RESEARCH ARTICLE

A Relationship of Dickkopf-1 with Glutathione in Iraqi Patients with Unstable Angina as a Model of Pharmaceutical Compounds

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ABSTRACT

Background: The importance of inflammation in coronary artery disease and the inflammatory biomarkers linked to adverse outcomes have recently been the subject of numerous investigations. In addition to examining the relationship between dickkopf-1 and high mobility group box-1 with other biochemical markers, this study seeks to ascertain the relationship between dickkopf-1 and high mobility group box-1 in patients whose angina pectoris is not stable.

Methods: The work includes 50 patients (20 female and 30 male) and 40, 20 female and 20 males as controls who attended Al Ramadi Teaching Hospital between November 2021 and April. The sample age ranged between 52.16 ± 8.423 years in comparison to 49.58 ± 8.098 for the controls.

Results: The serum DKK-1 levels (ng/mL) rose in unstable angina (UA) patients (135.527.13 for UA patients vs. 36.614.07 for the control group), so UA patients had higher DKK-1 release during platelet aggregation because DKK1 is involved in platelet-induced endothelial cell activation, so DKK1 causes inflammation. Additionally, the GSH was significantly lower in those having UA than in controls.

Conclusion: Serum DKK-1 levels was significantly higher for those with UA compared to the healthy and there is a significant drop in GSH levels in people with UA than in controls.

Keywords: Coronary artery disease, DKK-1, HMGB1, GSH, Inflammation.

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INTRODUCTION

Unstable angina (UA) is fast platelet aggregation on vascular smooth muscle and endothelial cells leading to an abrupt decrease in coronary flow. The process begins by atherosclerotic plaques and progresses when potential pathogenic factors were present.[1] At the site of plaque injury, transient episodes of thrombotic vessel occlusion may develop, leading to angina at rest, resulting in a brief vascular occlusion that may last about 10 to 20 minutes. Chest pain of unstable angina may be more intense and linger longer than with usual stable angina or discomfort that worsens but does not progress to myocardial necrosis.[2] (Un)stable angina, STEMI and non-ST-elevation myocardial infarction (NSTEMI) is caused by coronary plaque development, instability, or rupture, with or without luminal thrombosis and vasospasm. Dickkopf-1 (DKK1) is a protein secreted and identified as a direct inhibitor of the Wnt/B-catenin signaling pathway.[3] WNTs are cysteine-rich glycoproteins that are released and act as short-range ligands activating signaling pathways which mediated by the receptor.[4] Wnt proteins control many cellular processes like cell proliferation, migration, differentiation, genetic stability, and apoptosis.[5] DKK1 production serves as a feedback mechanism for limiting Wnt/b-catenin pathway activation.[6] DKK1's capabilities of inhibiting Wnt/B-catenin activity depends on its direct interaction with the Wnt co-receptor LRP5/6 (low-density lipoprotein receptor-related protein 5 or 6) or indirect interaction by binding to Kremen-1/2 on the cell membrane.[7] Dickkopf-1 inhibits Wnt signaling by a compelete inhibition of LRP5/6 interaction with Wnt and formation of a transmembrane protein Krm (Kremen) complex enhancing LRP5/6 receptor endocytosis and degradation. As a result, the signal via the Wnt/catenin cascade is deactivated now.[8] Glycine, glutamate, and cysteine are the three amino acids making up the tripeptide glutathione (GSH).[9] GSH is thought to be the most significant intracellular antioxidant. It can either work directly by removing reactive oxygen and nitrogen species

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from the body or indirectly by serving as an electron donor to enzymes that remove reactive oxygen species (ROS).[10] The high electron-donating capability of glutathione sets it apart from all other antioxidants. Recent investigations demonstrated significantly lower GSH levels in CAD patients compared to controls, indicating that low GSH levels may be linked to stronger oxidative stress defenses throughout the development of CAD.[11] C-Reactive protein is the family of pentraxin of proteins[12] composed of five identical 23-kDa monomers. Each monomer contains a calcium-dependent phosphocholine binding site.[13] It is mainly produced in the liver,[14] and under pro-inflammatory stimulation, is perhaps produced as an acute phase reactant responding to inflammation or ischemic tissue damage, which the local or systemic producing pro-inflammatory cytokines like interleukin-1 (IL-1) causes, interleukin-6, or tumor necrosis factor-α (TNF- α).[15,16]

MATERIALS AND METHODS

The serum was acquired before the procedures. The research was performed between November 2021 and April 2022, involved 50 patients 20 females and 30 males. They were admitted to the emergency unit at Al Ramadi Teaching Hospital because they experienced unstable angina following their visit to the cardiology department based on their ECG readings, troponin testing, physical examination, and clinical indicators. Commercial kits from Roche, Switzerland, calculated the next parameters: hs-cTnT, CPK-MB, hs-CRP, urea and creatinine, while DKK-1 and GSH were determined by ELISA technique (Melson Company, China).

