Original Article

Apelin-13: A Promising Biomarker for Multiple Sclerosis?

Murat Alpua, Yakup Turkel, Ersel Dag, Ucler Kisa[1]

Department of Neurology, [1]Department of Biochemistry, Kirikkale University, Faculty of Medicine, Kirikkale, Turkey

Abstract

Objectives: Recent studies have shown that Apelin 13 may have a neuroprotective property. Therefore it can be used as a biomarker for multiple sclerosis. Our purpose to assess serum apelin-13 levels in adult patients with multiple sclerosis and healthy controls. **Patients and Methods:** Subjects consisted of 42 relapsing remitting multiple sclerosis patients and 41 controls. Demographic characteristics including age, gender, duration of disease and Expanded Disability Symptom Scale (EDSS) were recorded. In serum samples obtained from the patients and controls, serum apelin-13 levels were measured with Enzyme Linked Immunosorbent Assay (ELISA) method. **Results:** Serum apelin-13 levels were significantly higher in the patients groups than the healthy controls ($P = 0.003$). Pearson analysis did not show any significant correlation between EDSS, disease duration and apelin-13 levels. **Conclusion:** The results of our study have been showed statistically significant higher levels of serum apelin-13 in multiple sclerosis patients compared to controls. Further studies with larger patients populations and healthy controls should be done to clarify to use serum apelin levels as a biomarker for multiple sclerosis.

Keywords: Apelin-13, Expanded Disability Status Scale, relapsing-remitting multiple sclerosis

Introduction

Multiple sclerosis (MS) is a heterogeneous autoimmune disorder, with various clinical and pathologic features reflecting distinct pathways to neuronal tissue injury. Inflammation, demyelination, and axonal injury are the main pathologic mechanisms leading to identifiable clinical findings.[1] However, the etiology of MS is still unknown. The immune system is involved in the demyelination and neuronal cell damage processes.

Recent studies have revealed that there is an association between oxidative stress and axonal degeneration in MS, and there is much evidence of the relationship of oxidative stress with neural damage in MS.[2,3] There are various mechanisms associated with oxidative stress in MS pathogenesis. As an example of an inflammatory mechanism, axonal injury is triggered by microglia-derived reactive oxygen and nitrogen species.[4] Myelin disruption and subsequent myelin phagocytosis occur in active MS lesions. In the central nervous system, iron is stored as ferritin in the myelin sheets. Some experimental studies revealed that iron can amplify oxidative damage in lesions of the central nervous system. When myelin breakdown occurs, iron spreads into the extracellular area and triggers oxidative stress.[5]

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Apelin is a peptide hormone secreted by adipose tissues; it is found in various tissues including brain, uterus, testes, lungs, and also the cardiovascular system tissues. This peptide participates in different functions, including fluid homeostasis, immune responses, regulation of cardiovascular functions, and cell proliferation.[6] Some studies have showed that apelin depresses the oxidative stress in several tissues.[7,8] The most active form of apelin is apelin-13. In addition, apelin-13 is suggested to have a protective role in focal cerebral ischemia–reperfusion injury by inhibiting oxidative stress in rats,[9] and apelin-13 can also be reportedly used as a biomarker of oxidative stress. Therefore, we can identify high oxidative stress in MS patients by detecting the serum levels of apelin-13.

Disease biomarkers have always been of interest in MS. Despite the new developments in MS treatment, no absolute biomarker can predict the course of the disease yet. The first biomarkers of MS are oligoclonal bands and higher IgG index in cerebrospinal fluid. Thus far, many molecules have been checked for their

Address for correspondence: Dr. Murat Alpua, Department of Neurology, Faculty of Medicine, Kirikkale University, Kirikkale, Turkey.
E-mail: dr.muratalpua@yahoo.com

