan Sharaf El-Deen Mohammed[1], Manal Mohamed Kamal[2], Hala Mostafa ElBadre[3], Amal Hosni[4], Azza Abo Elfadl[4], Mohamed Ahmed Mostafa[5], Reham Ibrahim El-Mahdy*[3]

Abstract

Background: Oxidized low-density lipoprotein (ox-LDL) has an important role in the genesis of coronary atherosclerosis. Lectin-like ox-LDL receptor 1 (OLR1) contributes to the uptake and internalization of ox-LDL. Genetic polymorphisms have been associated with coronary artery disease (CAD). Here we explore the association of plasma levels of ox-LDL and 3′ UTR OLR1 (rs1050286) SNP with CAD risk and in-hospital adverse outcomes.

Methods: A case-control study enrolled 192 patients with ST-segment elevation myocardial infarction (STEMI), 100 patients with unstable angina, and 100 healthy controls. Baseline, clinical characteristics, and risk scores of the patients were determined. Plasma ox-LDL and other biochemical variables were measured. All subjects are genotyped for OLR1 (rs1050286) by RT-PCR with TaqMan SNP genotyping assay.

Results: Plasma ox-LDL was higher with enhanced sensitivity and specificity in identifying patients with STEMI and was found as a significant independent risk factor for CAD in those two groups. Levels of ox-LDL were increased with increasing poor prognostic factors in STEMI patients that are associated with an increased incidence of some adverse events and in-hospital mortality. Elevated STEMI risk was associated with T allele of OLR1 (rs1050286) (odds ratio of 4.9, 95% CI: 2.6-9.4, p< 0.001). STEMI patients who have T allele exhibited higher risk scores, coronary multivessel narrowing, and elevated incidence of in-hospital major adverse clinical events.

Conclusions: These results suggest that plasma ox-LDL, as well as T allele of ORL-1 (rs1050286), is associated with the increased risk for developing STEMI and the associated adverse clinical outcomes.

Keywords: Coronary artery disease, genotyping, OLR1, outcomes, Oxidized low-density lipoprotein.

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Introduction

Cardiovascular diseases (CVD) remain the leading cause of disability and mortality worldwide (1). In particular, atherosclerosis and underlying vascular dysfunction with the ongoing process of coronary plaque formation are well characterized in the

1: Department of Internal Medicine, Faculty of Medicine, Assiut University, Assiut, Egypt.
2: Department of Medical Physiology, Faculty of Medicine, Assiut University, Assiut, Egypt.
3: Department of Medical Biochemistry and Molecular Biology, Faculty of Medicine, Assiut University, Assiut, Egypt.
4: Department of Clinical Pathology, Faculty of Medicine, Assiut University, Assiut, Egypt.
5: Department of Anesthesia, ICU and Pain Relief, South Egypt Cancer Institute, Assiut University, Assiut, Egypt.
***Corresponding author: Reham Ibrahim El-Mahdy; Tel: +2 01002714637; E-mail: reham.elmahdy@aun.edu.eg.**
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pathophysiological basis of CVD (2).

The underlying mechanism of atherosclerosis is complex involving genetic and environmental contributions. Moreover, several common cardiovascular risk factors, including age, hypertension, diabetes mellitus, gender, dyslipidemia, smoking, obesity, and behavioral risk factors are all inevitably implicated (3).

Patients who had coronary artery disease (CAD) remain at increased risk of major adverse cardiovascular events (MACEs) even with the use of early interventions and new pharmacological treatment that has greatly improved the prognosis (4, 5).

Despite CVD risks have been thoroughly investigated over the past years; there are still many residual risks to be resolved (6). Therefore, the early stratification of the overall risk and underlining of high-risk individuals is important for more thorough monitoring and intensive treatment.

Oxidized low-density lipoprotein (ox-LDL) is a pro-atherosclerotic mediator that has an important role in the pathogenesis and progression of coronary atherosclerosis by triggering inflammatory and oxidative stress responses and enhancing endothelial dysfunction (7). Atherosclerosis-related cells as endothelial cells, smooth muscle cells, and monocytes express lectin-like oxidized low-density lipoprotein receptor (LOX-1) that is the main scavenger receptor highly specific to ox-LDL. This receptor protein mediates the binding, internalization, and degradation of ox-LDL (7, 8).

The activation of the ox-LDL/LOX-1 signaling pathway leads to foam cell formation, increases reactive oxygen radicals, vascular smooth muscle cell proliferation, collagen deposition, platelet activation, and induced endothelial dysfunction, all characteristic pathophysiological background of atherosclerosis (8).

Ox-LDL receptor 1 (OLR1) gene in humans encodes the receptor LOX-1 (9). Under physiological conditions the expression of LOX-1 is minimal, and the OLR1 gene can be up-regulated in various inflammatory and oxidative states associated with several pathological conditions (9, 10).

Genetic variations, such as single nucleotide polymorphisms (SNPs) have been known to influence the expression of receptors at the gene level and have a role in disease susceptibility. Several SNPs in the OLR1 gene have been found and can affect the expression of LOX-1 and have been associated with carotid atherosclerosis (11, 12) and correlated to increased risk of ischemic stroke (13).

OLR1 (rs1050286) is located in the 3′-untranslated region (3′ UTR), it was earlier reported that the 1050286 SNP can influence LOX-1 expression by altering its regulator binding site and thus modifying protein homeostasis (14).

There are limited studies about the association of (rs1050286) SNP and the susceptibility and severity of CAD. Hence, the present study was conducted to explore the potential association of functional SNP rs1050286 of the OLR1 gene as well as the levels ox-LDL with CAD risk and in-hospital adverse outcomes in acute coronary syndrome patients presented with ST-segment elevation myocardial infarction (STEMI) and unstable angina (UA).

Materials and Methods

Participants' examination

This case-control study included 292 patients categorized into 192 patients diagnosed as ST-segment elevation myocardial infarction (STEMI) and 100 patients with unstable angina (UA). Patients were recruited from the Internal Medicine (critical care unit) and Cardiology Departments, Faculty of Medicine, Assiut University, during the period from June 2018 till November 2019 with the exclusion of patients having bleeding disorders, renal impairment, and orthopnea. The research was registered in the Clinical Trials with an identification number of NCT03830138. We adjusted the size of the sample to achieve 80% power and 5% confidence of significance (type 1 error).

Unstable angina was defined according to ESC guidelines (2015) (15) as an acute

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coronary syndrome with the absence of biochemical evidence of myocardial damage (did not include troponin-positive patients). It is characterized by prolonged (>20 minutes) angina at rest; or new onset of severe angina; or angina that is increasing in frequency, longer in duration (15).

A total of 100 unrelated healthy volunteers matched to the included patient by age were selected in this study as controls. All participants gave informed written consent to contribute to in the present study. The study was conducted following The Code of Ethics of the Declaration of Helsinki for experiments in humans.

The entire patient subjected to

History taking including stressing upon risk factors, the character, onset, and duration of chest pain, previous history of ischemic heart disease, and co-morbid conditions.

