Circulating thrombomodulin levels in familial Mediterranean fever

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ABSTRACT

Increments in circulating thrombomodulin levels reflect endothelial cell injury. Thrombomodulin can also be synthesized by several inflammatory cells including monocytes, neutrophils, and thrombomodulin itself can modulate the inflammatory response. In this study, we assessed circulating thrombomodulin concentrations in patients with familial Mediterranean fever (FMF). Twenty-five patients with FMF (F/M: 14/11) (mean age: 31.1 ± 9.7 years) and 25 healthy controls (F/M: 13/12) (mean age: 34.6 ± 7.0 years) were involved in the study. Thrombomodulin levels were measured by commercially available enzyme-linked immunosorbant assay (ELISA) (Immunoassay of thrombomodulin Diagnostica Stago, Asnieres-Sur-Seine, France). Twenty of the patients were in attack-free period and the remaining five had been during acute FMF attacks. Thrombomodulin levels were higher in the study group (20.9 ± 12.1 ng/ml) than healthy controls (14.1 ± 8.4 ng/ml) ($p < 0.05$). Circulating thrombomodulin levels were also higher in attack-free FMF patients (22.4 ± 12.9 ng/ml) than controls. This study disclosed for the first time significantly higher increments in the circulating levels of thrombomodulin in FMF. This observation could be a consequence of injured endothelium and/or activated inflammatory cells.

Introduction

Familial Mediterranean fever (FMF) is a recessive disorder characterized by self-limiting episodes of fever and serosal, synovial and cutaneous inflammation. Neutrophils are the dominant cells in the inflammation of FMF regulating the inflammatory response. During acute attacks of FMF, neutrophil accumulation and increased release of proinflammatory cytokines and chemokines such as tumor necrosis factor-alpha (TNF-alpha), interleukin-1 (IL-1), and IL-8 have been described (1-3). Endothelial cells play key roles in the initiation and perpetuation of the inflammatory response via expressing cell adhesion molecules and secreting proinflammatory cytokines. Endothelial cell damage is expected to occur during the neutrophil migration to adjacent tissue (3, 4).

Thrombomodulin is a vascular endothelial surface glycoprotein that is present on the luminal surface of endothelial cells. It has been previously reported that thrombomodulin is present in degraded forms in circulating blood plasma, and increased thrombomodulin levels reflect endothelial cell injury since the fragments of thrombomodulin detected in the blood were not secreted by endothelial cells under physiologic conditions (5-8). Thrombomodulin has also been reported to be synthesized by several cells, including monocytes, neutrophils, mesothelial cells and synovial lining cells (9) and can also modulate the inflammatory response (10). However, thrombomodulin levels have not been investigated in FMF. In this study we assessed serum thrombomodulin levels in FMF patients and healthy controls.

Patients and method

Patient selection

Twenty-five FMF patients (F/M: 14/11, mean age: 31.1 ± 9.7 years) diagnosed according to the Tel-Hashomer criteria (11) in the Department of Rheumatology, Hacettepe University between 2001-2003 and 25 healthy controls (F/M: 13/12, mean age: 34.6 ± 7.0 years) were consecutively involved in this study. Twenty patients were in attack-free period while the remaining five had acute attacks during blood

Table I. Thrombomodulin (TM) levels in familial Mediterranean fever (FMF) patients and healthy controls (N = 25).

Groups		TM levels (ng/ml)	P*
FMF patients	Overall patient group (n = 25)	20.9 ± 12.1	< 0.05
	Attack-free patients (n = 20)	22.4 ± 12.9	< 0.05
	Patients with acute attacks (n = 5)	14.8 ± 5.4	-
Healthy controlS		14.1 ± 8.4	

*p values indicate the comparison between all FMF patients *vs.* healthy controls and attack-free FMF patients *vs.* healthy controls.

sampling. The mean duration from the last attack of FMF in the attack-free group was 5.7 ± 1.53 months, and all patients in the attack-free period in our study were free of any disease manifestation of FMF for at least 3 months.