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potential use as biomarkers to aid in MS diagnosis. For example, clinically isolated syndrome (CIS) patients with higher serum levels of chitinase-3-like proteins have a higher conversion rate to clinically definite MS.[10] Besides, some microRNAs in peripheral blood,[11,12] which are included in T-cell regulation, could be promising novel biomarkers to identify the conversion of CIS to MS. In addition, levels of serum neurofilaments were higher in patients with CIS than in controls, and there was an association between higher levels of neurofilaments and increased disability and the number of T2-hyperintense, gadolinium-enhancing lesions. However, the prognostic value of these biomarkers in individuals needs a more comprehensive validation through cohort studies in the future.[13]

Early treatment initiation for MS is one of the currently most discussed topics in neurology because recent treatment options can delay the onset of MS after the initial demyelinating episode and may reduce the degree of MS-related disability.[14] Therefore, early detection and differential diagnosis of MS are crucial. The aim of our study was to investigate the possible protective and diagnostic roles of apelin-13 in MS.

Materials and Methods

The patients were chosen from among the registered patients of Neurology Clinic affiliated to the Kirikkale University, Faculty of Medicine. Doctrines of the actual version of the Helsinki Declaration were followed; ethical committee approval of institution was approved, and the protocol of the study was explained to the patients. After obtaining detailed information, each patient signed an informed consent form.

Forty-two adults with a clinical diagnosis of relapsing-remitting MS and 41 healthy controls were involved in the study. There was no family history of MS in healthy controls. All MS patients were diagnosed in accordance with the McDonald's 2010 revised criteria. The mean disease duration of MS patients was 4.5 years (range: 1–13 years). All MS patients were receiving immunomodulators or immunosuppressive therapy. The mean duration of drug use was 5.6 years (range: 1–14 years). When the samples were drawn, there were 32 patients receiving interferon, three patients receiving glatiramer acetate, six patients receiving fingolimod, and two patients receiving teriflunomide therapy. On gadolinium-enhanced magnetic resonance imaging, no lesions were observed in the patients. All patients were in the stable phase of the disease. The sample extent of our investigation was selected as our patient population. Previous similar studies were also taken into consideration. To the extent possible, age- and sex-matched volunteer family members and volunteer healthy workers without a history of neurodegenerative and/or psychiatric diseases were taken as controls.

Demographic characteristics, including age, gender, disease course, and Expanded Disability Symptom Scale (EDSS), were recorded.

Exclusion criteria were as follows: neurodegenerative diseases such as Parkinson's disease, dementia, history of any vascular or systemic disease, diabetes mellitus, epilepsy, and substance or drug abuse.

Blood samples were collected intravenously from both patients and healthy controls. The samples were taken from patients during the stable phase of the disease. Serum was separated from the blood samples by centrifugation at 1000 g for 20 min and was kept frozen at −80°C until the use for analyses to measure apelin-13 levels. Serum apelin-13 levels were measured by the Human apelin-13 ELISA Kit (Sunred Biological Technology, Baoshan District, Shanghai) with enzyme-labeled immunometric assay method (reference interval: 0–8000 pg/mL).

The results were analyzed using SPSS version 16.0. $P < 0.05$ was considered to indicate statistical significance. Continuous variables were presented as mean ± standard deviation. Categorical variables were expressed as proportions. The Student's *t*-test was used to test the differences in continuous variables and Chi-square test for categorical values. Relationships among the apelin with EDSS and disease duration were examined using Spearman's correlation in all participants.

Results

Table 1 summarizes the characteristics of the participants. The mean age and sex distribution did not differ between the patients with MS and controls. Serum apelin-13 levels were significantly higher in patients than in controls [$P = 0.003$; Table 2]. Spearman's analysis did not show any significant correlation between EDSS, disease duration, and apelin-13 levels [Table 3]. Table 4 shows the sensitivity and specificity of the apelin-13 values in ROC analysis. The confidence intervals around the apelin-13 values are mentioned in Table 5.