Blood Collection

In 5 mL venous blood was obtained from each patient and control group and allowed to coagulate for 15 minutes at room temperature (20–27°C) prior to centrifuging for 15 minutes at 4000 xg, separated into two parts, the first part was used immediately to estimate the variables: hs-cTnT, CPK-MB, hs-CRP, urea and creatinine, while the second part was divided into two equal volumes, each volume was transferred to a separate Eppendorff tube and stored at -20°C for later use in estimation of DKK-1 and GSH.

Statistical Analysis

GraphPad prism version 7 was used to analyze the data. The data appeared in simple mean, standard error of mean (SEM) and standard deviation (SD). Students-t-test was used for testing the significant different in different means (quantitative data) by the variance between two independent means. The statistical significance was used when the *p-value* became equal or lower than 0.05. We calculated the Pearson correlation between two quantitative variables with its t-test examining the correlation significance whose value (r) are either positive (direct correlation) or negative (inverse) with value <0.3 without correlation, 0.3-<0.5 as weak correlation, 0.5-<0.7 moderate strength, >0.7 strong. Also, the r^2 was measured (The coefficient of determination), i.e. if value of r=0.58, then r^2=0.34, then 34% of the variation in y is obtained by x values or *vice-versa*. We used the receiver operator curve (ROC). The zone beneath the ROC curve explains how useful a tested parameter to differentiate the three groups (one of which is a control group). So, the ROC analysis compares the chosen parameters to others. When the area is close to one (ideal test), it is beneficial for discrimination.

RESULTS

The present study includes 50 patients with 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 the patient's group in reference to the controls, whereas the mean of BMI significantly increased in the patients group (32.23 ± 4.925 vs 23.38 ± 1.155) in reference to the control group as Table 1 shows.

This study indicates that DKK-1 (ng/mL) serum levels is critical in the development of cardiovascular diseases, were serum levels of DKK-1 (135.8 ± 27.13 vs 36.6 ± 14.07) in UA patients as compared to HCs respectively, this finding supported by recent study High DKK1 level were found to be linked to cardiovascular mortality on patients having acute coronary syndromes[17] (Table 1 and Figure 2).

The mean of BMI significantly increased in patients group (32.23 ± 4.925 *vs* 23.38 ± 1.155) in reference to the control group as shown in Table 1 and Figure 3.

The findings in Table 1 and Figure 4 are supported by the findings of the recent studies,[18-20] which demonstrate that the (mmol/L) was significantly higher than the level of GSH of the control group than that of the patients (16.65 ± 1.741 vs 3.792 ± 1.349), respectively. So, compared to the healthy controls, UA patients reduced GSH levels.

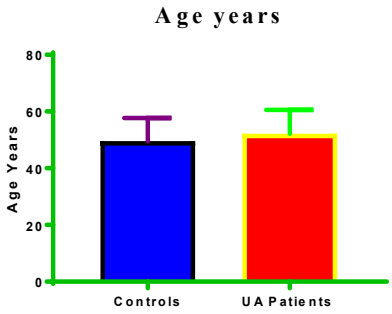

Figure 1: Mean + S.D for age in control and US patients

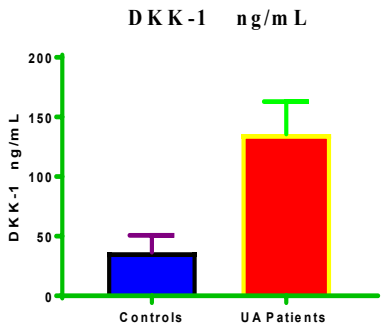

Figure 2: Mean+ S.D for DKK-1 in Control and UA Patients

Table 1: Baseline clinical parameters of unstable angina patients and controls

Parameter	*Healthy controls*			*US patients*			
	Mean	*SD*	*SEM*	*Mean*	*SD*	*SEM*	*p-value*
Age (in years)	49.58	8.098	1.28	52.16	8.423	1.191	0.1447
BMI (kg/m^2)	23.38	1.155	0.1826	32.23	4.925	0.6965	<0.0001
DKK-1 (ng/mL)	36.60	14.07	2.225	135.8	27.13	3.837	<0.0001
GSH (mmol/L)	16.65	1.741	0.2753	3.792	1.349	0.1908	<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
hs-CRP (mg/dL)	1.39	0.6543	0.1034	9.91	4.051	0.5729	<0.0001
Urea (mg/dL)	25.73	5.782	0.9143	34.5	10.62	1.502	<0.0001
Creatinine (mg/dL)	0.7275	0.1281	0.02025	0.866	0.2689	0.03922	0.0038

