

Serum complement (C3, C4) levels in patients with acute myocardial infarction and angina pectoris

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Abstract

Serum complement (C3, C4) levels in Libyan patients with acute myocardial infarction (AMI; 31 patients) and angina pectoris (AP; 11 patients) at the 1st day and 7th day of attack were estimated. A group of 26 healthy Libyans were taken as control subjects (CS). Serum C3 and C4 levels (mean \pm SD, mg/dl) were elevated at the 1st day in AMI as well as AP patients (C3 \rightarrow AMI1: 154.0 \pm 28.5, AP1: 152.0 \pm 45.0, CS: 132.0 \pm 8.0, ANOVA: $p=0.0072$; C4 \rightarrow AMI1: 38 \pm 13, AP1: 37 \pm 17, CS: 29 \pm 6, ANOVA: $p=0.0160$). No significant differences for the elevated C3 and C4 levels at the 1st day were observed between the two diseases groups (AMI1 vs AP1 \rightarrow C3: $p=0.879$, C4: $p=0.818$). At the 7th day, C3 and C4 levels were further elevated in AMI, while they remained at the similar elevated levels in AP (C3 \rightarrow AMI 7: 173.1 \pm 28.0, AP 7: 149.0 \pm 41.0, CS: 132.0 \pm 8.0, ANOVA: $p=0.0000$; C4 \rightarrow AMI 7: 46.0 \pm 7.0, AP 7: 36.0 \pm 15.0, CS: 29.0 \pm 6.0, ANOVA: $p=0.0000$). Again, no significance differences for the raised C3 and C4 levels at the 7th day was observed between AMI and AP patients (AMI 7 vs AP 7 \rightarrow C3: $P=0.059$, C4: $p=0.06$). The C3 elevation showed significant positive correlation in AMI group ($r=0.522$, $p=0.003$) while it was insignificant in AP patients ($r=0.037$, $p=0.915$). Regarding C4 levels, it was significantly correlated in AMI ($r=0.483$, $p=0.006$), and in AP, although it was positively correlated ($r=0.656$, $P=0.028$) the observed difference was not significant ($t=0.29$, $p=0.778$). In conclusion, serum C3 and C4 levels were more profoundly elevated in AMI compared to AP patients suggestive of an acute phase and inflammatory response.

Introduction

Acute myocardial infarction (AMI) is one of the major causes of death worldwide and atherosclerosis overweighs all other aetiologies¹⁻³. Many immunological changes and probable mechanisms such as basophils, immunoglobulin E (IgE), complements, neutrophil activation, immunoglobulin G (IgG) and T-lymphocytes have been implicated in the aetiopathogenesis of AMI⁴⁻⁸. The complement system is believed to play an important role in the pathogenesis of ischemia induced myocardial damage. Activated components of complement are potent chemotactic and stimulatory agents for neutrophil and hence initiate infiltration into the extravascular myocardial tissue. It has been reported that substances present in the mitochondria-rich subcellular fractions of heart muscle can activate both the classical and alternative pathway⁹.

The complement derived anaphylatoxins, C3a and C5a, may themselves contribute to the cardiac dysfunction accompanying myocardial ischemia. C3a, apart from eliciting chemotaxis, enhances

vascular permeability and provokes the release of mediators such as histamine, leukotriene, prostaglandins and platelet activating factor and also produces coronary constriction⁹. The formation of membrane attack complex (MAC) from complement was associated with loss of CD59 (protectin), an important sacrolemmal regulator of MAC, from infarcted area¹⁰. Thrombolytic therapy, with streptokinase (SK) and recombinant tissue type plasminogen activator (rTPA), activate the classical complement pathway and plasmin could be the common trigger for this phenomenon. A significant activation of the complement common pathway (from C3 to terminal components) was observed only with SK infusion and is attributable to the rapid formation of immunocomplex between SK and anti SK antibodies present in plasma as a consequence of previous streptococcal infections¹¹.

Serum complement level has been found to be retrospectively associated with the presence of diffuse atherosclerosis, and it was asserted that high serum C3 level is a predictive of future myocardial

infarction in male individuals who have no previous ischemic events¹². Among patients dying suddenly from early infarction, no consumption of complement could be detected and C3, in particular, was significantly elevated in cases of coronary fatalities¹³. Literature survey indicated that no work was done or reported on serum complement levels in Libyan patients with AMI and AP. The present study on serum levels of C3 and C4 was therefore undertaken during the 1st day of attack and at 7th day after attack in Libyan patients with AMI and angina pectoris (AP) and healthy control subjects (CS) for comparison.

