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Intracellular Neutrophil Myeloperoxidase in Myocardial infarction.

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Abstract:

A growing body of evidence suggests that inflammation may play a role in unstable angina and acute myocardial infarction. Neutrophil activation has been demonstrated in unstable angina and acute myocardial infarction. Myeloperoxidase is the major constituent of primary azurophil granules is neutrophil and discharged after activation. The chart of thirty-two patients (female 19 male 13) who were admitted in coronary care unit because of myocardial infarction and unstable angina pectoris were selected. Myeloperoxidase content (MPxI) had been determined using H1 hematology analyzer. In normal subjects this index is about 0 and negative values appear when the neutrophil are depleted of myeloperoxidase, which typically happens after neutrophil activation. Risk factors such as current smoking, hypertension, diabetes mellitus and high cholesterol level were recorded. The mean age of patients was 65 years old (female 66 male 64) with a range of 29 to 91. Leukocytosis and neutrophilia were present in 13 (40%) and 16 (50%) respectively. The range of ESR was between 1 to 28 (millimeter/hours) with mean = 10.4. The mean of MPxI was -3.04 (female -4.5 male -1.3, $P=0.27$). MPxI in patients who had positive and negative history of chronic stable angina was -5.14 and -2.3 ($P=0.64$) respectively. Because most of the patients had two or more risk factors, the relation between risk factors and MPxI, independently, was not possible to evaluate. There was no correlation between amount of creatin phosphokinase rising and age with MPxI values. During myocardial ischemia, neutrophil activity is increased. Further study is needed for determination whether neutrophil activation is caused by myocardial event or whether it is an independent, primary event.

Key words: Myocardial infarction, Inflammation, Myeloperoxidase index.

Introduction: Inflammation and oxidative stress may play a role in unstable angina and acute myocardial infarction. Evidence of inflammation in acute coronary syndromes has been provided not only by a high prevalence of inflammatory cells in unstable coronary plaque (1,2) and by the presence of activated circulating lymphocyte, monocytes and macrophages (3), but also by the evaluation of serum acute-phase protein in unstable angina and acute myocardial infarction (AMI) (4).

Advanced human atheroma contain high levels of the enzyme myeloperoxidase (MPO) that produces the pro-oxidant species, hypochlorous acid (HOCl). Stimulated phagocytes secrete this enzyme at inflammatory sites (5). Neutrophil activation has been demonstrated in unstable angina and in acute myocardial infarction (6). The release of the content of granules may lead to endothelial damage and enhanced procoagulant activity, but it is not known whether neutrophil activation is caused by myocardial ischemia-perfusion events in unstable angina or whether it is an independent, primary event. Myeloperoxidase is the major constituent of primary azurophil granules in neutrophils and is promptly discharged after activation by different agonists (7). Biasucci

and colleagues found significantly higher leukocyte intracellular MPO content in patients with coronary heart disease but circulating phagocytes release MPO by degranulation in acute coronary syndrome (8). Macrophages play a critical role in atherogenesis too (9), however, traditional sources of MPO include blood neutrophils but not tissue macrophages (5,10).

Several research have demonstrated that the drugs like magnolol, cyclosporin-A and tacrolimus that induce neutrophil inhibition, protect against myocardial ischemia-reperfusion injury in animal models (11-14).

The purpose of this research is to assess whether neutrophil activation having a role in myocardial infarction.

Materials and Methods: The chart of thirty three (female 20, male 13) patients who had been admitted with unstable angina and acute myocardial infarction, were reviewed.

