Markers of oxidative stress evaluated in patients with ischemic heart disease at the time of their first clinical manifestation

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(Ital Heart J 2002; 3 (Suppl 4): 49S-51S)

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Introduction

Oxidative stress and immune responses are involved in the onset and progression of atherosclerosis[1].

Several lines of evidence support the role of oxidized biomolecules as key antigens in atherosclerosis.

Detection of autoantibodies against malondialdehyde (MDA)-modified low-density lipoproteins has been used as a proof of *in vivo* immune response to oxidized proteins[2].

The plasma levels of MDA autoantibodies have been related to the extension of atherosclerosis in patients with coronary artery disease[3].

MDA autoantibodies may be potentially predictive for atherosclerosis but until now conflicting results have arisen from studies on this issue[4,5].

The aim of this study was to measure plasma levels of MDA autoantibodies and plasma levels of MDA expressed as thiobarbituric acid reactive substances (TBARS) in patients with new-onset unstable angina (UA) and in age-matched healthy control subjects.

Moreover, we investigated whether plasma levels of MDA autoantibodies are associated with oxidative stress measured in plasma as TBARS and/or with an increased prevalence of cardiovascular risk factors.

Methods

Patients. Twenty patients with new-onset UA and 20 age-matched healthy control subjects were enrolled for the study.

An interview to obtain incidence of major cardiovascular risk factors was performed.

Fasting blood collection to determine lipid profile, white blood cell count, fibrinogen and von Willebrand factor levels was routinely taken for each subject in the study.

Biochemical assays. Blood samples were collected in EDTA tubes, centrifuged at 2500 rpm and plasma was stored at -80°C until analysis.

MDA autoantibodies were determined by indirect Enzyme Linked Immuno Sorbent Assay-ELISA (reading at 490 nm).

MDA plasma levels were determined fluorimetrically (530/552 nm).

Statistical analysis. Statistical calculations were performed by StatView Software (Abacus Concepts, Berkeley, CA, USA).

Results are presented as means ± SD. A p value of < 0.05 was considered statistically significant.

Results

Plasma markers of inflammation showed a trend for more elevated levels in UA patients than in the control group: von Willebrand factor ($p = 0.08$), total white blood cell count ($p = 0.10$), fibrinogen ($p = 0.12$) (Table I).

More patients with UA had high titres for MDA autoantibodies, when compared to controls (30 vs 10%, levels > 75° percentile) (Fig. 1). Levels of MDA autoantibodies were not significantly increased in

Table I. Clinical characteristics of patients with unstable angina (UA) and controls.

Clinical characteristics	Patients with UA	Controls
Body mass index (kg/m^2)	27 ± 0.6	26.1 ± 0.7
von Willebrand factor (%)	172.4 ± 33	120.4 ± 16.8
Fibrinogen (mg/ml)	384.3 ± 20.7	331.4 ± 17.8
HDL (mg/dl)	38 ± 1.6	44.8 ± 2.1*
LDL (mg/dl)	133.5 ± 8.9	158.9 ± 10
Total white blood cell count (/mm^3)	7052.6 ± 366.6	6243.84 ± 324.4
Current smokers (%)	53	43
Family history of atherosclerosis (%)	30	7
Hyperlipidemia (%)	80	58
History of hypertension (%)	70	54

* = $p < 0.05$ vs patients with UA.

UA patients and did not have a consistent association with elevated levels of TBARS (Fig. 2).

Levels of MDA autoantibodies are not associated with clusters of risk factors for atherosclerosis.

Extension of atherosclerosis, according to the Gensini score, did not show any consistent association with the levels of TBARS ($p = 0.84$) and with MDA autoantibodies ($p = 0.41$).

In patients with UA, a significant association was present between TBARS levels and von Willebrand factor levels ($p < 0.05$); in addition, levels of MDA autoantibodies were correlated with HDL levels in the same group of patients ($p < 0.001$).

