

The Role of Some Inflammatory Markers (IL-6 and CRP) in the Pathogenesis of Acute Coronary Syndrome in Iraqi CCU for Heart Diseases

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Abstract

In this work an enzyme linked immunosorbent assay (ELISA) technique has been used for detection of some inflammatory markers in serum of acute coronary syndrome (ACS)-Patients Admitted to the cardiac care unit (CCU) of Iraqi Centre For Heart Diseases and Ibn AlNafees Teaching Hospital. The present method includes quantitative measurement of interleukine-6 (IL-6) and C-reactive protein (CRP), as their increase during symptoms may be responsible for identifying the mechanism of myocardial damag, in addition to their best performance than other quantitative tests perhaps due to their association with atherosclerotic process that belongs to the endothelial dysfunction. Aim of this study is to estimate the prevalence and correlation of IL-6 with CRP in ACS-patients presented with unstable angina/ non-ST elevation myocardial infarction (UA/NSTEMI) symptoms to be as new diagnostic parameters in Iraqi CCU. Seventy (70) ACS- patients with mean age (58.55 year \pm 9.98), from Jun.2009 to Feb. 2010 with diagnosis of UA/NSTEMI were included in this study. Proper history, physical examination, electrocardiograph (ECG), and Echocardiography (ECHO) were performed for all patients in addition to the routine laboratory works including fasting blood glucose, lipid profile, assay of transaminases activity (Aspartate and Alanine transaminase),and biomarkers analysis as cardiac troponin I and T, creatine kinase (CK and CK-MB) and myoglobin. Blood sample was collected from all patients for quantitative assay of IL-6 and CRP. All patients underwent diagnostic coronary angiography, were 66 of them with abnormal coronary outcome and four patients have normal coronary arteries, study include 39- male and 31 female. Seventeen of 67 patients (25.4%) had elevated serum level of IL-6 and fifty four of 62 patients (87.1%) had elevated serum level of CRP. Statistically found strong and significant (SS) correlation between IL-6 and CRP (assessed by Spearman's rho correlation coefficient, $P < 0.01$). The significant proportion of UA/NSTEMI patients that had elevated serum levels of IL-6 and/or CRP, in addition to the strong correlation with coronary angiographic findings make these inflammatory markers to be considered as risk stratification factors and good predictors for coronary artery disease independent of other traditional risk factors for cardiovascular disease (CVD).

Key words: ACS. IL-6. CRP.

