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ORIGINAL ARTICLE

The Possible Role of Apelin and Adiponectin in Multiple Sclerosis Pathogenesis

Apelin ve Adiponektinin Multipl Skleroz Patogenezindeki Olası Rolleri

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Abstract

Introduction: Adipocytokines are described as molecules synthesized from adipocytes, and in addition to their roles in metabolic disorders, these cytokines have recently been the focus of interest in autoimmune diseases. In this comparative study, we aimed to determine the levels of apelin and adiponectin molecules between relapses/remissions of MS and control subjects.

Methods: In this study, we include 34 relapsing-remitting MS (RRMS) patients and 20 healthy controls to evaluate apelin and adiponectin serum levels via the Enzyme-linked immunosorbent assay method.

Results: Between the RRMS and control groups, these molecule levels were statistically insignificant. On the other hand, significant differences were noted in terms of apelin and adiponectin levels between genders.

Discussion and Conclusion: Adipocytokines such as adiponectin, leptin, visfatin, adipsin, apelin, and ghrelin have already been examined in neuroinflammatory and neurodegenerative conditions. Despite the limited features of our study, the measured difference between apelin and adiponectin, especially during relapse, suggests that it may be possibly involved in this pathogenesis. Moreover, a significant difference was noted between serum adiponectin, apelin levels, and sex. We found higher levels in females compared to males in the remission period. The reason for this situation is yet to be identified, but we assumed that this may be attributed to the difference between fatty acid metabolism in men and women. In addition, this result correlates with the theoretical fact that MS affects women three times more often than men, and the prognosis in women is generally better than in men, which raises curiosity about the possible neuroprotective role of these molecules.

Keywords: Adiponectin; Apelin; Multiple sclerosis; Neuroimmunology; Neuroinflammation

Multiple sclerosis is a disease characterized by neurodegeneration and neuroinflammation via central nervous system (CNS) demyelination and axonal loss caused by autoreactive immunocytes and molecules.[1] This disease is mostly affecting the young population, aged 20 to 50 years old. It is noted to be three times more common in females than in

Our study presented as a poster and only of the abstract section in PACTRIMS 2017.

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males. Although the exact cause of MS is still unknown, environmental, genetic, epigenetic, and immunological factors are important in the complexity of this disease.[2]

Studies suggest that cytokines and chemokines may play an important role in MS immunopathogenesis.[3,4] In the cytokine family, adipocytokines such as apelin, adiponectin, resistin, ghrelin, leptin, visfatin, adipsin, chemerin are produced from adipose tissue and are associated with some metabolic and autoimmune conditions such as inflammation, inflammatory response, insulin resistance, and metabolic syndrome.[5] There have been very few studies about adiponectin's role in MS; furthermore, studies examining the role of apelin in this pathogenesis remain to be limited.[6–12] In humans, the apelin gene is located on the Xq25–26.1 chromosome and is encoded as a prepropeptide composed of 77 amino acids. Apelin is synthesized in many organs such as the CNS, heart, lung, and breast tissue.[13,14] APJ mRNA in the brain is especially produced by white matter glial cells.[15,16] The adiponectin gene (AMP1) is localized on chromosome 3q27. The molecular structure is composed of the C-terminal globular domain, which is similar to complement 1q (C1q) and the collagen-like N-terminal fibrous domain.[17] Adiponectin is present in plasma at a much higher concentration than other cytokines and hormones. It constitutes 0.01% of the total plasma proteins.[18]

In this current study, we intend to compare the serum levels of these molecules between patients with relapse and remission periods of MS and the control subjects.

Materials and Methods

Study Design and Data Collection

Patients who were diagnosed with multiple sclerosis in Gazi University Faculty of Medicine, Neurology Department, MS Unit between 2014 and 2015 were included in this study. In total, 34 MS patients were included in this study (14 patients were in relapse, 20 patients were in remission period), whereas 20 healthy individuals (control) with age and gender similarities were included. On the other hand, according to the latest McDonald criteria, patients without any systemic disease who met the definite diagnosis of MS constitute MS patient study group, and volunteer healthy individuals were the control group.

