



Science

THE POSSIBLE EFFECTS OF POLYAMINES IN MULTIPLE SCLEROSIS PATIENTS ON NEW LESION DEVELOPMENT AND DISABILITY

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Abstract

Background: Multiple sclerosis (MS) is a demyelinating autoimmune disease characterized by the infiltration of T cells into the central nervous system. Polyamines, which contribute to cell proliferation, hypertrophy and tissue development, have some tissue-specific roles in brain tissue. So, we aimed in this study to present the possible effects of polyamines on MS.

Method: Thirty- five patients with MS and 35 sex and age-matched control were included in this study. Arginine decarboxylase (ADC), ornithine decarboxylase (ODC) and agmatinase levels were measured by ELISA kits.

Results: The patient group had higher ODC and agmatinase levels than controls. The correlation analyses between ODC, ADC, and agmatinase levels and disease duration were revealed that there was a negligible positive relationship between disease duration and agmatinase, with negligible negative relationship between disease duration and ODC and ADC. Additionally, there were showed respectively moderate and weak positive correlations between EDSS (Expanded Disability Status Scale) scores and newly developed lesions and ODC and agmatinase levels.

Conclusions: Elevated polyamine synthesis in MS patients was presented by detecting increased ODC, ADC and agmatinase levels compared to controls. Besides this increased polyamine synthesis in MS patients was also related with disease duration, number of newly developed lesions and disability.

Keywords: Multiple Sclerosis; Polyamine Synthesis Pathway; Ornithine Decarboxylase; Arginine Decarboxylase; Agmatinase.

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1. Introduction

Multiple sclerosis (MS) is a demyelinating autoimmune disease characterized by the infiltration of T cells into the central nervous system (CNS) due to impaired blood brain barrier (BBB) (1). MS, the most common inflammatory demyelinating disease in the CNS, is the most common cause of morbidity in young adults, too (1). There are 4 subforms of MS. Relapsing-remitting MS (RRMS) is the most common MS form characterized by relapses of neurological symptoms due to transient focal inflammation in the CNS. The secondary progressive MS (SPMS) initially develop in the course of relapsing-remitting (RR) disease, with or without MS episodes on its course. Histopathologic features in RRMS are characterized by focal inflammation in perivascular localization. There is also a deterioration in the BBB, which interferes with the communication between the cells in the CNS (2,3). The typical lesions of MS are plaques with demyelination that can be observed in both white and gray matter. Demyelination, axonal degeneration and scar formation (sclerosis) are observed in inactive MS lesions. Besides, MS has features of both inflammation (in RR phase) and neurodegeneration (in progressive phase) (4). The cause of neurodegeneration is gross axonal and neuronal loss. Remyelination is the most well-known and valid method of tissue healing in the CNS. Likewise, the regeneration of damaged neurons and their axons in the CNS is very limited, but the lost myelin sheath is more effectively repaired (5). Polyamines are organic cations naturally found in plants, animals and microorganisms. The synthesis of polyamines, started from L-arginine, takes places in two ways. L-arginine is converted to ornithine by arginase or agmatine by arginine decarboxylase (ADC). Ornithine is then decarboxylated by ornithine decarboxylase (ODC) to produce putrescine, which can then be further converted to spermidine and spermine. Agmatine, an intermediate metabolite, is converted to putrescine via agmatinase (Fig. 1). The rate-limiting enzyme in this pathway is ODC.