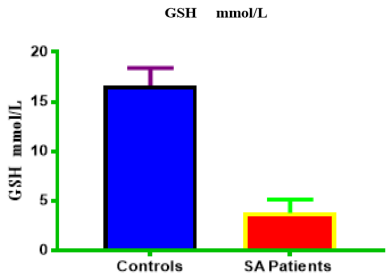

Figure 3: Mean + S.D for GSH in control and UA patients

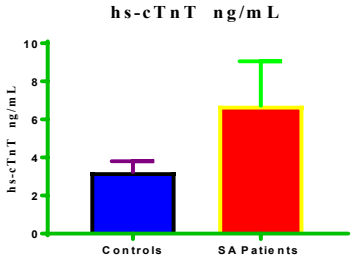

Figure 4: Mean + S.D for hs-cTnT in Control and SA Patients

The increase ($p < 0.05$) in serum levels of hs-cTnT (ng/mL) in UA Patients (6.723 ± 2.331 vs 3.231 ± 0.5756) was significant compared with the control group, as in Table 1 and Figure 5. These agree with a study that indicated patients with unstable angina had a negative cTn marker test result.[21] In addition, there was a 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) in reference to the controls, as shown in Table 1, and Figure 6.

There was a significant rise ($p < 0.05$) in levels of hs-CRP (mg/L) in patients group in reference to control group (9.91 ± 4.051 *vs* 1.39 ± 0.6543), respectively, as in Table 1 and Figure 7.

The study found out significant increases ($p < 0.05$) in level of urea (mg/dL) in the patients group compared with control group (34.5 ± 10.62 *vs* 25.73 ± 5.782), respectively as in Table 1 and Figure 8. Yet results of creatinine level showed that the patients and control groups are significantly different, as in Table 1.

This article showed the DKK-1 correlations and other variables with results in Table 2.

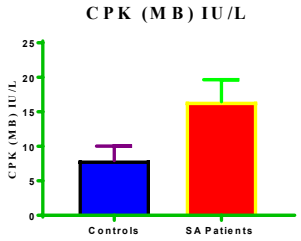

Figure 5: Mean+ S.D for CPK in control and SA patients

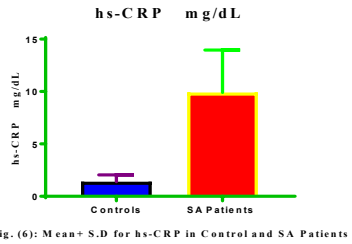

Figure 6: Mean + S.D for hs-CRP in control and SA patients

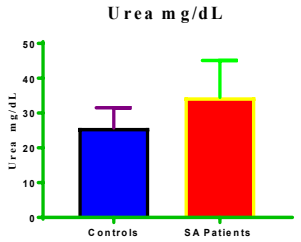

Figure 7: Mean+ S.D for urea in control and SA patients

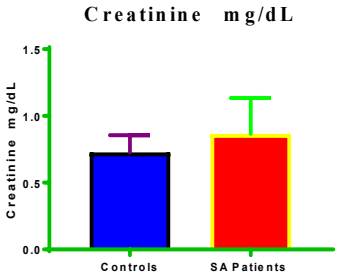

Figure 8: Mean+ SD for creatinine in control and SA patients

Table 2: Correlation of DKK-1 with studied parameters

Parameter	*(DKK-1 ng/mL)*	*p-value*
DKK-1 (ng/mL)	1.000	0.000
GSH (mmol/L)	-0.874	<0.0001
Age (years)	0.147	0.168
hs-cTnT (pg/mL)	0.629	<0.0001
CPK-MB (IU/L)	0.743	<0.0001
hs-CRP (mg/dL)	0.789	<0.0001
Urea (mg/dL)	0.465	<0.0001
Creatinine (mg/dL)	0.344	0.001

