

Serum Nitric Oxide Levels in Patients with Probable Alzheimer Disease and Vascular Dementia

[Alzheimer Hastalığı Olasılığı ve Vasküler Demans Bulunan Hastalarda Serum Nitrik Oksit Düzeyleri]*

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ABSTRACT

Aim: Nitric oxide is involved in pathological processes that lead to tissue damage partly because of its free radical nature. Oxidative stress and vascular dysfunction are recognized contributors in the pathogenesis of Alzheimer disease and vascular dementia. We investigated the serum concentration of nitric acid in 20 patients with probable Alzheimer disease, 20 patients with probable vascular dementia and in 19 control subjects. We also aimed to determine the association between this concentration and cognitive impairment tested by Mini-Mental State Examination in the disease groups.

Materials and methods: Serum nitric oxide concentration was determined by a colorimetric Griess reaction. Cognitive impairment was tested by the Mini-Mental State Examination score. Body mass index was calculated for each subject.

Results and Conclusion: We suggest that serum nitric oxide concentration is unrelated with the risk for Alzheimer disease and vascular dementia and that it is not a good marker of dementia and cognitive impairment.

Keywords: Alzheimer disease, vascular dementia, nitric oxide, cognitive impairment

ÖZET

Amaç: Nitrik oksit serbest radikal yapısından dolayı doku hasarına neden olarak patolojik süreç içerisinde yer almaktadır. Oksidatif stress ve vasküler işlev bozukluğu, Alzheimer Hastalığı ve vasküler demansın patogeneze katkıda bulunmaktadır. Çalışmada 20 olası Alzheimer hastası, 20 olası vasküler demansı olan hasta ve 19 kişiden oluşan kontrol grubunda serum nitrik oksit derişimleri ölçülmüştür. Ayrıca gruplarda nitrik oksit derişimleri ile kognitif bozukluk arasındaki ilişki Mini-Mental Durum Muayenesi ile incelenmiştir.

Gereç ve Yönetimler: Serum nitrik oksit derişimi kolorimetrik Griess reaksiyonu kullanılarak belirlenmiştir. Kognitif bozukluk Mini-Mental Durum Muayenesi skoru ile değerlendirilmiştir. Her birey için vücut kitle indeksi hesaplanmıştır.

Bulgular ve Sonuçlar: Serum nitrik oksit derişimi ile Alzheimer Hastalığı ve vasküler demans riski bakımından bir ilişki bulunmamıştır. Sonuç olarak nitrik oksit demans ve kognitif bozukluk için iyi bir belirteç değildir.

Anahtar Kelimeler: Alzheimer hastalığı, vasküler demans, nitrik oksit, kognitif bozukluk

Introduction

Nitric oxide (NO) is a free radical messenger molecule produced by neuronal nitric oxide synthase (nNOS), endothelial NOS (eNOS) and inducible NOS (iNOS) [1]. NO can damage tissues in part by oxidative stress, the cytopathologic consequence of a disbalance between antioxidant defenses and free radical production that lead to cellular death [2]. Oxidative stress and vascular dysfunction are recognized as contributors in the pathogenesis of Alzheimer (AD) and vascular dementia (VD) [3,4].

The principal source of NO in the vascular system is the constitutively expressed eNOS of endothelial cells. NO is continuously released at low levels, and produces vasodilation [5]. It also causes aggregation and activation of platelets [6], attenuates adhesion of leukocytes to the endothelium [7] and inhibits proliferation and migration of vascular smooth muscle cells [8]. In general, NO is associated with an atheroprotective effect [9]; and vascular risk factors, such as hyperglycemia, LDL-cholesterol, triglycerides, hypertension increase the vulnerability of developing dementia [10,11].

NO has been implicated in neurodegeneration and neuronal cell death through its neurotoxicity in AD and other neurodegenerative dementias [12]. In AD patients β -amyloid (A β) stimulates microglial and astrocytic NO production [13-15].

Significantly lower serum NO concentration was observed in patients with probable AD and VD than in controls [16]. Decreased plasma NO and increased homocysteine and asymmetric dimethylarginine concentration was found in AD [17].

