Biochemical Markers of inflammation and their relationship to Acute Coronary Syndrome in local Libyan Patients

by

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Abstract

The aim of this study is to measure inflammatory markers in local Libyan patients with acute Coronary Syndrome (ACS) and unstable angina pectoris (UAP).

Materials and Methods: The study was carried out in 40 patients, (20 with acute myocardial infarction (AMI), and 20 with unstable angina pectoris (UAP). We measured blood levels of Fibrinogen and C-reactive protein (CRP), WBC and ESR as markers of inflammation, and lipid profiles (Total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides), as conventional risk factors. Cardiac enzymes creatine kinase (CK) and lactate dehydrogenase (LDH) were also measured.

Results and Discussion: We observed significantly increased levels of all inflammatory markers in patients with ACS compared to control (P<0.005). CRP was increased (P<0.005) from 1.8 mg/l (normal) to 10.5 mg/l (AMI), and 8.75 mg/l (UAP), and fibrinogen (P<0.005) from 2.85 g/l (normal) to 4.03 g/l (UAP) and 3.99 g/L (AMI). We also observed that markers of inflammation were directly correlated with the duration of hospitalization (R=0.45256, P=0.0034) for CRP, and (R=0.41497, P=0.007) for Fibrinogen, and inversely correlated with the LVEF (R= -0.90578, P=0.000) for CRP, and (R=-0.82043, P=0.0002) for Fibrinogen. No relationship exists between inflammatory markers and in hospital mortality.

Conclusion: The study has demonstrated that the inflammatory markers were significantly high in subjects with ACS as compared with control group. This has highlighted the need to use these markers as a baseline measure in patients at increased risk of disease. Also it could predict in hospital complications and hospitalization duration as well as the magnitude of cardiac damage.
**Key words:** Acute coronary syndrome (ACS), unstable angina pectoris (UAP), acute myocardial infarction (AMI), fibrinogen, C-reactive protein; left ventricular ejection fraction (LVEF)

**Introduction**

Inflammation is closely linked to atherosclerosis and acute coronary syndrome (ACS). Chronic and long lasting inflammation stress, present both systemically or in the vascular wall, can trigger ACS. Markers of inflammation in ACS may help to improve risk stratification and identify patient groups who might benefit from particular treatment strategies. Some of them are very well known indicators of inflammation: C-reactive protein (CRP), WBC count, ESR, fibrinogen. The use of CRP and other novel inflammatory markers may significantly add to our ability to correctly identify patients presenting with ACS who are at high risk for future cardiovascular events. Measurement of certain inflammatory markers can help identify high-risk patients, monitor disease activity, and provide a therapeutic guide for reducing the inflammatory component of the disease. The present work was taken up to know the possible role of some inflammatory markers in ACS in local Libyan patients.

**Materials and Methods:**

All patients admitted to the Coronary Care Unit (CCU) at 7th October Hospital, Benghazi during the period from 1st December 2006 to 30th May 2007, with the diagnosis of Acute Coronary Syndrome (ACS) were selected. We studied 40 patients (31 males and 9 females) with (ACS), this group was classified into two subgroups according to WHO criteria, group I with Acute Myocardial Infarction (20 patients, 17 males and 3 females), group II with Unstable Angina Pectoris (UAP-20, 14 males and 6 females). These groups were compared with 20 age and sex matched healthy control subjects all without history of ACS.

In all cases Electro Cardiogram (ECG), was performed in the acute phase of the disease (on admission) to confirm the diagnosis with ACS. NO patients with acute infection were included in our study, i.e., patients had no signs of infectious or inflammatory disorders. All patients underwent complete detailed history assessment (including gender, age, history of hypertension (HTN), history of diabetes mellitus...
(DM), smoking habit, obesity, previous myocardial infarction, and any other cardiac diseases) and a complete physical examination done by an experienced physician at the department. HTN was defined as current blood pressure (BP) > 140/90 mmHg. DM was defined as previously diagnosed disease, or fasting blood glucose level > 130 mg/dl. Smokers were defined as those currently smoking during the study period.

