Original article / *Araştırma*

Xanthine oxidase, adenosine deaminase and vitamin E levels in patients with schizophrenia

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ABSTRACT

Objective: *Neuronal damage caused by free radicals is believed to be effective in pathogenesis of several psychiatric disorders. This belief is due to the toxic effects of free radicals that play a role in oxidative stress. Considering that the brain is one of the most sensitive organs to the oxidative damage, the importance of oxidative stress in psychiatric disorders will become more apparent. Additionally, high oxygen use in the brain, and its structure rich in lipid, which is one of the most sensitive molecules to the free radical damage, and its having the average antioxidant system yield support oxidative stress theory in the pathogenesis of psychiatric disorders. This study aimed to determine xanthine oxidase (XO), adenosine deaminase (ADA) and vitamins E levels in patients with schizophrenia and control groups, and to investigate the relationship between schizophrenia and the parameters by comparing the measured parameters with each other.* ***Methods:*** *Our study sample included 30 patients diagnosed with schizophrenia. The control group consisted of 30 healthy volunteers matched by sex with similar age and smoking habits. In the patient group and the control group, adenosine deaminase, xanthine oxidase, and vitamin E were measured manually using spectrophotometric methods.* ***Results:*** *Serum xanthine oxidase levels in the schizophrenic group were significantly higher than the control group levels. Serum vitamin E and adenosine deaminase levels in the schizophrenia group were significantly lower than the levels of the control group.* ***Discussion and Conclusion:*** *The fact that mechanism of schizophrenia pathogenesis which has a wide variety of clinical symptoms and a disease process is yet to be elucidated reveals the importance of this kind of studies. In this study, low levels of antioxidant vitamin E and adenosine deaminase, and high levels of xanthine oxidase suggest that oxidative stress-mediated neuronal damage may play a role in the pathogenesis of schizophrenia. Therefore, we believe that further research with larger sample groups should be conducted.* ***(Anatolian Journal of Psychiatry 2016; 17(6):476-481)***

Keywords: *schizophrenia, xanthine oxidase, adenosine deaminase, vitamin E*

Şizofreni hastalarında ksantin oksidaz, adenozin deaminaz ve E vitamini düzeyleri

ÖZ

Amaç: *Serbest radikallerin neden olduğu nöronal hasarın birçok psikiyatrik bozukluğun oluşumunda etkili olduğu düşünülmektedir. Bu düşünce oksidatif streste rol oynayan serbest radikallerin toksik etkilerinin olmasından kaynaklanmaktadır. Beynin oksidatif hasara en duyarlı organlardan biri olduğu düşünüldüğünde, oksidatif stresin psikiyatrik bozukluklardaki önemi anlaşılacaktır. Ayrıca beyindeki yüksek oksijen kullanımı serbest radikal hasarına en duyarlı moleküllerden biri olan lipitten zengin yapısı, ortalama antioksidan sistemine sahip olması psikiyatrik bozuklukların oluşumunda oksidatif stres kuramını desteklemektedir. Araştırma amacımız, şizofreni hastalarında ve kontrol gru-*

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bunda ksantin oksidaz (XO), adenozin deaminaz (ADA) ve E vitamini düzeylerini belirlemek, ölçülen parametreleri birbirleriyle karşılaştırarak şizofreni ile arasındaki ilişkiyi araştırmaktır. ***Yöntem:*** *Araştırmanın örneklemini şizofreni tanısı konmuş olan 30 hasta oluşturdu. Cinsiyet açısından eşleştirilen, yaş ve sigara içme bakımından benzer 30 gönüllü sağlıklı kişi kontrol grubunu oluşturdu. Hasta ve kontrol grubu serum ADA, XO ve vitamin E ölçümleri manuel olarak spektrofotometrik yöntemle yapıldı.* ***Sonuçlar:*** *Şizofreni grubunun serum XO düzeyleri kontrol grubuna göre anlamlı ölçüde yüksek bulundu. Serum E vitamini ve ADA düzeyi şizofreni grubunda kontrol grubuna göre anlamlı ölçüde düşük bulundu.* ***Tartışma ve Sonuç:*** *Çok çeşitli klinik belirtilere ve bozukluk sürecine sahip olan şizofreni oluşum düzeneğinin henüz aydınlatılamamış olması bu çalışmaların önemini ortaya koymaktadır. Çalışmamızda antioksidan olan E vitamini ve ADA düzeyinin düşük, XO düzeylerinin yüksek bulunması; şizofreninin patogenezinde oksidatif stres aracılı nöronal hasarın da rolünün olabileceğini göstermektedir. Bu nedenle daha geniş örnek grupları ile araştırmalar yapılması gerektiğini düşünüyoruz.* ***(Anadolu Psikiyatri Derg 2016; 17(6):476-481)***

