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The effects of thrombomodulin and activated protein C on the pathogenesis of multiple sclerosis

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ABSTRACT

Background: Various molecules of the coagulation cascade are thought to have varying roles in the pathophysiology of multiple sclerosis (MS). We aimed to find new information about the effects of the coagulation cascade molecules to develop new therapeutic strategies for MS.
Materials and methods: Patients with MS were chosen from among patients who were followed up at our hospital. We examined the thrombomodulin (TM) and activated protein C (APC) serum levels in patients with MS and the healthy controls. The patient groups were determined as relapsing-remitting MS (RRMS) or secondary progressive MS (SPMS) according to the McDonald criteria and between ages of 18 and 70.
Results: A total of 244 participants, 122 patients with multiple sclerosis and 122 healthy volunteers were included in the study. There was no statistically significant difference in the APC and TM levels between the patients and the healthy controls ($p > 0.05$), between the patients with RRMS and SPMS ($p > 0.05$), and between the first day of acute relapse and 10th day of methylprednisolone therapy in the patients with RRMS ($p = 0.334$; $p = 0.363$). We detected a statistically positive correlation only between the expanded disability status scale (EDSS) scores and TM levels in the patient group ($p = 0.009$).
Conclusion: Treatment with methylprednisolone decreases EDSS score in RRMS relapse. The increase in EDSS is related to level of TM. The changes in level of TM and APC may be indicator for prognosis of MS or treatment modalities to MS.

1. Introduction

Multiple sclerosis (MS) is a disease induced by complex relations of various genetic and environmental factors and is characterized by inflammatory demyelination of the central nervous system (CNS)(Griffin et al., 2006; Ropper, 2009). MS is a generalized disease characterized by neuroinflammation, oligodendrocyte death, and endothelial dysfunction. Apoptotic cell death, glutamate toxicity, and oxidative stress are among the causes of MS pathology (Genc, 2007).

Recent studies revealed the presence of cerebral fibrin deposits and tissue coagulation abnormalities in animal models of MS (EAE) (Adams et al., 2004). In patients with MS, disruption of endothelial cell integrity and migration of inflammatory cells in the blood cause extravasation of fibrinogen (Adams et al., 2004, Genc, 2007). In in vivo and in vitro studies, activated protein C (APC) helps maintain endothelial cell integrity (Esmon, 2006). In addition, APC decreases inflammatory cell adhesion and blood–brain barrier migration into cerebral endothelial cells (Festoff et al., 2012). Blood-related monocytes and activated microglias play an important role in cell injury (Genc, 2007). APC also suppresses proinflammatory cytokines and monocytes (Yuksel et al., 2002).

Thrombomodulin (TM) functions as a cofactor in the thrombin-induced activation of protein C in the anticoagulant pathway. Thrombomodulin is mainly found on the endothelial cell surface. TM causes changes in substrate specificity and acts as an anticoagulant and anti-inflammatory protein (Toda et al., 2013). The protein C system is an important anticoagulant mechanism. Protein C activated by TM has many functions such as inactivation of coagulation factors Va and VIIIa and inhibition of thrombin formation (Esmon, 1989). Thus, APC has anti-inflammatory, anti-apoptotic, and endothelial barrier–stabilizing effects and plays a role in cytoprotection (Neyrinck et al., 2009).

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In this study, we aimed to study the role of TM and APC in the pathophysiology of MS and different types of MS. The results will be helpful for the development of new treatment strategies.

2. Materials and methods

2.1. Patients

We examined patients admitted to the MS policlinic in 2013. Inclusion criteria were 18–70 years old, nonuse of immunomodulatory drugs within the last 6 months, and definitive diagnosis of relapsing-remitting MS (RRMS) or secondary progressive MS (SPMS) according to the 2010 McDonald criteria. The patients with relapsing-remitting MS were divided into relapse and remission groups. The RRMS relapse group was treated with methylprednisolone for 10 days. Blood samples were collected on the first day of before treatment and the 10th day of treatment. The RRMS remission group was composed of patients who had not been treated with immunomodulators within the last 6 months for any reason. Exclusion criteria were treatment with immunomodulatory drugs within last 6 months and history of neurodegenerative and other neuroinflammatory disease. We collected blood samples from relapsing-remitting patients with MS in remission, in the acute relapse phase (before treatment) and on the 10th day of acute relapse treatment (1000 mg IV methylprednisolone treatment). Secondary progressive MS patients are collected from patients admitted to policlinics for MS. The control group was composed of healthy hospital staff at the Istanbul Medeniyet University Goztepe Research and Educational Hospital between the age of 18 and 70 years. We collected blood samples from all participants and evaluated the TM and APC levels.

2.2. Statistical analysis

For the statistical analysis, NCSS (Number Cruncher Statistical System) 2007&PASS (Power Analysis and Sample Size) 2008 Statistical Software (Utah, USA) was used. To evaluate the study data, the Mann-Whitney *U* test was used for descriptive statistical methods (mean, standard deviation, median, frequency, rate, minimum and maximum) and for quantitative data to compare the parameters of the two groups without normal distribution. The Wilcoxon signed-rank test was used to compare the parameters without normal distribution within the group. Fisher's exact test and the Fisher-Freeman-Halton exact test were used to compare the qualitative data. Spearman's correlation analysis was used to evaluate the relationship between the parameters. The significance level was $p < 0.01$ and $p < 0.05$.