الخلاصة

تم في هذا العمل استعمال اختبار الامتصاص المناعي الإنزيمي للكشف عن بعض المؤشرات الالتهابية في أمصال مرضى متلازمة الشرايين الإكليلية الحاد الراقدين في وحدات العناية بالقلب. واشتملت الدراسة على الطرق الكمية لقياس الحريك الخلوي-6 (أو البين ابيضاضي) وبروتين الطور الحاد (أو بروتين سي الفعال)، حيث إن زيادتهم بالدم أثناء حدوث الأعراض محتمل أن يكون المسؤول عن التعريف بالية الضرر بعضلة القلب، هذا بالإضافة إلى أدائهم المفضل عن بقية الاختبارات الكمية وذلك لعلاقتهم بتصلب الشرايين الذي يعود إلى الاختلال الوظيفي للبطانة. الهدف من الدراسة لتقدير انتشار وارتباط الحريك الخلوي-6 و بروتين سي الفعال في مرضى متلازمة الشرايين الإكليلية الحاد مع أعراض الذبحة الغير مستقرة واحتشاء العضلة القلبية الغير مصحوبة بارتفاع ST-segment لتكون هذه الاختبارات كأدوات تشخيص جديدة في وحدات العناية بالقلب في العراق. شملت الدراسة سبعون مريضاً بالمتلازمة معدل أعمارهم (58.55 year \pm 9.98) ابتداءً من حزيران 2009 ولغاية شباط 2010. تم أخذ التاريخ المرضي وإجراء الاختبارات الفيزيائية، تخطيط كهربائية القلب وتخطيط صدى القلب للمرضى. عينات الدم جمعت من كافة المرضى لقياس الحريك الخلوي-6 و بروتين سي الفعال. جميع المرضى خضعوا للقسطرة الإكليلية التشخيصية التي أظهرت نتائج غير طبيعية للشرايين الإكليلية ل(66) مريض و أربعة مرضى لديهم شرايين إكليلية طبيعية. شملت الدراسة 31 ذكر و 39 أنثى. 17 مريض (25.4%) من أصل 67 لديهم ارتفاع بمستوى الحريك الخلوي-6 (اعتبر التركيز 25 بيكوغرام/مل كنقطة قطع لارتفاع مستوى الحريك الخلوي-6 بالدم) و 54 مريض (87.1%) من أصل 62 لديهم زيادة بمستوى بروتين سي الفعال (باعتبار التركيز 3.83 ملغم/لتر كنقطة قطع لزيادة مستوى بروتين سي الفعال بالدم). احصائياً وجد ارتباط معنوي قوي بين الحريك الخلوي-6 و بروتين سي الفعال ($P < 0.01$) لعموم مرضى المتلازمة وكذلك بين الـ 66 مريض ذوي الإصابات الإكليلية. النسبة المعنوية من مرضى الذبحة الغير مستقرة واحتشاء العضلة القلبية الغير مصحوبة بارتفاع ST-segment الذين لديهم زيادة بمستويات الحريك الخلوي-6 و/ أو بروتين سي الفعال وارتباطهم القوي بنتائج القسطرة الإكليلية يجعل هذه المؤشرات الالتهابية كعوامل خطيرة وتوقع جيدة لأمراض الشرايين الإكليلية مستقلين عن عوامل الخطورة المتعارف عليها في أمراض القلب الوعائية.

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Introduction

ACS is "any group of clinical symptoms compatible with acute myocardial ischemia," which includes UA (angina is coronary insufficiency and an important distinction between stable angina and UA is that the former is exacerbated by activity or emotional stress and relieved by rest and/or nitroglycerin; in contrast, UA occurs at rest) and myocardial infarction (MI), with or without ST- elevation (NSTEMI is associated with myocardial necrosis and resultant release of cardiac biomarkers and the ECG usually shows ST-segment depression or new T-wave inversion; in contrast, STEMI, which was once characterized as Q-wave MI, is associated with myocardial damage, with both elevated serum cardiac biomarker levels and ST-segment elevation on ECG^[1,2,3]^[4]. The differences between UA and MI in prevalence, etiology, severity, pathophysiology, clinical presentation, treatment, and outcomes^[5]. Patients presenting with symptoms that are consistent with an ACS require urgent evaluation because these conditions carry a high risk of avoidable complications, such as sudden death and MI^[6]. It caused by one of two events: rupture of the fibrous cap of an atheromatous plaque or endothelial (intimal) erosion of the cap, both of which lead to the subsequent development of a thrombus and decreased myocardial perfusion^[7-10], but research has shown that inflammation plays a central role^[8,11,12], as shown in figure(1) demonstrating that causative agents of necrosis are the risk factors which play a great role in the inflammatory response. The risk markers that are indicative of an adverse prognosis include recurrent ischemia, extensive ECG changes at rest or during pain, the release of biochemical markers (creatin kinase or troponin) arrhythmias and hemodynamic complications during episodes of ischemia. Risk stratification is important because it guides the use of more complex pharmacological and interventional treatment. In those patients with UA and non-Q-wave MI, early diagnosis results in the admission of these patients to the CCU or to a monitored bed in a higher dependency area^[13]. Increasing age is associated with a significant increase in adverse outcomes in patients with UA/NSTEMI. Diabetic patients with UA/NSTEMI are approximately 50% higher risk than non diabetics. Patients with extra cardiac vascular disease (i.e., those with either cerebrovascular disease or peripheral arterial vascular disease) also appear to have approximately 50% higher rates of death or recurrent ischemic events^[14]. Cardiac cath.