Clinical examination was done by searching for hemodynamic instability, signs of left ventricular (LV) dysfunction, and the presence or absence of systemic diseases.

Killip classification was done for all considering physical examination and the development of heart failure in order to predict their risk of mortality. Individuals with a low Killip class are less likely to die within the first 30 days after their myocardial infarction than individuals with a high Killip class (16).

All patients underwent resting echocardiography with special stress on the measurement of Ejection Fraction (by M-mode), wall motion abnormalities, and intracardiac masses.

Coronary angiography was performed within 2 hours of diagnosis of STEMI and it was performed electively in UA patients. This was done using the Seldinger technique for femoral artery puncture and insertion of 6F sheath throw which 6F Judkin's left & right diagnostic catheters were advanced over 0.035 guidewire to cannulate the Left Main and Right coronary arteries followed by injection of radiocontrast under fluoroscopy and cine with left anterior oblique (LAO) or right anterior oblique (RAO) caudal and cranial projection.

TIMI (thrombolysis in myocardial infarction) risk score algorithm scoring system developed to predict the risk of death from any cause at 30 days in patients with STEMI, with a score ranging from 0 to 14 (17).

GRACE risk scoring (Global Registry of Acute Coronary Events) which estimates mortality risk within 6 months to 3 years of myocardial infarction was done (18).

Laboratory investigations

Including blood picture, kidney function tests, liver function tests, hepatitis B surface antigen, hepatitis C antibody test, HIV antibody ELISA test, prothrombin time and prothrombin concentration, serum glucose, HbA1c, troponin I, creatine phosphokinase (CPK), creatine phosphokinase-MB (CPK-MB), triglyceride (TG), total cholesterol, low-density lipoprotein cholesterol (LDL-c), and high-density lipoprotein cholesterol (HDL-c).

Assay of ox-LDL plasma level and genotyping of OLR1 (rs1050286) SNP

A total of 5 ml peripheral venous blood was collected from each participant and distributed into two vacutainer tubes. A portion of 2.5 ml was collected in EDTA-containing tubes and stored at −80 °C till DNA extraction time for subsequent genotyping. The remaining portion of 2.5 ml was evacuated into EDTA containing vacutainers, centrifuged for 15 minutes at 1200 RCF for plasma separation, and stored at -20 °C until the assay of ox-LDL.

The biochemical assay of ox-LDL in human plasma samples was measured using an enzyme-linked immunosorbent assay (ELISA) technique. The ELISA kit provided by Cloud Clone Corporation (USA, Catalog No E-01160hu) according to the operational guidelines.

The genomic DNA was isolated from whole peripheral blood samples using a QIAamp DNA blood extraction kit (Qiagen, Germany) in accordance with the enclosed instructions. All DNA samples were quantitated using the Nano Drop®-1000 spectrophotometer (Nanodrop Technologies, Inc., Wilmington, USA).

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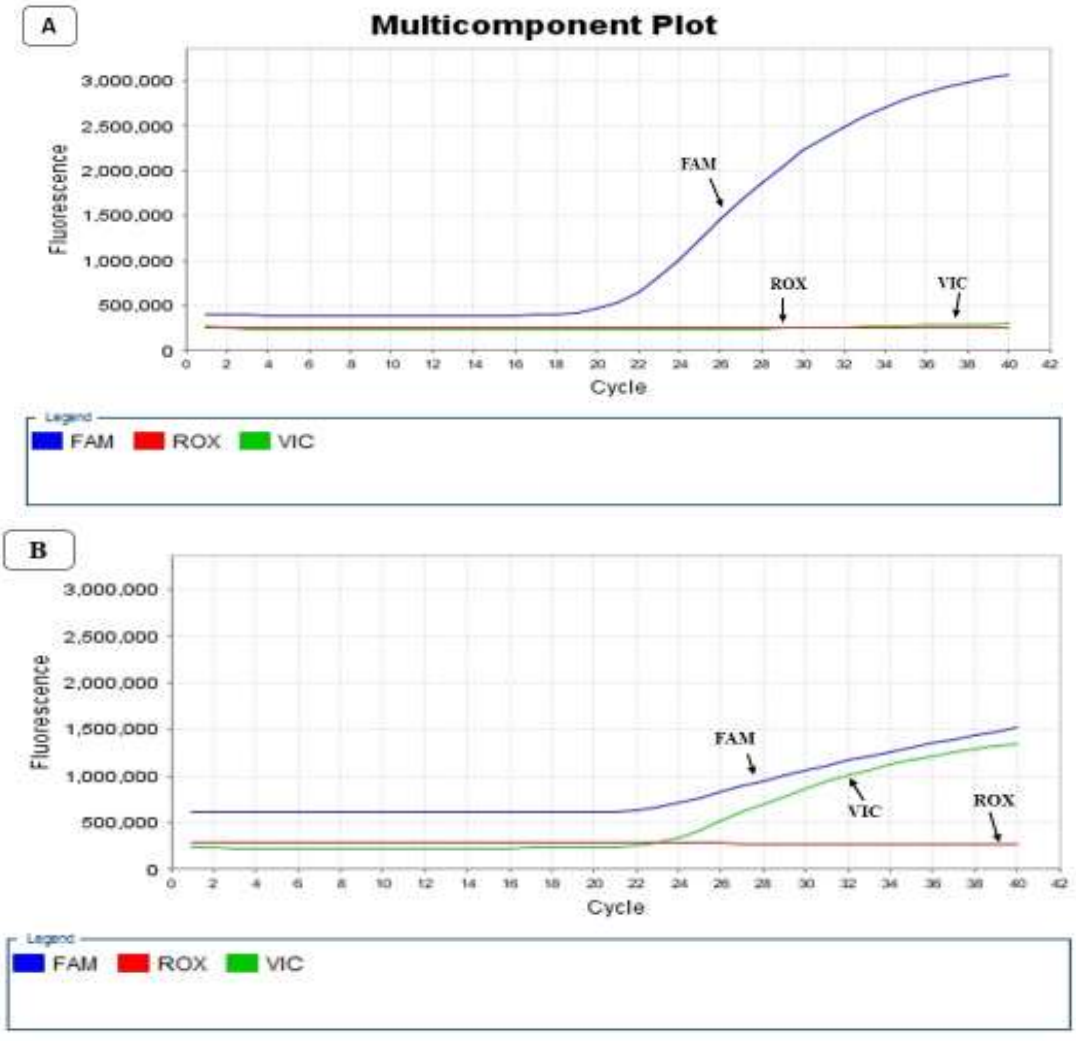
OLR1 3′UTR C188T SNP genotyping was done using real-time RT- PCR with TaqMan SNP genotyping assay (Assay ID was C__7433809_30, Catalog number 4351379) and its rs1050286 supplied by Thermo Fisher Scientific, Waltham, MA, USA. PCR was performed using Step one 7500 real-time PCR system (Applied BioSystem, CA, USA). The amplification was carried out using 25 µl PCR reaction mixture contained 12.5 µl of 2X TaqMan® Genotyping Master Mix, 1.50 µl of 20X specific TaqMan® SNP genotyping assay, 5 µl (20 ng) of whole blood genomic DNA template, and 6 µl of nuclease-free water. The 20X specific TaqMan® SNP genotyping assay included sequence-specific forward and reverse primers for amplification of the polymorphic target sequence and two alleles specific TaqMan® MGB probes; One labeled with VIC® dye for detection of the allele 1 sequence and the other probe labeled with FAM™ dye to identify the allele 2 sequence. The ROX was the passive reference dye.