Demographic information of patients and healthy volunteers were recorded. MS relapses were defined with the support of neurological examination and neuroradiological studies. At the time of sampling, all patients were receiving treatment. The treatment received by MS patients was only interferon beta-1a, interferon beta-1b, and glatiramer acetate. Approval was obtained from the ethics committee of the Keçiören Training and Research Hospital for the study (number 846 dated 27.05.2015). All the individuals who formed the study and control group were informed about the study and thus provided their consent.

Experimental Procedure

Enzyme-linked immunosorbent assay (ELISA) was used for the quantitative measurement of apelin (Bioassay Technology Laboratory Human Apelin (AP) ELISA Kit) and adiponectin (Bioassay Technology Laboratory Human Adiponectin/ACRP 30 (ADP) ELISA Kit) in serum samples with in vitro technique. Peripheral blood samples (10 ml) were collected from the patients and healthy controls and centrifugated at 3000 rpm for 5 minutes to separate the serum. The serum samples were stored at −20° C until analysis. After diluting the standard medium that contained the adiponectin and apelin antibodies, serum samples were added into the wells and incubated at room temperature for 2.5 hours. Soon after, these were washed and then incubated with a biotin antibody for 1 hour. After washing, streptavidin was added, left for 45 hours, and washed again; thereafter, one-step reagent was added, and it was incubated again for 30 min. The concentration of adiponectin and apelin was then determined spectrophotometrically. Absorbance values at 450 nm were determined using an ELISA reader.

Statistical Analysis

Analysis of the data was done using the IBM SPSS 21.0 statistical package program. Pearson chi-square test was used to compare qualitative data as well as descriptive statistical methods (frequency, percentage, mean, standard deviation, median, min-max) while evaluating study data. The normal distribution of the data was evaluated using Kolmogorov–Smirnow and Shapiro–Wilk tests for the data which have not fit the normal distribution. Mann–Whitney U and Kruskal–Wallis tests were used to compare groups. Spearman's Rho test was used in the analysis of the relationship between variables. The relationship between variables according to age was examined via independent samples t-test. The statistical significance level was set at $p<0.05$.

Results

As per our findings, it was determined that 37% of the participants were in the control group, 25.9% were relapse group, and 37% were in the remission groups. Further, 63% of the participants were female, while 37% were male. The average age of the participants was found to be 30.6±7.4, with the youngest being 19 and the oldest being 48 (Table 1).

Table 1. Distribution of participants

	n	%
Group		
Control	20	37.0
Relapse	14	25.9
Remission	20	37.0
Total	54	100.0
Gender		
Female	34	63.0
Male	20	37.0
Total	54	100.0

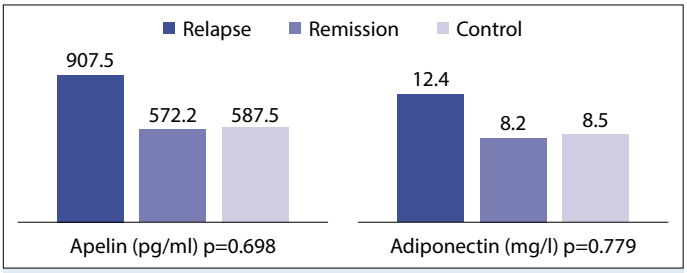

Figure 1. Comparison of apelin (pg/ml) and adiponectin (mg/l) between groups. There has not been any statistically significant difference between relapse MS, remission MS, and control groups, but apelin and adiponectin levels were found to be insignificantly higher in relapse group than in remission MS group and controls.