Discussion

The potential diagnostic role of apelin has been studied for various conditions so far.[15-17] Apelin-13 has also been investigated as a potential biomarker of many conditions in various studies [Table 6]. Apelin is an oxidative stress biomarker, and the presence of oxidative stress in the pathogenesis of MS suggests that apelin may be used as a biomarker for MS. However, to the best of our knowledge, there has been no study about apelin-13 in patients with MS so far, and our study is the first study to investigate the serum apelin-13 levels in patients with MS.

Serum apelin levels are considered indicative of oxidative stress in various medical conditions. Several studies have suggested that oxidative stress has an important role in the inflammatory processes and MS pathogenesis.[18,19] In accordance with the literature, our present study showed that relapsing–remitting MS patients have significantly higher serum apelin-13 levels than healthy controls. These results again showed that oxidative stress plays a role in MS; however, we believe that apelin has no protective effect on neuronal tissues, particularly in MS as intended.

Table 1: The demographics and apelin level of patients with multiple sclerosis and control group

	Mean±SD		*P*
	Patients (*n*=42)	Controls (*n*=41)	
Age, year	35.21±8.27	32.41±10.31	0.17
Gender (female/male)	30/12	26/15	0.48
MS duration, year	5.64±3.70		
EDSS (median)	1 (0–6)		
Apelin-13 (pg/ml)	46.46±21.01	33.67±17.09	0.003

MS=Multiple sclerosis, EDSS=Expanded Disability Symptom Scale, SD=Standard deviation

Table 2: The mean value and the standard deviation of apelin levels in patients and controls

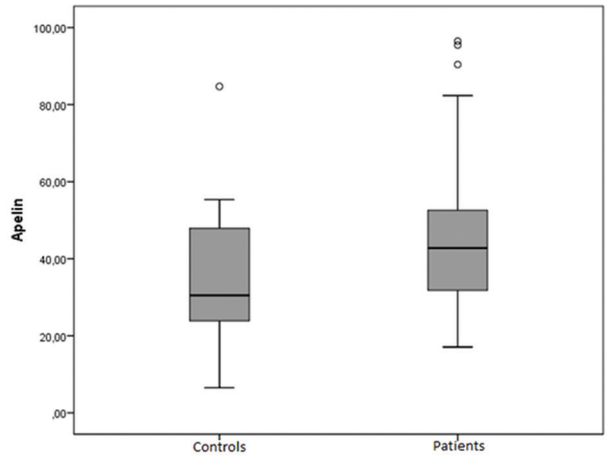

Table 3: The correlations between Expanded Disability Symptom Scale, disease duration, and apelin

Spearman's rho	Apelin	Duration
EDSS		
Correlation coefficient	0.080	0.540
P	0.620	0.000
n	42	42
Apelin		
Correlation coefficient		−0.173
P		0.272
n		42

EDSS=Expanded Disability Symptom Scale

Table 4: The ROC analysis of Apelin-13 values

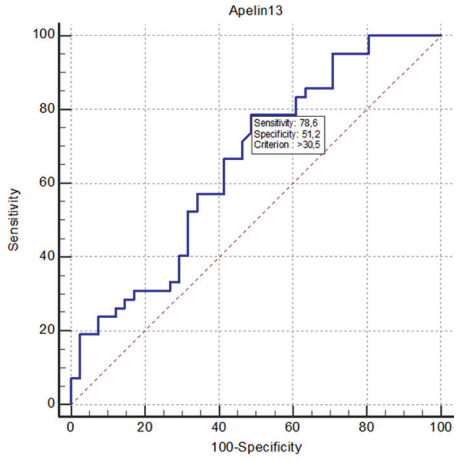

Table 5: The confidence intervals around the apelin-13 values

	Group		
Apelin	Controls	Mean	33.6710
		95% CI for mean	
		Lower bound	28.2752
		Upper bound	39.0668
		Median	30.5000
		SD	17.09483
		Minimum	6.51
		Maximum	84.71
	Patients	Mean	46.4602
		95% CI for mean	
		Lower bound	39.9117
		Upper Bound	53.0088
		Median	42.7600
		SD	21.01451
		Minimum	17.09
		Maximum	96.47

