An Association of High Mobility Group Box-1 with Lipid Profile in Iraqi Patients with Unstable Angina

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Abstract
Background: Numerous studies have recently looked into the function of inflammation in coronary artery disease and the relationship between inflammatory biomarkers and unfavorable outcomes. This study is focus into the association between Lipid Profile of serum High mobility group box 1 (HMGB1) protein and known risk factors for Unstable Angina. Methods: The study involved 50 participants 20 female and 30 male and 40 controls 20 female and 20 male who came to Al Ramadi Teaching Hospital issues between November 2021 and April. The average age of patients was 52.16±8.423 years, compared to 49.58±8.098 years for controls. Results: In our investigation, we found that UA patients had much higher TG, LDL, and VLDL levels than controls, while controls had significantly lower levels of HDL. Total cholesterol, TG, LDL, and VLDL levels in patients with unstable angina were shown to be greater than in the control group. Conclusions: The study found that blood levels of LDL, TG, and VLDL, which are risk factors for potential heart and blood vessel illnesses, are increasing in people with unstable angina. Patients with unstable angina had increased serum HMGB-1 levels, and all of these indicators can be utilized to identify these patients.
Keywords: Coronary artery disease, HMGB1, Lipid Profile, Unstable Angina, inflammation.

1. Introduction

Acute myocardial ischemia brought on by severe coronary artery stenosis or occlusion brought on by thrombosis is known as acute coronary syndromes, which is a wide term for a group of diseases that can cause unstable angina (UA), acute myocardial infarction (AMI), or sudden cardiac death. Unstable angina pectoris (UAP), non-ST elevation myocardial infarction (N-STEMI), and ST-elevation myocardial infarction are just a few of the clinical conditions that are included in the term "ACS" (1). (STEMI) (2) A disease characterized by symptoms and signs of acute myocardial ischemia, or a reduction in cardiac blood flow brought on by coronary artery atherosclerosis, is referred to as ACS. A third of the population in affluent nations experience unstable angina pectoris (UAP), a typical consequence of coronary heart disease (CHD), before the age of 70. (3) The diagnosis of UA is made using clinical criteria based on the length and severity of angina. The prognosis for adverse outcomes in UA patients varies, depending on factors such AMI, recurrent angina, necrosis biomarkers, ventricular function, and the need for myocardial revascularization. (4)
High mobility group box protein 1 has been linked to the pathogenesis of other vascular disorders. A prior study found that HMGB1 increases the production and secretion of monocytes, other inflammatory cytokines, and cell adhesion molecules by vascular endothelial cells. (5) In addition to assisting in the exploration of biological pathways, lipid analysis aids in the discovery of potential diagnostic, predictive, and monitoring biomarkers. In chronic inflammatory systemic disorders, such as atherosclerosis-related cardiovascular events like UAP and MI, lipids can also be exploited to develop new therapeutic tools and learn more about homeostatic and inflammatory pathways. (6) Circulating troponin levels can provide additional risk information beyond what is learned from modifiable cardiovascular risk variables because troponins (cTnI and cTnT) are indicators for heart cell death (7-8). It has been demonstrated that evaluating troponins 6–12 hours result in better risk categorization and earlier diagnosis, due to cTnI levels increase 2-3 hours after MI and can be elevated for up to 10 days. (9) In tissues with high and fluctuating energy requirements, like muscle and the brain, creatine kinase (CK) is a crucial regulator of cellular energy balance. It catalyzes the transfer of the high-energy phosphate moiety (P). (10) In the emergency unit, the assessment of serum CK is a routine test in the diagnosis of ACS, including acute MI and UA. This paper's major objective is to assess blood lipid levels and investigate the connection between individuals with unstable angina and the High Mobility Group Box 1. this paper aimed to examined whether serum HMGB1 and lipid profiles levels are related to risk factors for unstable angina, via evaluation their levels in serum for individuals with unstable angina.

2. Materials and Methods

The serum was acquired before the procedures. The study, which was conducted between November

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2021 and April 2022, involved 50 patients 20 female and 30 males. They were admitted to the emergency unit at Al Ramadi Teaching Hospital because they experienced with unstable angina following their visit to the cardiology department, which was based on their ECG readings, troponin testing, physical examination, and clinical indicators. The following parameters were measured using colorimetric enzymatic methods by commercial kits from Roche, Switzerland: hs-cTnT, CK-MB, total cholesterol, triglycerides, HDL, LDL, and VLDL; while HMGB1 was determined by ELISA technique (Melson Company, China).