Table 2. Comparison between apelin and adiponectin with clinical status

	Patients (Relapse + Remission) (n=34)	Control (n=20)	p
Apelin (pg/ml)	763.0 (-183.3 -4350.0)	587.5 (-225.0 -4446.7)	0.872
Adiponectin (mg/l)	10.0 (-1.2 -59.2)	8.5 (-1.8 -53.2)	0.747

Table 3. Comparison between apelin and adiponectin in groups and gender

	Female (Mean±SD)	Male (Mean±SD)	p
Apelin (pg/ml)			
Relapse	962.0±1.065.6	1.632.2±1.748.6	0.410
Remission	1.807.7±1.674.0	531.4±342.0	**0.031***
Control	1.176.5±1.835.9	2.226.5±1.792.3	0.222
Adiponectin (mg/l)			
Relapse	13.6±14.8	23.3±21.6	0.373
Remission	23.5±22.7	6.4±7.6	**0.036***
Control	15.5±21.6	26.9±20.4	0.256

The body mass index (BMI) of the patients was calculated. With the metric system, the formula for BMI is weight in kilograms divided by height in meters squared.[19] In the multiple sclerosis group (n=34), the mean BMI for women was 25.2 kg/m^2, whereas the mean BMI for men was 24.3 kg/m2. In the control group, the mean BMI value for women was calculated as 24.6 kg/m^2, whereas for men, it was 23.8 kg/m^2. No significant BMI difference was noted between the groups and between the genders.

The mean apelin level of the participants was 1.334.7–1.521.6 pg/ml, while the mean adiponectin level of the participants was found to be 17.4–19.1 mg/l. No statistically significant difference was found between sex and between the patient or healthy control groups in terms of apelin and adiponectin levels (Table 2). When we compared the subgroups, no statistically significant difference was not observed, but the levels of these molecules were noted to increase in the relapse period (Fig. 1).

No statistically significant difference was determined between sex in terms of apelin and adiponectin levels in the control or relapse groups, except for in the remission group. Females' apelin and adiponectin levels were found to be higher than males in the remission period (Table 3).

Discussion

Different types of lesions present heterogeneity of immunopathological and structural patterns of demyelination in MS, suggesting that MS is a neurological syndrome that contains inflammation, degeneration, and regeneration simultaneously.[20] Recently, the disease is a much more complex process involving all arms of the natural and acquired immune systems with slowly progressive neurodegeneration mechanisms besides acute inflammation.[1,20] In this complex immunopathogenesis mechanism, we intend to focus on adiponectin and apelin. We measured serum adiponectin and apelin levels in patients with MS

and healthy controls to determine some information as regards disease activity.

Even if the results of serum apelin and adiponectin levels were insignificant, they also weren't below the normal range in healthy controls. Results suggested that these molecules may have been at a baseline level to compensate for the harmful effects of the inflammatory process. Moreover, hypothetically in the relapse period, they may have a possible role in limiting the inflammation and neurodegeneration. There was a significant difference between serum adiponectin, apelin levels, and sex. We found higher levels in females compared to males in the remission period. The reason for this situation remains unclear, but we assumed that this was because of the sex hormone differences between males and females. Males have higher fatty acid metabolism than females via androgen hormones. Although, theoretically, MS is more common to affect females three times as often as males, the prognosis of the disease in females is generally better than in the male gender.[2] For these reasons, we thought that adiponectin may have been one of the contributors to provide a better prognosis in female MS patients due to its anti-inflammatory properties.