Anahtar sözcükler: *Şizofreni, ksantin oksidaz, adenozin deaminaz, E vitamini*

INTRODUCTION

Reactive oxygen radicals cause damage to cells by different mechanisms. Neuronal membrane in the high amount of unsaturated fatty acid content is exposed to attack by reactive oxygen species, and this causes lipid peroxidation. These effects damage anti-inflammatory and antioxidant functions, and affect neuronal membrane that provides a physiological defense against free radicals. It is suggested that neuronal damage caused by free radicals is effective in the pathogenesis of several psychiatric disorders. This assumption stems from the toxic effects of free radicals implicated in oxidative stress. The fact that brain is one of the most sensitive organs to the oxidative damage reveals the significance of oxidative stress in psychiatric disorders. Additionally, high oxygen use in the brain, and its structure rich in lipid, which is one of the most sensitive molecules to the free radical damage, and its having the average antioxidant system yield support oxidative stress theory in the pathogenesis of psychiatric disorders.[1-4] As a matter of fact, some studies have shown that there were changes both in the peripheral blood and the brain tissue in the antioxidant system in patients with schizophrenia.[5] In recent years, it has been suggested that the enzyme is also associated with psychiatric disorders. One of these enzymes is Xanthine Oxidase (XO) which is an important enzyme in the purine and adenosine metabolism, playing an important role in oxidetive stress as well. However, XO is one of the most important enzymatic sources of superoxide radical and hence one of the main sources of oxidative stress. XO is striking as a common ground for oxidative stress and the hypothesis of adenosine in schizophrenia.[6] The other enzyme, adenosine deaminase (ADA), is mainly involved in T cell activation and formation of cellular immunity and required for the provision of the normal immune response. There are studies in which ADA levels increased and decreased in psychiatric disease. A large number of researchers demonstrated that ADA is a marker of cellular immunity and increased serum levels in various disease accordingly.[7-9] Another group associated with psychiatric disorders is vitamins. The most powerful antioxidant of these is vitamin E. It neutralizes the oxygen free radicals, prevents peroxidation of membrane phospholipids and unsaturated fatty acids.It is also considered to exist in the area close to the free radical generating enzyme in the cell membrane. Especially, low levels of vitamin E are associated with increased lipid peroxidation in patients, and impoverish antioxidant defense systems. Therefore, it can be said that the patients are sensitive to lipid peroxidation.[4,9] These results are consistent with the concept of free-radical mediated neurotoxicity in schizophrenia. However, there are very few studies conducted with prooxidants in the context of schizophrenia. Beeecause of the possible roles of XO, ADA and Vitamin E in oxidative stress, it was especially intended to determine XO, ADA and the vitamin E levels in patients with schizophrenia and control groups in this study. In addition to this, the relationship between schizophrenia and measured parameters was investigated by comparing the parameters with each other.

METHODS

Selection of study group and evaluation

The study sample consisted of 30 patients who have at least two years history of schizophrenia and diagnosed with positive dominant schizophrenia symptoms. They were followed up during two months and admitted to psychiatric wards according to Diagnostic and Statistical Manual of Mental Disorders-Text Revised (DSM-5) criteria.[10] Duration of the disease varied from 2 to 30 years with average: 12.8 years. The control group consisted of 30 healthy volunteers matched in terms of sex, having similar age and

Table 1. Gender and smoking rates of groups

		Patient		Control	
		n	%	n	%
Gender	Female	11	36.7	12	40
	Male	19	63.3	18	60
Smoking	Smoker	20	66.7	18	60
	Non-smoker	10	33.3	12	40

smoking habits (Table 1). All participants provided written informed consent, and the study was approved by the Ethics Committee of Cumhuriyet University.

