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The investigation of the relationship among xanthine oxidase, interleukin-6 and body mass index in patients with an ischemic period of acute myocardial infarction: A novel correlation

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ABSTRACT

Some cardiovascular risk factors stimulate the entrance of inflammatory cells into the arterial wall; these cells are important sources for cytokines, including interleukine-6 (IL-6). For myocardial ischemia, a superoxide radical scavenger mechanism is conducted via xanthine oxidase (XO). In this study, the relationship among IL-6, XO, and body mass index (BMI) were studied in 45 patients with acute myocardial infarction (AMI) and 30 patients in a control group. Plasma IL-6 was measured using a sandwich enzyme-linked immunoabsorbent assay (ELISA) test; XO activity was measured using Prajda's method. The mean XO levels in the patient and control groups were 0.25 ± 0.15 and 0.14 ± 0.06 U/m L, respectively. The mean IL-6 in the patient and control groups was 24.13 ± 14.8 and 14.98 ± 8.34 pg/mL, respectively. In comparison with the control group, both XO and IL-6 were significantly higher ($p<0.005$). In correlation analysis for the patient group, a significant correlation was found between XO and BMI ($r:0.3$, $p<0.05$). However, no correlation existed among IL-6, XO, and BMI in either the patients with AMI, or the control group. IL-6 and XO increased in patients with AMI, but no correlation existed between them, indicating that IL-6 and XO are independent markers for insufficient endothelial function in ischemic myocardium. The correlation between XO and BMI in patients with AMI is a new finding requiring further investigation.

Key Words: Myocardial Infarction, Interleukine-6, Xanthine Oxidase, Body Mass Index.

INTRODUCTION

Acute myocardial infarction (AMI) is irreversible myocardial cell necrosis originating from insufficient tissue perfusion and ischemia (1). The important role of inflammation and intimal damage has been accepted in the development of coronary atherosclerosis, which is the most common cause of myocardial ischemia (2). Hypercholesterolemia and some cardiovascular risk factors stimulate the activation of inflammatory cells (monocytes and lymphocytes) and lead to their entrance into the walls of the arteries. These cells are important sources for cytokine and growth factors that may increase endothelial damage (3). During AMI, leukocytes migrate to damaged tissue. After this migration, myocytes and monocytes become active and release inflammatory substances including interleukine-1 (IL-1), tumor necrosis factor-α (TNF-α), interferon γ, and interleukine-6 (IL-6) (4). The role of IL-6 in immune response has been characterized to a greater extent than for other cytokines. Some studies have shown that the application of IL-6 led to increased lipolysis, during which the level of fatty acids and glycerol increased while the level of triacylgliserols (that is, triglycerides) decreased (5-9). On the other hand, some studies have reported that IL-6 releases during muscle contraction (10-13), and that IL-6 may have an impact on endothelial cells and increase lymphocyte adhesion.

Xanthine oxidase (XO) catalyzes the oxidation of hypoxanthine to xanthine and the oxidation of xanthine to uric acid, which appears as a secondary product in a superoxide molecule (14,15). The superoxide ions can cause oxidative stress in tissues (16). Some studies have reported that XO activity increases during cardiac failure (17). On the other hand, it has been immunohistochemically shown that XO is released by myocytes (18). Some studies have indicated that the inhibition of XO has enabled correction of endothelial dysfunction and decrease of oxidative stress markers in patients with heart failure (19, 20).

According to Berne's hypothesis, there is equilibrium between consumption and intake of oxygen. If myocardial consumption of oxygen increases, (e.g., during exercise), oxygen pressure in cardiac myocytes decreases, adenine nucleosides are destroyed, and adenosine diffuses toward the outside of the cell (21). Adenosine leads to vasodilation of cardiac arteries, increasing the blood flow in the myocardium and allowing oxygen pressure in the myocardium to return to its normal level (22). Adenosine in circulation is converted to hypoxanthine, which is metabolized to uric acid by xanthine dehydrogenase (XDH) in normal conditions. Conversely, XO catalyzes hypoxanthine to uric acid if hypoxia occurs (23).

In this study, whether or not IL-6 and XO are independent from (or correlated with) each other and their relationship to body mass index (BMI) were investigated for the ischemic period of AMI.

EXPERIMENTAL SECTION

Study population

This study involved 45 patients with AMI and 30 individuals in a control group. The mean ages for the patient and control groups were 59.7 ± 10.6 and 58.7 ± 10.1, respectively. Patients who had undergone major surgery or thrombolytic treatment in the last month, a prolonged history of rheumatism, or oncologic diseases or chronic infections were excluded.