provided for detailed assessment of the anatomy and physiology of the heart and vasculature and to determine the nature and extent of a suspected cardiac problem in a symptomatic patient in whom surgical, electrophysiologic, or interventional therapy is anticipated. Also coronary angiography is the most widely used imaging tool to evaluate luminal, obstructive disease in coronary arteries, but the modality is not optimal for identifying plaque^[15]. The function that cytokines induce can both turn on or turn off particular immune responses^[16]. IL-6 is an 'upstream' pro-inflammatory cytokine that stimulates hepatocytes to synthesize acute phase response proteins such as C-reactive protein (CRP) and fibrinogen^[17,18]. Kumari and his colleague propose a role for IL-6 in the pathogenesis of coronary heart disease (CHD) through a combination of autocrine, paracrine and endocrine mechanisms at which an autocrine and paracrine activation of monocytes by IL-6 in the vessel wall contribute to the deposition of fibrinogen. IL-6 decreases lipoprotein lipase activity and monomeric LPL levels in plasma, which increases macrophage uptake of lipids^[19]. Lindmark et al agreed with Manginas that IL-6 is a strong independent marker of increased mortality in UA patients, as IL-6 with both pro- and anti-inflammatory mediators affecting both B-cell immunoglobulin production and T-cell cytotoxic activity, and this indicates a possible role for IL-6 in progression of coronary artery disease (CAD)^[20,21]. The local inflammatory response is accompanied by a systemic response known as the acute- phase response. CRP is a prototype positive acute-phase protein whose serum level increase 1000- fold during an acute- phase response. A calcium-binding pentameric protein consisting of five identical, non-covalently linked, 23-kDa subunits^[22,23]. Its name for its ability to bind and precipitate the C- polysaccharide of pneumococcus. CRP is expressed in atherosclerotic plaque^[24] and may enhance expression of local adhesion molecules^[25], increase expression of endothelial plasminogen activator inhibitor- one (PAI-1), reduce endothelial nitric oxide bioactivity^[26], and alter low density lipoprotein- cholesterol (LDL-c) uptake by macrophages^[27]. CRP has been found within thin cap atheromas and immunohistochemical deposition of CRP within plaques corroborates the concept that inflammation is an important component to plaque instability reflected by serum CRP^[28]. Accordingly, elevated CRP levels could be linked to increased long-term mortality^[29].

was free of haemolysis, no turbidity and not lipemic.

Materials

IL-6 and CRP ELISA-kit (from DRG International Inc., USA/ Germany) is an *in vitro* sandwich type assay for the quantitative measurement of human IL-6 and CRP in serum, plasma, urine and culture supernatants. The principle of the assay is based on a solid phase enzyme-linked immunosorbent assay. The laboratory investigations were performed in the Research Centre for Biotechnology and the Specialized Centre of Endocrinology and Diabetes.

Statistical Analysis

The results were expressed using Software Statistical Package for Social Science (SPSS) version 11.5 and 12. For the estimation of result's differences between the studied groups consider the (S) at $P < 0.05$, 0.01 . Also by using special ELISA software (4.01 Bio-Rad Lab., Inc.) to draw the standard curves of: IL-6 and CRP.

Results and Discussion

Traditional risk factors of this study revealed the following data: smoking (7 (10%) smokers and 7(10%) previously smoking), $p < 0.01$; hypertension (53(75.7%) hypertensive, $p < 0.01$); diabetes mellitus (34(48.6%) diabetic, $p > 0.05$); 28 patients have both DM and hypertension; family history of cardiac disease (47(67.1%), $p < 0.01$). Also found 66 patients with abnormal angiographic outcome and only four ACS-patients with normal cath. findings. Data of table (1) shows significant differences in the serum levels of IL-6 and CRP ($P < 0.05$, 0.01 respectively) between ACS-patients and apparently healthy persons. Table (2) reveal the prevalence of elevated levels of CRP in the serum of ACS-patients having positive risk factors (positive family history of cardiac diseases, smokers, diabetic, and hypertensive patients); while the number of patients with positive risk factors that have increased level of IL-6 was less in comparison to those with normal levels of IL-6 this may be due to the usage of some medications by the patients (as anti-inflammatory- Aspirin and clopidogrel, antihyperlipidemic- Statines and Fibrates, antihypertensive angiotensine converting enzyme inhibitor (ACEI) and hypoglycaemic agents) that causes these differences in the serum levels of IL-6 in relation to the risk