Collecting of serum samples

For measurement of the serum sample of thrombomodulin, all serum samples were obtained between 9.00-10.00 in the morning from resting subjects who had been required to fast. Care was taken to avoid platelet activation, by the use of atraumatic needle punctures, a butterfly needle and minimal stasis with tourniquet release before blood withdrawal into 10 ml syringes. Smokers were asked to abstain from smoking since the previous night (as of 12.00 midnight). Plasma was prepared for measurement of thrombomodulin by collecting blood into 3.13% trisodium citrate, which has been centrifuged within 20 minutes of venipuncture at 3,000g. All samples for measurement of thrombomodulin were stored at -80ºC until assayed.

Measurement of thrombomodulin

Thrombomodulin was measured following a microenzyme immunoassay technique using commercial kits (immunoassay of thrombomodulin-Diagnostica Stago, Asnieres-Sur-Seine, France) in accordance with the manufacturer's instructions. Briefly a 5 µl sample of serum of standard thrombomodulin and 300 µl of Fab'monoclonal antithrombomodulin antibody (MFTM-6)- horseradish peroxydase conjugate 10 g/L BSA, 0.1 mol/L NaCl, and 10 mmol/L ethylendiamine tetra acetate were incubated with a polystyrene ball which had been coated with the monoclonal anti-thrombomodulin antibody MFTM-4 at room temperature after 60 min of incubation, the polystyrene ball was washed twice with 4 ml of 5 mmol/L Na-phosphate buffer pH 7.0 containing 50 mmol/L NaCl and was incubated with 300 µl hydrogenperoxide (0.075g/L) as a substrate for 30 minutes at room temperature. The reaction was stopped by adding 800 µl sulfuric acid (1.75 mmol/L) at the absorbance at 450 nm was measured by a spectrometer.

Statistical analysis

Unless otherwise stated, values are expressed as mean ± SD. All data was stored in Statistical Package for Social Sciences (SPSS) software, version 11.0 (SPSS, Chicago, IL). Differences between the groups were analyzed by Mann Whitney U test. P value less than 0.05 ($p < 0.05$) was accepted as significant.

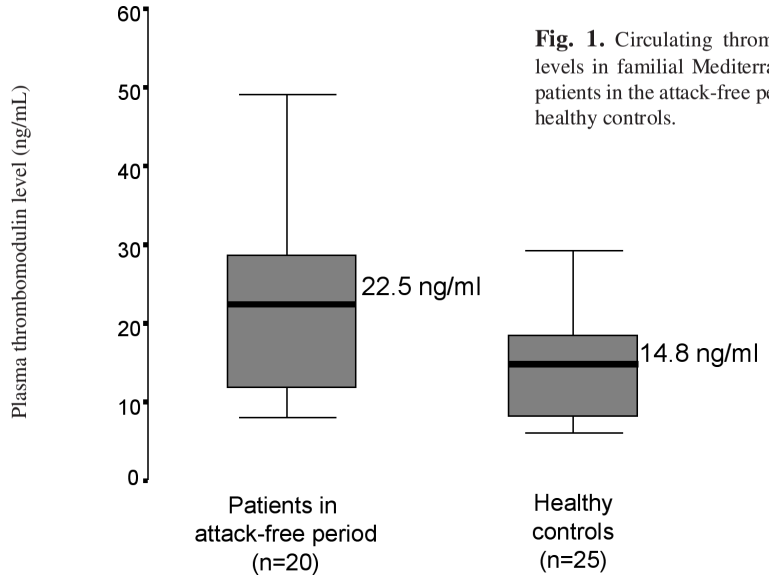

Fig. 1. Circulating thrombomodulin levels in familial Mediterranean fever patients in the attack-free period and in healthy controls.