CI=Confidence Interval, SD=Standard deviation

Table 6: Previous studies related to apelin-13

Zhang DL, Liao H, Wei YY, Zhang QD, Wang ZG. Elevation of serum apelin-13 is positively correlated with ADMA in patients on maintenance hemodialysis. Clin Nephrol 2009;71:405-12

Selimoglu Sen H, Kaplan I, Abakay O, *et al.* Serum apelin-13 levels in patients with pulmonary embolism. Clin Appl Thromb Hemost 2016;22:543-7

Du FH, Li X, Li R, Xu L, Ma RR, Liu SF, *et al.* Elevation of serum apelin-13 associated with proliferative diabetic retinopathy in type 2 diabetic patients. Int J Ophthalmol 2014;7:968-73

Yavuz YC, Sevinc C, Deniz MS, *et al.* Role of circulating serum apelin-13 levels in glomerulonephritis: A pilot study. J Clin Exp Nephrol 2015;1:2

Until now, oligoclonal bands have been the most important biomarkers for MS and have been used to support the diagnosis MS so far. Recently, with the new revision of the McDonald's criteria, oligoclonal bands in cerebrospinal fluid (CSF) can be viewed as substitution for the dissemination in time requirement. For example, with the new criteria, if patients have lesions or symptoms in more than one location that fulfill the dissemination-in-space requirement, they can be diagnosed with MS without waiting if they test positive for oligoclonal band in CSF. However, a major disadvantage of this biomarker is that it can be detected by only CSF examination.[20] Although there have been many studies about biomarkers in MS, they could not identify an absolute serum biomarker to aid early

detection or diagnosis of MS.[21] In our study, we showed that serum apelin levels in RRMS patients are significantly higher than in healthy controls; therefore, they can be used as a serum biomarker for MS in clinical practice.

The only limitation of our study is the small number of participants, which is due to a small number of patients applying to our center. However, the exclusion criteria had to be met to obtain reliable results in this study. In the future, multicenter studies with a large number of volunteers seem promising and are warranted to provide more valuable results.

Conclusion

Our current study suggests the use of apelin-13 as a biomarker for MS, and unlike other studies that advocated a neuroprotective role of apelin-13 either using animal models or in other conditions,[22,23] our study was not able to confirm any neuroprotective role of apelin-13 in MS. We suggest that more comprehensive studies may more clearly reveal the biomarker role of apelin-13 in MS in the future.

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Conflicts of interest

There are no conflicts of interest.