2.1 Blood Collection

Five mL of venous blood was obtained from each patient and control group and allowed to coagulate for 15 minutes at room temperature (20-27°C) before being centrifuged for 15 minutes at 4000 xg, separated into two parts, the first part was used immediately to estimate the variables: hs-cTnT, CK-MB, total cholesterol, triglycerides, HDL, LDL, and VLDL, while the second part was transferred to Eppendorff tube and stored at -20 °C for later use in estimation of HMGB1

2.2 Statistical Analysis

Analysis of data was carried out using the GraphPad prism version 7. Data were presented in simple measures of mean, standard deviation (SD) and standard error of mean (SEM) values. The significance of difference of different means (quantitative data) was tested using Students-t-test for difference between two independent means. Statistical significance was considered whenever the P value was equal or less than 0.05. Pearson correlation was calculated for the correlation between two quantitative variables with its t-test for testing the significance of correlation. The correlation coefficient value (r) either positive (direct correlation) or negative (inverse correlation) with value <0.3 represent no correlation, 0.3-<0.5 represent weak correlation, 0.5-<0.7 moderate strength, >0.7 strong correlation. In addition to correlation the r2 was calculated (The coefficient of determination), i.e., when value of r=0.58, then r2=0.34, this means that 34% of the variation in the values of y may be accounted for by knowing values of x or vice versa. Receiver operator curve (ROC) analysis was also employed. The area under the ROC curve gives an idea about the usefulness of a tested parameter in differentiating between three groups (one of which is a control group). In this context the ROC analysis helps in comparing selected parameters to others. The closer the area to one (ideal test) the more useful it is in discrimination.

3. Results

The present study includes 50 patients with unstable angina (UA) (20 female & 30 male), and 40 healthy individuals (20 female & 20 male). The ages of both groups approximately had the same values Figure 1. The mean of age has no significant difference in patient's group as compared with control group, whereas the mean of BMI significantly increased in patients' group (32.23±4.925 vs 23.38±1.155) in comparison to the control group as shown in table 3.1.

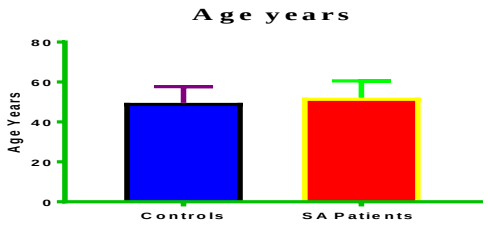


Fig. (1): Mean+ S.D for Age in Control and SA Patients

Table 3.1 Baseline clinical parameters of unstable angina patients and controls.

	Healthy controls			SA patients			
Parameter	Mean	SD	SEM	Mean	SEM	SD	p-value
Age years	49.58	8.098	1.28	52.16	8.423	1.191	0.1447
HMGB1 pg/ml	5480	2043	323.1	34629	8485	1200	<0.0001
hs-cTnT ng/mL	3.231	0.5756	0.09101	6.723	2.331	0.3297	<0.0001
CPK-MB IU/L	7.938	2.097	0.3315	16.48	3.17	0.4483	<0.0001
T. Cho. mg/dL	168.2	32.13	5.08	174.6	34.31	4.852	0.3668
TG mg/dL	117.1	35.55	5.621	164.7	76.12	10.76	0.0004
HDL mg/dL	44.33	7.116	1.125	38.62	9.981	1.412	0.0031
LDL mg/dL	91.05	18.57	2.974	101	24.29	3.435	0.0373
VLDL mg/dL	23.4	7.14	1.129	32.93	7.552	1.068	<0.0001
LDL/HDL	2.111	0.5473	0.08654	2.759	0.8797	0.1244	0.0001
T. Cho./HDL	3.847	0.7956	0.1258	4.832	1.66	0.2348	0.0009

The results of present study revealed a significant difference (p <0.05) in serum HMGB-1(pg/mL) concentration in patients with unstable angina (34629±8485 vs 5480±2043) compared to control group, as shown in table 3.1 and (figure 2).

