

*Ibrahim C. Haznedaroğlu
Yunus Erdem
Ahmet Uğur Yalçın
Nilgün M. Sayınalp
Ünal Yasavul
Çetin Turgan
Şali Çağlar
Semra DüNDAR
Şerafettin Kirazlı*

Departments of Nephrology and Hematology, Hacettepe University School of Medicine, Ankara, Turkey

Circulating Thrombomodulin as a Molecular Marker of Endothelium Damage in Renal Transplant Recipients

Dear Sir,

Thrombomodulin (TM) is a cell surface glycoprotein located at the luminal surface of vascular endothelium as a membrane-bound high-affinity thrombin receptor [1]. It inactivates thrombin and stimulates anticoagulatory pathways. TM therefore belong to the anticoagulant defence system against thrombosis and plays a crucial role in the regulation of blood coagulation and fibrinolysis *in vivo* [2]. Consequently, quantitative and/or qualitative impairment of TM could possibly play a pathogenetic role in the thrombogenesis and endothelial damage [3].

A smaller soluble form of TM has been isolated from human blood and urine [4]. The structure of soluble TM is not known but is thought to be similar to the soluble protein obtained after proteolytic modification of TM with elastase [5], a cleaved form of tissue TM with loss of part of the transmembrane domain and the cytoplasmic tail [6]. Therefore soluble TM, which exists in circulating plasma as heterogeneous fragments, appears to be derived from injured endothelial cells or to be proteolytically cleaved from TM by proteases [7]. Soluble TM is cleared from the circulation by the kidneys and liver [2].

The study was a cross-sectional determination of TM, a major component on the endothelial surface, concentrations in plasma from renal transplant recipients and healthy volunteers. Seventeen renal transplant recipients with normal graft function

(serum creatinine <2 mg/dl; 8 females, 9 males, age 31 ± 2 years, mean transplantation duration 34 ± 6 months), 7 renal transplant recipients in chronic rejection (serum creatinine ≥ 2 mg/dl; 2 females, 5 males, age 39 ± 3 years, mean transplantation duration 44 ± 11 months) and 15 nonsmoking healthy volunteers (7 females, 8 males, age 26 ± 9 years) with normal renal function were included in the study. Patients selected for this study were on triple immunosuppressive treatment with prednisolone, azathioprine and ciclosporine A and were not receiving drugs affecting coagulation and fibrinolysis.

After a 30-min period of rest in the sitting position, blood samples were drawn from large antecubital veins. All venipunctures were carried out without venous stasis. TM concentrations in plasma from patients and healthy volunteers were measured by the solid phase 'sandwich' ELISA method. Plasma TM was measured by a two-site ELISA with two monoclonal anti-human TM antibodies (ELISA, Asserachrom Thrombomodulin, Diagnostica Stago, France). The intra-assay coefficient of variation was 6% and the interassay coefficient of variation 8%, computed from results of pathological plasma samples. As the distribution of the TM data is not homogenous, we used the nonparametric Kruskal-Wallis test for the analysis of variances. Post hoc comparisons were performed by the Mann-

Whitney U test with downward-adjusted p values. Significant p values were assigned to be lower than 0.017 (0.05/3). Results are expressed as median (IQR).

Median plasma TM concentrations in recipients with good graft function, in chronic rejection and in the control group were 8.3 (7.8), 18.9 (10.8) 2.0 (0.8) ng/ml, respectively. TM concentrations were higher both in renal transplant recipients in chronic rejection and with normal graft functions compared to the control group ($p < 0.0001$ and $p < 0.001$, respectively). TM concentrations were notably increased in renal transplant patients in chronic rejection compared to renal transplant recipients with normal renal function ($p < 0.004$).

High serum TM concentrations reflecting endothelial injury have been previously reported in uremic patients during hemodialysis and patients with orthotopic liver transplantation [8, 9]. In our study, increased plasma-soluble TM concentrations were observed in renal transplant recipients compared to the control group. We also observed that increments in TM concentrations were more prominent as graft function declines in renal recipients. It might be concluded that increased plasma soluble TM concentrations may be a sign of endothelial injury in renal transplant recipients. Higher levels of TM antigen may indicate increased endothelial damage to the graft kidney vascular bed during a chronic rejection period.

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