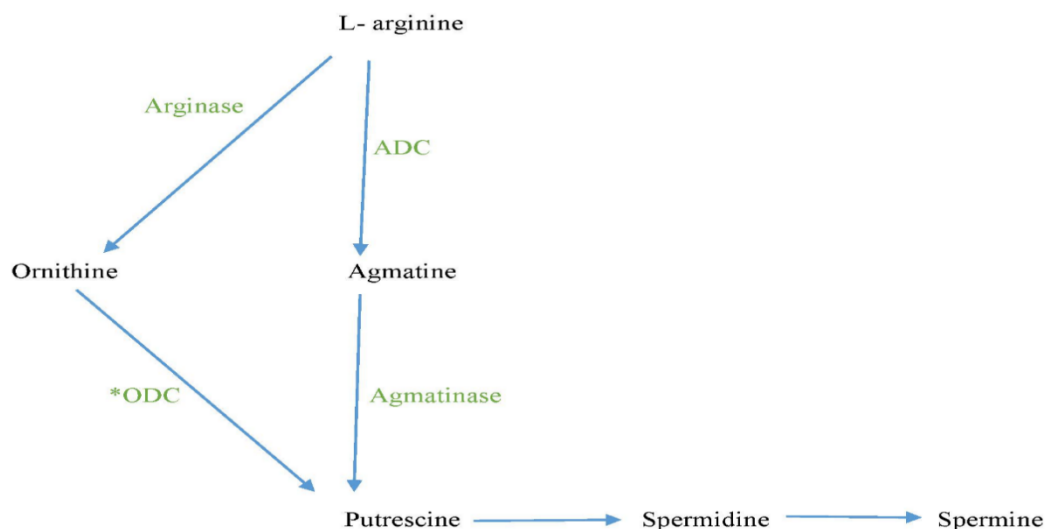


Figure 1: The polyamine synthesis pathway starting from L- arginine. Abbreviations: ADC: arginine decarboxylase; ODC: ornithine decarboxylase.

*The rate-limiting enzyme

Polyamines contribute to cell proliferation, hypertrophy and tissue development. In addition to its functions in other tissues, polyamines also have a variety of tissue-specific roles that affect neuronal excitability by regulating ion channels and receptors in brain tissue (9-14). However,

despite all this information, the functions of the endogenous polyamines in CNS are still unknown. For these reasons, we believe that some effects of polyamines may occur during the inflammation that occurs in MS disease and in the subsequent regeneration process.

The main aim of this study is to determine the effects of polyamines on the duration of disease, subforms and number of newly developed T2 or contrast-enhancing lesions by comparing ODC, ADC and agmatinase levels in the serum of MS patients with healthy controls.

2. Materials and Methods

2.1. Selection of The Patient and Control Group

Thirty-five patients who applied to X University's Hospital of Neurology Department and diagnosed with MS were enrolled in the study between 01.08.2017 and 31.08.2017. There were no gender or age restrictions among the patients. The McDonald's 2010 diagnostic criteria was used in confirming MS diagnosis. The degrees of patient's disability were calculated using Expanded Disability Status Scale (EDSS). The date of first diagnosis was determined to estimate the patient's disease duration. The treatments on the date, when the blood samples were taken from the patients were recorded. New cranial and cervical magnetic resonance imaging (MRI) was performed to detect newly developed T2 lesions. Cranial MR imaging was performed on each patient with a Siemens Symphony Magnetom 1.5 tesla device. The 5 cc contrast agent containing gadolinium was used intravenously to patients for contrast-enhanced cranial and cervical MRI. The newly developed T2 and contrast-enhancing lesions are expressed by both an expert radiologist and two different expert neurologists. All participants' rights were protected and a written informed consents were obtained before the procedures according to the Helsinki Declaration.

Patients aged under 18, or patients diagnosed with systematic acute/chronic inflammatory / autoimmune or infectious/ hematological disease, acute myocardial infarction or psychiatric disorders, and cancer or severe liver /heart/ renal failure were not included in the study.

The control group was composed of 35 healthy controls without diagnosed any uncontrolled systemic disease, who had similar age and gender in our patient group. For all patients and controls included in the study, the study form was filled in a question-and-answer manner.

The ethics committee approval number for this study is 2016- 01/01.

2.2. Collection of Blood Samples

A 10 ml blood samples were taken from the patient and control groups from the right antecubital vein into tubes without anticoagulant.

The blood samples were centrifuged at 1610 g for 10 minutes and then acquired serums were divided into Eppendorf tubes and the suitable parameters were stored at -80 ° C for study. The samples were evaluated two times in order to prevent any errors that may occur during the experiment and the averages of the values obtained for each sample were taken.

2.3. Ornithine Decarboxylase, Arginine Decarboxylase and Agmatinase Assays

Using Elisa kit, ornithine decarboxylase, arginine decarboxylase and agmatinase determinations were performed.

In the equation of the standard curve drawn by the absorbances read against the standard concentrations, the concentration of each of the enzymes was calculated as (pg/ml).

2.4. Statistical analysis

In all statistical analyzes conducted within the scope of this research; 35 in the patient group and 35 in the control group a total of 70 individuals took part in this study. In the analysis of the data, IBM SPSS Statistics 22 statistical package programs were used.