Materials and Methods

A total of 31 AMI patients (sex: 27 males, 4 females; age range: 36-80 years with mean age 62 years) were obtained from Coronary Care Unit (CCU) at Seventh October Hospital, Benghazi, Libya and Jamahiriya Hospital, Benghazi, Libya during September to December 1998. The diagnosis of AMI was based on clinical assessment (medical history, ECG changes) and laboratory evaluation with serial determination of cardiac enzymes¹³⁻¹⁵. A group of 11 admitted AP cases were included as the disease control group (sex: 6 males, 5 females; age range: 48-88 years, with mean age 61 years). Their diagnosis was based on medical history and ECG changes¹³⁻¹⁵. According to the personal questionnaire, none of the AMI and AP patients were atopic, i.e., none had personal or family history of asthma, eczema, allergic rhinitis, drug allergy and/or food allergy and none had any connective tissue disorder. A group of 26 randomly selected clinically healthy Libyan adults were included in the study as control subjects (CS) (sex: 16 males, 10 females; age range: 38-82 years with mean age 58 years). All CS subjects were non smokers and had no history of allergic disorders.

Venous blood specimen was collected in tube with or without anticoagulant as required for routine hematological tests and biochemical investigations and special immunological assays. Blood specimens were allowed to clot at room temperature for 30 minutes and sera were separated by centrifugation at 2,500 rpm for 12-15 minutes. The separated sera were aliquoted and kept frozen at -30^o C until analyzed for the parameters of interest during the 1st day of attack and at 7th day after attack.

Complements (C3, C4) assay: Serum C3 and C4 levels were quantitatively assayed by using commercially available immuno kit of BioMerieux, France (Cat.No. C3: 714012A, C4: 720372A)

based on the principle of radial immunodiffusion as described by Mancini et al¹⁶.

Statistical analysis: The statistical significance of the results was evaluated by Student's t-test, Paired t- test, One-way analysis of variance (ANOVA) and correlation coefficients¹⁷. All computing analyses were done using statistical package for social sciences (SPSS) programme.

Results

Table 1 shows the mean \pm SD values of serum C3 and C4 levels at the 1st and 7th day in AMI and AP patients and in CS together with observed range, 95% CIM, ANOVA and Student's t-test.

The increased C3 levels at the 1st day of heart attack in AMI patients (mean: 154 mg/dl) were highly significant compared with CS individuals (p=0.000). The elevation in C3 among AP patients at the 1st day (mean: 152 mg/dl) were also significant when compared with CS group (p=0.03), while there was no significant difference for the elevated C3 levels between AMI and AP patients (P=0.879). At the 7th day the elevation in C3 levels in AMI patients (mean: 171 mg/dl) was still highly significant compared with CS subjects (p=0.000) and high C3 levels in AP patients (mean: 149 mg/dl) was significant when compared with CS group (p=0.039). Again there was no significant difference in C3 levels at the 7th day between AMI and AP patients (p=0.059). In AMI group, C3 elevation at the 7th day was significantly higher than that in the 1st day (t= -3.39, p=0.002) and there were significant positive correlation (r=0.522; P=0.003), while in case of AP patients the difference in increment of C3 levels at the 1st and 7th day were insignificant (t=0.17, p=0.871) and not correlated significantly (r=0.037, p=0.915).

Regarding C4 at the 1st day, it was observed that high C4 level in AMI patients (mean: 38 mg/dl) was highly significant compared with CS subjects (p=0.002). The elevated C4 level in AP patients (mean: 37 mg/dl) was significant compared with CS subjects (p=0.047) and there was no significant difference in C4 levels between AMI and AP patients (p=0.818). At the 7th day, the elevation of C4 levels among AMI patients (mean: 46 mg/dl) were highly significant in comparison with CS (p=0.000); the elevated C4 level in AP patients (mean: 36 mg/dl) failed to reach to significant level compared with AMI patients (p=0.06) and CS subjects (p=0.062). There was low positive correlation between C4 levels in AMI patients at the 1st day and 7th day (r=0.483; p=0.006) and the

Table I: Serum levels of C3 and C4 in AMI patients, AP patients at the 1st and 7th day and control subjects

Statistical Analysis	C3 level (mg/dl)				C4 level (mg/dl)			
	AMI (n=31)	AP (n=11)	CS (n=26)	ANOVA: F(P)	AMI (n=31)	AP (n=11)	CS (n=26)	ANOVA: F(P)
1st day analysis								
Observed range	69 – 210	99 – 240	116 – 146		9 – 67	8.5 – 62	16 – 43	
Mean ± SD	154 ± 28.5	152 ± 45	132 ± 8	5.3284(0.007)	38 ± 13	37 ± 17	29 ± 6	4.4086(0.016)
95% CIM	144 – 165	122 – 183	129 – 135		33 – 43	26 – 48	26 – 32	
7th day analysis								
Observed range	111 – 219	87 – 201	116 – 146		9 – 69	8.5 – 59	16 – 43	
Mean ± SD	171 ± 28	149 ± 41	132 ± 8	16.950(10.000)	46 ± 15	36 ± 15	29 ± 6	13.4126(0.000)
95% CIM	161 – 181	122 – 177	129 – 135		40 – 51	26 – 46	26 – 32	
Student's t-test								
Groups	t value	df	P		t value	df	P	
1st day analysis								
AMI vs CS	3.85	55	0.000		3.20	55	0.002	
AMI vs AP	0.15	40	0.879		0.24	40	0.815	
AP vs CS	-2.26	35	0.03		-2.06	35	0.047	
7th day analysis								
AMI vs CS	6.86	55	0.000		5.39	55	0.000	
AMI vs AP	1.95	40	0.059		1.94	40	0.06	
AP vs CS	-2.14	35	0.039		-1.93	35	0.062	