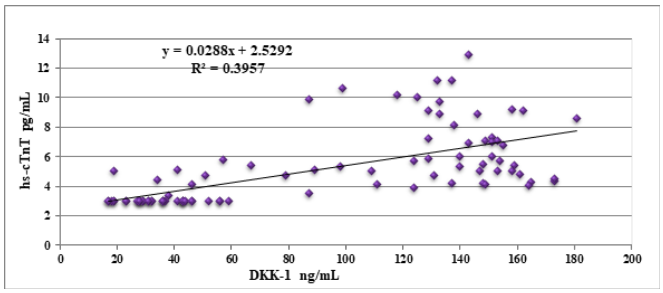

Figure 9: Correlation between DKK-1 and hs-cTnT

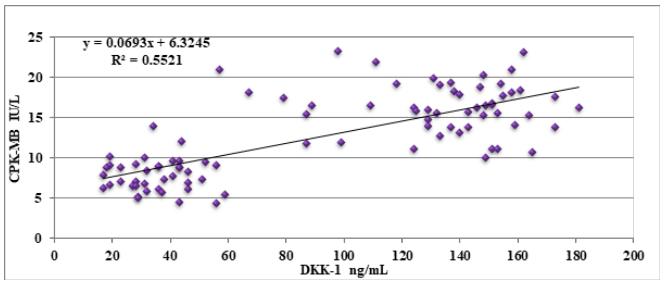

Figure 10: Correlation between DKK-1 and CPK-MB

Results showed that DKK-1 did not correlate with age, while, there are a strong positive correlation with hs-cTnT, CPK-MB (r = 0.629, r = 0.743 at p < 0.01, respectively) as shown in Figures 9 and 10, respectively,

A strong correlation between DKK-1 and hs-CRP (r= 0.789 at p < 0.01) which Figure 11 shows. Besides, there was a strong negative correlation of DKK-1 with GSH (r=-0.874 at p < 0.01), as shown in Figure 12.

Receiver Operating Characteristic Curve Analysis

The experimental data within the parameters of current research were evaluated using ROC curves, and the data are given in Table 3.

Perhaps the speculative AUC result is significant when the test value more than 0.7. The parameters hs-CRP and GSH were among the standards that are highly valid with an excellent strategy to discriminate between the healthy and those with unstable angina, [AUC = 1, p < 0.0001, 95% CI: 1 to 1 and SE: 0] as shown in Figure 13, [AUC = 1, p < 0.0001, 95% CI: 1 to 1 and SE: 0] as shown in Figure 14 for above parameters respectively, therefore, it is possible to state that these parameters are definitely functional for the diagnosis of unstable angina disease

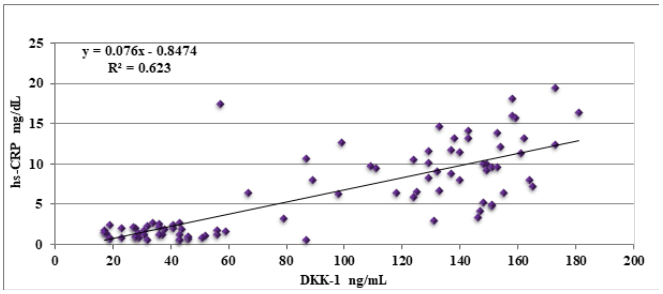

Figure 11: Correlation between DKK-1 and hs-CRP

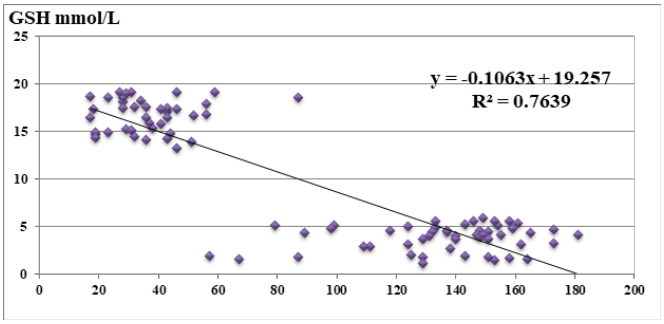

Figure 12: Correlation between DKK-1 and GSH

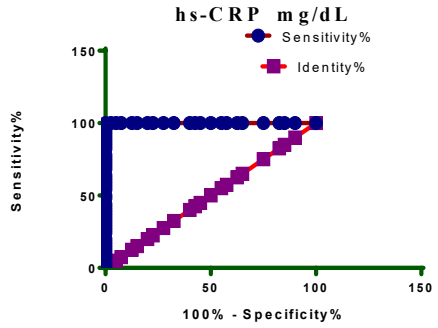

Figure 13: Area under curve of hs-CRP in UA patients

Results of this study revealed that DKKP-1 is very critical with values [AUC =0.9978, p < 0.0001, 95% CI: 0.9931 to 1.002, SE: 0.00239] as shown in Figure 15. In addition, the cardiac enzymes hs-cTnT and CPK-MB they also provide an excellent test. [AUC = 0.9735, p < 0.0001, 95% CI: 0.9458 to 1.001, SE: 0.01415] as shown in Figure 16 and [AUC = 0.988, p < 0.0001, 95% CI: 0.9724 to 1.004, SE: 0.007956] as shown in Figure 17, respectively