The probability of making the first type of error of study is $\alpha = 0.05$, the probability of making second type error is $\beta = 0.30$, the statistical power is $1-\beta = 0.70$, and the effect size is calculated as 0.837 (Cohen's d). Baseline demographic and laboratory data were compared across patient and control groups using chi square test for categorical variables and Kruskal-Wallis test for continuous variables. In the analysis of characteristic and laboratory data of the individuals participating in the research; mean, standard deviation (SD), minimum and maximum (min - max) values and percentage (%) were calculated. Since parametric test hypotheses were not available in the study, nonparametric analysis techniques were used in the analysis of data. In this context, the Mann-Whitney U Test was used in the comparison between the two independent groups. The Spearman correlation coefficients were calculated when the relationship between ODC, ADC and agmatinase levels and MS subtypes and drug types was determined and the Eta correlation coefficients was used while the relationship between ODC, ADC and agmatinase levels and disease duration, EDSS and number of newly developed lesions was revealed. Besides the values for correlations were graphically indicated. A $p < 0.05$ was accepted to be statistically significant.

3. Results

The baseline demographic and clinical characteristics and laboratory parameters of the patient and control group were compared in Table 1. There was no statistically significant difference in age or gender between the two groups. The mean age in the patient group was 34.66 ± 6.73 while it was 33.69 ± 6.76 in the control group. There were an equal number of men and women in the patient and control groups. While there were 31 RRMS patients in the patient group ,4 of them were SPMS. The mean duration of disease was 5.96 ± 5.11 , while the number of newly developed lesions was 2 in the patient group. The mean EDSS value of the patients was 2.77 ± 1.51 . The number and percentage of patients according to drug use were shown in Table 1. In addition, there was no statistically significant difference in ADC levels between the the patient and control groups($p=0.11$). However, the patient group had higher levels of ODC and agmatinase (Table 1) ($p=0.03, p=0.003$).

Table 1: The comparison of baseline demographic, clinical and laboratory characteristics of the control and patient groups

		Patient group (n=35)	Control group (n=35)	p
Age	Mean ± SD	34.66 ± 6.73	33.69 ± 6.76	0.63
Gender, n (%)	Female	21(60%)	21(60%)	1.00
	Male	14(40%)	14(40%)	
MS subtype, n (%)	RRMS	31(88.6%)	-	-
	SPMS	4(11.4%)	-	-
Drug types, n (%)	IFN β-1b	5(14.3%)	-	-
	IFN β-1a (44 mcgr)	12(34.3%)	-	-
	IFN β-1a (once a week)	1(2.9%)	-	-
	Fingolimod	6(17.1%)	-	-
	Immunosupresant	3(8.6%)	-	-
	Teriflunomide	3(8.6%)	-	-
	Glatiramer acetate	4(11.4%)	-	-
	Dimethyl fumarate	1(2.9%)	-	-
Duration (year)	Mean ± SD	5.96 ± 5.11	-	-
ODC (pg/ml)	<i>Median</i>	<i>145.47</i>	<i>115.02</i>	<i>0.03</i>
	<i>(min - max)</i>	<i>(60.89 - 1479.05)</i>	<i>(2.71 - 437.76)</i>	
ADC (pg/ml)	Median	171.13	111.13	0.11
	(min - max)	(0 - 2876.13)	(0 - 1009.88)	
Agmatinase (pg/ml)	<i>Median</i>	<i>216.30</i>	<i>130.79</i>	<i>0.003</i>
	<i>(min - max)</i>	<i>(78.85 - 2390.75)</i>	<i>(0 - 1428.57)</i>	
EDSS	Mean ± SD	2.77± 1.51	-	-
	(min - max)	(1 - 6.5)	-	-
New lesion	Median	2	-	-
	(min - max)	(0 - 14)	-	-

Italicized words show statistical significance. Abbreviations: ADC: arginine decarboxylase, EDSS: Expanded Disability Status Scale, IFN: interferon, MS: multiple sclerosis, ODC: ornithine decarboxylase, RRMS: relapsing-remitting MS, SPMS: seconder progressive MS.

When the relationship of ODC, ADC, and agmatinase levels were compared to MS subtypes and drug types in the patient group, there was found no statistically correlation between ODC, ADC, and agmatinase levels and MS subtypes and drug types (Table 2).

Table 2: The correlations between ODC, ADC and agmatinase levels and MS subtypes and drug types

N=35	Eta Correlation Coefficients
ODC -MS subtypes	0.04
ADC - MS subtypes	0.01
Agmatinase -MS subtypes	0.03
ODC- Drug types	0.02
ADC- Drug types	0.01
Agmatinase- Drug types	0.00

Abbreviations: ADC: arginine decarboxylase, MS: multiple sclerosis, ODC: ornithine decarboxylase.

