

Original Research Article

THE IMPORTANCE OF INCREASED SERUM ORNITHINE LEVELS IN THE PATHOGENESIS OF ALZHEIMER AND PARKINSON'S DISEASES

Running title: Arginine catabolism effects on neuroinflammation.

ABSTRACT

Background : The aim of this study was to investigate the levels of enzymes and ornithine involved in the synthesis of polyamines in patients with Alzheimer's and Parkinson's disease and to see their positive or negative effects on the modulation of the immune system

Methods: Thirty-five healthy subjects as control group and 35 patients with Alzheimer's and Parkinson's disease were included in this study. Determination of Ornithine decarboxylase, Arginine Decarboxylase and Agmatinase levels were evaluated by using Enzyme Linked Immunosorbent Assay (Elisa kit). Ornithine levels were measured spectrophotometrically.

Results: When ornithine levels of Alzheimer and Parkinson patients were compared to the control group, differences were found as significant ($p < 0.05$). On the other hand, when Ornithine decarboxylase, Arginine Decarboxylase, Agmatinase levels of Alzheimer and Parkinson patients were compared to the control group, the differences were found as insignificant ($p > 0.05$).

Conclusion: Although the enzyme levels in the pathway of polyamine synthesis in Alzheimer's and Parkinson's diseases do not change, the increase in ornithine level will not contribute to the fight

21 mode of the immune system. On the contrary, it may change plasticity by increasing osmolality.

22 **Key words:** Alzheimer, Parkinson, Polyamine, Ornithine, Agmatine, Immunomodulation

23 INTRODUCTION

24 Alzheimer's and Parkinson's disease are two of the most common neurological diseases that
25 cause neurodegeneration and affect many people [1]. The most valid hypothesis explaining the
26 development of Alzheimer's disease (AD), the most common neurological disease, is the amyloid
27 cascade hypothesis. According to this hypothesis, amyloid- β peptides (A β) accumulate in the
28 cerebral blood vessels and brain parenchyma, and accumulation of hyperphosphorylated tau
29 proteins in neurons leads to lethal disease, progressive loss of consciousness, functional impairment
30 and memory loss [2,3]. Parkinson's disease, which is the second most common neurological disease
31 that occurs after death of dopaminergic neurons in Substantia nigra pars compacta, causes rigidity,
32 tremor and hypokinesia [4,5]. In addition, α -synuclein protein, which accumulates in neurons, plays
33 a key role in Parkinson's disease. Lewy particles, which are caused by excessive accumulation of this
34 protein in neurons, lead to pathology leading to disease progression in cholinergic and
35 monoaminergic neurons in the brain. The diagnosis of idiopathic Parkinson's disease can be
36 determined by applying these two major neuropathologies (neuronal loss in specific areas of the
37 substantia nigra and widespread intracellular protein (α -synuclein) accumulation [6]. In Alzheimer's
38 disease, a different pattern of α -synuclein pathology was found to accumulate mainly in the limbic
39 region of the brain [7]. In vitro experiments have shown that the presence of polyamines in α -
40 synuclein accumulation and fibril formation is effective and this effective sequence has been shown

41 as spermin>spermidine>putrescine [8]. Although the mechanism of brain atrophy and neuronal loss
42 is not fully known, there is a growing body of evidence recently that the lack of arginine and
43 suppressing immunity play a critical role in the pathogenesis of AD [9]. The cells responsible
44 primarily for the immune system in the brain are microglia cells and macrophage-like immune cells
45 found in the brain parenchyma, which are involved in the modulation of the brain's inflammatory
46 response [10]. The microglia are activated immediately after the ischemia. Circulating monocytes are
47 rapidly transformed into macrophages in the brain via blood-brain barrier due to inflammation
48 [10,11]. Microglia and macrophages are known to be essential cells in inflammation after cerebral
49 ischemia. Polyamines have a negative regulatory effect on macrophage activation through complex
50 associations with NO metabolism. In macrophages, NO is an intermediate product in the L-arginine
51 oxidation process [12].