References

1. Dendrou CA, Fugger L, Friese MA. Immunopathology of multiple sclerosis. Nat Rev Immunol 2015;15:545-58.
2. Gilgun-Sherki Y, Melamed E, Offen D. The role of oxidative stress in the pathogenesis of multiple sclerosis: The need for effective antioxidant therapy. J Neurol 2004;251:261-8.
3. Sayre LM, Perry G, Smith MA. Oxidative stress and neurotoxicity. Chem Res Toxicol 2008;21:172-88.
4. Nikić I, Merkler D, Sorbara C, Brinkoetter M, Kreutzfeldt M, Bareyre FM, *et al*. A reversible form of axon damage in experimental autoimmune encephalomyelitis and multiple sclerosis. Nat Med 2011;17:495-9.
5. Hametner S, Wimmer I, Haider L, Pfeifenbring S, Brück W, Lassmann H, *et al*. Iron and neurodegeneration in the multiple sclerosis brain. Ann Neurol 2013;74:848-61.
6. Carpéné C, Dray C, Attané C, Valet P, Portillo MP, Churruca I, *et al*. Expanding role for the apelin/APJ system in physiopathology. J Physiol Biochem 2007;63:359-73.
7. Foussal C, Lairez O, Calise D, Pathak A, Guilbeau-Frugier C, Valet P, *et al*. Activation of catalase by apelin prevents oxidative stress-linked cardiac hypertrophy. FEBS Lett 2010;584:2363-70.
8. Chun HJ, Ali ZA, Kojima Y, Kundu RK, Sheikh AY, Agrawal R, *et al*. Apelin signaling antagonizes ang II effects in mouse models of atherosclerosis. J Clin Invest 2008;118:3343-54.
9. Wu G, Li L, Liao D, Wang Z. Protective effect of Apelin-13 on focal cerebral ischemia-reperfusion injury in rats. Nan Fang Yi Ke Da Xue Xue Bao 2015;35:1335-9.
10. Hinsinger G, Galéotti N, Nabholz N, Urbach S, Rigau V, Demattei C, *et al*. Chitinase 3-like proteins as diagnostic and prognostic biomarkers of multiple sclerosis. Mult Scler 2015;21:1251-61.
11. Keller A, Leidinger P, Steinmeyer F, Stähler C, Franke A, Hemmrich-Stanisak G, *et al*. Comprehensive analysis of microRNA profiles in multiple sclerosis including next-generation sequencing. Mult Scler 2014;20:295-303.
12. Siegel SR, Mackenzie J, Chaplin G, Jablonski NG, Griffiths L. Circulating microRNAs involved in multiple sclerosis. Mol Biol Rep 2012;39:6219-25.
13. Disanto G, Adiutori R, Dobson R, Martinelli V, Dalla Costa G, Runia T, *et al*. Serum neurofilament light chain levels are increased in patients with a clinically isolated syndrome. J Neurol Neurosurg Psychiatry 2016;87:126-9.
14. Fujihara K. BENEFIT 8-year results provide further support for the long-term value of early treatment of multiple sclerosis. J Neurol Neurosurg Psychiatry 2014;85:1179.
15. Ma WY, Yu TY, Wei JN, Hung CS, Lin MS, Liao YJ, *et al*. Plasma apelin: A novel biomarker for predicting diabetes. Clin Chim Acta 2014;435:18-23.
16. Selimoglu Şen H, Kaplan I, Abakay Ö, Sezgi C, Yilmaz S, Taylan M, *et al*. Serum apelin 13 levels in patients with pulmonary embolism. Clin Appl Thromb Hemost 2016;22:543-7.
17. Zhou Y, Wang Y, Qiao S. Apelin: A potential marker of coronary artery stenosis and atherosclerotic plaque stability in ACS patients. Int Heart J 2014;55:204-12.
18. Ferretti G, Bacchetti T, Principi F, Di Ludovico F, Viti B, Angeleri VA, *et al*. Increased levels of lipid hydroperoxides in plasma of patients with multiple sclerosis: A relationship with paraoxonase activity. Mult Scler 2005;11:677-82.
19. Kemp K, Redondo J, Hares K, Rice C, Scolding N, Wilkins A, *et al*. Oxidative injury in multiple sclerosis cerebellar grey matter. Brain Res 2016;1642:452-60.
20. Stangel M, Fredrikson S, Meinl E, Petzold A, Stüve O, Tumani H, *et al*. The utility of cerebrospinal fluid analysis in patients with multiple sclerosis. Nat Rev Neurol 2013;9:267-76.
21. Comabella M, Sastre-Garriga J, Montalban X. Precision medicine in multiple sclerosis: Biomarkers for diagnosis, prognosis, and treatment response. Curr Opin Neurol 2016;29:254-62.
22. Kasai A, Kinjo T, Ishihara R, Sakai I, Ishimaru Y, Yoshioka Y, *et al*. Apelin deficiency accelerates the progression of amyotrophic lateral sclerosis. PLoS One 2011;6:e23968.
23. Zeng XJ, Yu SP, Zhang L, Wei L. Neuroprotective effect of the endogenous neural peptide apelin in cultured mouse cortical neurons. Exp Cell Res 2010;316:1773-83.