On the other hand, the correlation analyses between ODC, ADC, and agmatinase levels and disease duration were showed that there was a negligible positive relationship between disease duration and agmatinase, with negligible negative relationship between disease duration and ODC and ADC (Table 3). In addition, there were found respectively moderate and weak positive correlations between EDSS scores and newly developed lesions and ODC and agmatinase levels. (Table 3). There was not any statistically significant correlation between EDSS scores and newly developed lesions and agmatinase levels (Table 3).

Table 3: The correlations between ODC, ADC, and agmatinase levels and disease duration, EDSS and number of newly developed lesions

Parameters	Values	ODC	ADC	Agmatinase
Duration	Correlation Coefficient	-0.05	-0.05	0.10
	Sig. (2-tailed)	0.79	0.77	0.57
EDSS	Correlation Coefficient	0.52	0.12	0.86
	Sig. (2-tailed)	0.01	0.62	0.01
New lesion	Correlation Coefficient	0.27	0.09	0.28
	Sig. (2-tailed)	0.13	0.57	0.11

Abbreviations: ADC: arginine decarboxylase, EDSS: Expanded Disability Status Scale, ODC: ornithine decarboxylase.

4. Discussion

Polyamines were previously reported to have an effect on neuritogenesis (16), brain development (17), neuronal survival (18), and peripheral nerve regeneration (19). Elevation in major polyamine levels contributes to cell proliferation in neuronal tissue (20). Among them, the importance of spermidine was showed previously (21). Spermidine has been identified to reduce the production of pro-inflammatory cytokines by microglia (22). Besides, spermidine and spermine were revealed to be essential in mediating protection against oxidative stress caused by hydrogen peroxide in fibroblasts of rats (23). It is known that the impairment of spermidine metabolism has a role in the pathogenesis of various diseases (24,25). Additionally, it has been shown that administration of spermidine in experimental autoimmune encephalomyelitis disease in animal models may be beneficial for suppressing signs by reducing the reactive oxygen radicals responsible for the pathogenesis of MS (26).

According to the results of our study, ODC, ADC and agmatinase enzyme levels increased in the MS patient group compared to the control group. These increases were statistically significant for ODC and agmatinase levels but not for ADC level (Table 1). These results suggest that the pathway of polyamine synthesis leads to major polyamine synthesis and induces both the proliferation process and spermidine production which has anti-inflammatory property (Fig. 1). In this way, it may be trying to both suppress focal inflammation in the perivascular area and repair the myelin damage by proliferation. Additionally, there was no significant relationship between ODC, ADC and agmatinase levels and disease subtypes and drug types (Table 2). It has been accepted that there are minor changes in the pathophysiological process between MS subtypes (27). As is known, medications used in MS treatment have different mechanisms of action. Conversely, the results of our study showed that the polyamine synthase pathway is active in MS patients independent of the subtype of the disease and the drug type.

Moreover, in our study, it was also found that there was a linear relationship between disease duration and agmatinase levels. However, the relationship between ODC and ADC levels and disease duration were inversely (Table 3). Although the level of agmatinase increases, if it is considered that the ODC enzyme is the rate-limiting enzyme in the polyamine synthesis pathway, these results suggest that polyamine synthesis is reduced in the later stages of MS disease. This may be the reason why the relapses in the later stages of the disease leave sequelae rather than complete recovery.

In addition, there was a weak but positive correlation between the newly developed lesions and ODC and agmatinase levels (Table 3). This can be explained by the effort of body to suppress the inflammation around the newly developed lesions or correct the resulting damage by increasing the polyamine synthesis. Besides, the EDSS scores of patients and ODC and agmatinase levels were found to be moderately positive correlated (Table 3). Based on this result, it can be claimed that EDSS scores are more influenced by newly developed lesions rather than the disease duration.

Our study has several limitations. Firstly, the patient number of this study was relatively small because it is a one-centered study. For this reason, in the correlations analyzes, the relationship between ODC, ADC and agmatinase and disease subtypes, drug types, disease duration, newly developed lesions and EDSS scores could not be assessed in detail. Moreover, the levels of other enzymes and proteins in the polyamine synthesis pathway could not be measured.

5. Conclusions and Recommendations

In conclusion, increased polyamine synthesis in MS patients was revealed by detecting elevated levels of ODC, ADC and agmatinase compared to controls. In addition, this study showed that polyamine synthesis in MS patients was also associated with disease duration, number of newly developed lesions and EDSS score. In the future, there is a need for more extensive and detailed randomized controlled studies.

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