52 Polyamines are molecules having 2, 3 or 4 amino groups. They are widely found in living organisms
53 because they have a key role in the survival of life [13]. The major polyamines synthesized by several
54 enzymes from the amino acid of L-Arginine are putrescine, spermidine, spermine and agmatine (Fig
55 1). Polyamines play an important role in biological processes because of their interaction with many
56 different receptors, protein kinases, nucleotide cyclases. It has been reported that the increase in
57 the levels of polyamines may be associated with diseases such as cancer, as they have a key role in
58 growing and dividing the cell [14,15,16]. However, due to their interaction with systems such as
59 polyamines, catecholamines, GABA, nitric oxide (NO), glutamate, they are also associated with many
60 psychiatric disorders, especially schizophrenia [16,17,18]. The survival of the cells that make up the
61 organism depends on the presence of polyamines. It is known that neurodegenerative diseases

62 caused by cell death develop due to the differentiation of polyamine metabolism [19,20,21]. Many
63 researchers have shown that agmatine has neuroprotective potential and develops a cognitive
64 function in various animal models of central nervous system damage such as neurotrauma and
65 neonatal ischemia [22,23,24,25,26]. The degraded polyamine metabolism indirectly causes the
66 degradation of nitric oxide synthase. Increased agmatine synthesis causes suppression of NO
67 synthesis in immunocytes and leads to defense deficiency, while decreased levels result in increased
68 NO synthesis resulting in neurodegeneration and death of neurons (Figure 1) [27,28,29] .

69 Our aim in this study was to reveal the possible roles of polyamines in the pathogenesis of Alzheimer
70 and Parkinson diseases. For this purpose, the levels and effects of arginine decarboxylase, ornithine
71 decarboxylase, agmatinase and ornithine involved in the pathway of polyamine synthesis on the
72 immune system were examined.

73 MATERIALS AND METHODS:

74 Patient and Control Group

75 The 35 patients, who applied to the Cumhuriyet University Medicine Faculty Neurology Policlinic
76 and took diagnosis with alzheimer and parkinson, were included in the study as experimental group.

77 It was not made any restriction in terms of the gender and age. Our control group was chosen from
78 the 35 healthy individuals who have not any systemic diseases (diabetes, hypertension and
79 neurodegenerative disease).

80 Blood Samples Collection

Comment [DPE1]: Patients of all ages and both gender were included in the study.

81 Blood samples were taken from the controls and patients who were made the diagnosis of
82 alzheimer and parkinson and not receive any therapy. The serums were obtained by centrifugation
83 at 1610 x g for 10 minutes of the blood samples were preserved in 80 0C for to study.

84 Detection of enzyme levels

85 Ornithine, arginine decarboxylase, ornithine decarboxylase and agmatinase levels have been
86 measured in serum of alzheimer and parkinson patients. Ornithine level were calculated
87 spectrophotometrically at 515 nm using the method defined by Chinard and the value was given as
88 $\mu\text{mol/ml}$ [30]. Arginine decarboxylase, ornithine decarboxylase and agmatinase levels were
89 determined by ELISA kit according to the manufacturer's protocol (SunRed, China).

90 Statistical Analysis

91 Mann Whitney U test was used for statistical analysis of the data. The data were stated in the
92 tables as the arithmetical average $\pm$ Standard deviation and the level of significance was taken as
93 0,05

94 RESULTS

95 There wasn't any significant difference between patient groups and control group in terms of
96 gender and age ranges ($P>0,05$) (Table 1). When ADC, ODC and Agmatinase enzymes, which were
97 determined at serum levels, were compared according to the control group, no statistically
98 significant difference was found between the two disease groups (Alzheimer and Parkinson) ($P>0,05$)
99 (Table 2).

100 On the other hand, serum ornithine levels increased in both groups when compared with the control
101 group, and they were statistically significant. $P < 0.05$ (Table 2).

102 DISCUSSION:

103 In this study, enzymes (ADC, ODC and Agmatinase) and ornithine levels in polyamine synthesis
104 pathway were measured in serums of Alzheimer and Parkinson's patients. When the levels of the
105 enzymes were compared with the control as a result of analysis, no significant difference was found
106 between the two groups of patients and control ($p > 0.05$). Ornithine was higher and statistically
107 significant in both groups when compared to the control group ($p < 0.05$). Enzyme and ornithine
108 levels in the polyamine synthesis pathway have been investigated in this study for the first time in
109 serums of Alzheimer and Parkinson's patients. Most of the studies carried out to date regarding
110 polyamines and their relation to these diseases are in the form of experimental animals and
111 postmortem studies. In one postmortem study, the amount of spermidine increases in the temporal
112 cortex (70%) and reduction in levels of putrescine (28%) were found in patients with AD [19]. In
113 another postmortem study, there was no difference in spermidine and spermine concentrations in
114 the basal ganglia of parkinson, huntington's disease (HD) and progressive supranuclear palsy (PSP)
115 patients and it was found to decrease with age [31]. Changes in the homeostasis of polyamines play
116 an important role in the emergence of many diseases such as cell growth, senility, memory
117 performance, neurodegenerative diseases, metabolic diseases and cancer [32,33].