These patients had been admitted in coronary care unit because of myocardial infarction and unstable angina pectoris. Myeloperoxidase content was determined using a H1 hematology analyzer that measures leukocyte differential count, as well as blood cell count. Leukocytes containing myeloperoxidase such as neutrophil and eosinophil

granulocytes, reduce hydrogen peroxide in water and free oxygen. The H1 measures the scattering and absorption of light produced by leukocytes while passing in single file through a well collimated light beam. The measurement of scattered light is proportional to the cell size, where are the amount of absorbed light is a function of cell staining intensity too (i.e., of myeloperoxidase activity). The H1 computer software calculates the index, named the myeloperoxidase intracellular index (MPxI) which quantifies the mean myeloperoxidase activity of the whole neutrophil population. The MPxI is an index that expresses in arbitrary units the mean absorption of light of the neutrophil population of each sample. In normal subject this index is about 0. Positive values appear when the neutrophils are rich in myeloperoxidase and negative values appear when the neutrophils are depleted of myeloperoxidase which typically happens after neutrophil activation.

The result of leukocyte, neutrophil count, erythrocyte sedimentation rate, (ESR), C reactive protein (CRP), creatin phosphokinase (CPK) and lactate dehydrogenase (LDH) levels were recorded. Positive finding indicative of previous chronic stable angina and risk factors such as current smoker, hypertension, cholesterol above than 200

milligram/deciliter (mg/dl) and diabetes mellitus were also recorded.

The data were tested for statistical significance by Mann-Witney and T-test.

Results: The mean age of patients was 65 years old (female 66, male 64), with the range of 29 to 91. Fifty six percent of patients were male. Leukocytosis was defined as 2SD above the mean and neutrophilia was defined when absolute neutrophil count (ANC) > 8000 in deciliter which were present in 13 (40%) and 16(50%) patients (Fig 1) respectively. ESR were checked in 14 patients with mean = 11 (millimeter/hours) (male 5 female 17). Other data are shown in Table 1 and Fig 1 & Fig 2. The mean value of MPxI was -3.4 (female- 4.5 male - 1.3) (P=0.27). MPxI in patients had positive and negative history of chronic stable angina (CSA) were - 4.7 and -2.6 (P= 0.64) respectively. MPxI value in patients who had previous history of CSA was different in males and females (P= 0.05) but no any different in males and females without history of CSA (P= 0.88). Because most of the patients had two or more risk factors, the relation between risk factors and MPxI was not possible to evaluate independently. There was no correlation between amount of CPK rising and age with MPxI values.

Table 1. Clinical characteristics of study patients

	AMI (n=27)	UA (n=5)	Total (n=32)
Male gender	16 (59)	2 (40)	18 (56)
Age			
Mean	63	67	65
Range	29-80	54-91	29-91
History			
Previous CSA or MI	12 (44)	3 (60)	15 (47)
Risk factors			
Current smoker	19 (70)	1 (20)	20 (63)
Hypertension	16 (59)	2 (40)	18 (56)
Cholesterol >200mg/dl	6 (22)	2 (40)	8 (25)
Diabetes	2 (7)	0 (0)	2 (6)

Data presented are number (%) of patients

Fig 1: Number of neutrophils (PMN) in patients with acute myocardial infarction (AMI) (Mean \pm SD) (9484 \pm 5083 cell/mm³) and unstable angina (UA) (Mean \pm SD) 4007 \pm 1995 cell/mm³)