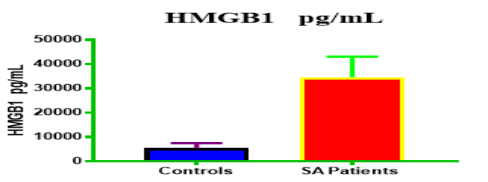


Fig. (2): Mean+ S.D for HMGB1 in Control and UA Patients

Results of this study showed a significant increase ($p < 0.05$) in serum levels of hs-cTnT (ng/mL) in UA Patients (6.723±2.331 VS 3.231±0.5756) compared with control group, as shown in table (3.6) appendix (figure 7). Our results also showed that there was significant increase ($p < 0.05$) in serum levels of CPK-MB (IU/L) in UA Patients (16.48±3.17 VS 7.938±2.097) when compared to control group, as shown in table (3.1) (figure 3).

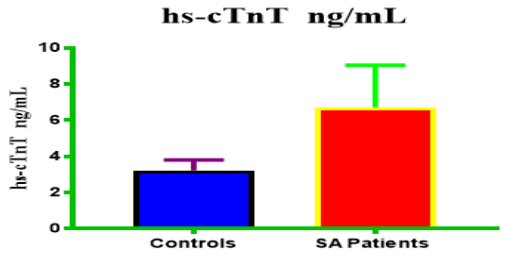


Fig. (3): Mean+ S.D for hs-cTnT in Control and UA Patients

Our results demonstrated a slight increase in troponin and CPK-MB serum levels. However, these elevated levels were within the normal range value of these biomarkers, which indicates there are no necrosis signs in UA patients, as shown in table (3.1) (figure 4).

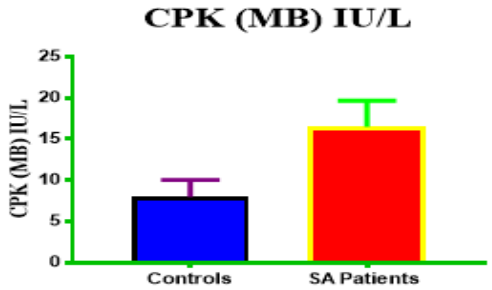
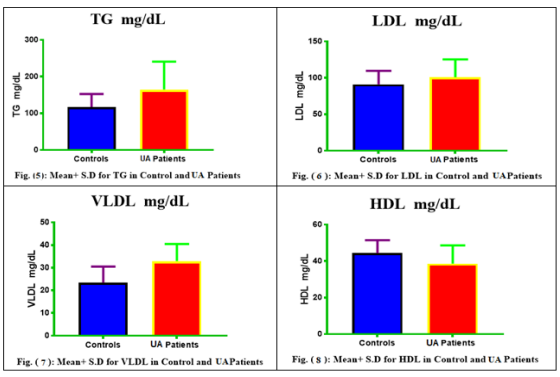


Fig. (4): Mean+ S.D for CPK in Control and UAPatients

In our findings observed significant increased levels of TG, LDL, VLDL and significant decreased level of HDL in UA cases as compared to controls as shown in table (3.1) (figures 5-8) respectively. Our findings were consistent with a recent study that demonstrated lipid metabolism abnormalities. In patients with unstable angina, total cholesterol, TG, LDL, and VLDL levels were shown to be higher than in the control group (11). Moreover, a study on Iraqi patients with unstable angina observed increased levels of LDL, TG, and VLDL in the blood, which are considered risk factors for potential heart and blood vessel disorders (12).



The findings revealed that there was a non-significant difference in T. Cholesterol levels (mg/dL) between patients and control group (174.6±34.31 vs 168.2±32.13) as shown in table 3.1 and (figure 9).

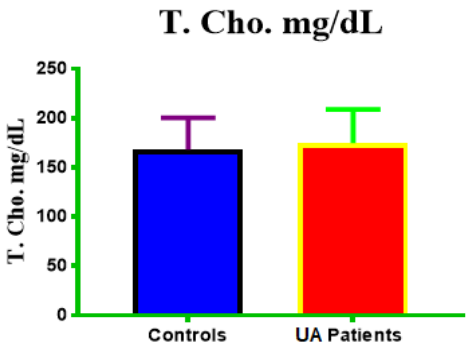
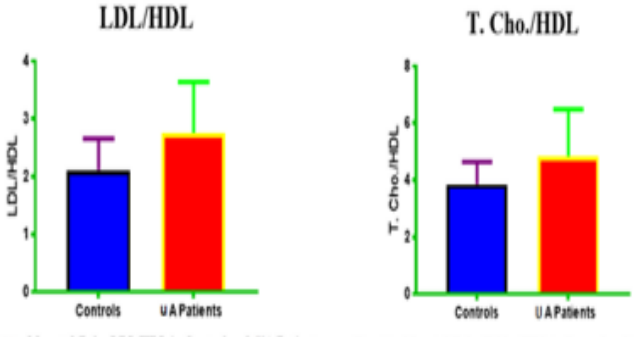