118 In the polyamine synthesis pathway, two major molecules play an important role in the
119 modulation of the immune system. These are ornithine and agmine molecules. Ornithine is the

Comment [DPE2]: were

Comment [DPE3]: increased

Comment [DPE4]: begin with capital letters-
Parkinson's, Huntington's

precursor molecule of the synthesis of major polyamines (putresin, spermidine and spermine), the precursor of both L-glutamate and GABA molecules as well as the precursor of L-proline required for the synthesis of connective tissue for wound healing (Figure 1). In this study, ornithine levels were increased in both groups of patients compared to the control and statistically significant ($p<0.05$). The major metabolic ways in which the increased ornithine can go, are L-proline, L-glutamate, GABA, polyamine synthesis and urea cycle. Since the ODC levels, the rate limiting enzyme in polyamine synthesis, did not change in both patient groups, the polyamine synthase pathway would not be active (Table 2).

The entry of the increased ornithine into the urea cycle will contribute to the formation of excessive urea and thus the increase of osmolarity in many regions of the brain such as the cerebellum, cerebral cortex and brain stem [34]. Chronic osmotic pressure caused by ornithine also modifies plasticity in the hippocampal region by mediating tonic inhibition of the GABA receptor family [18,35,36]. On the other hand, the increase of L-proline synthesis for collagen production, which is necessary for connective tissue, means that immune system cells pass into the fix mode (wound healing). Thus, increased L-proline synthesis will only contribute to the synthesis of collagen [37].

The main problems of both Parkinson and Alzheimer's patients are mainly a cluster of α -synuclein and AB. Extra collagen accumulation will also increase the negative effect. This approach could not

be verified for the time being because L-prolin levels could not be measured due to the limitations of the study. Another molecule that is effective in the modulation of immune cells is agmatine. The active immunocytes (microglia, macrophage) increase NO production in the M1 (fight-killing program) mode. They do this via inducible nitric oxide synthase (iNOS). NO is a twisted sharp knife;

Comment [DPE5]: write in full

Comment [DPE6]: write in full- Nitric oxide

141 The first is the main events caused by their decreasing concentration. These are reduced vascular
142 perfusion, blockage of granulocytes in the blood vessels in the inflamed area, inhibition of pro-
143 inflammatory reactions, and thickening of the capillary membranes [38,39,40]. All these adverse
144 events lead to neurodegeneration and neuronal cell death due to inadequate O₂ and glucose
145 transport to the resulting neuronal and glial cells [41]. In the second, high concentrations of NO
146 inhibit many complexes in the respiratory chain and lead to an increase in the synthesis of highly
147 toxic compound peroxynitrite (ONOO⁻). This highly toxic compound causes mitochondrial damage
148 and leads to mitochondrial dysfunction leading to neurodegeneration and neuronal cell death [42].
149 When the level of arginine is reduced to an undetectable level in regions with inflammation, it is
150 mean that Arginine metabolism is a central pathway for macrophages in the immune system [43].
151 When inflammation occurs, the macrophages must be directed to one of the "fight" or "fix" modes.
152 Fight is a killing program (eg pathogen) and fix is a wound healing program. The Fight pathway
153 activated by macrophages results in the formation of nitric oxide (NO) and cell proliferation is
154 inhibited [44]. When the fix pathway is activated, proliferation and healing process is initiated by
155 ornithine formation (via polyamine and collagen) [45,46,47,48]. Both the M1 (fight) and M2 (fix)
156 pathways use arginine, but the NO and Ornithine pathways can not be active at the same time
157 because the metabolites of a pathway inhibit the enzymes of the other pathway (Şekil 1). The fact
158 that ADC enzyme levels which are effective in the synthesis of agmatine in both groups of patients
159 do not change and that there is no difference in agmatinase enzyme in the destruction pathway will
160 not change the amount of agmatine (Tablo 2). Solubulous amyloid-Beta oligomers are responsible
161 for synapse loss and neurodegenerative development in Alzheimer's disease [49]. B-Amyloid 1-40

Comment [DPE7]: spelling-vascular

Comment [DPE8]: oxygen

Comment [DPE9]: please re-frame this statement

162 peptide regulates glutamate release by acting on glutamergic terminals [50], while B-amyloid

163 plaques lead to increased intracellular **Ca** concentration and death of neurons by directly affecting

164 ~~on~~ NMDA receptors [51].