**Specimen collection and preparation:**
One venous blood sample was collected, by drawing 10 cc of blood from a vein, usually from the inside of the elbow or the back of the hand, and the blood is collected in a syringe and then in commercially available tubes (K\textsubscript{2}EDTA for CBP and ESR, 0.11 M trisodium citrate for fibrinogen, and plain tubes for other serum variables such as cardiac enzymes (Aspartate Aminotransferase (AST), Creatine Kinase –MB (CK-MB) and Lactate Dehydrogenase (LDH), and lipid profiles (serum cholesterol, triglyceride (TG), high density lipoproteins- cholesterol (HDL-C), and low density lipoproteins-cholesterol (LDL-C). All the samples were obtained from patients within the first 24 hours of acute onset of symptoms. CBP, ESR, and fibrinogen were performed immediately after collection, the samples in the plain tubes separation of serum was performed within 1 hour by centrifugation for 10 minutes and samples were stored at -30 ºC until analyzed.

**Measurement of Left Ventricular Ejection Fraction:**
Measurement was made of left ventricular end-diastolic diameter (EDD) and end-systolic diameter (ESD) from M-mode, guided by parasternal short-axis image (upper left) to optimize medial-lateral beam orientation.
LV ejection fraction was calculated using the formula: \( EF = \frac{EDD - ESD}{EDD} \times 100 \)

Results

Levels of inflammatory markers in different subgroups and control group.

We demonstrated significant higher levels of CRP, Fibrinogen, WBC and ESR in patients with AMI and UAP as compared to the control as Table II shows (\( P=0.0001 \)).
Table II. Inflammatory Markers in the Local Libyan subjects

<table>
<thead>
<tr>
<th>Inflammatory Markers</th>
<th>AMI</th>
<th>UAP</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (cell count/m m³)</td>
<td>11480±107*</td>
<td>10359±101.7*</td>
<td>8949±94.5</td>
</tr>
<tr>
<td>ESR (mm/hr)</td>
<td>55±7.4 *</td>
<td>56±7.4 *</td>
<td>25±5</td>
</tr>
<tr>
<td>Fibrinogen (g/I)</td>
<td>3.81±1.95*</td>
<td>3.25±1.8**</td>
<td>3.15±1.7</td>
</tr>
<tr>
<td>CRP (mg/I)</td>
<td>40.8±6.4*</td>
<td>32.9±5.7*</td>
<td>12.6±3.5</td>
</tr>
</tbody>
</table>

Values are expressed as mean ±SD

* p<0.001
** p<0.05

We found there were a positive correlation between serum levels of CRP and WBC count (R=0.74488, P=0.0002). We also found a positive correlation between serum levels of CRP and ESR (R=0.60137, P=0.005). There was a significant correlation between serum levels of CRP and fibrinogen levels (R=0.75600). A significant negative correlation between serum levels of CRP and left ventricular ejection fraction (LVEF) (R=-0.90578, P=0.000) was observed. Patients with the highest levels of CRP at day of admission were having the lowest estimated ejection fraction (EF) by echocardiogram. Fig (3-1).
We also found there were a significant negative correlation between serum levels of fibrinogen and left ventricular ejection fraction (LVEF), \( R = -0.82043, \ P = 0.0002 \). Fig (3-2).

**Fig (3-1):** Correlation between CRP levels and LVEF
Fig. (3-2): Correlation between fibrinogen levels and LVEF

We found a positive correlation between serum levels of CRP and the duration of hospitalization (R=0.45256, P=0.0034) Fig3-3.
Those patients with higher levels of CRP and fibrinogen have a tendency to have a longer hospital stay; in other word this may reflect more in-hospital complication than patients with lower levels.

**Fig. (3-3):** Show the relationship between CRP levels and days of hospitalization.
A good statistical relationship is found between serum levels of fibrinogen and the duration of hospitalization ($R=0.41497$, $P=0.007$). Fig (3-4)

There were no significant correlation between serum levels of CRP and serum levels of triglycerides (TG), $R=0.04376$, $P=0.8547$. Also no significant correlation between CRP and HDL-C, $R=0.17927$, $P=0.4495$. No significant correlation between serum levels of CRP and serum levels of cholesterol, $R=0.18818$, $P=0.4269$. No significant correlation between serum levels of CRP and serum levels of (LDL-C), $R=0.01133$, $P=0.6552$. In AMI and UAP there was no significant correlation between the inflammatory markers CRP and fibrinogen with the cardiac enzyme CK-MB where P value > 0.05 ($P=0.968$) for CRP, as shown by scattered diagram.(Figs.3.5 and 3.6)
Fig. (3-5): Relationship between CRP levels and CK-MB levels