Fig. (9): Mean+ S.D for T.Cho. in Control and UA Patients

Whereas the data of this study revealed 759a significant difference ($p < 0.05$) in the levels of LDL/HDL and T. Cho. /HDL between patients and control group (2. ±0.8797 vs 2.111±0.5473) (4.832±1.66 vs 3.847±0.7956) as shown in table 3.1 and figures (10 and 11) respectively.



3.1 Correlation of High Mobility Group Box-1 with Studied Parameters

From table 3.2 we can see there were many significant correlations had indicated between HMGB1 with studied parameters,

Table (3.2): Correlation of High Mobility Group Box-1 with Studied Parameters

Parameter	r (HMGB1 pg/mL)	p-value
HMGB1 pg/mL	1.000	0.000
Age years	0.114	0.285
hs-cTnT pg/mL	0.695	<0.0001
CPK-MB IU/L	0.833	<0.0001
T.Cho. mg/dL	0.078	0.466
TG mg/dL	0.310	0.003
HDL mg/dL	-0.245	0.020
LDL mg/dL	0.176	0.099
VLDL mg/dL	0.501	<0.0001
LDL/HDL	0.308	0.003
T.Cho./HDL	0.273	0.009

This study reveals a strong correlation of HMGB1 with CPK-MB and hs-CRP at ($P < 0.01$) ($r= 0.833$ and $r= 0.703$ respectively), as shown in figures 12 and 13 respectively, while a moderate correlation between HMGB-1 with VLDL at ($P < 0.01$) ($r= 0.501$) as shown in figure 14, however, Results shown there is no correlation between HMGB1 with age, T. Cho., HDL and LDL.