Adipocytokines have been recently examined in molecule types in obesity, metabolic conditions, as well as in autoimmune diseases. Between these adipocytokines, some have recognized functions in these autoimmune processes, but generally we have insufficient information about its number and their relations to each other.[17,18,20] Adiponectin and apelin have been investigated in a wide spectrum of diseases such as chronic liver diseases, arthritis, diabetes mellitus, coronary artery disease, asthma, various malignancies, and autoimmune and neurological conditions.[21] In MS, even though these studies have some opposition, most of them suggested that adiponectin has an increasing pattern in relapses.[20,21] In a study, adiponectin with its fractions, visfatin, and omentin has not been considered a reason for relapse, but they also suggested that there have been some correlations when parameters adjusted with BMI and gender.[22] In 174 MS patients and 182 controls cohort, total radical-trapping antioxidant parameter, sulfhydryl (SH) groups, serum levels of zinc, and adiponectin have been determined to have a role in the pathophysiology of MS.[23] In a pediatric MS and control group cohort, it has been demonstrated that leptin levels were higher, but adiponectin levels were lower in the MS group compared to controls. Also, it has been suggested that these markers could serve as a potential prognostic target for the disease activity.[24] In a small cohort like our study, findings have shown that adipokines, particularly apelin and adiponectin, might be involved in MS pathogenesis, especially in the relapse period.[25] Another study that focuses on MS patients whose first relapse was optic neuritis or not, has suggested that leptin, adiponectin, resistin, monocyte chemoattractant protein-1 (MCP-1), and IL-8 have a high titer in RRMS patients than controls.[26] An analysis has demonstrated the increasing levels of adiponectin and its high molecular weight oligomers in MS. Also, they have suggested that the oligomerization process should be altered in MS patients. They are yet to find a significant difference between the relapse and remission period of these patients like in our study but suggested that these molecules bring a poor prognosis.[27] From a genetic perspective, a study has shown that adiponectin (especially in female sex) and leptin (especially in males) gene polymorphisms are associated with susceptibility to MS; also, they suggested that adiponectin gene polymorphism may have a role in primary progressive MS pathogenesis.[28] Besides them, a study with genome-wide single nucleotide polymorphisms technique has demonstated that adiponectin and leptin have insignificant effect on MS pathogenesis.[29] Studies of adiponectin have revealed that adiponectin inhibits the activation of NF-κB via TNF-α inhibition, thereby suppressing the inflammatory effect on the endothelium and exhibiting an anti-inflammatory status.[30,31]

Apelin, which has recently been examined for its neuroprotective effects in CNS, has been studied in only a few cases of MS before. In literature, apelin has been investigated for some effects on CNS in epilepsy, amyotrophic lateral sclerosis (ALS), and stroke in mouse models. Apelin/APJ system has been investigated as a neuroprotective regulator for hippocampal and cortical neurons against neuronal damage induced by glutamate-induced temporal lobe epilepsy models.[32] In an ALS study, apelin was identified as an endogenous neuroprotective factor using the superoxide dismutase enzyme SOD1G93A mouse models. APJ receptor was detected in spinal cord embedded neuronal cell bodies, and SOD1G93A mice showed apelin expression in the spinal cord decreased together with a paralytic phenotype, and this apelin deficiency has, in turn, accelerated the ALS progression. Contrary to expectations, they found that the expression of apelin in the spinal cord has decreased together with the progression of ALS.[33] Lastly, apelin was also studied in stroke. In ischemia/reperfusion (I/R) rat models, APJ receptor expression was noted to increase. Moreover, the expression of ionized calcium-binding adaptor molecule 1 (Iba1), glial fibrillary acidic protein, and high mobility group box protein 1 (HMGB1) in I/R rats was decreased by

apelin-13 treatment, indicating the inhibition of microglia, astrocytes, and other inflammatory cells to alleviating the neuroinflammation.[34]

There have been some limitations in our study. The most important limitation of our study was its small sample size. Also, patients haven't been categorized according to whether they had been received any treatment or not. Treatments that the patients received might have influenced results. Patients in the MS group were not categorized according to disease subtypes, and no classification was made according to their treatment regimens either.

Conclusion

Although this study has important limiting aspects mentioned above, it has been one of the few human studies to elucidate the effect of apelin and adiponectin in literature. Results suggested that these molecules may have been involved in the complex pathogenesis of MS.[35] Particularly, levels in the relapse periods had already drawn attention. Considering that MS has also been recognized as a neurodegenerative disease such as ALS or refractory epilepsy, the effects of apelin and adiponectin in the pathogenesis of MS can be revealed by conducting more detailed studies with broader series and further researches with more independent components. The results of these studies may have allowed us to find either biomarker for diagnosis and follow-up or new treatment options for this complex pathogenesis.

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