SERUM TUMOR NECROSIS FACTOR LEVELS IN ACUTE MYOCARDIAL INFARCTION AND UNSTABLE ANGINA PECTORIS*

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Tumor necrosis factor (TNF) enhances leukocyte adherence to vascular endothelium and increases procoagulant activity in the endothelial cells. Thus it may be implicated in the pathogenesis of acute vascular occlusions. To study the role of TNF in the early stages of acute myocardial infarction (AMI), we have measured circulating TNF levels in the sera of patients with AMI and unstable angina pectoris.

Blood samples were obtained within 6 hours after onset of chest pain and stored at -70°C until tested. A sensitive sandwich ELISA test was used for TNF measurement. C-reactive protein (CRP) levels were determined semiquantitatively. Immediate complications such as heart failure, arrhytmia and shock were also noted. Twenty-four patients with electrocardiographically and biochemically confirmed AMI and 14 patients with unstable angina pectoris were included in the study. TNF levels were serially assessed at the time of admission, 24, 48, 72 and 96 hours after onset of chest pain in 2 patients with AMI. Detectable TNF was found in 13 sera of AMI group (range:10-1510 pg/ml) and 4 sera of angina pectoris group (range:15-240 pg/ml). There was no correlation between the serum TNF levels and the occurence of complications and the extent of myocardial damage. CRP response was unrelated to TNF levels.

Unlike previous report serial measurements of TNF revealed that peak values were reached within 6 hours and diseppeared after 24 hours.

We concluded that TNF may contribute to the development of acute coronary artery occlusion by changing vascular endothelial cell characteristics rather than being a late consequence of myocardial infarction.

Key words: Tumor necrosis factor, myocardial infarction, unstable angina pectoris

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Tumor necrosis factor (TNF) is a 17 kda protein mediator predominantly secreted by monocyte/macrophages[1]. It induces fever[2] hemorrhagic necrosis of tumor tissue[3] and cancer cachexia[4]. Although TNF enables endothelial cells to gain new functions rather than causing direct injury[5], it may initiate coagulatory and inflammatory pathways in vascular beds[6]. Consistently it was shown that TNF was the major mediator in the development of endotoxin induced shock[7]. It renders cultured endothelial cells susceptible to cytotoxic antibodies found in the sera of Kawasaki disease[8]. Serum TNF levels were found to be elevated in Kawasaki disease[9], renal allograft rejection[10], and AMI[11]. It was suggested that high serum TNF levels in AMI was a result of extensive tissue damage[11]. To test the possibility of pathogenetic relationship between TNF and acute MI, we have measured serum TNF levels in patients with AMI and unstable angina pectoris, within 6 hours after onset of chest pain.

Materials and Methods

Patient groups: 24 patients (20 male, 4 female; age range 37-88; mean 58.24±9.84) with electrocardiographically confirmed acute MI and 14 patients (8 male 6 female; age range 44-67 mean 58±6.21) with unstable angina pectoris were studied.

Time after onset of chest pain, ECG findings, hemodynamic and electrophysiologic complications were recorded. A coronary angiography was performed in some of the angina cases within 2-4 days.

Serum studies: Blood samples were obtained at the time of admission i.e within 4-6 hours after the onset of chest pain and sera were stored at -70 °C until tested. TNF was measured by using a sensitive sandwich ELISA test (T cell sciences, Cambridge, USA). C-Reactive Protein (CRP), was determined semiquantitatively by latex agglutination test (Behring Werke AG, Germany). In 2 patients with AMI, TNF and CRP were measured serially at 6, 24,48,72 and 96 hours after the onset of chest pain. AII samples were studied in duplicate at the same day.

Statistical analysis: Results were expressed as mean ± standard deviation. Correlation coefficient between TNF and CRP levels was calculated by linear regression analysis. Statistical comparisons were made by X^2 analysis and student's t test.

Results

Serum TNF concentrations were raised in 13 out of 24 AMI and 4 out of 14 angina pectoris cases (Table I). TNF levels and localization and extent of myocardial ischemia are shown in (Fig.1).