Changes in cerebrospinal fluid (CSF) [18] and plasma nitrate levels have been reported [17,19], but a causal relationship between NO and dementia has not been demonstrated. The level of nitrates correlates negatively with the degree of cognitive disorder in AD patients [20]. On the contrary, Navarro et al. [21] and Milstien et al. [22] did not find significant differences in CSF and plasma nitrate levels between AD patients and controls. However, Kuiper et al. [23] reported decreased CSF nitrate levels in AD that were associated with decreased tetrahydrobiopterin (BH4) levels [24]. The mechanism by which decreased NO levels affect the pathogenesis of dementia remains unclear.

We investigated the serum NO concentration in patients with probable AD and probable VD and in control subjects and the association between this concentration and cognitive impairment in the disease groups.

Materials and Methods

We conducted a cross-sectional study in 20 patients (18 females and 2 males) with probable AD, 20 patients (17 females and 3 males) with probable VD, and 19 community-dwelling age-matched apparently healthy

(16 females and 3 males), asymptomatic persons without dementia. All subjects were aged 65 years or over.

The patients were institutionalized in a specialized unit at the Health-Care Hospice for person with disabilities in Sarajevo, Bosnia and Herzegovina. The clinical diagnosis was made by a senior staff neurologist and psychiatrist by NINCDS-ADRDA criteria for probable AD [25] and by NINDS-AIREN criteria for probable VD [26].

Body Mass Index (BMI) for each subject was calculated as weight in kilograms divided by height in meters squared. Height was measured with stadiometer and weight was measured with Toledo self-zeroing weight scale. Trained persons measured blood pressure using a mercury sphygmomanometer on the right arm after at least a 5-min rest. Subjects with a history of cardiovascular or thyroid disease, chronic inflammatory disease (asthma and rheumatoid arthritis), hepatic or renal insufficiency and cancer were excluded from the three groups.

Approval for the study was obtained from the local ethics committee. All procedures on human subjects were performed in the accordance with Helsinki Declaration of 1975. Informed consent was obtained from subjects and caregivers upon careful explanation of the study procedure.

Global cognitive function was tested with the MMSE test [27-29] which has been used for rapid screening of those with cognitive and/or intellectual deficit. It assesses orientation, short term memory, serial subtraction, constructional capacities and use of language. The total score is 30. A score of 24 is considered abnormal. A score of less than 17 is considered dementia. All patients in the AD and VD groups had A score of ≤ 12 while subjects in the control group had a score of 26-30.

The Hachinski ischemic score (HIS) helps differentiate patients with VD from those with AD.

The original scale consists of 13 items; each scale item was assigned a numeric value with double weighting applied to specific clinical features. A score of 7 or more means vascular dementia, A score of 0-4 means Alzheimer's dementia and a score of 4-7 means mixed dementia [29]. Our patients in the AD group had a score of 4 or less, and patients with VAD had a score 7 or more .

The plasma level of nitric oxide was determined by measuring plasma nitrite concentrations, a stabile metabolic product of NO with oxygen. Conversion of nitrate (NO₃⁻) into nitrite (NO₂⁻) was done with elementary zinc. Nitrite concentration in plasma was determined by Griess reaction [30]. Absorbance was measured at 546 nm. The results were expressed as $\mu\text{mol/L}$.

Determination of NO concentration in serum of patients and control group was done at the Institute for Physiology and Biochemistry of Faculty of Medicine in Sarajevo.

Statistical analysis was performed with SPSS, version 16.0. Data are presented as mean \pm SEM. Data distributi-

on was determined using the Kolmogorov-Smirnov test. Data were statistically analysed with the non-parametric Mann–Whitney U test. Additionally, Spearman correlations were used as measures of association for the continuous variables. Statistical significance was set at $p < 0.05$.

Results

The characteristics of the three groups are shown in Table 1. There were no differences in systolic and diastolic blood pressures and BMI between the groups. The AD and VD groups had significantly lower MMSE scores than the control group ($p < 0.0001$), but there was no difference in MMSE scores between the AD and VD groups.

Table 1. Characteristics of patients in AD, VD and control groups

	AD	VD	C
Age (years)	80.0±1.1	76.9±1.4	77.8±1.1
SBP (mmHg)	139.5±4.0	132.0±6.6	130.5±4.4
DBP (mmHg)	84.0±2.0	80.0±3.4	81.3±1.9
BMI (kg/m²)	25.1±1.1	23.9±0.5	25.6±0.8
MMSE score	4.3±0.8***	8.0±1.3***	27.6±0.3

*** $p < 0.001$

Data as mean ± S.E.M. SBP: systolic blood pressure; DBP: diastolic blood pressure; BMI: Body mass index; MMSE: Mini Mental State Examination.