The reaction mix was kept at 95 °C for 10 min for AmpliTaq Gold R, UP, enzyme activation, followed by 40 cycles. Each amplification cycle comprised of DNA denaturation at 95 °C for 15 s, primer annealing, and extension at 60 °C for 1 minute. After PCR amplification, the fluorescence measurements were made during the plate read. The real-time PCR instrument software values based on the fluorescence signals from each well and then determined which alleles were in each sample (Fig. 1). Four Non-template controls (NTCs) were incorporated in each plate to guarantee the accuracy of the genotyping.



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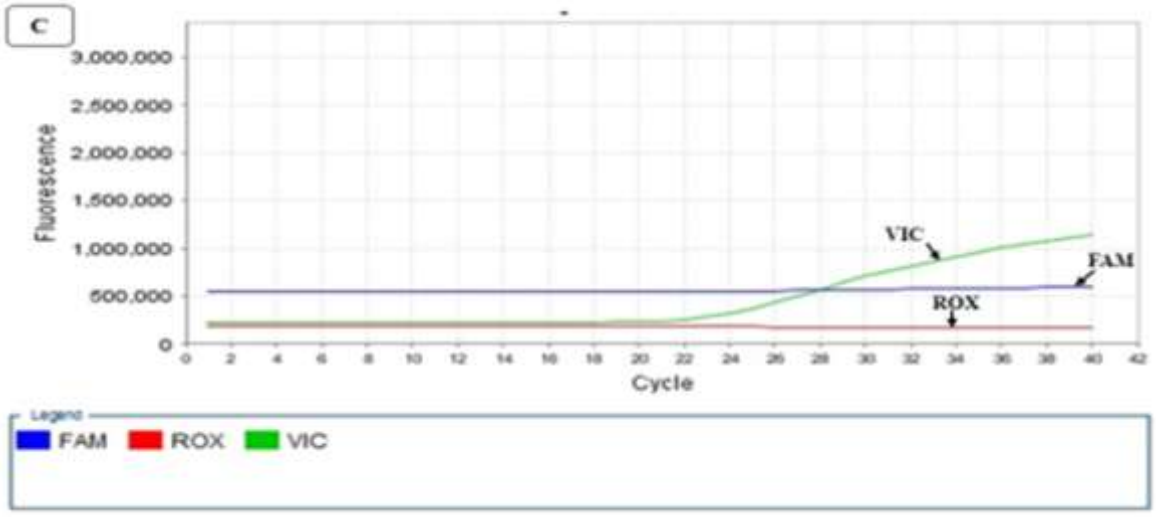

Fig. 1. Multicomponent plot created through Real-Time PCR demonstrating the wild TT genotype (A), heterozygous mutant CT genotype (B), and homozygous mutant CC genotype (C) in C188T 3′UTR of the OLR1 rs1050286.

Statistical analysis

The clinical and laboratory data were collected and processed by SPSS version 20 (SPSS Inc, Chicago, IL, USA) and Graph Pad Prism 6 Software (San Diego, California, USA). The expression of the continuous data was presented as mean±SD or median (IQR), while the expression of categorical variables was as numbers and percentages. For statistical assessment, Student's t-test was used for the comparison of means between the two groups. Mann-Whitney u test was performed for comparison of non-parametric variables. Categorical variables were examined by the Chi-square $\chi 2$ test. The one-way ANOVA was done to compare the means between the three groups. Pearson correlation coefficient was performed to test the correlation between different variables. The receiver operating characteristics (ROC) curve was created by using the MedCalc program to identify the sensitivity, specificity, area under the ROC curve (AUC), and cut-off value to test the differentiating efficacy of ox-LDL. Hardy-Weinberg calculation was done to examine the observed genotype frequencies of the tested SNP. Logistic regression analysis was used to detect the independent predictor variables. The two-tailed P-value of less than 0.05 was considered statistically significant.

Results

Patient characteristics

Demographic and clinical data of study groups are shown in table 1. All groups were well-matched concerning their age (p= 0.169). The sex distribution of the groups differed significantly with a greater number of male patients with STEMI (n= 152) than female patients (n= 40) (p= 0.01). The BMI and proportions of smokers were greater among the two patient groups with acute coronary syndrome (ACS) compared with healthy controls (p= 0.001 for each). Patients with STEMI showed increased blood pressure, smoking index, diabetes duration, duration of hospitalization than UA patients (p= 0.046, p= 0.01, p= 0.001, and p= 0.016 respectively). No significant differences were found between patient groups regarding hyperlipidemia, percent of diabetics, or in-hospital mortality.

The distribution of occluded arteries showed that 68.8% of STEMI patients had occlusion in the left anterior descending artery, followed by right coronary artery (52.1%), the left main vessels involvement was more common in UA

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patients (84%). Coronary arterial dominance pattern varies significantly among ACSpatients presented with STEMI and UA (p= 0.001). The right dominant coronary artery system was present in 77.1% of STEMI and 66% of UA patients. Left dominance was found in 10.4% of STEMI and 34% of UA patients whereas co-dominant circulation was detected in 12.5% of STEMI patients (Table 1).

Table 1. Demographic and clinical data of all groups enrolled in the study.

Parameters	STEMI (n= 192)	UA (n= 100)	Control group (n=100)	p- value
Age (years) mean±SD	56.2±8.9	57.5±6.1	54.9±5.6	0.169
Sex N (%)				
Male	152 (9.2%)	43 (43%)	60 (60%)	0.01*
Female	40 (20.8%)	57 (57%)	40 (40%)	
BMI mean±SD	32.5±8.8	28.4±2.5	27.5±3.2	0.001**
Cardiovascular risk factors				
Smoking N (%)				
Yes	101 (52.6%)	32 (32%)	25 (25%)	0.001**
No	91 (47.4%)	68 (68%)	75 (75%)	
Diabetes N (%)				
Yes	48 (25%)	28 (28%)	-	0.579
No	144 (75%)	72 (72%)	-	
Hypertension N (%)				
Yes	28 (14.6%)	24 (24%)	-	0.046*
No	164 (85.4%)	76 (76%)	-	
Hyperlipidemia N (%)				
Yes	135 (70.3%)	74 (74%)	-	0.507
No	57 (29.7%)	26 (26%)	-	
Smoking index (pack/year)	88±29.4	51.4±27.9	50.8±27	0.01*
Diabetes duration (years)	8.2±3.9	6.9±0.5	-	0.001**
Diseased vessels N (%)				
LM	12 (6.2%)	84 (84%)	-	0.001**
LAD	132 (68.8%)	28 (28%)	-	0.001**
LCx	44 (22.9%)	33 (33%)	-	0.064
RCA	100 (52.1%)	6 (6%)	-	0.002**
Diagonal	36 (18.8%)	0 (0.0%)	-	0.001**
OM	20 (10.4%)	0 (0.0%)	-	0.001**
Dominance N (%)				
Right	148 (77.1%)	66 (66%)	-	0.001**
Left	20 (10.4)	34 (34%)	-	
Co-dominant	24 (12.5%)	0 (0.0%)	-	
Duration of hospitalization	3.7±1.2	3.6±0.4	-	0.016*
In hospital mortality N (%)				
Yes	10 (5.2%)	3 (3%)	-	0.385
No	182 (94.8%)	97 (97%)	-	