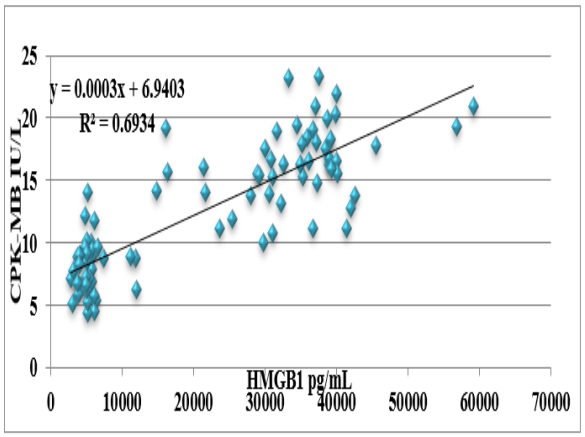


Figure (12): Correlation of HMGB1 with CPK-MB

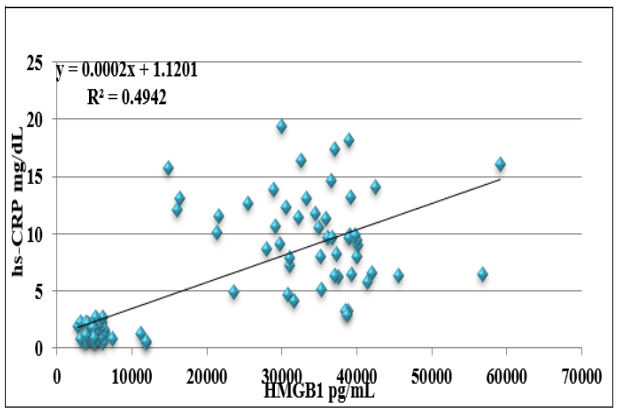


Figure (13): Correlation of HMGB1 with hs-CRP

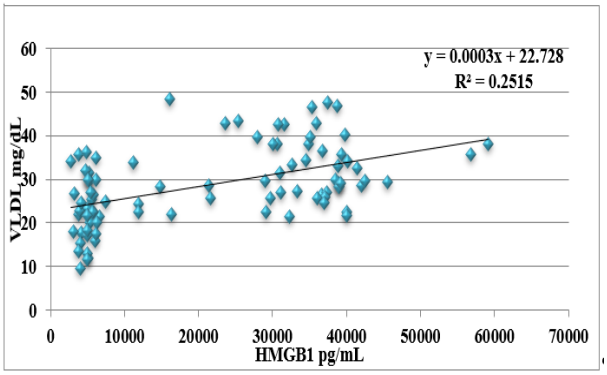


Figure (14): Correlation of HMGB1 with VLDL

An HMGB-1 finding shows a weak positive correlation with TG, LDL/HDL, T. Cho. /HDL, Urea and Creatinine (r= 0.310 at p 0.003, r= 0.308 at p 0.003, r= 0.273 at p 0.009, r= 0.433 at p <0.0001, r= 0.309 at p 0.004, respectively)

3.2 Receiver Operating Characteristic Curve Analysis

In this study, the receiver operating characteristic (ROC) curve and the area under curve (AUC) were used as an effective measure of accuracy. This curve is crucial in determining the optimal cut-off values for evaluating the diagnostic ability of tests to identify the true state of subjects (13). The experimental data within the parameters of current research were evaluated using ROC curves, and the details are provided in table 3.3

Table (3.3): The area under the ROC curve for Studied Parameters

Parameter	AUC	Std. Error	95% confidence interval	P-value
Age years	0.6033	0.06086	0.484 to 0.7225	0.0936
HMGB1 pg/ml	1	0	1 to 1	<0.0001
hs-cTnT ng/mL	0.9735	0.01415	0.9458 to 1.001	<0.0001
CPK-MB IU/L	0.988	0.007956	0.9724 to 1.004	<0.0001
T. Cho. mg/dL	0.533	0.06222	0.4111 to 0.6549	0.5920
TG mg/dL	0.707	0.05463	0.5999 to 0.8141	0.0008
HDL mg/dL	0.7095	0.05407	0.6035 to 0.8155	0.0007
LDL mg/dL	0.579	0.06134	0.4588 to 0.6992	0.2029
VLDL mg/dL	0.8083	0.04538	0.7193 to 0.8972	<0.0001
LDL/HDL	0.7285	0.05287	0.6249 to 0.8321	0.0002
T. Cho./HDL	0.7075	0.05495	0.5998 to 0.8152	0.0008

It is possible to say that the speculative AUC result is significant if the test value is higher than 0.7. The parameters HMGB1, hs-cTnT and CPK-MB were among the standards with the highest validity and displayed an excellent and spectacular strategy for discriminating between healthy people and patients with unstable angina, [AUC = 1, P < 0.0001, 95% Confidence Interval (CI): 1 to 1 and SE: 0] as shown in figure 12, [AUC = 0.9735, P < 0.0001, 95% CI: 0.9458 to 1.001, SE: 0.01415] as shown in figure 13, [AUC = 0.988, P < 0.0001, 95% CI: 0.9724 to 1.004, SE: 0.007956] as shown in figure 14. for above parameters respectively, therefore, it is possible to state these parameters are definitely functional for the diagnosis of unstable angina disease.

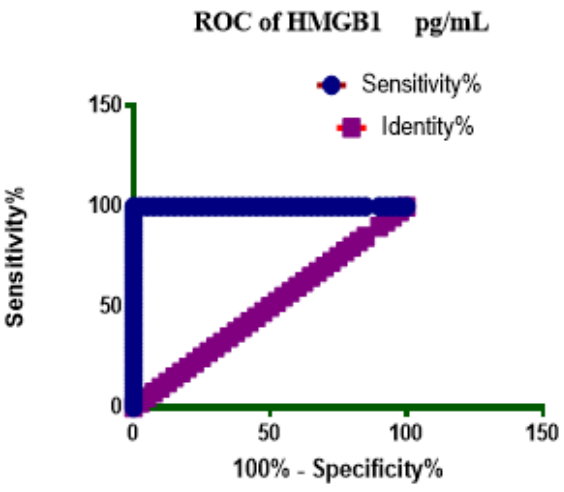


Figure (12): Area under Curve of HMGB-1 in UA Patients.