P value >0.05 (P=0.656) for Fibrinogen
In our population study 35% were not hypertensive (non-HTN) and 65% were hypertensive defined as a BP of 140/90 or higher. We found that hypertensive patients have high CRP levels as compared to non-hypertensive patients as shown in the diagram (P=0.000).

Individuals with hypertension had a significantly greater likelihood of CRP elevation than people with normal blood pressure.

The other risk factor for heart disease is diabetes. 40% of our subjects taken were not diabetic while 60% was diabetic and they have high CRP levels (P=0.001) as seen in the diagram:

**Discussion:**

Inflammation is closely linked to atherosclerosis and ACS. Chronic and long lasting inflammation stress, present both systemically or in the vascular wall, can trigger ACS.\(^1\). The use of CRP and other novel inflammatory markers may significantly add
to our ability to correctly identify patients presenting with ACS who are at high risk for future cardiovascular events. There was a statistically significant correlation between the high levels of CRP, levels of fibrinogen and the duration of hospitalization (R=0.45256, R=0.41497). Our observation in local Libyan subjects is in agreement with the previous study by Zebras et al who showed that CRP levels are higher after AMI and after UAP. They also showed that CRP was strongly predictive of long-term death from MI (D/AMI), for patients presenting with ACS. However, it can be used to monitor the severity and progression of some well-defined cardiovascular diseases, for example, it can predict serious events in patients with coronary artery disease (CAD) who are hospitalized with ACS or MI. Many trials have confirmed the association between high levels of CRP and risk of future coronary events such as MI and sudden cardiac death. In the European Concerted Action on Thrombosis and Disabilities study, elevations of mean CRP levels by 20% or more was found in patients after an MI. CRP levels are higher in survivors of MI with or without a demonstrable coronary lesion, CRP may serve to represent the inflammatory burden. In the monitoring Trends and Determinants in Cardiovascular Disease trial, a long-term prospective study of cardiovascular risk, patients with the highest CRP levels had 2.6 times the risk of MI. In another study, post infarction angina occurred in only 14% of patients with a normal level. By comparison, 64% of patients admitted with high CRP levels and evidence of post infarction angina; nearly 42% required revascularization, and 21% had recurrent MI. Measurement of CRP may have practical clinical significance in management of patients hospitalized for suspected ACS.

So, patients who are hospitalized for the treatment of ACS and have high CRP concentration, have significantly more ischemic episodes in the hospital than patients with lower CRP levels. Patients with ACS with elevated CRP levels are prone to greater plaque instability. We suggest that higher CRP levels could be predictors for poor outcome, whereas low CRP levels are suggest a good outcome. Ejection fraction (EF) indicates the degree of myocardial damage. EF is good indicator for short and long term prognosis. In our study those patients with higher levels of CRP and fibrinogen at time of admission where having lower EF, this may indicate that these markers are sensitive indicators for the magnitude of myocardial damage and injury.
Left Ventricular Ejection Fraction (LVEF) is an important prognostic factor in post myocardial infarction and a sensitive marker of left ventricular function. Correlation between the two main inflammatory markers in the present study (CRP and fibrinogen) were estimated in which there is an inversely relationship, \( R = -0.90578, P = 0.000 \) and \( R = -0.82043, P = 0.002 \) for CRP and fibrinogen respectively. These finding were in disagreement with Brunetti et al (2006), who showed that CRP peak concentrations did not correlate with ejection fraction and angiographic findings, but correlate with incidence of ( Major adverse cardiac events). So we conclude that patients with high inflammatory markers, have less left ventricular ejection fraction which in turn reflect a poor outcome of the disease states. In our present we found no correlation between inflammatory marker CRP and CK-MB cardiac enzyme in these patients. These findings are in agreement with that of Auer (2002) who showed there was no correlation between CRP and creatine kinase(CK), CK-MB isoenzyme, or troponin I positivity as markers for the extend of the myocardial injury during the observation period.  

