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Cerebrospinal Fluid Analysis of Pericytic Mediators in Clinically Isolated Syndrome and Multiple Sclerosis: A Preliminary Study

Klinik İzole Sendrom ve Multipl Skleroz Hastalarının Beyin Omurilik Sıvılarında Perisitik Mediatörlerin Analizi: Pilot Çalışma

Tuncay Gündüz[1], Tuba Tanyel-Kiremitçi[2], Canan Ulusoy[3], Murat Kürtüncü[1], Recai Türkoğlu[2]

[1]Department of Neurology, İstanbul University İstanbul School of Medicine, İstanbul, Turkey
[2]Department of Neurology, Haydarpaşa Numune Training and Research Hospital, İstanbul, Turkey
[3]Department of Neuroscience, Institute of Experimental Medicine, İstanbul University, İstanbul, Turkey

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ABSTRACT

Objectives: In many studies, blood brain barrier has been shown to be compromised in multiple sclerosis patients. Pericytes play an active role in ensuring the continuity of the blood brain barrier along with a series of cells. In this study, the effect of pericytic dysfunction on the development of demyelinating plaques in patients with multiple sclerosis was investigated.

Material and Method: Concentrations of pericyte dysfunction mediators (PDGFbb, MMP9, TIMP3 and ADAM17) in cerebrospinal fluid of patients with clinically isolated syndrome (CIS), relapsing remitting multiple sclerosis (RRMS) and healthy control group were measured by ELISA and oligoclonal bands (OCB) were investigated. We aimed to determine whether the concentration of these mediators differed between groups and whether they correlated with lesion load, number of attacks, and EDSS scores.

Results: Concentrations of all four mediators were similar in patients with CIS and RRMS. However, both groups were found to be higher than the healthy group. In the CIS and RRMS groups, the levels of the mediators were not correlated with any parameters examined. However, the levels of PDGFbb (p=0.045), MMP9 (p=0.037), and TIMP3 (p=0.033) were higher in OCB positive patients than in those without OCB, whereas ADAM17 levels remained unchanged.

Conclusion: This study shows that pericytes may play a role in the pathogenesis of MS from early stages of the disease. The presence of higher levels in patients with OCB suggests that pericyte dysfunction may be associated with OCB formation.

Keywords: Multiple sclerosis, clinically isolated syndrome, pericyte, cerebrospinal fluid, neuroinflammation

ÖZ

Amaç: Multipl skleroz'da kan beyin bariyerinin bozulduğu pek çok çalışmada gösterilmiştir. Perisitler bir dizi hücre yanında kan beyin bariyerinin devamlılığının sağlanmasında aktif rol üstlenir. Bu çalışmada multipl skleroz hastalarında perisit disfonksiyonunun demyelinizan plakların gelişimindeki etkisi araştırılmıştır.

Gereç ve Yöntem: Klinik izole sendrom (KİS), relapsing remitting multipl skleroz (RRMS) ve sağlıklı kontrol grubundan alınan beyin omurilik sıvılarında perisit disfonksiyon mediatörlerinin (PDGFbb, MMP9, TIMP3 ve ADAM17) konsantrasyonları ELISA ile ölçüldü ve oligoklonal bantlar (OKB) araştırıldı. Bu mediyatörlerin, gruplar arasındaki farklılıkları lezyon yükü, atak sayısı ve EDSS skorları ile korelasyon gösterip göstermediği belirlendi.

Bulgular: Her dört mediyatörün konsantrasyonları KİS ve RRMS hastalarında benzer bulundu. Bununla beraber her iki grupta sağlıklı kontollerden yüksek bulundu. KİS ve RRMS grubunda, mediyatörlerin seviyelerinin bakılan hiçbir parametre ile korelasyon göstermediği saptandı. Buna karşın, beyin omurilik sıvılarında OKB bulunan hastalarda PDGFbb (p=0,045), MMP9 (p=0,037) ve TIMP3 (p=0,033) düzeylerinin OKB bulunmayanlara göre daha yüksek olduğu, ADAM17 seviyelerinin ise değişmediği görüldü.

Sonuç: Bu çalışma perisit disfonksiyon mediyatörlerinin MS'in patogenezinde hastalığın erken dönemlerinden itibaren rol alabileceğini göstermektedir. Oligoklonal band bulunan hastalarda daha yüksek seviyelerdeki mevcudiyeti, perisit disfonksiyonunun OKB oluşumu ile bir ilgisi olabileceğini akla getirmektedir.