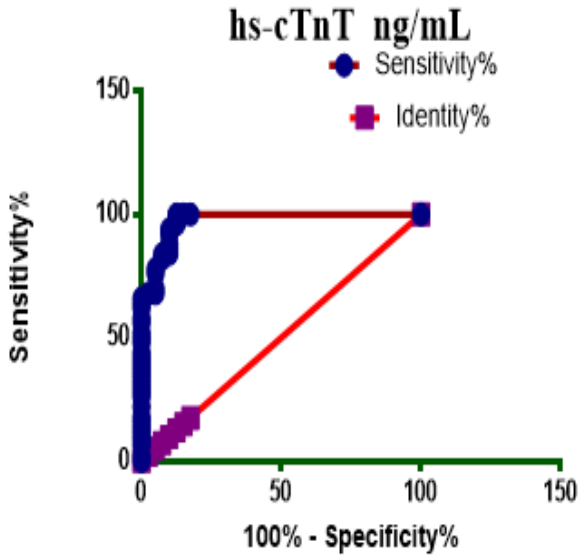


Figure (13): Area under Curve of hs-cTnT in UA Patients.

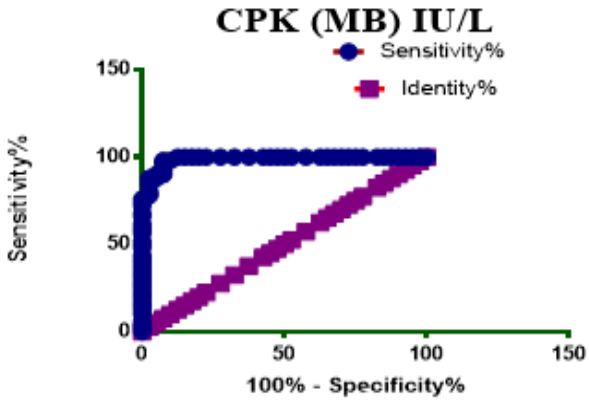


Figure (14): Area under Curve of CPK-MB in UA Patients

While Age, T. Cho., T.G., HDL, LDL, VLDL, LDL/HDL and T. Cho. /HDL not display any strategy for discriminating between healthy people and patients with unstable angina, as shown in in table 3.3 and figures 15-22 respectively.

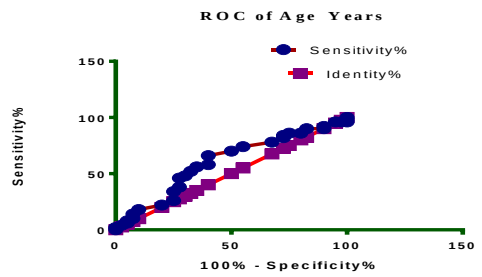


Figure (15): Area under Curve of Age in UA Patients.

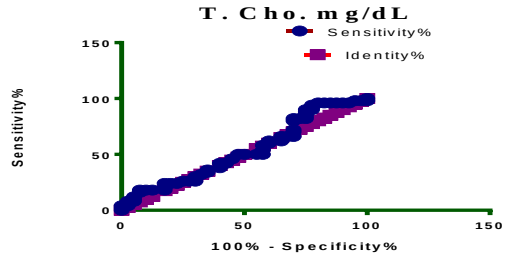


Figure (16): Area under Curve of T. Cho. in UA Patients.

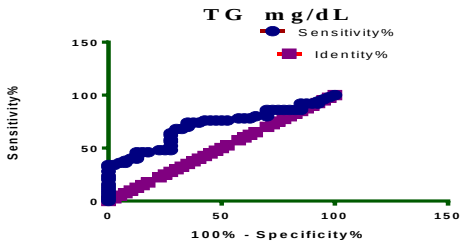


Figure (17): Area under Curve of TG in UA Patients.

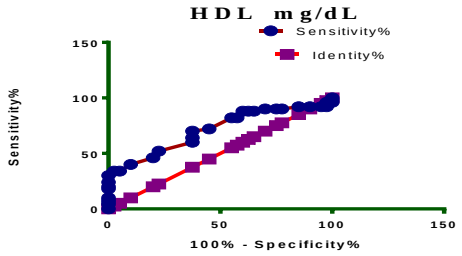


Figure (18): Area under Curve of HDL in UA Patients.