Data are presented as mean ± standard deviation or number and percentage. STEMI: ST-segment elevation myocardial infarction; UA: unstable angina; BMI: body mass index; LM: left main; LAD: left anterior descending; LCx: left circumflex; RCA: right coronary artery; OM: obtuse marginal. *Statistically significant difference (p< 0.05).
** Statistically significant difference (p< 0.01).

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Plasma ox-LDL

Plasma ox-LDL mean values were significantly higher in patients with STEMI compared with UA and controls (p< 0.001 for each) (Fig. 2A). To test the power of ox-LDL to distinguish patients with STEMI and UA from healthy individuals, a ROC analysis was performed. The cut-off of ox-LDL was 577.5 ng/ml which conveys the best sensitivity (88.02%) and specificity (97%) for predicting patients who have a higher risk for STEMI (ROC area under the curve that represents the overall accuracy of the test was 0.942) (Fig. 2B).

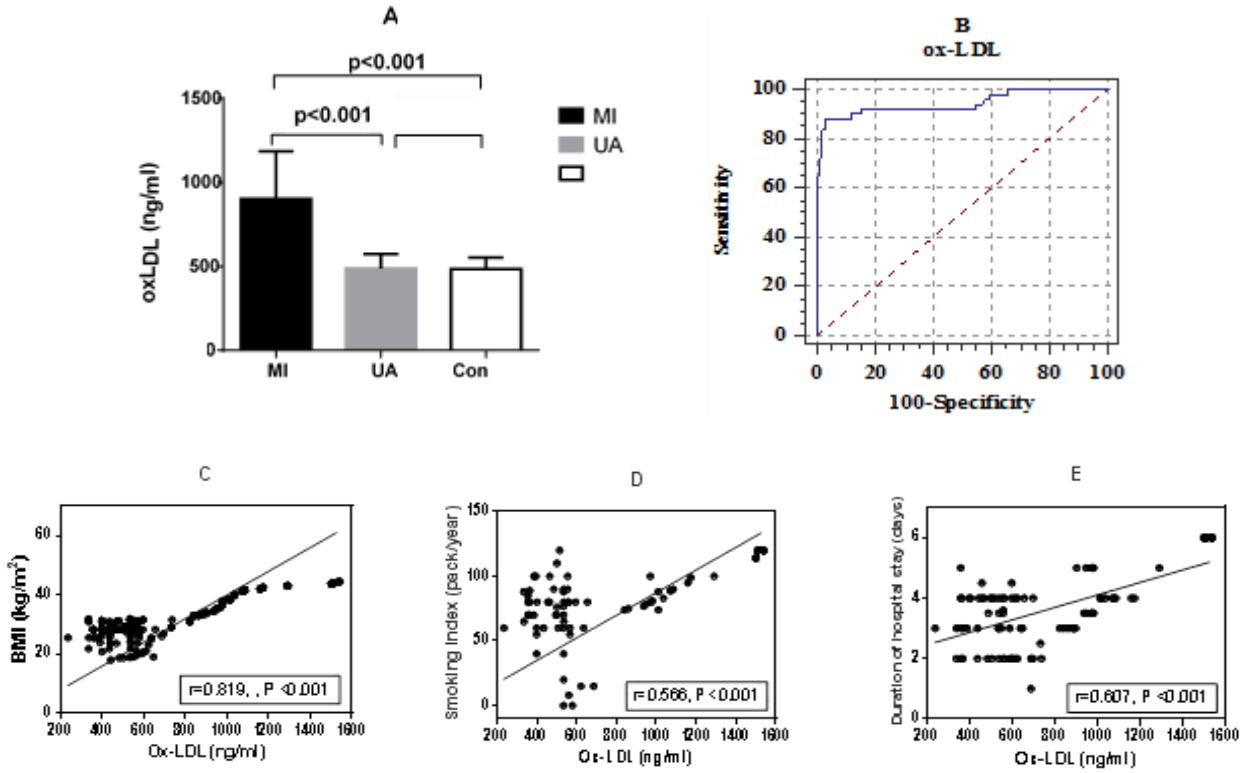

Fig. 2. The plasma levels of ox-LDL in studied groups. (A) The ox-LDL level was significantly higher in STEMI compared with UA and controls (p< 0.001 for each). (B) ROC curve for plasma level of ox-LDL, ox-LDL could differentiate STEMI from UA and controls. The AUC, sensitivity, specificity, and cut-off values were 0.942, 88.02%, 97%, and 577.5 respectively. A positive correlation between plasma ox-LDL levels with BMI (C), smoking index (D), and duration of hospital stay (E) in coronary artery disease patients. (r= 0.819, r= 0.566, and r= 0.607, p< 0.001) for each.

Plasma ox-LDL in relation to patient characteristics

We then analyzed whether the ox-LDL associate with disease severity and adverse outcome. STEMI Patients who showed a significantly increased number of diseased vessels, a higher Killip class, increased incidence of high-risk according to the TIMI and GRACE risk scores, or increased mortality had a significantly high ox-LDL level (p< 0.001 for each) (Table 2). Among patients who had arrhythmias, patients who experienced atrial fibrillation had a lower concentration of ox-LDL while two patients who experienced asystole had the highest mean level. UA patients with diabetes, hyperlipidemia, and an increased number of diseased vessels had significantly higher ox-LDL concentration increased (p= 0.001, p 0.017, and 0.016 respectively) (Table 2).

Significant positive correlations were found between plasma ox-LDL in all patients with BMI (Fig. 2C), smoking index (Fig. 2D), andduration of hospital stay (Fig. 2E), (r= 0.819, r= 0.566, and r= 0.607, p< 0.001 for each).

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Table 2. Plasma level of ox-LDL and its relation to the examined prognostic factors, angiographic characteristics, risk scores and outcome in STEMI and UA.

