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Endothelial Apoptosis and Arterial Thrombosis: Expression of CD31 and CD146 in Recent and Old Thrombotic Events

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ABSTRACT

Background: Apoptosis (programmed cell death) of vascular lining endothelium is one of the causes of increased thrombogenicity. The luminal release of apoptotic endothelium-derived microparticles can cause the activation of tissue factor (TF).

Subjects and Methods: This study included 76 cases of recent and old arterial thrombosis and 15 age and sex-matched controls. Endothelial-derived CD146 positive microparticles were statistically higher among cases of old thrombosis vs. controls ($p<0.001$). They were also statistically higher among old thrombosis than recent thromosis cases ($p<0.05$). No statistical difference was found between recent thrombosis cases and controls regarding CD146. CD31 was found to be insignificantly raised among recent and old thrombotic cases in comparison to controls.

Results: Nineteen diabetics were among the patients' group but no statistical difference was detected regarding CD31 or 146 levels in comparison to non-diabetic patients. Similarly, 8 hypertensive cases were included with no statistical difference regarding CD31 or CD146 in comparison to normotensive cases. Endothelial microparticle detection among old cases of arterial thrombosis may indicate the persistence of the danger of rethrombosis.

Key Words: *Apoptosis - Thrombosis - Endothelial-derived CD146, CD31.*

INTRODUCTION

Clinical manifestations of atherosclerosis are the consequences of atherosclerotic plaque rupture that triggers thrombus formation. Tissue factor (TF), the most potent known initiator of the blood clotting system, is highly dependent on its activity on the presence of phosphatidyl serine (PS) (Mallat et al., 2000). Luminal endothelial cell apoptosis with shedding of PS rich microparticles might be responsible for thrombus formation on eroded plaques without rupture (Farb et al., 1998). These circulating microparticles can be identified by anti-CD31 and CD146 antibodies (Tegui and Mallat, 2001). So, the aim of this work was to study the presence of thrombogenic circulating microparticles in cases of recent and old arterial thrombosis as evidenced by circulating CD31 and CD146 exhibiting particles.

SUBJECTS AND METHODS

This study included 76 patients with arterial thrombosis above the age of 25 years with no history of anemia, infection, auto-immune disease, malignancy, hormonal therapy for females, renal problems or trauma related thrombosis. These included: Thirty nine cases of recent thrombosis (within 48 hours of acute attack) and thirty seven cases of old thrombosis (three or more months after acute attack).

Cases were Sub-Grouped as Follows:

Eleven cases of recent peripheral artery thrombosis as evidenced by cold pulseless limb and Duplex study.

Twelve cases of recent cerebral thrombosis as evidenced clinically by signs of lateralization, CT scan and/or MRI. Embolic cases were excluded.

Sixteen cases of acute myocardial infarction with acute chest pain, ECG findings, raised cardiac enzymes.

Eight cases of old peripheral thrombosis as evidenced clinically and by Duplex, history of amputation or recent graft surgery (within one week of surgery).

Sixteen cases of old cerebral thrombosis as evidenced clinically and by CT scan.

Thirteen old myocardial infarction as evidenced clinically, by ECG and normalization of cardiac enzymes.

All Cases were Subjected to:

Full history taking including reference to special habits and clinical exam Blood count-lipid profile-liver and kidney functions-uric acid-Hb A1c-Serum soluble CD31 and CD146 level estimation:

Venous sample was collected and serum was separated. Annexin V was used to capture PS containing particles. CD31 and CD146 levels were estimated in nmol/L PS equivalent by ELISA using anti-CD31 and CD146 antibodies respectively.

Cases were compared to 15 age-matched healthy controls.

RESULTS

This study included 76 patients with an age range of 39 and 74 years with a mean of 51.1 years ±8 and a male to female ratio of 2.67:1. Recent thrombosis was found in 39 cases while old thrombosis was found in 37 cases. Fifteen controls were included with a mean age of 47.3±9.1 years.

A mean platelet count of 250000/cmm was found among recent thrombosis group, and 265000/cmm among the old thrombosis group. Neither differed from a mean of 234500/cmm count (±4690) among the control group with no statistical difference (p=0.05).

