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RESEARCH ARTICLE

Plasma Nitric Oxide Synthesis Activity at Acute Phase of Stroke and Stroke Subtypes[1]

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Plasma Nitric Oxide Synthesis Activity at Acute Phase of Stroke and Stroke Subtypes

Summary

This study aims at investigating plasma nitric oxide synthesis activity at acute phase of stroke and stroke subtypes. 34 acute stroke patients (21 males, 13 females) and 14 healthy individuals (10 males, 4 females) participated in the study. The plasma nitric oxide synthesis activity was determined by colorimetric assay in conformity with the kit procedure. Plasma nitrite+nitrate levels were determined as 0.83 (0.83-0.83) µM and 0.89 (0.76-1.12) µM for the patient group and the control group respectively. As for the patient subgroups, the levels were obtained as 0.83 (0.54-0.83) µM for large vessel disease and 0.83 (0.83-1.20) µM for small vessel disease. Nonetheless, there was no statistically significant relationship (p>0.005) among groups. Consequently, in this study, we may assert that plasma nitric oxide synthesis activity does not constitute an important criterion for stroke and stroke subtypes. However, more comprehensive investigations are required to determine definite effect of plasma nitric oxide synthesis activity on stroke and stroke subtypes.

Key Words: *Plasma Nitric Oxide Synthesis Activity, Stroke, Stroke Subtypes.*

Akut Faz İnme ve Alt Tiplerinde Plazma Nitrik Oksit Sentaz Aktivitesi

Özet

Bu çalışmada akut faz inme ve alt tiplerinde plazma nitrik oksit sentaz aktivitesinin araştırılması amaçlanmıştır. Çalışmada 34 akut inme hastası ile (21 kadın, 13 erkek) ve 14 sağlıklı birey (10 erkek, 4 kadın) yer almıştır. Plazma nitrik oksit sentaz aktivitesi kit prosedürüne bağlı olarak kolorimetrik ölçüm ile tayin edilmiştir. Plazma nitrit+nitrat düzeyleri hasta grubunda 0.83 (0.83-0.83) µM, kontrol grubunda 0.89 (0.76-1.12) µM olarak ve hasta alt gruplarında 0.83 (0.54-0.83) µM (büyük damar hastalığı) ve 0.83 (0.83-1.20) µM (küçük damar hastalığı) olarak belirlenmiştir. Gruplar arasında istatistiksel olarak anlamlı bir ilişki (p>0.005) bulunmamıştır. Sonuç olarak, bu çalışmada, plazma nitrik oksit sentaz aktivitesinin inme ve alt tipleri için önemli bir kriter olmadığını söyleyebiliriz, ancak, inme ve altiplerinde plazma nitrik oksit sentaz aktivitesinin kesin etkisinin belirlenmesi için daha kapsamlı çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: *Plazma Nitrik Oksit Sentaz Aktivitesi, İnme, İnme Alt Tipleri.*

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INTRODUCTION

Stroke is a clinical case occurring as a result of sudden cerebral function disruption due to pathology in brain vessels and is the third most important cause of death after heart diseases and cancer (1).

Nitric oxide (NO) is a multifunctional inorganic gas synthesized from L-Arginin by three different isoforms of nitric oxide synthesis. The final products of NO are nitrite (NO^{2-}) and nitrate (NO^{3-})

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in vivo (2-4). NO is the most important endothelial mediator and plays a vital role in the regulation of vascular tone and in saving vessel integrity. Studies showed that, because of its role on vascular endothelial, NO is effective on the development of myocardial ischemia, hypertension, atherosclerosis and cerebral ischemia (5,7). Under cerebral ischemia, high concentrations of NO is generated by the either calcium-dependent activation of the constitutive neuronal nitric oxide synthesis (nNOS) or the activation of the inducible form of nitric oxide synthesis (iNOS) in macrophages and or other cell types intervening in inflammatory and cytotoxic actions that lead to neuronal death. In contrast, the NO, generated by the activation of the constitutive endothelial nitric oxide synthesis (eNOS), may have protective effects that decrease the ability of platelets to aggregate, prevent leukocyte endothelial adhesion, and increase vascular dilation and cerebral blood flow (8).

In line with the information given above, after a stroke, resting on nitric oxide synthesis, NO production may play a protective and/or a destructive role (9). In studies on which the relationship among the stroke, nitric oxide synthesis activity and NO levels were investigated the results still show contradiction (7-14). In our study, we planned to investigate nitric oxide synthesis activity on acute phase of stroke and its subtypes using nitrite+nitrate levels which are one of the final products of NO that are synthesized in result of nitric oxide synthesis.

MATERIALS AND METHODS

This study included 34 acute stroke patients (21 males and 13 females; mean age 65) and 14 healthy individuals (10 males and 4 females; mean age 56) recruited from Neurology Department, Medicine Faculty, Eskisehir Osmangazi University. Informed consent, in accordance with the study protocol, approved by the ethics committee of Medical Faculty, Eskisehir Osmangazi University, Eskisehir, was obtained from each patient. The acute stroke patients were divided into two groups as large and small vessel disease in agreement with the computed tomography (CT) and magnetic resonance imaging (MRI) results. Healthy persons were selected among individuals who consist of middle age patients, who do not have personal or family history of stroke, cardiovascular diseases and do not use any vasodilator drugs with nitrate or whose metabolism releases nitrate as products.