Variables	Number of patients	Ox-LDL, ng/ml STEMI patients Mean±SD	p- value	Number of patients	Ox-LDL, ng/ml UA patients Mean±SD	p- value
Smoking N (%)						
Yes	101	1049.7±270	0.119	32	471.3±91	0.690
No	91	734.4±196		68	495.3±85	
Diabetes N (%)						
Yes	48	797±242	0.743	28	519.6±70	0.001**
No	144	951.4±280		72	475.2±90	
Hypertension N (%)						
Yes	28	895.4±377	0.083	24	457.5±91	0.469
No	164	918.2±263		76	497±85	
Hyperlipidemia N (%)						
- Yes	135	930.8±294	0.537	74	496.7±82	0.017
- No	57	827.8±250		26	461.7±98	
Number of diseased vessels						
None	0	-	<0.001***	4	454.5±41	0.016*
- One vessel	97	767.4±193		46	463.5±90	
Two vessels	62	919.9±270		50	512.5±81	
Three vessels	33	1254.6±223		0	-	
Killip classification						
Class I	84	669.3±143	<0.001***	100	487.6±87	NA
Class II	48	983.4±239		-		
Class III	28	1058.5±73		-	-	
Class IV	32	1243.4±235		-	-	
TIMI score						
Low	36	542.4±58	<0.001***	97	485.9±88	0.287
Intermediate	36	666.5±70		3	541±11.3	
High	120	1078±202		-	-	
GRACE risk score						
Low	44	638.6±218	<0.001***	100	487.6±87	NA
Intermediate	36	669.4±67		-	-	
High	112	1077.5±209		-	-	
MACE						
Heart failure	27	1260.4±237	0.183	3	476.3±101	0.061
Myocardial reinfarction	3	1073±3.8		-	-	
Cardiogenic shock	12	1163.2±256		3	521±23	
Cerebrovascular stroke	3	1015±3.6		2	685±70	
Arrhythmias						
Atrial fibrillation	4	886.3±6.2	<0.001***	1	595	0.347
Ventricular tachycardia	7	1329±195		-	-	
Heart block	7	966.8±504		2	535±5	
Asystole	2	1524±19.8		-	-	
In hospital mortality						
Yes	10	1519.2±16.6	<0.001***	3	588±49	0.177
No	182	866.3±252		97	484.5±87	

MACE: major adverse cardiovascular events; NA: not applied
** Statistically significant difference ($p < 0.01$).
*** Statistically significant difference ($p < 0.001$).

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Multivariate logistic regression analysis for ox-LDL and other risk factors

To detect if ox-LDL is an independent predictor of ACS patients presented with STEMI and UA, multivariate logistic regression analysis was used. We could identify that smoking has the highest odds ratio (OR) (2.784) with 95% confidence interval (CI)= 1.834-4.228, followed by HbA1c (OR of 1.394; 95% CI= 1.215-1,598). Furthermore, the levels of ox-LDL, LDL-C, and total cholesterol were also independent risk factor for CAD (OR of 1.022; 95% CI= 1.018-1.029, OR of 1.02; 95% CI= 1.012-1.028, and OR of 1.007; 95% CI= 1.001-1.012 respectively) in the two patient groups (Table 3).

Table 3. Logistic regression of predictors of coronary artery disease.

Variables	OR	95% CI		P-value
		Lower	Upper	
Smoking	2.784	1.834	4.228	<0.001***
Diabetes	0.857	0.497	1.479	0.579
Hypertension	0.541	0.294	0.994	0.048
Ox-LDL (ng/ml)	1.022	1.018	1.029	<0.001***
TG (mg/dl)	1	0.997	1.003	0.870
Total cholesterol (mg/dl)	1.02	1.012	1.028	<0.001***
LDL-C (mg/dl)	1.007	1.001	1.012	0.018*
HDL-C (mg/dl)	1.028	0.993	1.064	0.119
HbA1c	1.394	1.215	1.598	<0.001***

OR: odds ratio; CI: confidence interval.
*** Statistically significant difference (p< 0.001).

OLR1 (rs1050286) SNP genetic variants

For patients and controls, all genotype and allele distributions were consistent with Hardy-Weinberg equilibrium law. T allele was overrepresented in STEMI patients (87.5%) than in controls (60%). Significantly elevated STEMI risk was associated with T allele (OR of 4.9, 95% CI: 2.6-9.4, (p< 0.001) in the CT and the TT genotypes (OR of 4.76 and 4.32, 95% CI: 2.5-8.9 and 2.2-8.7 respectively) (p< 0.001), and in recessive model (CC vs TT+CT) with OR of 4.61 (95% CI: 2.6-8.3, p< 0.001). However, genotypes and allelic distributions in UA patients didn't show significant differences from controls or significant association with disease risk (Table 4).

Table 4. Distribution of genotypes and allelic frequencies of OLR1 rs1050286 3′UTR 188C>T gen polymorphism among STEMI, UA patients and controls subjects.

OLR1 rs1050286	STEMI (n= 192)	UA (n= 100)	Controls (n= 100)	OR (95% CI) STEMI vs. controls	OR (95% CI) UA vs. controls
	No. %	No.%	No.%		
Genotype					
CC (ref.)	24 (12.5)	34 (34.0)	40 40.0)	-	-
CT	100 (52.1)	45 (45.0)	35 (35.0)	4.76 (2.5-8.9) ***	1.5 (0.8-2.8)
TT	68 (35.4)	21 (21.0)	25 (25.0)	4.32 (2.2-8.7) ***	0.99 (0.47-2.1)
Allele C	124 (64.6)	79 (79.0)	75 (75.0)	1.05 (0.57-1.9)	1.2 (0.65-2.4)
Allele T	168 (87.5)	66 (66.0)	60 (60.0)	4.9 (2.6-9.4) ***	1.3 (0.73-2.3)
Dominant					
CT+CC (ref.)	124 (64.6)	79 (79.0)	75 75.0)	-	-
TT	68 (35.4)	21 (21.0)	25 (25.0)	1.4 (0.96-2.8)	0.79 (0.41-1.5)
Recessive					
CC (ref.)	24 (12.5)	34 (34.0)	40 40.0)	-	-
- TT+CT	168 (87.5)	66 (66.0)	60 (60.0)	4.61 (2.6-8.3) ***	1.2 (0.73-2.3)

STEMI: ST-segment elevation myocardial infarction; UA: unstable angina; OR: odds ratio; CI: confidence interval.
*** Statistically significant difference (p< 0.001).

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OLR1 (rs1050286) SNP genetic variants and patient characteristics

Diabetes duration, systolic blood pressure, CPK, CPK_MB at admission, glucose levels were higher among the STEMI patient carriers of the rs1050286 T allele (CT and TT genotypes compared with the CC carriers). BMI and diabetes duration were more in CT genotype compared with other genotypes. TT carriers showed less smoking index and more diabetes prevalence, higher heart rate, troponin, and total cholesterol levels at admission and more pulmonary arterial systolic pressure compared with the CC carriers (Table 5).

Table 5. The relation between the different genotype variants of OLR1 rs1050286 3′UTR 188C> T gene with clinical characteristics laboratory finding, **number of diseased vessels,** risk scores, and outcomes in STEMI patients.