Total cholesterol mean of 146.8 (±103.6) mg/dl was detected among the recent group. This did not differ significantly neither from the old group (mean of 130.6±45.2mg/dl) nor from the control group (mean of 208.1± 87.5mg/dl).

LDL cholesterol levels still did not show any significant difference among the three groups (mean of 148.7±41.5mg/dl) in the recent group 32.6±29.6mg/dl among the old group and 134.8±34.6mg/dl among the control group).

HDL levels showed a mean of 45.5±14.2 mg/dl among the recent group, 41.6±14.6mg/dl among the old group and 49.8±13.9mg/dl among the control group with no statistical difference between the groups (p>0.05).

Serum triglycerides still did not differ significantly among the groups with a mean of 162.3±88.6mg/dl among the recent group, 137.7±73.7mg/dl among the old group and 116.6±84.3mg/dl among the controls.

Among our patients, 50 cases were smokers, half of whom smoked two or more packs per day. Duration of smoking showed a mean of 15±4.3 years.

Nineteen cases were known diabetics, eight receiving insulin and 11 on oral therapy with a mean Hb A1c of 9%. Eight cases were known hypertensive, four of whom were controlled on therapy.

CD31 level among the recent group showed a mean of 13.1±6.4nmol/L, among the old group showed a mean of 12±6.6mol/L and 11.9± 5.4nmol/L among the control group with no statistical difference. CD31 levels were not statistically different among patients who were smokers and patients who were not diabetic and non-diabetic cases, nor were they different among hypertensive vs, normotensive cases.

CD146 showed a mean of 428.7±121.9 nmol/L among the recent thrombosis group, 675±223.4nmol/L among the old thrombosis group and 382.7±120.2nmol/L among the control group. Difference between the old group and controls was highly significant (p<0.01) and was also significant between the old and the recent group (p<0.05).

CD146 levels did not show a significant difference between smoking and non-smoking cases, diabetic and non-diabetic cases, nor was a significant difference found between hypertensive and non-hypertensive cases.

DISCUSSION

Apoptosis, through its procoagulant ad proadhesive potentials, may play a critical role in both plaque and blood thrombogenicity and may be an important step in the transition from stable to unstable atherosclerotic disease. Circulating apoptotic microparticles could be a valuable marker in thrombus formation and hence the instability of the atherosclerotic plaque. All cell types involved in atheromatous

plaque are involved in apoptosis, particularly apoptotic macrophages. Vascular endothelial cell apoptosis promotes the coagulation process (Iwakura et al., 2000).

Among the first morphological changes after initiation of apoptosis of the endothelium is membrane blebing with shedding of microparticles, loss of focal adhesion sites and retraction from the matrix followed by detachment from arterial wall. A significant number of these shed cells results (Mallat and Tedgui, 2000).

CD146 (S-Endo1-associated antigen) (Mel-CAM) (MUC 18) is a transmembrane glycoprotein that is constitutively expressed in the whole of the human endothelium, some dendritic cells and smooth muscle cells (Bardin et al., 1996). It is localized at areas of cell-cell junction (Bardin et al., 2001). The enumeration of circulating endothelial cells released in the blood after vascular injury represents a direct exploration of this process of apoptosis and shedding. Monoclonal antibody that recognizes CD146 allows the detection of high numbers of these cells or smaller particles of their residues in thrombotic, infectious, or immunological disorders with insignificant levels in normal subjects, thus a useful marker for vascular wall injury (Dinat-George and Sampol, 2000).

CD31 (Platelet Endothelial Cell Adhesion Molecule 1) (PECAM1) is a cell adhesion molecule of the Ig superfamily expressed on vascular endothelium, platelets, monocytes, neutrophils and subsets of T lymphocytes. It has been implicated in leukocyte-endothelial cell interactions and monocyte and neutrophil recruitment. Simon et al., 2002, showed that apoptosis disabled CD31-mediated cell detachment from phagocytes.

The presence of these antigens in blood in the context of arterial thrombosis implicates the existence of apoptotic endothelial-derived microparticles with potential thrombogenic activity (Tedgui and Mallat, 2001). Tissue factor may be found encrypted in these microparticles resulting in the enhancement of Factor VIIa enzymatic activity (Morrissey, 2001).