Anahtar Kelimeler: Multipl skleroz, klinik izole sendrom, perisit, beyin omurilik sıvısı, nöroinflamasyon

Corresponding Author/Sorumlu Yazar: Tuncay Gündüz **E-mail:** drtuncaygun@gmail.com
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INTRODUCTION

Disruption of the blood-brain barrier (BBB) is an important physiopathological aspect of multiple sclerosis (MS), which enables hazardous humoral and cellular immunological elements to gain access into the central nervous system (1). BBB integrity is maintained by the neurovascular unit, which is made up of astrocytes, endothelial cells and pericytes (2). Although involvement of glial cells and endothelial inflammation in BBB disruption and MS pathogenesis has been extensively studied (1), little is known about the contribution of pericytes to the formation of active demyelinating lesions. Pericyte dysfunction is known to lead to the release of various mediators of inflammation (e.g. matrix metalloproteinase 9; MMP9), which lead to the disruption of the BBB (2). Platelet-derived growth factor (PDGF) bb–PDGF receptor β signaling pathway in pericytes is crucial for BBB functioning (3). Interaction of metalloproteinases (e.g. MMP9 and a disintegrin and metalloproteinase-17; ADAM17) with their inhibitors (e.g. tissue inhibitor of metalloproteinases-3; TIMP3) modulate pericyte functions and thus BBB permeability (4, 5). Notably, mice with inborn pericyte dysfunction show defective remyelination in response to the experimental MS model induced by lysolecithin (6). Moreover, several mediators released by pericytes and/or endothelial cells (e.g. PDGFbb, MMP9, TIMP3, and ADAM17) have been shown to be associated with MS physiopathology (7-9). These findings suggest a link between pericyte dysfunction, onset of BBB disruption and formation of demyelinating lesions and bring forward the notion of using pericytic mediators as biomarkers or therapeutic targets of MS.

METHODS

To investigate the significance of pericytic mediators, cerebrospinal fluid (CSF) samples were collected from 18 patients with clinically isolated syndrome (CIS) and 19 patients with relapsing remitting MS (RRMS). All samples were collected during the remission period. CSF samples of CIS patients were collected after the cessation of steroid treatment within the first two months of the first clinical episode. None of the patients were under immunosuppressive or immunomodulating treatment during sampling. RRMS patients had higher number of relapses, MRI lesions and EDSS scores than CIS patients, as expected (Table 1). Oligoclonal bands (OCB) were investigated in all patients. Twenty age/gender-matched healthy individuals, whose CSF samples had been collected for differential diagnosis of acute-onset headache, were used as controls. These individuals did not have any disorders, had normal neurological and systemic examination, brain MR imaging, CSF cell count, protein and glucose content, total blood count and blood chemistry findings. They also gave prompt response to non-steroidal anti-inflammatory drugs and remained healthy for a follow-up period of six months. CSF samples were kept at −80°C until use and thawed at the same time prior to experiments. CSF levels of PDGFbb, MMP9, TIMP3, and ADAM17 were measured by ELISA as per manufacturer's instructions (Abcam, Cambridge, UK). A standard curve was constructed based on a range of standard concentrations. The unknown concentrations in the samples were calculated from the standard curve and results were expressed as pg/ml or ng/L. $p<0.05$ was considered as significant in statistical tests. Ethics committee approval was received for this study from the ethics committee of İstanbul University (11.01.2016 – 2015/KK/96).

RESULTS

Both CIS and MS patients had significantly higher PDGFbb ($p=0.022$), MMP9 ($p=0.039$), TIMP3 ($p=0.010$), and ADAM17 ($p=0.018$) levels than healthy controls. However, no significant differences could be found between CIS and MS patients by Tukey's post-hoc test (Figure 1). Spearman tests, performed to seek a correlation between CSF levels of pericytic mediators and age, disease duration, number of relapses, number of brain MRI lesions and EDSS scores failed to identify a significant cor-

Table 1. Clinical and demographic features of clinically isolated syndrome (CIS) patients, multiple sclerosis (MS) patients and healthy controls (HC)

	CIS (n=18)	MS (n=19)	HC (n=20)	p
Gender (women/men)	10/8	12/7	11/9	0.850*
Age	26.4±7.9	29.9±7.1	28.6±6.3	0.512**
Disease duration (years)	-	5.9±1.3	-	-
Number of relapses	1.0 ± 0.0	6.4±3.1	-	NA
Number of MRI lesions	1.8±1.0	7.1±1.9	-	<0.001***
Number of patients with CSF OCB	10	13	-	0.640*
EDSS score	1.7±0.7	3.1±1.0	-	<0.001†

CSF: cerebrospinal fluid; OCB: oligoclonal bands; EDSS: expanded disability status scale; NA: not applicable
Numerical values are signified in the form of average ± standard deviation.
*Chi-square test; **ANOVA; ***Student's t-test; †Mann-Whitney U test