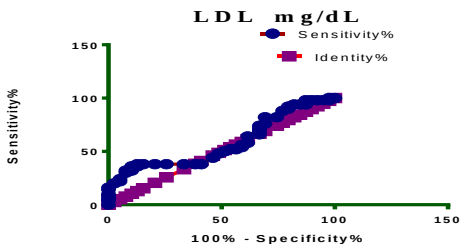


Figure (19): Area under Curve of LDL in UA Patients.

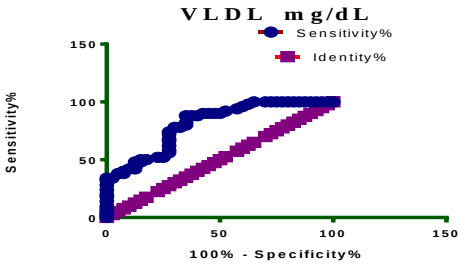


Figure (20): Area under Curve of VLDL in UA Patients.

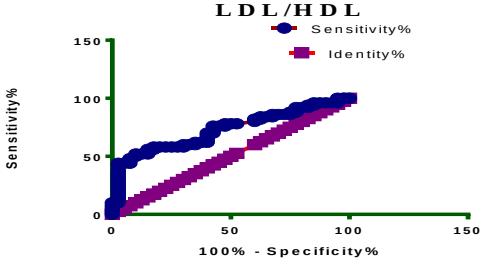


Figure (21): Area under Curve of LDL/HDL in UA Patients.

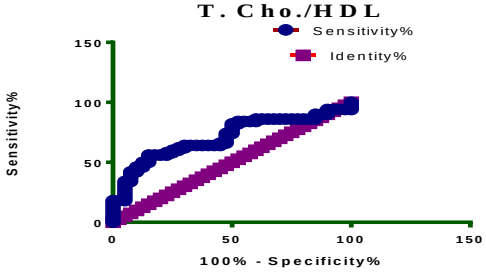


Figure (22): Area under Curve of T. Cho./HDL in UA Patients.

4. Discussion

According to a study that produced findings that were comparable to those of the other studies, those who have ST-Segment Elevation Myocardial Infarction have higher levels of HMGB1 than people who are healthy. Additionally, the study presents evidence for the first time linking greater HMGB1 levels and a higher risk of mortality in STEMI patients who were sampled at admission. These results suggest that plasma HMGB1 may represent a potential biomarker for mortality in STEMI patients (14). A study on patients with unstable angina found that their plasma levels of HMGB1 were considerably higher than those of healthy individuals. Elevated plasma HMGB1 levels in patients with unstable angina may signify atherosclerosis of the coronary arteries. As a result, it can be used as both a diagnostic and a standalone risk factor for clinical assessment in young patients with chest discomfort (15), which is consistent with our findings. Patients with unstable angina are those who do not have myocardial necrosis but do have myocardial ischemia. High-sensitivity cardiac troponin assays have a number of important analytical differences from conventional cardiac troponin assays that may alter how unstable angina is detected and how frequently it occurs (16). According to our findings, serum levels of troponin and CPK-MB have slightly increased. The fact that these higher levels fell within the biomarkers' normal range values, however, shows that there are no symptoms of necrosis in UA patients. Our study's hs-cTnT results were consistent with one showing that people with unstable angina had negative cTn marker test results. (17) Increased levels of LDL, TG, and VLDL in the blood, which are thought to be risk factors for potential heart and blood vessel diseases, were found in a study on Iraqi patients with unstable angina (12). Additionally, a recent study showed abnormalities in lipid metabolism. Total cholesterol, TG, LDL, and VLDL levels in patients with unstable angina were shown to be greater than in the control group (11). These results are in line with our results, in which we found that UA cases had much higher levels of TG, LDL, and VLDL and significantly lower levels of HDL than controls.

5. Conclusion

According to the research, patients with unstable angina had higher serum levels of HMGB-1; this finding explains the importance of these biomarkers and their implications for the pathogenesis of unstable angina. Serum levels of HMGB1 are important, crucial factors in the development of unstable angina in Al- Ramadi city and can be utilized to predict its occurrence. Additionally, levels of hs-cTnT and CPK-MB revealed a substantial positive connection with blood levels of HMGB-1, suggesting that these levels could be used as novel indicators to identify patients with unstable angina.

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