And also the same finding were obtained by Tsakiris et al(2006). This may be explained by the fact that CRP levels peaked around 50 hours after the onset of pain, a time when CK-MB which peaked after about 15 hours, had already returned to normal. So, this will suggest that measurement of CRP may be useful in case in which the diagnosis of infarction is only suspected late.

Elevated blood pressure (BP) is a prevalent independent risk factor for cardiovascular disease. Although the mechanisms underlying the development of cardiovascular disease in people with hypertension are still not completely understood, an accumulating body of evidence suggests that inflammation may play an important intermediary role. This is particularly important because elevated levels of inflammatory markers such as C-reactive protein (CRP) independently predict increased cardiovascular (CV) risk. Elevated levels of CRP have been linked to several atherogenic conditions, including hyperglycemia, insulin resistance, and overt diabetes. In the Helsinki Heart Study, the combination of systolic blood pressure (SBP) >150 and CRP >5mg/L was found to be additive for the risk of myocardial infarction and coronary death compared to elevated SBP alone. We found no statistically significant relationship between CRP level and serum level of TG, \( P = 0.8547, R = 0.04376 \). Also no correlation were found between CRP and HDL-C level.
P=.4495, R=-.17927. As well as there were no statistically significant correlation between CRP and serum cholesterol level, and LDL-C level p=.4269, R=.18818. And p=.6552, R=-.01133. respectively. These findings is are agreement with Liet al. (2002)\(^\text{14}\) who showed no correlation between plasma levels of CRP and serum total cholesterol(TC) as well as high-density lipoprotein cholesterol (HDL-C). Early studies indicated that cholesterol levels decrease significantly after ACS. However, most studies were small or did not measure low-density lipoprotein cholesterol (LDL-C) directly, and many used non fasting or retrospective data. More recent studies suggest less pronounced changes in cholesterol levels after ACS. The LDL-C levels decreased in the 24 h after admission, followed by an increase over the subsequent 2 days these changes did not seem to be clinically meaningful. Similar changes were observed for total cholesterol and smaller changes for high-density lipoprotein cholesterol; fasting triglyceride levels did not change. Mean lipid levels vary relatively little in the 4 days after an ACS and can be used to guide selection of lipid-lowering medication.\(^\text{14}\) So, the CRP level is a stronger predictor of cardiovascular events than the LDL-C level and that it adds prognostic information to that conveyed by Framingham risk score, in particular when LDL-C is low.\(^\text{15,16}\) Elevated CRP may be just as important as elevated LDL-C levels; and that furthermore, high CRP levels may identify high-risk patients who would be missed by just measuring cholesterol levels. Indeed, patients with high CRP but normal cholesterol apparently had a higher risk than those with normal CRP and high LDL-C levels. In other word, CRP is an independent marker of cardiovascular risk, and may be a partial explanation for why some patients develop significant coronary artery disease despite normal cholesterol levels.

**Study limitation, clinical application and suggestion for future study:**

We could not include more inflammatory markers for the present because of non-availability of reagents and kit specific for such markers. The second limitation of the study is the small number of patients included in the study.

**Clinical application:** We suggest measuring CRP levels in patients who –on the basis of multiple risk factors scoring- 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". Such knowledge might spur both the doctor and the patient to adopt more aggressive risk-reducing measures. The use of CRP and other novel inflammatory markers significantly add to our ability to correctly identify patients presenting with ACS who are at high risk for future cardiovascular events. The predictive value of CRP appears to be independent of, and in addition to, troponin. Individuals with evidence of heightened inflammation may benefit most from an aggressive modification of lifestyle and intensification of proven preventive therapies such as aspirin and statins. Moreover, the benefits of an early invasive strategy may also be greatest among those with elevated levels of inflammatory biomarkers. Measuring fibrinogen and CRP levels can identify patients at increased risk for coronary events. CRP levels are a better measure and more reproducible than fibrinogen levels.

**Scope for future study** these findings support the hypothesis that inflammatory markers are important predictors of prognosis in ACS. The results of this preliminary study had paved for studies with larger number of Libyan subjects with a view to further evaluate these markers in long term prognosis.

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