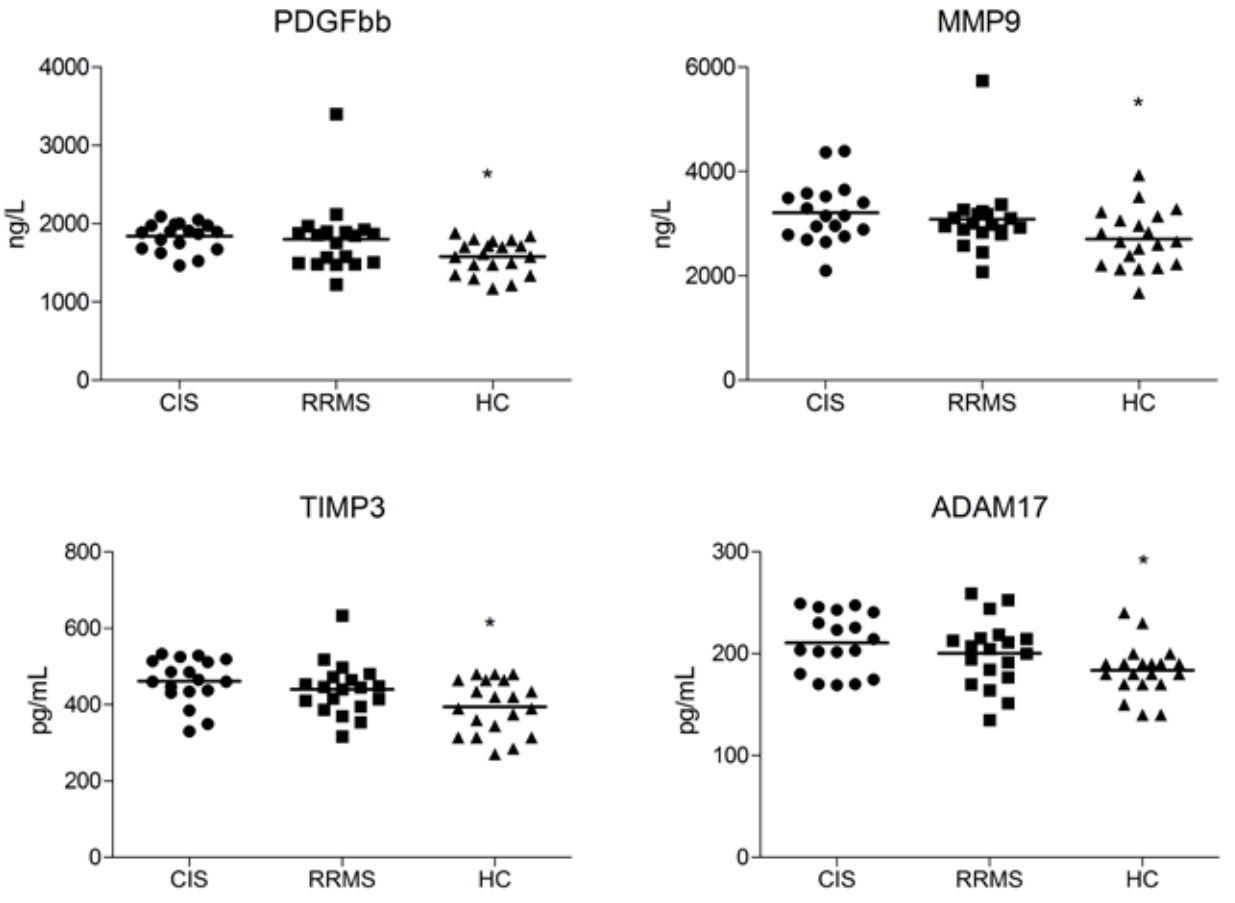

Figure 1. Cerebrospinal fluid PDGFbb, TIMP3, ADAM15, and MMP9 levels of clinically isolated syndrome (CIS) patients, relapsing remitting multiple sclerosis (MS) patients and healthy controls (HC). Horizontal lines indicate mean values.
*p<0.05 by ANOVA

relation. CSF levels of PDGFbb (p=0.045), MMP9 (p=0.037) and TIMP3 (p=0.033) were higher in MS patients with pattern 2 or 3 OCB than those without CSF OCB. By contrast, ADAM17 levels were comparable among OCB positive and negative groups (p=0.499) (Figure 2).