Multivascular atherosclerosis reduces life expectancy. After an initial myocardial infarct, life expectancy is 13.9 years, after an initial stroke, it is 8.8 years, and after peripheral arterial disease it is 16 years, going down to 1.5 to 1.8 years if later complicated by myocardial infarction (Habrel and Dembowski, 1999).

Our results revealed a highly significant elevation of the soluble CD146 in arterial thrombosis as compared to controls ($p<0.01$). We also found a highly statistical difference when comparing CD146 levels between controls and old peripheral thrombosis group ($p<0.01$) and between controls and old coronary thrombosis group (0.01) with a very high significant difference between controls and old thrombosis group ($p<0.001$). Among the recent thrombosis patients, a significant difference was detected between controls and recent cerebral thrombosis group ($p<0.05$).

Our findings are in line with the detection of elevated levels of circulating procoagulant microparticles 8 days after acute coronary ischemia reported by Van Belle et al., 1998, who observed persistent intracoronory thrombi 24 hours to 30 days after the ischemic episode. They propose that persistence of these high levels may be a useful indicator of the persistence or recurrence of ischemic events.

Soejima et al. (1999), reported that plasma tissue factor antigen levels are significantly elevated in patients with unstable angina compared to stable angina cases and was associated with poor prognosis.

Mallat et al. (1999), analyzed the presence of shed membrane apoptotic microparticles in extracts form 6 human atherosclerotic plaques and 3 underlying arterial walls with detection of marked tissue factor expression in close proximity to apoptotic cells with significant tissue factor thrombogenic activity.

Mallat et al. (2000) found high levels of CD146 positive microparticles in acute coronary syndrome cases in comparison to stable angina and non coronary chest pain patients. They collected their samples on days 0 to 8 of diagnosis but no follow up of samples on later dates was performed.

In our study, soluble CD31 levels did not differ from patients to controls thus contradicting findings by Mallat et al. (2000) detecting high levels of CD31 positive microparticles in acute coronary cases. However, Seebruany and Gerbel (1999), found that soluble PECAM1 plasma

levels was identical between acute myocardial infarction cases and controls, with significant rise 3 hours after thrombolysis (p=0.02) followed by significant decrease 24 hours after attempted reperfusion.

Our results depended on samples taken very early after diagnosis and at least three months after acute event.

A statistically significant difference (p<0.05) was found comparing soluble CD31 levels among controls vs. thrombosis patients when grouped according to affected arterial bed. Cerebral thrombosis group was higher vs. coronary thrombosis group (p<0.01) and still higher when compared to peripheral thrombosis group (p<0.05). Zaremba and Losy, (2002), found increase in soluble PECAM1 level in serum and CSF within 24 hours of the onset of stroke which is confined to the endothelial cells of the blood brain barrier. Thus, PECAM1 may play a role in regulation of inflammatory cell migration in CNS (Qing et al., 2001).

In addition to their direct effect in promotion and amplification of the coagulation cascade, endothelial derived microparticles may be responsible for dissemination of the procoagulant and pro-inflammatory potentials to sites remote from the micro environment of their formation (Satta et al., 1994).

Synthesis of TF is increased in vitro by several factors including shear stress, hypoxia, oxidized lipoproteins and anionic phospholipids (Conner, 1994).

Among our patients, 50 cases were smokers forming a hypoxic stress. However, no significant difference between smoking and non-smoking cases. Hypertension can form a shear stress (Tricot et al., 2000). PECAM1 endothelial cell expression was found to be upregulated in response to experimentally induce hypertension with endothelial cell injury as reported by Suzuki et al. (2001). However, no difference as detected between our hypertensive patient group (eight cases) and our non-hypertensive group (68 cases) neither in CD31 nor in CD146 levels.

Diabetes increases oxygen intermediates with activation of protein kinase C, a major intracellular intermediate in cell apoptosis pathway (Schwartz et al., 1992).

No difference was found in our study between the nineteen diabetic patients included in the study and the non-diabetic cases. Thus the vascular endothelium can no longer be viewed as a static physical barrier. Prolonged and exaggerated endothelial activation leads to dysfunction. Apoptosis of the vascular endothelium is an important factor in thrombogonicity. Detection of circulating procoagulant micro-particles as a result of apoptosis is suggested to be done repeatedly to detect its power in the prediction of persistence or recurrence of ischemic events.

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