Collection of blood samples

5 ml blood samples were taken from schizophrenia patients and the individuals constituting the control group. Blood samples were centrifuged at 4000 rpm for 15 minutes at 4°C. Afterwards, the serum samples obtained were stored at -80°C for a period required for detection of relevant parameters.

Statistical analysis

Independent t test was conducted to determine statistical differences between groups with the aid of SPSS software version 11.0 (SPSS, Chicago, IL, USA). Statistical significance was defined as $p< .01$ and $p<0.05$ for all tests.

Biochemical analysis

Serum ADA in control and patients groups was determined using the Giusti and Galanti method based on the Bertholet reaction.[11] Briefly, the indophenol complexes formed when ammoniac was released from adenosine and was quantified using a spectrophotometer at a wavelength of 620 nm. One unit of ADA was defined as the amount of enzyme required to release one micromole of ammonia per minute from adenosine at standard assay conditions. ADA activity was expressed as units per liter (U/L) in the serum.

Serum XO activity was measured by the method of Prajda and Weber, where activity is measured by the determination of the amount of uric acid formed from xanthine.[12] Serum samples (100 ml) were incubated for 30 min at 37°C in 3 ml of the phosphate buffer (pH 7.5, 50 nM) containing xanthine (4 mM). The reaction was stopped by the addition of 0.1 ml 100% (w/v) TCA, and the mixture was then centrifuged at 1780 g for 20 min. Uric acid was determined in the supernatant by measuring the absorbance at 292 nm against a blank and expressed as mIU/ml. A calibration curve was constructed using 10–50 mU/ml concentrations of standard XO solutions (Sigma X-1875, Sigma-Aldrich, St. Louis, MO). One unit of activity was defined as 1 mmol of uric acid formed per minute at 37°C and pH 7.5.

Determination of vitamin E was made based on Martinek method.[13] Briefly, ferrous iron was reduced to ferric iron with the effect of 2,4,6-tripyridyl-s-triazine (TPTZ). Capped centrifuge tubes were used for the determination of vitamin E. 1.0 ml ethanol was placed in the sample tube and 1.0 ml serum was added into the tube. After 1.0 ml addition of xylene into the tubes, they were closed tightly and shaken for 30s. For determination of vitamin E, the tubes were centrifuged at 4000 rpm with the centrifugal supernatant obtained (vitamin E in the supernatant is stable for 24 hours at +4°C). Afterwards, 0.5 ml of the supernatant was pipetted in reaction vessel, 0.5 mL. TPTZ reagent was added on it and it was read against blank at 460 nm at 15 seconds. After spectrophotometry was blinded at 600 nm against blind, 0.1 ml $FeCl_3$ reagent was added into the reaction vessel, and mixed, and the absorbance values were read at 600 nm at 15 seconds. This procedure was repeated at 460 and 600 nm, respectively for each sample. The blind and standards were studied as examples. Owing to lack of carotene in standard, standard absorbance was only read at 600 nm. Absorbance determination at 460 nm was used in order to eliminate serum carotene. Serum vitamin E concentration=sample absorbance at 600 nm (0.4 x sample absorbance at 460 nm)/Standard absorbance x 1%.

RESULTS

XO serum levels were significantly higher in the schizophrenia group compared to the control group ($p<0.01$). On the other side, serum ADA and vitamin E levels were significantly lower in

Table 2. Age groups, xanthine oxidase and adenosine deaminase enzymes and vitamin E levels

	Patient Mean±SD	Control Mean±SD	t	p
Age	29.77±5.39	29.63±5.25	0.097	0.923
Xanthine oxidase (U/L)	0.71±0.13	0.53±0.1	6.009	<0.001
Adenosine deaminase (U/L)	17.12±3.18	20.22±3.1	-3.838	<0.001
Vitamin E (mg/dl)	0.96±0.14	1.09±0.19	-3.172	<0.005

the schizophrenic patients than those of healthy controls $p<0.01$ and $p<0.05$ consecutively (Table 2).