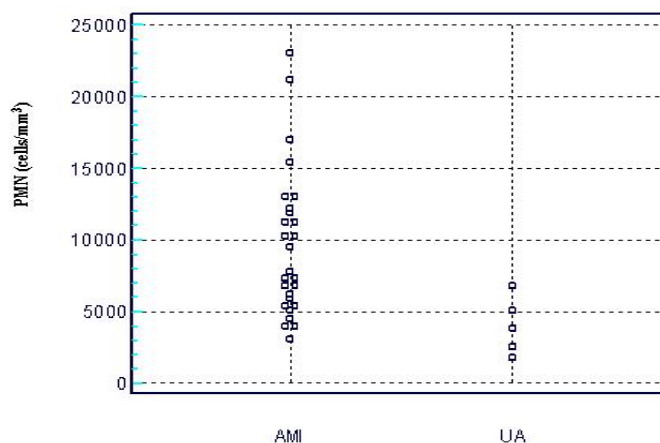
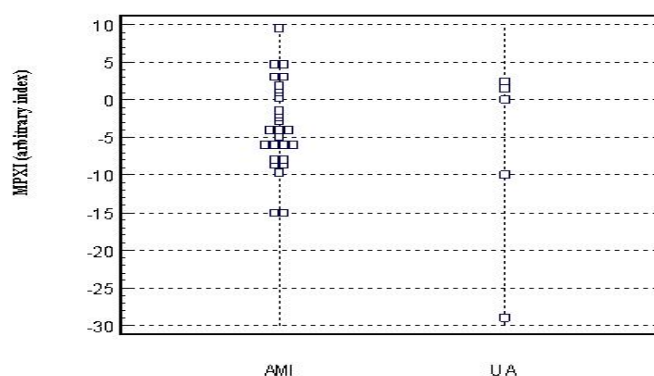


Fig 2: Myeloperoxidase intracellular index (MPXI) values in patients with acute myocardial infarction (AMI) (median=-4 [range 9.5 to -15]) and unstable angina (UA) (median=0 [range 2.4 to -29])



Discussion:

Our study demonstrates that circulating neutrophils in patients with acute myocardial infarction and unstable angina have a low myeloperoxidase content, indicative of a significant release of myeloperoxidase from neutrophils related to their activation which is in agreement with previous studies (7-9, 15).

In previous studies, neutrophil activation was observed and related to ischemia-reperfusion. In experimental animals it was observed as a component of reperfusion injury after 3 hours of coronary occlusion (16) or 90 minutes of low flow perfusion with 20 minutes of reperfusion (17). Neutrophil activation has also been demonstrated in unstable angina and acute myocardial infarction by Mehta et al (18) who observed a 15-fold increase in levels of peptide b-beta, a marker of elastase release and a constituent of primary granules such as myeloperoxidase in unstable angina and by Mazzone et al (19) who showed significant transcardiac expression of CD 11b/CD18 integrin in neutrophils in unstable angina patients. There was no correlation between MPxI and CPK level, the same as Dinermann et al results (20).

Leukocytosis and neutrophilia were demonstrated in these patients, however, we could not rule out the possibility of the increased number of granulocytes and their degranulation in acute myocardial infarction may be secondary to the ongoing necrosis or to transient episodes of reperfusion.

The finding that no further decrease of the MPxI was observed after thrombolysis in patients with AMI suggests that neither prolonged periods of ischemia and reperfusion nor thrombolytic agents or thrombus remodeling are sufficient to cause a profound reduction in the neutrophil MPxI in the periphery (15). The previous study (15) showed that MPxI increased of the same magnitude as that observed in AMI and it suggests that myocardial ischemia and reperfusion may not be the only stimuli responsible for neutrophil activation. However in active variant angina, a human model of ischemia, not associated with plaque instability or thrombus formation, does not result in activation of peripheral circulating neutrophils (4). It is possible that short episodes of ischemia may not be sufficiently strong to cause myeloperoxidase release in a large enough number of neutrophils to allow detection of the phenomenon in peripheral blood, or alternatively,

activated neutrophils may be rapidly sequestered (6,19). Neutrophil activation in unstable angina may also result from inflammatory mediators such as the complement system, aggregated immunoglobulins or immune complexes or from inflammatory cytokines and fibrin degradation products (7). A protective effect of the myeloperoxidase deficiency against cardiovascular damage was shown by Kutter et al (21).

Conclusion:

Our finding and the growing evidence that inflammation plays a major role in AMI suggest that neutrophil activation may be related to the inflammatory component of unstable angina and acute myocardial infarction and the drugs that inhibit neutrophil may protect heart from AMI, although more prospective study in a larger number of patients is needed to evaluate.

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