As shown in Figure 1 patients in the AD group had significantly higher NO concentration than those in the VD group ($p = 0.001$). There was no significant difference in NO concentration between the AD and VD groups and the control group.

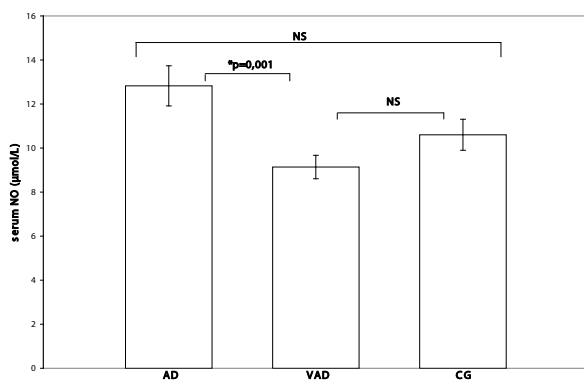


Figure 1. Serum nitric oxide concentration in AD, VD and control groups.

Bars show means and error bars show ±SEM; NS-Not significant

A negative but not significant correlation was noted between NO concentration and MMSE score in the AD and VD groups ($r = -0.1$ and $r = -0.19$; respectively). The control group showed a positive but not significant correlation between NO concentration and MMSE score.

Discussion

We found a significantly higher serum NO concentration in the AD group than in the VD group and no difference in serum NO concentration between patients with AD and VD groups and the control group.

Overproduction or deficiency of NO play an important role in the development of neurodegenerative damage in dementias. The excess of NO observed in our AD group may reflect overproduction of NO by astrocytes and microglia which could be neurotoxic. Studies so far have shown that accumulation of A β in the brain induces NO overproduction in microglia and astrocytes [31]. The neurotoxic effect of NO excess is mediated by mitochondrial dysfunction and ATP depletion leading to neuron apoptosis [32]. In AD disease A β activates CD4+ T cells, which produces various cytokines that activate microglial cells [33] with release of NO [34] and production of TNF- α , which also potentiates NO production [34]. Aggregation of reactive astrocytes in the proximity of A β has been demonstrated, and presence of cytokines can directly induce NO production or act with A β to induce iNOS expression [13, 35, 36]. Neurofibrillary tangles (NFT) may also participate in NOS activation and NO production in AD [37, 38].

The deficit of endothelial NO may produce dysfunction of vascular endothelium and hypoperfusion of the brain. The decreased NO concentration observed in our VD group reflects underproduction of NO [39]. On the other hand, NO prevents platelet and leukocyte adhesion to the endothelium, a process that may down-regulate proinflammatory events [40, 41]. There is increasing evidence that cerebral blood flow decreases with aging, which produces endothelial cell dysfunction and reduction of NO release. Considerable evidence demonstrates that endothelial dysfunction is a common feature of aging [42, 43]. Chronic cerebral hypoperfusion resulting from aging and vascular risk factors such as hypertension, hyperglycemia or hyperlipidemia can stimulate a rapid release of NO via eNOS activation [44]. This effect is considered to be a homeostatic response and reduces the damage caused by hypoperfusion. However, such chronic eNOS activation can result in endothelial cell dysfunction and disorders in NO release from the endothelium, and finally, basal NO levels diminish and are unable to regulate normal vascular perfusion, block granulocyte adherence in blood vessels or prevent proinflammatory reactions. Such a phenomenon may promote the basement membrane thickening [45-47].

Corzo et al. [16] found significant lower serum NO concentration in patients with probable AD and VD than

in the control group. They suggested that decreased serum levels of total NO in dementia, either probable AD and VD, seems to be unrelated to genetic risk factors, or serum A β and ApoE levels. However, progressive NO decline seems to be associated with an HDL cholesterol-related atheroprotective effect in dementia. Selley et al. [17] found an association between decreased plasma NO and increased homocysteine and asymmetric dimethylarginine concentration in AD.

We found a negative but not significant association between cognitive decline and serum NO levels, which might be due to a relative small sample size. Similar to our results Corzo et al. [16] found a weak, also a negative association between cognitive decline tested by MMSE scores and NO levels.

In conclusion, our results suggest that serum level of nitric oxide is not related with the risk for AD and VD and that it is not good marker of dementia and cognitive impairment.

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