3. Results

In the study, 122 of the 244 participants had MS, and the other 122 were volunteer hospital staff without any health problems. The mean age was 41.13 ± 10.71 in the patient group and 37.09 ± 12.62 in the control group. Most participants were female: 65.9% of all participants, 78% of the RRMS patient groups, 50% of the SPMS patient group, and 59% of the healthy controls (Table 1).

In the comparison of the patient and control groups, there was no statistically significant difference in terms of the APC and TM levels ($p > 0.05$).

The patients' expanded disability status scale (EDSS) scores increased with age ($p=0.002$) and duration of disease ($p=0.001$) (Fig. 1). In patient group, there was statistically significance in EDSS score between RRMS and SPMS (p‹0.01). EDSS scores were significantly higher in the SPMS group than in the RRMS group and increased with duration of disease (Fig. 2). In RRMS relapse subgroups, there was a statistically significant difference in the EDSS scores between 1st day of relapse and 10th day of treatment of relapse (p:0.03). The EDSS scores decreased significantly after methylprednisolone treatment (Table 2). In addition, In the present study, we found that EDSS scores were statistically correlated TM levels ($p=0.009$); but not with APC (p:0.232) in the patient group (Table 3 and Fig. 3).

When we compared sex with the EDSS scores and levels of APC and TM, there was no statistically significant difference ($p > 0.05$). However, there was statistically significance between age and EDSS score (p:0.001). Age was also higher in SPMS group than RRMS. This was also statistically significant (p:0.001).

In the patient group, there was no statistically significant difference between APC and TM levels in the RRMS and SPMS groups ($p > 0.05$). In the RRMS relapse subgroup, there was no statistically significant difference in the APC and TM levels between the first day of relapse and 10th day of methylprednisolone treatment ($p > 0.05$). In the present study, there was a statistically positive correlation between EDSS scores and TM levels in the patient group ($p=0.009$; Table 3 and Fig. 3) In RRMS group, there was no statistical relation between levels of TM and APC and EDSS score, duration of disease ($p > 0.05$). In SPMS group, there was no statistical relation between levels of TM and APC and EDSS score, duration of disease ($p > 0.05$). There was no significant change in the APC and TM levels by age and duration of disease ($p > 0.05$).

In the control group, there were no statistically significant changes in the APC and TM levels with sex and age ($p > 0.05$). However, the APC and TM levels decreased with age.

4. Discussion

MS is an autoimmune disease triggered by miscellaneous relations between various genetic and environmental factors and characterized by progressing inflammatory demyelination of the

Table 1
Evaluation of protein C and thrombomodulin levels in all groups included in the study (whole RRMS patients, RRMS patients in remission, MS patients on first day of acute relaps, MS patients on 10th day of acute relaps, SPMS patients, control group).

	N (%)	Female n (%)	Age Mean ± SD (median)	Length of dx Mean ± SD (median)	EDSS Mean ± SD (median)	Protein C Mean ± SD (median)	TM Mean ± SD (median)
All participants	244	161 (%65.9)	38.56 ± 10.72 (39.5)	8.36 ± 6.21 (7.0)	1.58 ± 1.35 (1.0)	65.84 ± 65.63 (40.7)	5.55 ± 5.33 (3.2)
RRMS	100	78 (%78)	38.76 ± 9.45 (39.0)	7.67 ± 5.76 (6.0)	1.19 ± 0.71 (1.0)	65.25 ± 68.78 (40.7)	5.35 ± 5.34 (3.0)
RRMS remission	85	66 (%77.6)	39.44 ± 9.59 (40.0)	7.66 ± 5.70 (6.0)	1.06 ± 0.66 (1.0)	64.61 ± 69.51 (38.7)	5.25 ± 5.33 (3.0)
RRMS relapse 1st day	15	12 (%80)	35.67 ± 8.30 (35.0)	8.40 ± 6.68 (5.0)	2.07 ± 0.65 (2.0)	68.87 ± 60.51 (47.6)	5.25 ± 5.20 (3.9)
RRMS relapse 10th day	15	12 (%80)	35.67 ± 8.30 (35.0)	8.40 ± 6.68 (5.0)	1.77 ± 0.75 (1.5)	61.42 ± 81.43 (31.5)	4.76 ± 4.97 (3.2)
SPMS	22	11 (%50)	46.45 ± 8.07 (45.5)	14.18 ± 6.95 (13.5)	4.95 ± 1.33 (5.5)	77.12 ± 70.79 (54.9)	5.75 ± 4.94 (3.5)
Healthy control	122	72 (%59)	37.09 ± 12.62 (38.5)	–	–	65.31 ± 58.60 (39.8)	6.03 ± 5.51 (3.4)