factors, or may be due to the release of CRP immediately in the acute state while the optimal time for measuring cytokines is unknown because of the short half life of the cytokines in addition this findings indicate that the association between IL-6 levels and future cardiovascular events was independent of major cardiac risk factors; also table (2) demonstrate that seventeen of 67 patients (25.4%) had elevated level of IL-6 (25 pg/ml is the cutoff point for IL-6 representing Mean serum level of IL-6 \pm S.D in apparently healthy control and any level more than this value considered elevated) and fifty four of 62 patients (87.1%) had elevated level of CRP (3.83 mg/L is the cutoff point for CRP representing Mean serum level of CRP \pm S.D in apparently healthy control and any level more than this value considered elevated) were 50 patients with abnormal cath. outcomes have elevated level of CRP this results provides prognostic value to the CRP in addition to the traditional cardiac risk factors. Therefore, in a high-risk patient, an elevated CRP level should even further alert both the physician and the patient to the need for aggressive risk-lowering strategies^[33] so the AHA/CDC recommends measuring CRP levels in patients who appear to have a moderately elevated risk of cardiovascular events. In these patients, an elevated CRP measurement would indicate that the risk may very well be much greater than "moderate." In the present study also found (SS) correlation between IL-6 and CRP (assessed by Spearman's rho correlation coefficient, $P < 0.01$) this (SS) correlation was also documented by Biasucci et al stating that elevated CRP in the plasma of most UA refer to the important role of an inflammation in ACS and has indirect mark for increased IL-6 production^[34]. In addition this strong correlation between the inflammatory markers may refer to the underlying cause of this type of cardiac disease which is stated by many studies one of them is the results of Hashmi and his colleague showing increased concentrations of the proinflammatory cytokines IL-17, IL-6, IL-8 and CRP, these findings point towards the role of inflammation in the form of increased activity of those interleukins in an UA and AMI patients^[35]. Furthermore, elevated CRP levels predict recurrent ischemia and death in patients with stable and UA^[36-38], this is because CRP plays a direct pathogenic role in arterial disease^[39]

Table 1: Demography of the inflammatory markers in the studied groups

Parameters	Studied groups	No.	Mean \pm Std. D	Min	Max
hs-CRP** mg/L	ACS-patients	62	13.16 \pm 8.25	0.40	31.20
	Healthy control	18	2.66 \pm 1.17	0.10	4.80
IL6* pg/ml	ACS-patients	67	27.80 \pm 22.61	0.00	153.74
	Healthy control	18	15.28 \pm 9.80	0.00	23.61

** F- test and Anova significance < 0.01

*Significance < 0.05

Table 2: Correlation between the inflammatory markers and the risk factors in ACS-Patients

Inflammatory Markers	IL-6		CRP	
Cutoff points* (Mean serum level of the marker in apparently healthy individuals \pm S.D)	>25 pg/ml	\leq 25 pg/ml	>3.83 mg/L	\leq 3.83 mg/L
Age (mean \pm SD)	62.6 \pm 11.7	57.08 \pm 9.33	58.11 \pm 9.08	60.5 \pm 11.2
Male (no.)	12	25	30	5
Female (no.)	5	25	24	3
Smokers (no.)	2 (7 previously smoking)	5	4 (5 previously smoking)	2 (1 previously smoking)
Hypertensive patients (no.)	12	38	41	7
Diabetic patients (no.)	9	24	29	4
Patients with +ve Family history	10	35	37	6

*Normal reference value of CRP: 0.068-8.2mg/L⁽⁴⁰⁾

Conclusion

The significant proportion of patients with UA/NSTEMI that had elevated serum levels of IL-6 and CRP, and their strong correlation with coronary angiographic findings make these markers to be considered as risk stratification factors and good predictors for coronary artery disease independent of other traditional risk factors for cardiovascular disease.

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