Comment [DPE10]: write in full

165 The absence of an increase in the amount of agmatine, the natural antagonist of the receptors,

166 naturally inhibits blockage of these receptors.

167 Agmatine activates eNOS while inhibiting iNOS from NOS enzymes. **studies** on experimental animal

Comment [DPE11]: end the other statement with a full stop and begin this new one with a capital letter- Studies

168 models showed that application of agmatine in brain cells damaged by lipopolysaccharide reduced

169 and corrected the damage with these properties [52,53,54,55].

170 As a result, polyamine synthesis enzymes whose levels are not altered in Alzheimer and

171 Parkinson's disease can not contribute to the prevention of neurodegeneration. Increased ornithine

172 levels may contribute to proline, glutamate, GABA and urea synthesis and increase osmolarity and

173 impair plasticity. Exposing **these darker aspects** with more extensive studies may allow new

Comment [DPE12]: these grey areas

174 approaches to be introduced in the treatment of such diseases

175 Acknowledgments: **This research did not receive any specific grant from any funding agency in**

176 **the public, commercial or not-for-profit sector.** |

Comment [DPE13]: acknowledgement talks about people that helped you in this research. So you are mentioning their names here.. It has nothing to do with grants.

177 Conflict of Interest/Disclosure Statement: The authors have no conflict of interest to report.

178

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Table 1. The gender and age ranges of patients and control

	Alzheimer		Parkinson	
	Control	Patient	Control	Patient
Gender				
Male	25	25	19	19
Women	10	10	16	16
Age (X ± S)	77 ± 11	74 ± 14	67 ± 12	68 ± 11

Data expressed as mean ± standard deviation

Table 2. The serum Ornithine, Arginine Decarboxylase, Ornithine Decarboxylase and Agmatinase levels in patients and controls

	Alzheimer			Parkinson		
	Control	Patient	P	Control	Patient	P
ADC	3.07±0.22	3.08±0.13	>0.05	3.11±0.21	3.07±0.22	>0.05
(pg/ml) (n=35)						
Agmatinase	3.06±0.21	3.02±0.21	>0.05	3.09±0.21	3.06±0.13	>0.05
(pg/ml) (n=35)						
ODC	3.13±0.15	3.11±0.23	>0.05	3.11±0.21	3.07±0.2	>0.05
(pg/ml) (n=35)						
Ornithine	0.12±0.02	0.16±0.02	<0.05	0.14±0.03	0.16±0.02	
<0.05						
(μmol/ml)						
(n=35)						

331

332

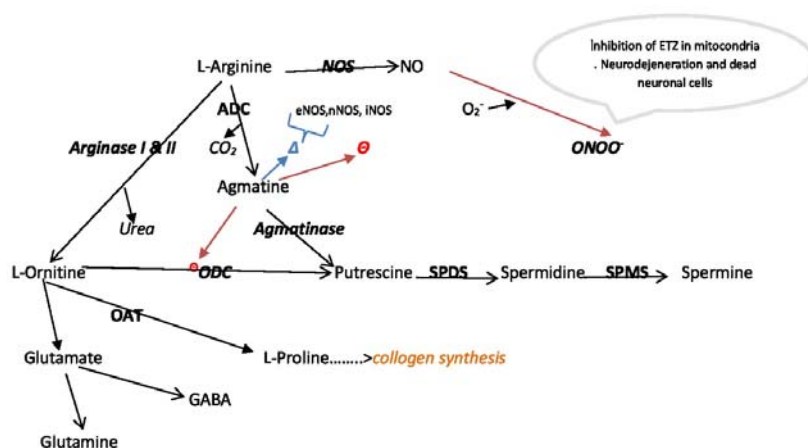


Figure 1: Regulation of NO synthesis and polyamines synthesis by agmatine. Nitric oxide synthase (NOS); endothelial NOS (eNOS); neural NOS (nNOS); inducible NOS (iNOS); Arginine decarboxylase (ADC); Ornithine decarboxylase (ODC); Spermidine synthase SPDS; Spermine synthase SPMS; γ -aminobutyric acid (GABA); Ornithine amino transferase (OAT); Ornithine decarboxylase (ODC); inhibition; $\oplus$: Activation Δ :

333