	Carriers of CC	**Carriers of CT**	**Carriers of TT**	**CC vs. CT**	**CC vs. TT**	**CT vs. TT**
	(n= 24)	**(n= 100)**	**(n= 68)**	**P1**	**P2**	**P3**
Age (years)	60.6±7.2	56.8±9.3	53.8±8.5	0.078	0.944	0.036*
BMI (Kg/m²)	26.9±9.2	35.8±6.9	29.6±9.2	0.004**	0.958	<0.001***
Smoking N (%)						
Yes	10 (41.7%)	57 (57%)	34 (50%)	0.176	0.482	0.371
No	14 (58.3%)	43 (43%)	34 (50%)			
Smoking index (pack/year)	83.5±6.9	87.3±24	72.7±38	0.181	0.019*	0.02*
Diabetes N (%)						
Yes	4 (16.7%)	16 (16%)	28 (41.2%)	0.936	0.03*	0.001**
No	20 (83.3%)	84 (84%)	40 (58.8%)			
Diabetes duration (years)	3.5±0.1	12±3.9	6.5±1.7	0.02*	0.005**	0.003**
Hypertension N (%)						
Yes	4 (16.7%)	16 (16%)	8 (11.8%)	0.936	0.540	0.441
No	20 (83.3%)	84 (84%)	60 (88.2%)			
Heart rate (bpm)	94.2±20	106.8±22.3	98.2±25	0. 133	0.004**	0.023*
Systolic blood Pressure (mm Hg)	120.4±24.3	134.8±26.8	139±39	0.001**	0.002**	0.522
EF (%)	51.8±9.4	49.4±10.7	49.5±8.6	0.658	0.448	0.112
PASP (mm Hg)	33.2±12.6	27.7±10.2	30.6±8.2	0.135	0.036*	0.554
Ox-LDL (ng/ml)	715±235	997.1±252	823.3±293	0.517	0.372	0.035*
Troponin at Admission (ng/ml)	0.04 (0.01)	0.16 (1.2)	1.1 (1.25)	0.361	<0.001***	0.001**
CPK at admission (U/L)	250 (115)	1084 (1531)	987 (938)	0.029*	<0.001***	0.115
CPK_MB at Admission (IU/L)	41 (59)	100 (165)	143 (147)	0.001**	<0.001***	0.079
TG (mg/dl)	214.8±92	262.3±82	213.4±86	0.80	0.488	0.564
Total cholesterol (mg/dl)	191.3±41	264.5±87	219.8±70	0.230	<0.001***	<0.001***
LDL-C (mg/dl)	142.9±57.7	173.4±79	147.9±58.7	0.097	0.149	0.004**
HDL-C (mg/dl)	44.5±6.6	47.5±7.4	47±6.3	0.352	0.950	0.133
Creatinine (mg/dl)	1±0.46	0.9±0.27	0.92±0.4	0.159	0.371	0.102
Glucose (mg/dl)	158±36	162.9±98	188.2±68.9	0.01*	0.001**	0.428
HbA1c	4.2 (2.8)	5 (2)	4.5 (3.1)	0.158	0.886	0.208
Number of Diseased vessels						
One vessel	20 (83.3%)	35 (35%)	42 (61.8%)	0.002**	0.331	0.002**
Two vessels	0 (0.0%)	44 (44%)	18 (26.5%)			
Three vessels	4 (16.7%)	21 (21%)	8 (11.8%)			
KILLIP						

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Classification						
I	20 (83.3%)	24 (24%)	40 (58.8%)	<0.001***	0.10	<0.001***
II	0 (0.0%)	0 (40%)	8 (11.8%)			
III	0 (0.0%)	24 24%)	4 (5.9%)			
IV	4 (16.7%)	12 (12%)	16 (23.5%)			
TIMI score						
Low	12 (50%)	0 (0.0%)	24 (35.3%)	<0.001***	0.415	<0.001***
Intermediate	4 (16.7%)	20 (20%)	12 (17.6%)			
High	8 (33.3%)	80 (80%)	32 (47.1%)			
GRACE risk score						
Low	16 (66.7%)	8 (8%)	20 (29.4%)	<0.001***	0.004**	<0.001***
Intermediate	4 (16.7%)	16 (16%)	16 (23.5%)			
High	4 (16.7%)	76 (76%)	32 (47.1%)			
Mace						
Heart Failure	0 (0.0%)	19 (57.6%)	8 (66.7%)	0.002**	0.038*	0.462
Myocardial Reinfarction	0 (0.0%)	3 (100%)	0 (0.0%)			
Cardiogenic Shock	0 (0.0%)	8 (24.2%)	4 (33.3%)			
Cerebrovascular Stroke	0 (0.0%)	3 (100%)	0 (0.0%)			
Arrhythmias						
Atrial Fibrillation	0 (0.0%)	0 (0.0%)	4 (5.9%)	0.352	0.145	0.001**
Ventricular Tachycardia	0 (0.0%)	0 (0.0%)	7 (10.3%)			
Heart Block	0 (0.0%)	2 (3%)	4 (5.9%)			
Asystole	0 (0.0%)	1 (1%)	1 (1.5%)			
In hospital mortality						
Yes	0 (0.0%)	6 (6%)	4 (5.9%)	0.219	0.224	0.975
No	24 (100%)	94 (94%)	64 (94.1%)			

Data presents as mean±SD or median (IQR) and range.

BMI: Body mass index; EF: ejection fraction; PASP: pulmonary arterial systolic pressure; Ck: creatine kinase; HbA1c: glycosylated hemoglobin, MACE: major adverse cardiovascular events

*Statistically significant difference ($p < 0.05$)

** Statistically significant difference ($p < 0.01$).

*** Statistically significant difference ($p < 0.001$).

We investigated whether genetic variation in OLR1 (rs1050286) is associated with the severity of the disease and adverse outcomes. Our results showed a significant percentage of STEMI patients who are carriers of CT and TT genotypes had higher GRACE risk scores for acute coronary disease, and experienced heart failure as a major adverse cardiovascular event compared with the CC genotype. Additionally, STEMI patients with CT genotype demonstrated a higher incidence of more than one coronary vessel diseased, a higher Killip class, an increase in the proportion of patients identified as high-risk according to the TIMI risk score compared to other 2 genotypes carriers, while more incidence of arrhythmias in STEMI patients who are TT genotype than other 2 genotypes (Table 5).

In UA cases, CT and TT genotypes were associated with increased BMI, a higher prevalence of smoking, elevated levels of CPK, CPK_MB at admission when compared with patients with CC genotype (Table 6). Unstable angina patients with CT genotype had a significantly high percentage of two vessels diseased when compared to the other two genotypes. No differences were found between different genotype carriers in patients with UA concerning different risk scores or adverse cardiovascular events (Table 6).

Plasma ox-LDL level varies significantly according to rs1050286 polymorphism. STEMI patients who are CT genotype carriers presented with higher plasma ox-LDL levels than TT genotype carriers ($p = 0.035$) (Table

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5). In patients with UA, both CT and TT carriers have increased ox-LDL levels compared with CC carriers (p= 0.007 and p= 0.001 respectively) (Table 6).