Results

There was not any difference regarding age and gender between the study group and controls ($p > 0.05$). Mean disease duration of the all patients were 14 ± 11 years (min-max: 2-40 years). Serum thrombomodulin levels were 20.9 ± 12.1 ng/ml and 14.1 ± 8.4 ng/ml in FMF patients and healthy controls, respectively. Subgroup analysis revealed that circulating thrombomodulin levels were 22.4 ± 12.9 ng/ml and 14.8 ± 5.4 ng/ml in attack-free FMF patients and in patients with acute FMF attacks, respectively. The difference regarding circulating thrombomodulin levels between the whole patients group and the controls, and between patients in attack-free period and the controls were statistically significant ($p < 0.05$) (Table I) (Fig. 1). Because of the small number of the patients with acute attacks, we did not perform further analysis including patients with acute FMF attack.

Discussion

In this study, we demonstrated that circulating levels of thrombomodulin, an endothelial injury marker, were significantly elevated in patients with FMF. To our knowledge this is the first study indicating endothelial cell injury during the course of FMF. The endothelium is a highly dynamic layer that is

involved in a multitude of physiologic functions, including the vasomotor tone, the trafficking of cells and nutrients, the maintenance of blood fluidity, and the growth of new blood vessels (4). The strategic location of the endothelium allows it to sense changes in hemodynamic forces and blood born signals and respond by releasing a number of vasoactive factors/substances and remodeling vascular structure. Endothelial cells play active roles in some connective tissues disorders and vasculitis (12-15). It is suggested that FMF may cause some endothelial cell abnormalities (16). Endothelial cells are important in the nature of the FMF inflammatory attacks (2). We have recently demonstrated elevated soluble vascular endothelial growth factor receptor-1 (sVEGFR-1) levels in FMF patients with and without FMF attacks compared to healthy controls (17). Direskeneli *et al.* showed that intercelular adhesion molecule 1 (ICAM 1) that is a cell surface glycoprotein of the immunoglobulin super family and is expressed in endothelial cells, is markedly up regulated in FMF attacks and plays an important role in neutrophil extravasation. This represents neutrophil endothelial cell adhesion and subsequent migration to the serosal surface, triggering the inflammatory response (3). On the other hand, endothelial cell damage can occur during the neutrophil migration to adjacent tissue (4, 16). Therefore, our findings suggest that endothelial cell injury can take place during the course of FMF. The majority of patients in our study group were in attack-free periods during blood sampling. Moreover, thrombomodulin levels were significantly higher in attack-free subgroup of patients than healthy controls. We along with others we have previously demonstrated a subclinical inflammatory state in attack-free FMF patients (3, 18, 19). Increments in the circulating thrombomodulin levels in the attack-free FMF patients in this study may represent injured endothelium together with ongoing subclinical inflammatory activity.

Thrombomodulin itself has some pro- and anti-inflammatory properties. The molecule is chemotactic for neutrophils and monocytes, increases IL-1β and tumor necrosis factor-α-induced neutrophil chemotaxis. N terminal domain of thrombomodulin may play a direct role in modulating inflammation like polarization, complement activation, lymphocyte, neutrophil and platelet homing, and regulation of apopitotic pathways (10, 20). Besides the endothelium, other cells including mesothelial cells of the peritoneum and circulating neutrophils, monocytes and platelets can be the source of circulating thrombomodulin (21-24). However, thrombomodulin levels were comparable in healthy controls and in FMF patients with acute attack. Thrombomodulin has a quite short half-life in the plasma although the exact period cannot be determined due to the heterogeneity of its dynamics. The plasma half-life of a long-acting recombinant human thrombomodulin was only 2-3 days after subcutaneous injections (25). All patients in the attack-free period in our study were free of any disease manifestation of FMF for at least 3 months. Hence, our results suggested that TM was released mainly from the endothelial cells, not from the cells of inflammatory nature such as neutrophils, reflecting subclinical inflammation in the attack-free periods of FMF. However this hypothesis needs to be confirmed in a larger group.

In conclusion, thrombomodulin levels are elevated in attack-free FMF patients. Our results suggest that thrombomodulin is released mainly from the endothelial cells, and support the hypothesis of subclinical inflammation in attack-free periods of FMF. However, since the number of patients with acute attack was very limited, our results should be confirmed by further studies with larger number of FMF patients in both acute attack and attack-free periods. Moreover specific markers of endothelial activation and/or injury such as soluble E-selectin should also be studied concurrently.

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