DISCUSSION

This work examined the correlations of DKK-1 and other variables. The results revealed that the DKK-1 did not correlate with age, while a strong positive correlation with HMGB1 with statistically significant and not ignored (r = 0.814 at p < 0.01) with a strong positive correlation with hs-cTnT, CPK-MB (r = 0.629, r = 0.743 at p< 0.01, respectively), and a strong correlation between DKK-1 and hs-CRP (r= 0.789 at p < 0.01), yet a moderate positive correlation between emerged, whereas DKK-1 shows that the correlation with GSH (r=-0.874 at p < 0.01) is strongly negative.

Table 3: The zone beneath the ROC curve for studied parameters

Parameter	*AUC*	*Std. Error*	*95% confidence interval*	*P-value*
Age (in years)	0.6033	0.06086	0.484 to 0.7225	0.0936
BMI (kg/m^2)	0.981	0.01271	0.9561 to 1.006	<0.0001
DKKP-1 (ng/mL)	0.9978	0.00239	0.9931 to 1.002	<0.0001
GSH (mmol/L)	1	0	1 to 1	<0.0001
hs-cTn (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
hs-CRP (mg/dL)	1	0	1 to 1	<0.0001
Urea (mg/dL)	0.7618	0.05063	0.6625 to 0.861	<0.0001
Creatinine (mg/dL)	0.6612	0.05836	0.5468 to 0.7755	0.0099

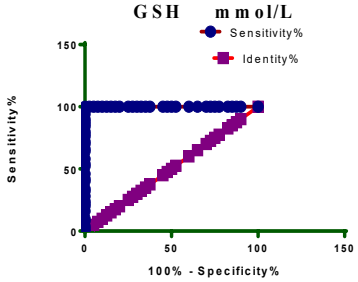

Figure 14: Area under Curve of GSH in UA Patients.

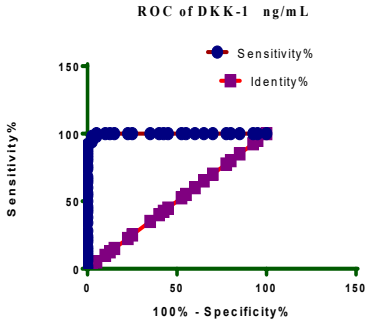

Figure 15: Area under Curve of DKK-1 in UA Patients

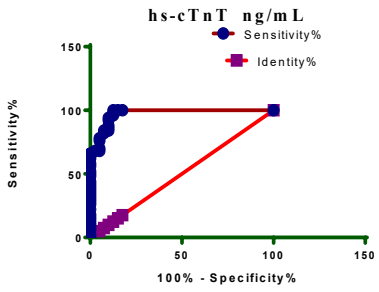

Figure 16: Area under curve of hs-cTnT in UA patients.

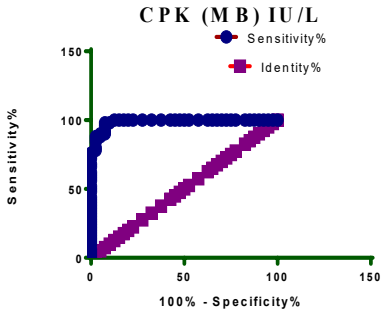

Figure 17: Area under curve of CPK-MB in UA patients

CONCLUSION

Serum levels of DKK-1 are critical in causation unstable angina in Al- Ramadi city; this variable is used to predict unstable angina and may treat unstable angina. Serum DKK1 level was inversely associated with GSH, which means DKK1 and GSH is perhaps significant in unstable angina in this clinical setting. This work has examined a new aspect of the complex interplay between inflammation and oxidative stress in angina, which is not unstable. The current study serum level of DKK-1 revealed a strong positive correlation with hs-cTnT, hs-CRP, CPK-MB, and each other. That means serum level of DKK-1 may be used as new variables to determine unstable angina in Iraqi patients. The current data have shown that serum DKK1 level was higher in unstable angina patients; this result has explained this biomarker's significance and implications for the pathophysiology of unstable angina. Current data showed lower GSH concentrations linked the development of unstable angina and illustrates the fat peroxidation significance implying unstable angina pathophysiology. Measuring fat peroxidation biomarkers in the blood is perhaps useful in testing unstable angina, so oxidative damage is critical in developing unstable angina.

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