DISCUSSION

Schizophrenia is a devastating mental disorder affecting approximately 1% of the world population with some variation in incidence.[14] It is viewed as a neurodevelopmental disorder characterized by positive symptoms, negative symptoms and cognitive dysfunction.[15] Although genetic diathesis and abnormalities in the neural circuitry have been proposed as bases for schizophrenia, its etiology remains unclear.[16]

Antioxidant enzymes such as SOD, GpX and CAT are commonly measured for quantifying the antioxidative defense in schizophrenia, along with vitamin E and C levels.[17] While the majority of studies have reported decreased antioxidant defense in patients with schizophrenia,[18-20] there are also some studies where the opposite has been reported.[21-23]

The control group in this study consisted of 30 healthy volunteers matched for sex and age with similar smoking habits, and the values of the individuals in experimental group were compared with the values of the individuals in this group (Table 1). It was found as a result of the study that the schizophrenic group had low levels of the antioxidant vitamin E (Table 2). This situation can be explained by the argument that low level vitamin E can be prooxidant for schizophrenia. As a matter of fact, a study on schizophrenia conducted in India found that plasma vitamin E and C levels were significantly lower in schizophrenic patients compared to healthy controls.[24] These results are in agreement with our study. Attempts to ameliorate oxidative damage and therapeutically induced extrapyramidal syndrome are of considerable importance. The use of antioxidants and PUFAs in the treatment of schizophrenia has yielded some positive results, but still remains experimental. Current results suggest that vitamin E supplementation may play a role in the treatment of schizophrenia within certain subgroups of patients. Considering the levels of XO (Table 2), it is clear that XO levels in the schizophrenic group were significantly higher than those of the control group. This can be explained with the key role of XO in oxidative stress. In fact, both the purine/adenosine metabolisms as well as oxidative stress have recently been discussed as causal factors in the etiology of schizophrenic psychosis.[25] Taken together, the majority of studies confirm that oxidative stress and oxidative damage are present in schizophrenia.[21-23,26] One of the parameters examined in this study was ADA levels (Table 2). ADA levels were significantly lower in the schizophrenic patients than those in the control group (Table 2). ADA is a significant indicator of active cellular immunity. For example, deficiency in ADA in humans manifests primarily as severe lymphopenia and immunedeficiency. In the light of these results, we can argue that schizophrenia may be associated with immunodeficiency and lymphopenia.[27]

Finally, there is a growing body of evidence that oxidative stress is involved in the pathology of schizophrenia. Oxidative stress in schizophrenia can be evaluated with a wide spectrum of biomarkers. However, this needs further confirmation. There are indications that different types of schizophrenia, and positive and negative symptoms can be distinguished by the levels of biomarkers.[28] This would be of great help in the diagnosis of schizophrenia and predicting the course of disease. On the other hand, the most important limitation of our study is absence of other psychiatric disorders to be compare and the difficulty to find patients who use the drug. Therefore, according to the study results, it can be mentioned that patients taking antipsychotic assessment are evaluated according to the control group.

Does the change in metabolite levels studied disorders connected, or is it a result of the the treatment remains as question needing to be answered. Therefore, comparisons must be made by looking at the metabolite levels in schizophrenia patients not use antipsychotics. Also, the new studies using different methods can be effective and appropriate to take into account the effects of other variables. Therefore we suggest that further studies, including larger, randomized control trials, are needed to fully elucidate the role of vitamin E supplementation, ADA, XO and different parameters in the treatment of schizophrenia.

Yazarların katkıları: *H.K.: Konuyu bulma, literatür tarama, planlama, araştırmanın yürütülmesi, istatistik, makaleyi yazma; S.B.: Konuyu bulma, literatür tarama, planlama, araştırmanın yürütülmesi, makaleyi yazma; D.K.: Literatür tarama, planlama, araştırmanın yürütülmesi; Ö.D.: Literatür tarama, planlama, araştırmanın yürütülmesi; K.D.: Literatür tarama, planlama, araştırmanın yürütülmesi; S.E.: Konuyu bulma, literatür tarama, planlama, araştırmanın yürütülmesi makaleyi yazma; E.E.E.: Konuyu bulma, literatür tarama, planlama, araştırmanın yürütülmesi, makaleyi yazma.*

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