Study protocol

Blood was drawn from each patient within 4 to 6 hours after their admission to the emergency department. Blood samples were centrifuged at 3000 rpm for 10 minutes, and the plasma samples were stored at –80°C in Eppendorf tubes (Eppendorf International, Hamburg, Germany) until analysis.

Blood sample analysis

Plasma IL-6 tests were run via sandwich ELISA (Cat. No. IM 1120, Beckman Coulter Chemical Company, USA). Briefly, in this procedure 100-µL plasma, controls and standards were put in wells and, following addition of 100-µL conjugate, shaken at 350 rpm for 2 hours at room temperature. After this, washing–aspiration was performed 3 times in an ELISA washer (Bio-Tek Instruments ELx50/16V), and 200-µL substrate was added to the wells. At this point, the solution (plasma/conjugate) was shaken at 350 rpm for 30 minutes in a dark room. The reaction was stopped with 50-µL stop solution, and absorbance was measured in 405 nm in ELISA reader (Bio-Tek Instruments ELx800). The concentrations of IL-6 were calculated as pg/mL. XO activity was tested using the method previously described by Prajda and Weber (24), which was based on uric acid production from xanthine via catalysis of XO assumed in the sample, and calculated as U/mL.

Statistical analysis

For statistical analysis, SPSS 10.0 (SPPS/IBM, Armonk, New York, US) was used. A student's *t* test was performed, and a $p<0.05$ was accepted as significant.

RESULTS AND DISCUSSION

The mean values of XO and IL-6 in the patient group were significantly high in comparison of those for the control group ($p<0.005$) (Table I). In correlation analysis for the patient group, a significant correlation was found between XO and BMI (r: 0.3, $p<0.05$, Figure 1). On the opposite side of this finding, no correlation was found among IL-6 and XO, age, and BMI. Furthermore, no correlation was found among all parameters in the control group.

Table I: Mean values, standard deviations, and statistical comparisons of age, BMI, XO, and IL-6 in the patient and control groups.

Parameters	Patient Group (50)	Control (30)	Student *t* test
Age	59.68 ± 10.56	58.75 ± 10.06	$p>0.05$
BMI	27.84 ± 4.25	26.24 ± 3.03	$p>0.05$
XO (U/mL)	0.25 ± 0.15	0.14 ± 0.06	$p<0.005$
IL- 6 (pg/mL)	24.13 ± 14.80	14.98 ± 8.34	$p<0.005$

AMI is the primary cause of mortality in adults worldwide. Active inflammation on a coronary atherosclerotic plaque is almost always present in AMI in addition to oxidative stress originating from myocardial ischemia (25). In fact, the significant increases of XO and IL-6 levels in our patient group would support the development of AMI (Table I). During AMI, myocardial damage causes the release of proinflammatory cytokines from vascular and myocardial tissues, and then these cytokines may play a role in biological processes related to cell growth and migration in order to fix damaged tissues.

The damage of myocardial tissue leads to functional and metabolic changes during myocardial ischemia and reperfusion (26, 27). Ischemia causes the depletion of adenosine triphosphate (ATP) within several seconds while increasing adenosine diphosphate, adenosine monophosphate, and adenosine. As a result, ischemia leads to the increase of adenosine and inosine, the destruction of which is a very important source for superoxide radicals (28). Eskurza et al. showed that XO was the important source for vascular endothelial reactive oxygen species, including superoxide anions, which reacted with nitric oxide and produced peroxynitrite. In this study, peroxynitrite caused vascular dysfunction as shown by the decreased bioavailability of nitric oxide (29).