DISCUSSION

Cerebrospinal fluid levels of pericytic mediators are higher in MS patients compared to healthy controls, starting shortly after the first clinical episode during the CIS stage indicating altered pericytic activity in early stages of MS. Absence of a significant difference between CIS and RRMS patients imply that CSF levels of pericytic mediators are unlikely to be used as biomarkers to predict conversion from CIS to MS. Additionally, MMP9 levels have been found comparable among MS and CIS patients (10). By contrast, OCB positive RRMS patients display elevated levels of PDGFbb, MMP9 and TIMP3, all of which participate in the BBB functions. MMP9 is a well-known mediator of BBB dysfunction (7). Although PDGFbb is required for pericyte generation, high levels of PDGFbb may lead to pericyte loss (3). Thus a likely hypothesis is that pericyte dysfunction emerges during the progression of MS further increasing the BBB permeability and ultimately leading to increased B cell access and enhanced OCB formation. TIMP3 is required for the BBB integrity and has previously been shown to be overexpressed in MS lesions (9). This mediator is possibly released in increasing amounts as a compensating factor to avert the BBB dysfunction in MS.

Pathogenic differences between OCB positive and negative MS patients have been scarcely investigated. OCB positivity has been associated with increased lesion load and disability in some studies (11). Our results suggest that pericytic dysfunction, and ensuing BBB dysfunction might be one of the underlying causes of OCB formation.

Many other pericytic mediators such as chemokines, cytokines and adhesion molecules were not investigated since they are produced by a variety of cell types and therefore are not useful in dissecting specific involvement of pericytes (2). Evidently, mediators involved in this study may also be produced by