Blood samples were taken from stroke patients at acute phase of stroke before drug treatment. Plasma nitric oxide synthesis activity was determined by using Nitric Oxide Synthase Assay Kit (EMD Chemicals, Calbiochem, Darmstad). The assay kit is based on a modified Griess method that quantifies the combined levels of nitrite and nitrate (both stable NO metabolites) as an indicator of nitric oxide synthesis activity (15,16). Statistical analyses of the data were made by Statistical Package for Social Sciences (SPSS) 15.0 and SigmaStat 3.5 software programs. The data, consisting of independent groups and do not distribute normally, analysed by Mann-Whitney U test. The average values are expressed as median (%25-%75). The probability values below 0.05 ($p<0.05$) is accepted statistically significant.

RESULTS

Plasma nitrite+nitrate levels were determined as 0.83 (0.83-0.83) µM in the patient group, whereas the levels were found as 0.89 (0.76-1.12) µM in the control group. However, between the patients and the controls, there was no statistically significant difference ($p>0.05$) (Table 1). Additionally, in the subgroups of the acute stroke patients, the plasma nitrite+nitrate levels were determined as 0.83 (0.54-0.83) µM and 0.89 (0.83-1.2) µM for the large and

Table 1. Plasma Nitric Oxide Synthesis Levels in Patients and Controls and Stroke Subtypes

Group	Plasma Nitric Oxide Synthesis Activity (Nitrite+Nitrate) (µM)	Statistic
Patients	0.83 (0.83-0.83)	p>0.05
Controls	0.89 (0.76-1.12)	
Large Vessel Disease	0.83 (0.54-0.83)	p>0.05
Small Vessel Disease	0.83 (0.83-1.20)	

small vessel diseases respectively. There was also no statistically significant difference between stroke subtypes (p>0.005) (Table 1).

DISCUSSION

In our study, according to plasma nitric oxide synthesis activity a statistically significant difference was not observed among stroke patients, controls and the stroke subtypes. Ülker *et al*., found that serum NO levels, in patients with ischemic stroke, was higher compared to the patients in the control group. Nevertheless, as for the serum NO levels, stroke subtypes and lesion size, the relationship among these variables were not statistically significant. The results of the study shows similarities, in aspect of NO levels and stroke subtypes, to our study, however, the fact that stroke and NO levels were higher in the control group in Ülker *et al*.,'s study differed than our study's results.

In the study, carried out by Aygül *et al.*, it was reported that NO levels in ischemic stroke patients, compared to those in control group, exhibited an increase in the plasma and cerebrospinal fluid at acute phase of stroke. They explained the reason of this increase in terms of the ischemic effect and nitric oxide synthesis induction (11). Similarly, Castillo *et al.* established that NO metabolite levels increased significantly in cerebrospinal fluid, taken from acute stroke patients, compared to those in the control group and NO generation played a vital role on acute ischemic stroke (8). Taffi *et al*. suggested that changes in NO metabolism may be considered as markers of brain injury in patients with ischemic stroke. Furthermore, they stated that NO levels were significantly higher in the control group compared to stroke patients (9).

The nitric oxide synthesis activity in serum and plasma, belonging to the patients with acute stroke, or NO occurring in result of this activity or all sort of studies in which NO metabolites were investigated a clear result could not be obtained. This situation may stem from the different methods used in researches, changes on environmental and hereditary factors, presence of other diseases (i.e., diabetes, hypertension etc.), life styles or lack of sufficient patient groups. For instance Bozkurt *et al.*, reported that in healthy individuals who smoke has decreased serum NO levels and this decrease increased in the number of cigarettes smoked per day (17). Hereby, the fact that a standard method use in the determination of the products of NO metabolism, addition of hereditary and environmental factors which may play important role on the disease development into researches, consideration of other diseases and life styles which can affect the NO metabolism and enhancing the number of patients in researches will be effective on determination of real role of NO metabolism in stroke development.

The synthesis of NO by different nitric oxide synthesis isoforms caused new researches to be carried out in the level of tissue. In the study of Clavier *et al.* on rats, predisposed to stroke, it was indicated that the brain nitric oxide synthesis levels were not effective on the probability of stroke development (10). Nishikawa *et al*., carried out a study on cats, moving from the hypothesis that the excessive NO generation acted as a cell death agent in stroke, stated that nitric oxide synthesis inhibition decreased acute neuronal damage in focal ischemia and hence, NO had an important role in focal ischemia in brain damage mechanism (12). In their experimental ischemic stroke studies, Willmot *et al.* reported that nitric oxide synthesis inhibitors decreased infarct volume and thus, they could be used in the treatment of ischemic stroke (13). Eliasson *et* al. asserted that nNOS activation had cellular localization concerning ischemic stroke and NO synthesis was not a direct neurotoxicity source but it caused cell death in case that it converts into peroxynitrite (14). As it is seen, in the results of this study the different data were obtained, however; the relationship between stroke and NO metabolism could not be determined.

CONCLUSIONS

Consequently, in this study, we may claim that plasma nitric oxide synthesis activity does not constitute an important criterion for stroke and stroke subtypes. However, more comprehensive investigations are required to determine definite effect of plasma nitric oxide synthesis activity on stroke and stroke subtypes.

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