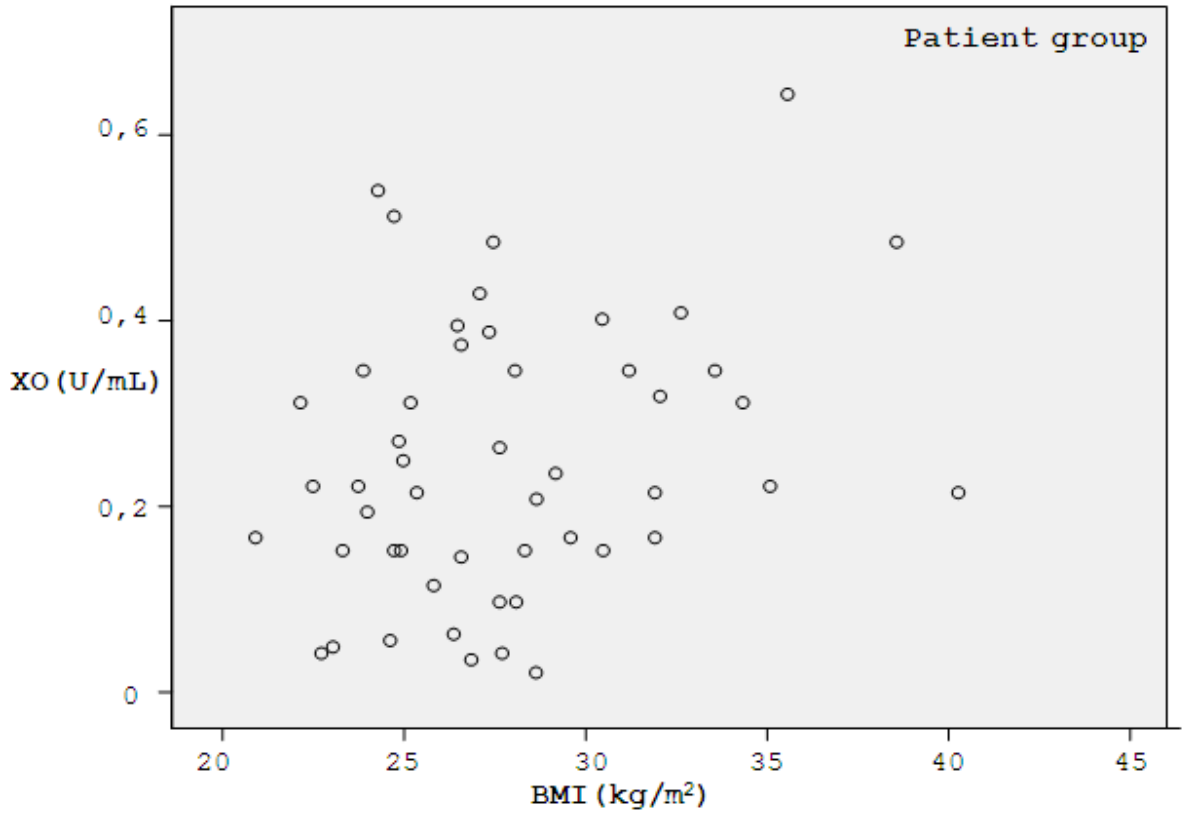

Figure 1: The significant correlation between XO and BMI in the patient group (r: 0.3, p<0.05).

XO is synthesized from XDH, which is found in 90% in normal tissue (30). XDH uses nicotine adenine dinucleotide as an electron receiver in the oxidation of hypoxanthine and xanthine. Alternatively, a superoxide radical scavenger mechanism is conducted by XO after conversion of XDH to XO in tissues affected by ischemia (31, 32). This may accelerate ATP deprivation, resulting in membrane Ca^{+2} gradients. Increased cytosolic Ca^{+2} activates Ca-dependent proteases, which selectively catalyze conversion of XDH to XO (33).

Cappola et al. showed that the activity of XO increased in ischemic myocardial tissue (34). Our results supported this finding. Additionally, a significant positive correlation existed between XO and BMI (r: 0.3, p<0.05); our study is the first to determine this relationship. No other publications have confirmed this result yet; however, the results of Nasser at al., which showed a negative correlation between Ca-ATPase and BMI in a study of 30 women with BMI ranging

from 20 to 40, may indirectly support our findings. The interpretation Nasser et al provided for their results was that decreased Ca-ATPase caused decreases in the Ca pumped from the cell and led to deterioration of the Ca membrane gradient. Sequelae of this included increased intracellular Ca, which was activated to fatty acid synthetase. This eventually led to increased accumulation of fat in the body (35). Similarly, our findings may be interpreted as indicating that lost ATP and ATPase dysfunction under ischemic conditions causes activation of Ca-dependent proteases catalyzing the conversion of XDH to XO.

Our study showed that XO and IL-6 plasma levels in patients with AMI were significantly elevated. Moe at al. suggested that increased IL-6 was a marker for cardiac ischemia (36). Some reports indicated that IL-6 levels in angina pectoris were significantly lower than they were in AMI (37, 38). Furthermore, Karpinski et al. showed that IL-6 was significantly high in patients 6 months after AMI (39).

In conclusion, IL-6 and XO increase in patients with AMI, but no correlation exists between them, indicating that IL-6 and XO are independent markers for insufficient endothelial function and ischemic condition in myocardium. The correlation between XO and BMI in patients with AMI, as a new finding, requires further investigations.

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