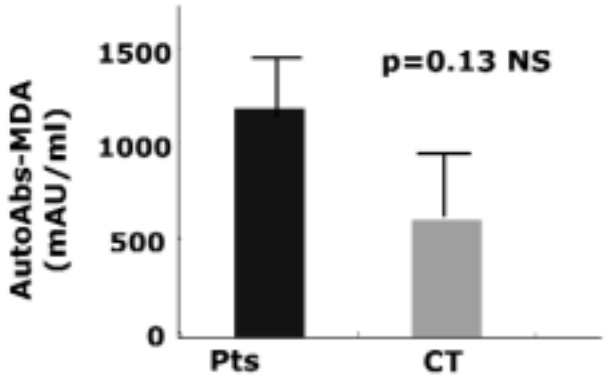

Figure 2. *Malondialdehyde (MDA) autoantibodies distribution in patients with unstable angina and controls (CT). AU = arbitrary units.*

Discussion

Mean plasma levels of TBARS were more elevated in UA patients than in control subjects.

More patients with UA had high titres for MDA autoantibodies, when compared to controls (30 vs 10%, levels > 75° percentile).

Levels of MDA autoantibodies were not associated with clusters of risk factors for atherosclerosis.

In UA patients, elevated levels of MDA autoantibodies were not predicted by an increased incidence of traditional risk factors for atherosclerosis, and did not have a consistent association with elevated levels of TBARS.

Our results do not support a direct association between MDA autoantibodies and the MDA antigen levels.

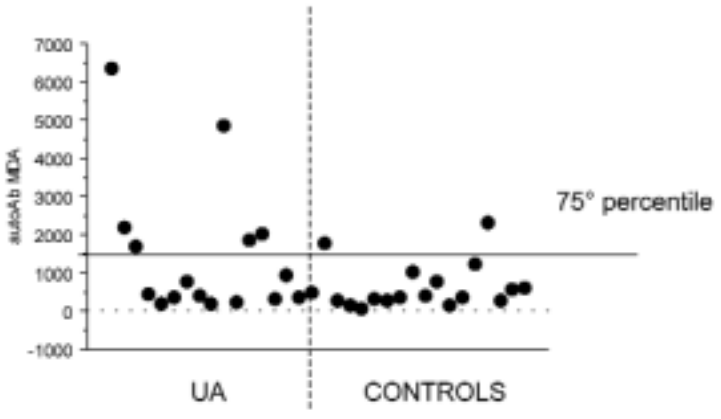

Figure 1. *Malondialdehyde (MDA) autoantibodies levels in patients with unstable angina (UA) and controls.*

In conclusion, determinants to mount an immune response against MDA in humans do not seem to be related to elevated levels of oxidative stress nor to a profile at risk for atherosclerosis, suggesting that other factors may be involved in triggering an immune response against MDA.

References

1. Maggi E, Chiesa R, Melissano G, et al. LDL oxidation in patients with severe carotid atherosclerosis. A study of in vitro and in vivo oxidation markers. Arterioscler Thromb 1994; 14: 1892-9.
2. van de Vijver LP, Steyger R, van Poppel G, et al. Autoantibodies against MDA-LDL in subjects with severe and minor atherosclerosis and healthy population controls. Atherosclerosis 1996; 122: 245-53.
3. Iribarren C, Folsom AR, Jacobs DR Jr, Gross MD, Belcher JD, Eckfeldt JH. Association of serum vitamin levels, LDL susceptibility to oxidation, and autoantibodies against MDA-LDL with carotid atherosclerosis. A case-control study. The ARIC Study Investigators. Atherosclerosis Risk in Communities. Arterioscler Thromb Vasc Biol 1997; 17: 1171-7.
4. Ahmed E, Trifunovic J, Stegmayr B, Hallmans G, Lefvert AK. Autoantibodies against oxidatively modified LDL do not constitute a risk factor for stroke: a nested case-control study. Stroke 1999; 30: 2541-6.
5. Boullier A, Hamon M, Walters-Laporte E, et al. Detection of autoantibodies against oxidized low-density lipoproteins and of IgG-bound low-density lipoproteins in patients with coronary artery disease. Clin Chim Acta 1995; 238: 1-10.