Table 6. The relation between different genotype variants of OLR1 rs1050286 3′UTR 188C> T gene with clinical characteristics. laboratory finding, **number of diseased vessels**, risk scores, and outcome in UA patients.

	Carriers of CC	**Carriers of CT**	**Carriers of TT**	**CC vs. CT**	**CC vs. TT**	**CT vs. TT**
	(n= 34)	**(n= 45)**	**(n= 21)**	**P1**	**P2**	**P3**
Age (years)	57.4±5.6	56.3±6.9	60.4±4.2	0.622	0.044	0.045*
BMI (Kg/m²)	25.5±2	30.7±1.5	29±4.2	0.001**	0.001**	<0.001***
Smoking N (%)						
Yes	3 (8.8%)	13 (28.9%)	16 (76.2%)	0.028*	0.001**	0.001**
No	31 (91.2%)	32 (71.1%)	5 (23.8%)			
Smoking index (pack/year)	80 (16.3)	80 (18.8)	79 (5)	0.278	0.069	0.516
Diabetes N (%)						
Yes	9 (26.5%)	14 (31.1%)	5 (23.8%)	0.653	0.826	0.542
No	25 (73.5%)	31 (68.9%)	16 (76.2%)			
Diabetes duration (years)	6.9±0.5	7±2	6.8±0.27	0.118	0.122	0.703
Hypertension N (%)						
Yes	0 (0.0%)	12 (26.7%)	12 (57.1%)	0.001**	0.056	0.017*
No	34 (100%)	33 (73.3%)	9 (42.9%)			
Heart rate (bpm)	80±10	79.7±4.5	82±19	0.051	0.054	0.023*
Systolic blood pressure (mm Hg)	127.4±9	128.5±15.5	134.8±20	0.715	0.069	0.169
EF (%)	60±2	65.7±0.5	64±2.6	0.691	0.503	0.802
PASP (mm Hg)	20.4±1.2	21±1.9	19.1±1.2	0.054	0.881	0.01*
Ox-LDL (ng/ml)	449.9±97	510.4±82	499.7±59	0.007**	0.001**	0.355
Troponin at admission (ng/ml)	0.009±0.006	0.009±0.007	0.008±0.006	0.387	0.309	0.056
CPK at admission (U/L)	163.8±20	208.9±10	193.8±12	0.002**	0.009**	0.518
CPK_MB at admission (IU/L)	16±1.4	16.3±4	17.5±1.2	0.041*	0.061*	0.079
TG (mg/dl)	187±44.8	242±58	219.3±56	0.003**	0.088	0.663
Total cholesterol (mg/dl)	180±20	179.6±30.5	156±30	0.059	0.01*	0.001**
LDL-C (mg/dl)	124.9±14.5	125.3±25	124.9±20	0.074	0.092*	0.004**
HDL-C (mg/dl)	48.7±7	44.5±7.3	43.6±5.8	0.209	0.057	0.413
Creatinine (mg/dl)	0.8±0.2	1±0.2	0.99±0.2	0.061	0.371	0.102
Glucose (mg/dl)	142.2±62	138±59.7	139±51	0.691	0.081	0.117
HbA1c	5.2 (0.2)	5 (0.3)	4.5 (0.8)	0.001**	0.083	0.001**
Number of diseased vessels						
None	0 (0.0%)	0 (0.0%)	4 (19%)	0.033*	0.001**	0.001**
One vessel	17 (50%)	12 (26.7%)	17 (81%)			
Two vessels	17 (50%)	33 (73.3%)	0 (0.0%)			
Three vessels	0 (0.0%)	0 (0.0%)	0 (0.0%)			
KILLIP classification						
I	34 (100%)	45 (100%)	21 (100%)	NA	NA	NA
II	0 (0.0%)	0 (0.0%)	0 (0.0%)			
III	0 (0.0%)	0 (0.0%)	0 (0.0%)			
IV	0 (0.0%)	0 (0.0%)	0 (0.0%)			
TIMI score						

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	Carriers of CC	Carriers of CT	Carriers of TT	CC vs. CT	CC vs. TT	CT vs. TT
	(n= 34)	(n= 45)	(n= 21)	P1	P2	P3
Low	34 (100%)	45 (100%)	20 (95.2%)	0.843	0.726	0.575
Intermediate	0 (0.0%)	0 (0.0%)	1 (4.8%)			
High	0 (0.0%)	0 (0.0%)	0 (0.0%)			
GRACE risk score						
Low	34 (100%)	44 (97.8%)	21 (100%)	0.840	NA	0.499
Intermediate	0 (0.0%)	1 (2.2%)	0 (0.0%)			
High	0 (0.0%)	0 (0.0%)	0 (0.0%)			
MACE						
Heart failure	0 (0.0%)	3 (50%)	0 (0.0%)	0.233	0.732	0.233
Myocardial reinfarction	0 (0.0%)	0 (0.0%)	0 (0.0%)			
Cardiogenic shock	1 (100%)	1 (16.7%)	1 (100%)			
Cerebrovascular stroke	0 (0.0%)	2 (33.3%)	0 (0.0%)			
Arrhythmias						
Atrial fibrillation	0 (0.0%)	1 (2.2%)	0 (0.0%)	0.308	NA	0.480
Ventricular tachycardia	0 (0.0%)	0 (0.0%)	0 (0.0%)			
Heart block	0 (0.0%)	2 (4.4%)	0 (0.0%)			
Asystole	0 (0.0%)	0 (0.0%)	0 (0.0%)			
In hospital mortality						
Yes	1 (2.9%)	2 (4.4%)	0 (0.0%)	0.729	0.428	0.327
No	33 (97.1%)	43 (95.6%)	21 (100%)			

Data presents as mean ± SD or median (IQR) and range
BMI: Body mass index; EF: ejection fraction; PASP: Pulmonary arterial systolic pressure; Ck: creatine kinase; HbA1c: glycosylated hemoglobin, MACE: major adverse cardiovascular events; NA: not applied
*Statistically significant difference ($p< 0.05$)
** Statistically significant difference ($p< 0.01$).
*** Statistically significant difference ($p< 0.001$).

Discussion

Patients with ACS presented with STEMI and UA had different pathophysiological and prognostic characteristics and varied in ischemic risk, comorbidities, and in-hospital adverse outcomes. The pathologic cause of STEMI is most often from occlusion of the coronary artery by thrombosis from plaque rupture. Whereas the most common cause of UA is reduced myocardial perfusion that results from coronary artery narrowing caused by a non-occlusive thrombus that developed on a disrupted atherosclerotic plaque without detectable cardiac biomarkers (19).