AMI: Acute myocardial infarction patients; AP: Angina pectoris patients; CS: Healthy control subjects; SD: Standard deviation; 95% CIM: 95% confidence interval for mean; ANOVA: One-way analysis variance; P: Probability; P≤0.05: Significant; P>0.05: Not significant.

higher C4 levels at 7th day reached a significant difference in comparison with the elevated C4 level at the 1st day. (t: -3.01; p=0.005). In AP patients C4 levels at the 1st day and 7th day were positively correlated (r=0.656, p=0.028) and the observed difference was not significant (t=0.29, p=0.778).

Discussion

We observed that the common complement component C3 and classical pathway complement component C4 were elevated in almost all patients in the 1st 24 hours post AMI and the elevations were significantly more pronounced one week after. The complement components increment was also found in AP patients, although in lesser extent, at the 1st day post ischemic event. In contrary to AMI, there was a non significant decline in C3 and C4 at 7th day compared with 1st day among AP patients.

Although some studies had demonstrated *in vivo* deposition of complement components including MAC in the myocardium of patients with AMI, others could not detect any significant complement changes in the plasma of uncomplicated AMI patients¹⁸⁻²⁰. It was explained that such activation at ischemic area might not cause detectable levels of complement catabolic peptides in peripheral circulation. The discrepancies between the above mentioned studies might be related to differences in the severity of the clinical situation of AMI patients

in each occasion²⁰. The substantial C3 and C4 increments in the 1st week of AMI in our study could be a result of persistence of the stimulus via irreversible infarcted tissue, while the backtracked C3 and C4 values in AP patients at the 7th day might be explained by lack of induction due to repair of reversible ischemic insult of myocardium. Another possible mechanism of complement activation in AMI includes the formation of oxygen radicals leading to the activation of C5 by converting C5 to a C5b - like metabolite which then reacts with C6 and initiates MAC formation²¹. Complement activation also could be initiated by infusion of thrombocytic agents, although the patterns of complement activation were different in different drugs. Streptokynase (SK) activates the entire system through the classic pathway and recombinant tissue-type plasminogen activator (rTPA) gives a similar degree of activation of the classic pathway component C4, but only slightly activates C3 and the terminal components from C5 through C9⁹.

Complement activation might lead to myocardial injury through the formation of MAC and the generation of anaphylatoxins as indicated in many studies²²⁻²⁷. These studies demonstrated the relationship between morphological and functional alterations brought about by the assembly of MAC on myocardial cells. Recombinant soluble complement receptor type 1 (sCR1) could prevent membrane deposition of MAC in an animal model and coincidentally, there was preservation of

myocardial ultrastructure and contractile function^{26, 27}. The hypothesis of anaphylaxis had been investigated and the results were consistent with the idea that resident cardiac mast cell activation might be involved in this phenomenon^{24, 25}. In vitro induction of human heart mast cell degranulation by recombinant human C5a anaphylatoxin had been provoked²⁸. In animals the intracoronary injection of the anaphylatoxin (C5a) produced myocardial ischemia characterized by an increase in coronary vascular resistance, contractile dysfunction, a myocardial accumulation of leucocytes primarily neutrophils and a marked release of histamine into the coronary venous blood^{24, 29}. Interestingly, the histamine release was not a contributory component to either the fall in contractile function or coronary blood flow alteration, since H₁ and H₂ histamine receptor blockade did not modify the C5a induced myocardial infarction^{24, 25}. Instead C5a induced myocardial injury was dependent on the production of TxA₂, leukotriene (LT), C4 and LTD₄³⁰. Furthermore, prior treatment with Lodoxamide tromethamine, a mast cell inhibitor, attenuated the C5a induced myocardial release of histamine and TxA₂ into the coronary circulation and blunted the ischemic response^{24, 25}. In man, consequences of complement activation and markers of mast cell degranulation have to be detected in peripheral circulation and/or in local injured tissue to provide evidence of anaphylactic reaction in ischemic and necrotic myocardial tissue.

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