There was no significant difference between AMI and angina pectoris groups with respect to mean TNF concentrations ($p=0.14$). The localization and extent of MI was not related to TNF elevation ($X^2=4.062$, $p=0.13$) (Fig.1). TNF levels and occurence of complications i.e arrhythmia, shock and heart failure are summarized in table II. Of 10 cases with complications 6 showed high levels of TNF. In 7 cases of AMI no complication was noted despite high levels of TNF. Mean TNF concentrations were not statisticaly different in 2 groups ($p>0.05$).

There was no significant relationship between TNF detection and presence of complications.($X^2=0.05$, $p=0.94$) (Table III). A total of 11 CRP positivity was found in 24 AMI and 5 in 14 angina pectoris cases (Table I). Difference between mean CRP concentrations was significant ($p>0.01$).There was no significant correlation between CRP and TNF levels in both groups. Serial meausurement of TNF and CRP in 2 cases with AMI is shown in (Fig.2.) TNF was highest in both cases at 6 th hour of chest pain and a rapid decrease was noted with no detectable TNF beyond 24 hours. CRP response seems to be unrelated to TNF elevation.

Table I: Statistical comparison of mean (SD) TNF and CRP concentrations and linear regression analysis of TNF versus CRP in study groups.

Patient Groups	n	No of cases with raised serum TNF	Mean TNF concent-rations of positive cases	p	No of cases with raised CRP	Mean CRP concent-rations of positive cases	p	TNF versus CRP r
AMI	24	13	289.23±421.27	0.14	11	68.18±40.00	0.0007	0.02
Unstable Angina Pectoris	14	4	53.75±49.22		5	15.60±18.29		-0.20

AMI: acute myocardial infarction.

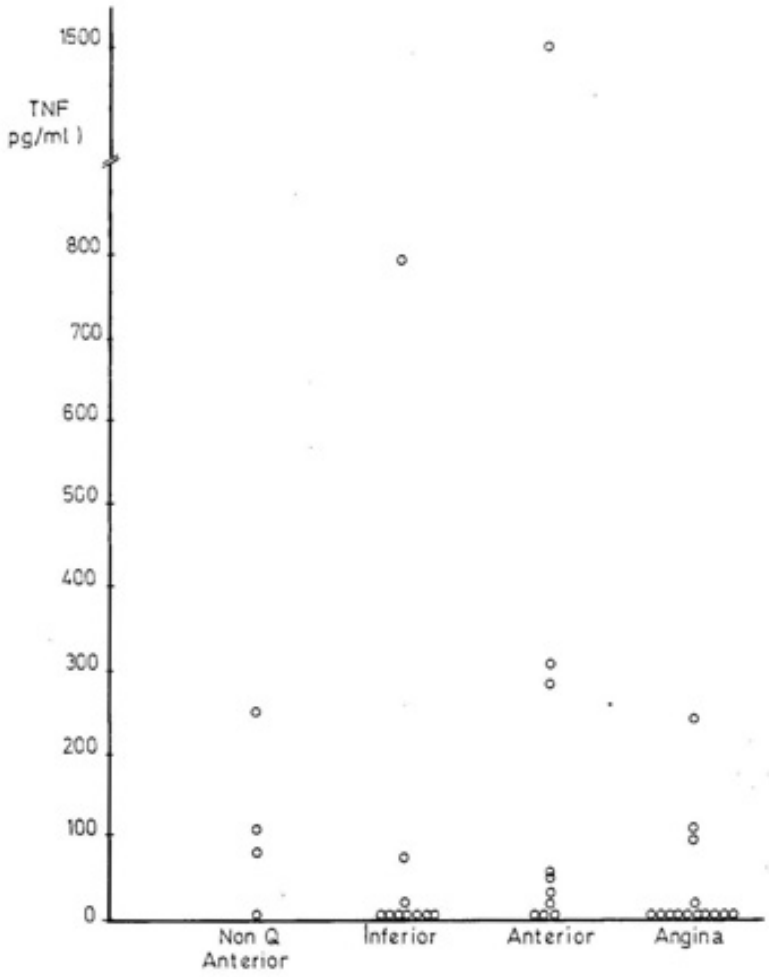

Fig.1. Serum TNF levels in AMI with different localizations in angina pectoris. X^2 analysis did not reveal significant relationship between TNF elevation and localization and extent of ischemia (X^2=4.062 D.F:2)