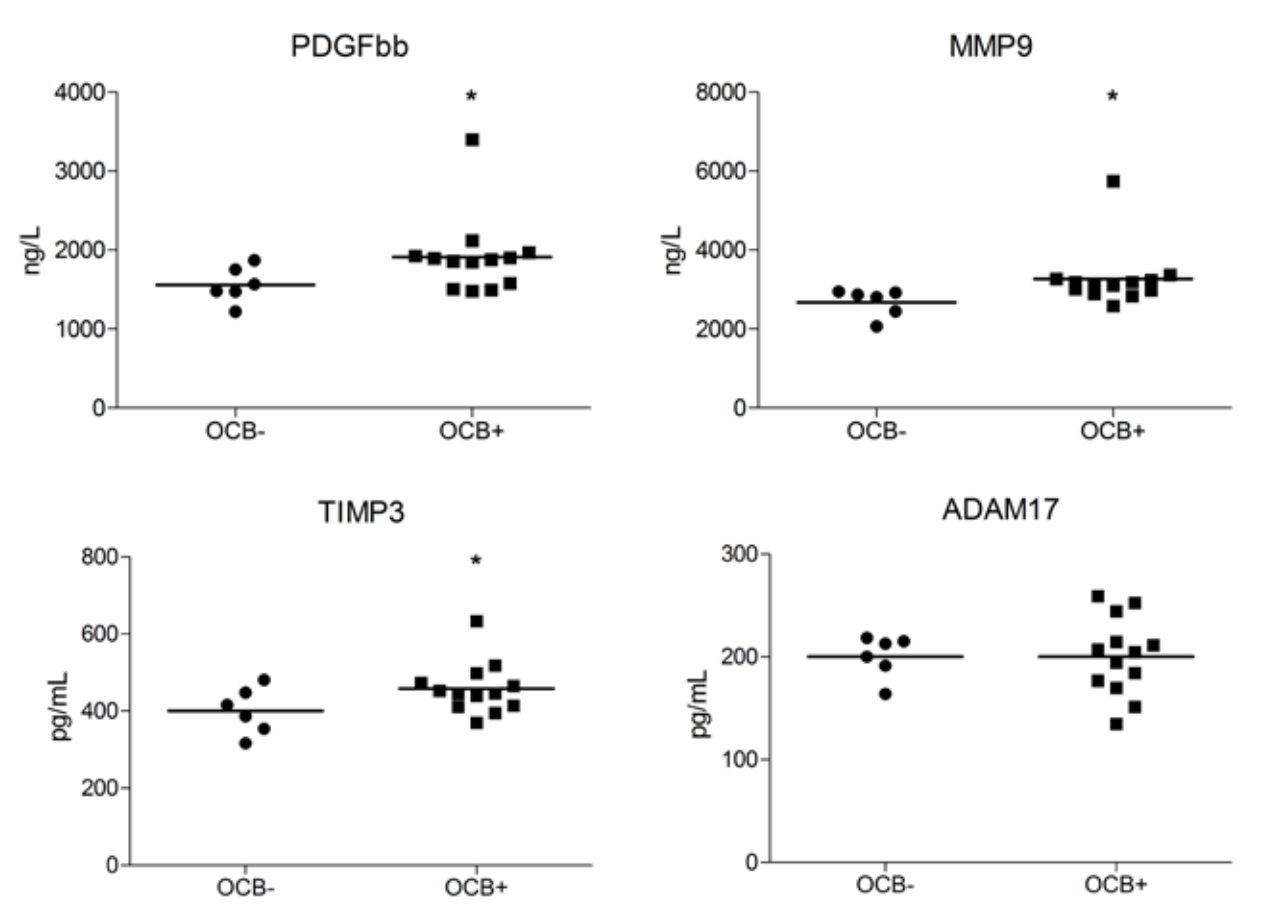

Figure 2. Cerebrospinal fluid PDGFbb, TIMP3, ADAM15, and MMP9 levels of oligoclonal band (OCB) positive (pattern 2 or 3) and OCB negative relapsing remitting multiple sclerosis (MS) patients. Horizontal lines indicate mean values.
*p<0.05 by Student's t-test

endothelial cells and even glial cells to a certain degree and finding a factor exclusively produced by pericytes is a major challenge.

Our preliminary study gives a hint on involvement of pericytes in MS pathogenesis in early stages and indicates that pericytes are probably not innocent bystanders. Increased levels of pericytic dysfunction mediators in OCB positive patients compared to OCB negative patients suggests the need for further investigations. Novel methods for specific assessment of pericytes are also required for better characterization of the neurovascular unit in MS.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of İstanbul University (11.01.2016 – 2015/KK/96).

Informed Consent: Written informed consent was obtained from patients whoparticipated in this study.

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REFERENCES

1. Minagar A, Alexander JS. Blood-brain barrier disruption in multiple sclerosis. Mult Scler 2003; 9: 540-9. [CrossRef]
2. Hill J, Rom S, Ramirez SH, Persidsky Y. Emerging roles of pericytes in the regulation of the neurovascular unit in health and disease. J Neuroimmune Pharmacol 2014; 9: 591-605. [CrossRef]
3. Niu F, Yao H, Liao K, Buch S. HIV Tat 101-mediated loss of pericytes at the blood-brain barrier involves PDGF-BB. Ther Targets Neurol Dis 2015; 2: pii: e471.
4. Shukla V, Shakya AK, Shukla M, Kumari N, Krishnani N, Dhole TN, et al. Circulating levels of matrix metalloproteinases and tissue inhibitors of matrix metalloproteinases during Japanese encephalitis virus infection. Virusdisease 2016; 27: 63-76. [CrossRef]
5. Candelario-Jalil E, Yang Y, Rosenberg GA. Diverse roles of matrix metalloproteinases and tissue inhibitors of metalloproteinases in neuroinflammation and cerebral ischemia. Neuroscience 2009; 158: 983-94. [CrossRef]
6. Muramatsu R, Kuroda M, Matoba K, Lin H, Takahashi C, Koyama Y, et al. Prostacyclin prevents pericyte loss and demyelination induced by lysophosphatidylcholine in the central nervous system. J Biol Chem 2015; 290:1 1515-25.
7. Fainardi E, Castellazzi M, Bellini T, Manfrinato MC, Baldi E, Casetta I, et al. Cerebrospinal fluid and serum levels and intrathecal production of active matrix metalloproteinase-9 (MMP-9) as markers of disease activity in patients with multiple sclerosis. Mult Scler 2006; 12: 294-301. [CrossRef]
8. Gold SM, Chalifoux S, Giesser BS, Voskuhl RR. Immune modulation and increased neurotrophic factor production in multiple sclerosis patients treated with testosterone. J Neuroinflammation 2008; 5: 32. [CrossRef]
9. Haddock G, Cross AK, Plumb J, Surr J, Buttle DJ, Bunning RA, et al. Expression of ADAMTS-1, -4, -5 and TIMP-3 in normal and multiple sclerosis CNS white matter. Mult Scler 2006; 12: 386-96. [CrossRef]
10. Ljubisavljevic S, Stojanovic I, Basic J, Vojinovic S, Stojanov D, Djordjevic G, et al. The role of Matrix Metalloproteinase 3 and 9 in the pathogenesis of acute neuroinflammation. Implications for disease modifying therapy. J Mol Neurosci 2015; 56: 840-7. [CrossRef]
11. Farina G, Magliozzi R, Pitteri M, Reynolds R, Rossi S, Gajofatto A, et al. Increased cortical lesion load and intrathecal inflammation is associated with oligoclonal bands in multiple sclerosis patients: a combined CSF and MRI study. J Neuroinflammation 2017; 14: 40. [CrossRef]