In this study, we demonstrated that ox-LDL is a significant independent risk factor for patients with ACS presented with STEMI and UA, among other significant independent risk factors investigated including smoking, HbA1c, total cholesterol, and LDL-C. This could be explained by the oxidative stress properties of ox-LDL that induce endothelial cell dysfunction, thus contributing to accelerate atherosclerosis (7). Ox-LDL has also pro-inflammatory effects that induce atherosclerotic plaque development, progression, and destabilization (20). Since ox-LDL can promote localized inflammatory reaction (21), therefore, Ox-LDL levels may reflect the degree of inflammatory responses and even the severity of coronary stenosis. Regarding the extent of the disease, we reported that the plasma levels of ox-LDL were higher in patients with STEMI than in UA and controls, which validated the previous results (22, 23). Besides, ROC curve analysis showed the diagnostic accuracy of ox-LDL with enhanced sensitivity and specificity in identifying patients with an increased risk of STEMI.

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In this study, we found that there were significant correlations between ox-LDL levels and BMI, smoking index in ACS patients. Besides, UA patients with diabetes mellitus or hyperlipidemia had higher values of ox-LDL. It has been observed that some cardiovascular risk factors as diabetes, hyperlipidemia, smoking, obesity are known to induce oxidative stress, the release of reactive oxygen species from different inflammatory cells, accelerate the oxidation of LDL that will enhance inflammation in the arterial wall subsequently (24).

Data from earlier studies found that ox-LDL is highly correlated to angiographically detected complex lesions that predict the adverse outcomes in patients with CAD (25, 26). This is consistent with the results in this article suggesting that ox-LDL levels may reflect the severity of ACS in the two patient groups and have a value in predicting adverse cardiovascular events. Since ACS patients, especially STEMI group with unfavorable prognostic factors concerning multivessel stenosis, high-risk criteria based on Killip classification or TIMI and GRACE risk scores, or with increased duration of in-hospital stay, had significantly higher levels of ox-LDL. We further found that increased ox- LDL levels were associated with increased risk of incidence of some adverse events and in-hospital mortality in STEMI patients. Therefore, Ox-LDL may provide an additional adverse effect on CAD patients. Several studies have demonstrated that elevated levels of ox-LDL in acute STEMI individuals relate to coronary plaque inflammation, instability, and disruption with coronary thrombosis (27, 28). In another study, ox-LDL had the strongest correlation with infarct size and with the clinical parameters of heart failure (29). Therefore, the result of the association between ox-LDL and the adverse outcomes found in our study is warranted.

The pathological effects of ox-LDL are mediated by the LOX-1 receptor, one of the major scavenger receptors that sense, take up, or degrade ox-LDL (7). The binding of ox-LDL/LOX- 1 involved in endothelial damage, inflammatory cells recruitment, foam cell formation, and rupture of atherosclerotic plaque (30). LOX-1 is encoded by the OLR1 gene; cellular expression of LOX-1 is minimal under physiological conditions but upregulated in case of oxidative, inflammatory, and atherosclerotic stimuli (10). Deletion of the OLR1 gene attenuates the progression of atherosclerosis (31). Many reports have related genetic variations of the OLR1 gene and atherosclerosis by its effect on LOX-1 expression (14, 32). Previous genetic studies evaluating the clinical relevance of OLR1 gene polymorphism and CAD have produced conflicting results, both a protective role against CAD and MI (11, 33, 34) and a risk role for MI (14, 35) were proposed.

In the study groups, we aimed not only to reveal the relationship between ACS risk in the two patient groups and outcome with ox-LDL concentration but also to study this relation with 3′ UTR OLR1 gene rs1050286 SNP. The results showed that the variant T allele of ORL1 3′ UTR rs1050286 was associated with increased odds of STEMI, OR=4.9, 95% CI: 2.6-9.4, and significantly elevated STEMI risk in the CT and the TT genotypes and the recessive (CC vs TT+CT) model. This indicated the T allele to be a risk factor for STEMI. Our finding was supported by Morini et al. (2016) (14) who reported that the presence of rs1050286 SNP on the 3′ UTR of OLR1 could significantly modify LOX-1 expression that might lead to the increased susceptibility to AMI and CAD. Our results are also in accord with previous studies on CAD patients, Mango et al. observed that patients with TT or CT genotypes at OLR1 3′ UTR polymorphism are at increased risk of acute STEMI with OR of 3.74 (32), and Zhang et al. who showed significant associations between AA genotype of ORL-1 (rs1050286) with an increased risk of carotid plaque (35). In contrast, Knowles et al. found that rs3736232 and rs1050286 SNP polymorphism has no association with the risk of CVD and the minor allele was associated with a lower odds ratio of CVD risk (33). In another study, the

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polymorphism within the OLR1 3′ UTR was significantly associated with IgM anti-ox-LDL and CC genotype showed the highest levels suggesting that the C allele is protective against coronary stenosis (36).

The postulated mechanism by which ORL1 rs1050286 SNP could affect the risk of CAD is that when SNPs occur in 3′- UTR-OLR1 rs1050286 it will alter the regulatory role of MicroRNAs (miR-24) on OLR**1** expression that may contribute to modify susceptibility to CAD and AMI (14), MiR-24 are noncoding RNAs located in the 3′-untranslated region control protein-coding genes thus influencing all cellular pathways which may lead to altered disease susceptibility (37). Another intriguing result is that the subjects who carried the T allele in the STEMI group exhibited significantly higher Killip class, GRACE, and TIMI risk scores with an increase in the proportion of patients harboring coronary multivessel narrowing. In our study genotypes were identified to have an association with the burden of STEMI so that T allele carriers either CT or TT are prone to the incidence of some in-hospital major adverse clinical events in STEMI patients. Notably, in this study, ten patients with STEMI patients died during hospitalization. Although the number was insufficient for statistical analysis, the genotypes of these patients were CT (six patients) and TT (four patients).

We found a concomitance of some CV risk factors associated with the T allele. BMI was higher among CT and TT genotypes in both STEMI and UA patients, T allele carriers were found to be significantly associated with diabetes duration, systolic blood pressure, and glucose levels among STEMI patients. Thus, under these CV risk factors, the genetic predisposition associated with the T allele could further enhance the risk and compromise adverse outcomes.

In all patients, CT and TT carriers have increased ox-LDL levels compared with CC carriers. Therefore, this may have an additive effect on the risk of ORL1 (rs1050286) variations. Additionally, ox-LDL can upregulate the LOX-1 receptor as other risk factors for CAD, and inflammatory stimuli can do (10).

The strengths of the current study are a large number of recruited patients from the critical care unit. However, some limitations cannot be ignored. First, we only evaluated the in-hospital adverse outcome of the patients; a long-term outcome assessment will provide more value to the tested variables. Second, as in other genetic studies, various confounders especially comorbidities may have developed and influenced the results.

In conclusion, the novel contribution of this investigation is the impact of plasma ox-LDL as well as genetic variation in ORL-1 (rs1050286) SNP on ACS risk and clinical outcome, especially in people with STEMI. Importantly, the determination of the ox-LDL level and genetic tests for ORL-1 (rs1050286) SNP variants will add further information to the established risk factors. This can help in identifying patients with in-hospital deteriorating outcomes who require more intensive monitoring and treatment.

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