Discussion

Recent evidences suggest that inflammatory, coagulatory and immune mechanisms contribute to the vascular injuries[5,6]. TNF is secreted by monocyte/macrophages in response to various stimuli and profoundly affects endothelial cell functions. It facilitates coagulation by inhibiting anticoagulant pathway[12], augmenting plasminogen activator inhibitor[13] and inducing cell surface expression of tissue factor procoagulant activity[12]. It also increases the adhesion of leukocytes and binding of lymphocytes to the endothelium[14].

The role of thrombus formation on atherosclerotic ground in the pathogenesis of myocardial infarction is well known[15]. TNF may be implicated both in the development of atherosclerosis [16] and progressive formation of thrombus[6]. It has been shown that monocytes play a predominant role in the initiation of fatty streaks that continue to grow by an ultimate migration of smooth muscle cells to vascular intima[17]. These cells express TNF on their surfaces[16]. The cytokine interleukin, (IL-1) has been suggested to contribute to the pathogenesis of atherosclerosis[18]. TNF induces de novo synthesis of IL-1 in endothelial[16] and smooth muscle cells[19]. Platelet activating factor is a major endogenous mediator in the pathogenesis of ischemic conditions[20]. Its synthesis is stimulated by TNF[6,20].

These evidences suggest that TNF may be implicated in the pathogenesis of myocardial infarction.

Although there is no direct evidence for the involvement of TNF in the pathogenesis of human AMI, elevated serum levels have been demonstrated previously by Maury and Teppo[11]. They have found that peak TNF concentrations were attained 33-76 hours after the onset of chest pain. TNF was virtually absent during the first 6-12 hours.

Serum concentrations were particulary raised in large infarcts with hemodynamic and electrophysiologic complications, whereas slightly increased in small uncomplicated infarcts and angina pectoris. They subsequently concluded that TNF is released into circulation as a result of extensive myocardial damage.

We have found that TNF is raised in samples obtained within 6 hours after the onset of chest pain approximately in half of the patients with AMI and 4 out of 14 patients with unstable angina pectoris (Table I). Serial measurement of TNF in two AMI cases at 6, 24,48,72, and 96 hours revealed that serum levels were highest at 6th hour and no TNF was measured after 24 hours (Fig.2).

TNF concentrations do not seem to be affected by the extent of infarction and presence of complications. Furthermore, 3 out of 4 detectable TNF levels in angina group were significantly high which were comparable to the AMI group.

These results are inconsistent with previous report in several aspects. First, we detected TNF at much earlier time after onset of chest pain; second, no correlation was noted between TNF concentration and occurence of complications (Table II and III). Neither localization nor the extent of infarcted areas significantly affected TNF raise in the sera of TNF positive cases (Fig.1).

Acute phase responses including CRP and leukocytosis following AMI has long been known. Consistently we have found higher CRP levels in AMI group. It has been shown that CRP and TNF levels was well correlated in AMI[21]. Our results suggested that TNF and CRP responses was not associated, since no correlation was present between TNF and CRP levels in both groups (Table I).

We concluded that TNF may play role in the development of both atherosclerosis and thrombus formation. It has been suggested that unstable angina pectoris and AMI are 2 elements in a continuing process[22]. Some mediators may trigger abrupt transition from chronic to acute disease state. TNF, possibly along with other related cytokinases may be locally secreted to play role in the formation of atherom plaque and platelet aggregation leading to progressive narrowing of coronary arteries with a final outcome of complete occlusion. This may explain significantly high levels of TNF in angiographically confirmed angina cases without biochemically, and electrocardiographically apparent tissue necrosis and early increase in AMI cases. Alternatively TNF is readily secreted immediately after ischemic injury rather than

Table II. Mean (SD) TNF concentrations in AMI with and without complications

Patient groups	n	Mean TNF concentration	p
AMI with complications*	6	140±125.17	0.16
AMI without complications	7	384.28±54.15	

* Arrhythmia, cardiac failure, shock

Table III. X^2 analysis of occurence of complications and TNF detectability

	No. of cases with detectable TNF	No. of cases without detectable TNF
Cases with complications	6	7
Cases without complications	4	7

X^2=0.005 p=0.94 D.F:1

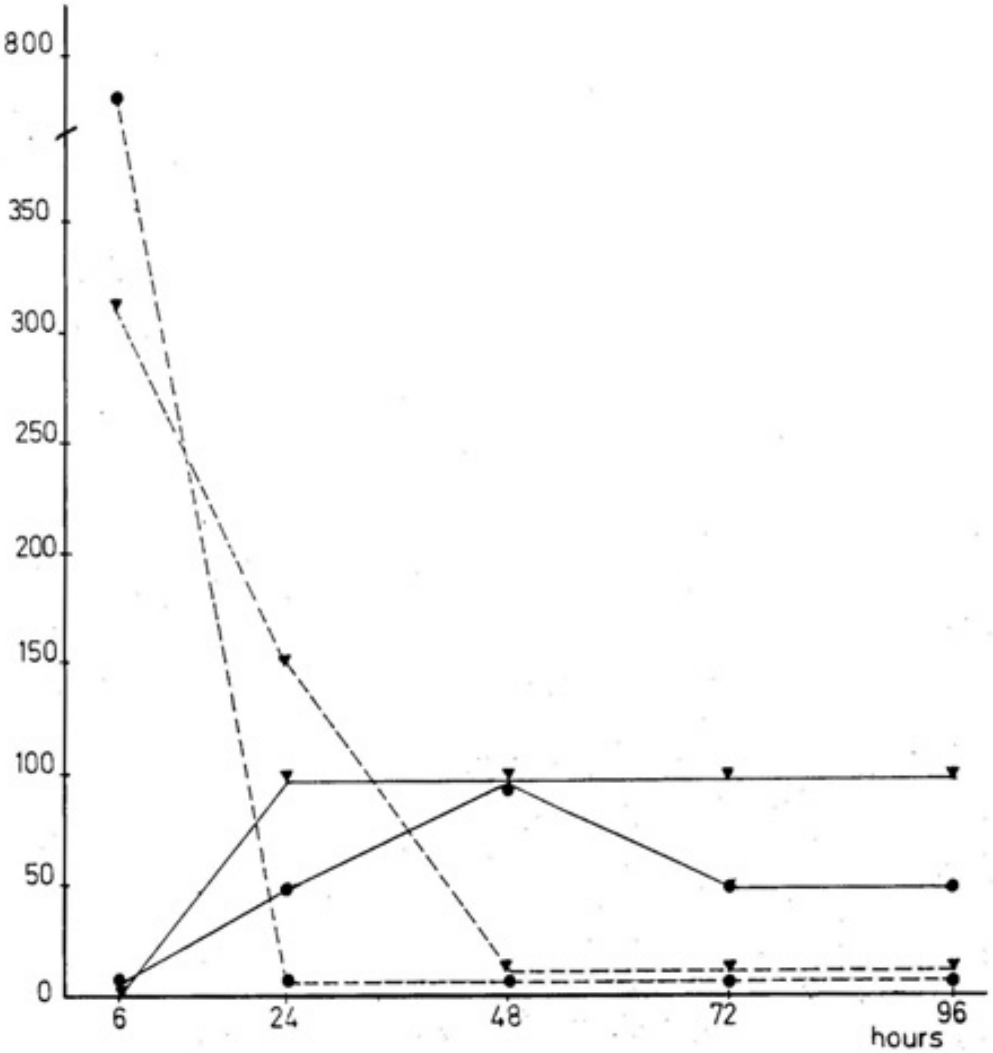

Fig.2. Serial measurements of TNF and CRP levels in two cases with AMI. Dashed line represents TNF concentrations (pg/ml), contanuous line represents CRP concentrations (mg/dl).

being a relatively late consequence of extensive tissue necrosis.

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