

Biological Markers in Newly Diagnosed Generalized Anxiety Disorder Patients: 8-OHdG, S100B and Oxidative Stress

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Purpose: Generalized Anxiety Disorder (GAD) is a chronic disease persisting for at least 6 months, characterized by excessive and continuous anxiety, which leads to evident problems and functional disorders. S100B is a glial protein that plays a role in intercellular communication regulating cell growth and differentiation, and intracellular signal transmission. This study aimed to analyze the serum S100B, 8-OHdG, and oxidative stress levels of patients newly diagnosed with GAD who had not started treatment, to better understand the underlying neurobiological basis of the etiology of GAD.

Patients and Methods: Forty-four patients diagnosed with GAD according to DSM-5 diagnostic criteria and 44 healthy controls were included in the study. The Beck Anxiety Inventory (BAI) was used to determine the anxiety levels of the GAD patients. The serum S100B, 8-OHdG, total oxidant status (TOS), and total antioxidant status (TAS) levels were measured in the patient and control groups.

Results: The 8-OHdG values of the GAD group were determined to be statistically significantly higher than those of the control group ($p=0.028$). No significant difference was determined between the GAD patients and the control group in respect of the TAS, TOS, and oxidative stress index (OSI) values ($p>0.05$). The S100B levels of the GAD group were found to be higher than those of the control group.

Conclusion: The results of this study showed that there could be DNA damage because of oxidative stress in GAD patients. There is a need for further studies to confirm the role of S100B protein in GAD etiology and pathogenesis.

Keywords: generalized anxiety disorder, oxidative stress, 8-OHdG, DNA damage, S100B

Introduction

Anxiety can emerge as an appropriate response to stressful situations. However, when it reaches levels that are difficult to control it is accepted as a pathological disorder and is known to play an important role in the pathogenesis of many psychiatric disorders.¹ Generalized anxiety disorder (GAD) is the most commonly seen anxiety disorder in primary healthcare institutions. Anxiety, which is experienced as excessive and uncontrollable worry about various subjects, remains the key diagnostic criterion for GAD.² This state of extreme anxiety causes individuals with GAD to experience various physical, emotional, and cognitive problems.^{3,4} In addition, non-specific symptoms are seen in daily life, such as restlessness, rapid tiring, concentration difficulties, irritability, muscle tension, or sleep disorders. Although it has been stated that many social, psychological, and biological factors play a role in the etiology, no factor has been identified as a definitive cause.³

Exposure to an extreme state of anxiety and chronic stress can affect the neurochemical transmission and activation of many cells in the brain such as microglial.⁵ S100B is a protein of acidic structure that binds zinc and calcium. It has been stated to be mainly in astrocytes in the nervous system and in greater concentrations in other glial cells. The levels of S100B

in biological fluids are used as a reliable biomarker of active neuronal cell distress.⁶ S100B is known to have important roles in cell growth and differentiation, calcium homeostasis, enzyme activity, and intracellular signal transmission and communication.⁷ However, the effect of S100B on brain tissue may vary according to the concentration. S100B at low concentrations (nanomolar) shows a neurotrophic effect, while over-production of these molecules (micromolar concentration) ultimately shows a neurotoxic effect. There are studies in the literature confirming the role of S100B in several nervous system diseases,⁶ and there is also a study which has associated high S100B levels with mood disorder.⁸

The diagnostic process of psychiatric diseases is based on clinical evaluations rather than biochemical tests. Therefore, evaluation of the systemic effects of the disease with biochemical tests would be valuable in terms of being able to predict the biological effects of GAD. Oxidative stress is a much-discussed factor in the etiology of diseases. It has been reported to play a role in the biological mechanisms underlying many psychiatric diseases, such as anxiety disorders, schizophrenia, and autism spectrum disorders.⁹ Chronic exposure to oxidative stress can cause structural and functional disorders in biomolecules through the accumulation of reactive oxygen species (ROS).¹⁰

One of the most well-known harmful effects of oxidative stress is DNA damage. 8-Hydroxy-2'-deoxyguanosine (8-OHdG) is one of the most studied biological markers of nucleic acid oxidation caused by ROS.¹¹ To be able to better understand the pathogenesis of GAD, it is important to investigate biological factors for the prediction of disease prognosis and to be able to better determine preventative strategies. S100B is a neurotrophic factor secreted in response to stress that modulates the proliferation and differentiation of neurons and glial cells. It is a neuronal survival protein that is always upregulated in acute stress situations.^{6,7} It's important to note that this situation can significantly impact the balance between oxidants and antioxidants, ultimately resulting in oxidative stress. Being mindful of this potential outcome can help us proactively mitigate and manage any adverse effects. Therefore, this study aimed to measure the serum S100B, and 8-OHdG levels, and the TOS, TAS, and OSI values of patients newly diagnosed with GAD who had not started treatment.

Materials and Methods

Study Population

This case-control study included 44 patients diagnosed with GAD and a control group of 44 age and gender-matched healthy subjects. The patient group comprised 44 GAD patients aged 18–65 years, who were drug-naïve and newly diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders-5 (DSM-5) criteria in the Harran University Psychiatry Clinic. The clinical diagnosis of GAD was made by psychiatrists. Data collection was carried out between October 2018 and April 2019. The study exclusion criteria for the patient group were defined as a psychiatric diagnosis other than GAD, taking any medication, or the presence of any neurological, genetic and/or other medical disease. The exclusion criteria for the control group were the presence of any neurological, genetic and/or other medical disorder, or having taken any medication in the last week. None of the patients or control group subjects had a history of tobacco or alcohol consumption.

The Beck Anxiety Inventory (BAI) was administered to the patient group. This scale is a widely used 21-item self-report scale used to assess anxiety levels. The Turkish validity and reliability study of the scale was conducted by Ulusoy et al in 1993.¹²

Approval for the study was granted by the Harran University Local Ethics Committee (decision no:18/10/08 date: 04.10.2018) and all procedures were in accordance with the Declaration of Helsinki and local laws and regulations. Written informed consent was obtained from all the study participants.

Laboratory Analysis

Blood samples (10 mL) were obtained from the antecubital vein of each study participant after overnight fasting. The samples were immediately centrifuged at 1600 g for 5 min to separate the serum. Then, the serum samples were stored at –80 °C until analysis. A commercial enzyme-linked immunosorbent assay (ELISA) kit was used to determine the level of 8-OHdG (E10140h, Cusabio, USA) and S-100B (E08065h, Cusabio, USA) in the serum samples according to the manufacturer's instructions. The TAS and TOS levels of the samples were measured using Rel Assay brand commercial kits (Rel Assay® Kit Diagnostics, Turkey) with the spectrophotometric method. The oxidative stress index (OSI) level was then calculated. OSI is an indicator of the level of oxidative stress and was calculated as the ratio of TOS to TAS.

Statistical Analysis

Data obtained in the study were analyzed statistically using SPSS for Windows 23.0 software. The conformity of the continuous variables and scale points to normal distribution was examined with the Shapiro–Wilk test. To examine the relationships between the groups, the Chi-square test was applied to categorical variables, and the Student's *t*-test was used for numerical variables with normal distribution and the Mann–Whitney *U*-test for variables not showing normal distribution. A value of $p < 0.05$ was accepted as the level of statistical significance.

Results

Equality between the groups in terms of gender was ensured, and so the age and gender distributions of the patients and control subjects were statistically similar. The demographic and clinical features of the patients and control subjects are shown in Table 1. The results of the BAI showed that the severity of anxiety was mild in 3 (6.8%) patients, moderate in 20 (45.4%), and severe in 21 (47.8%). There was no significant difference between the GAD and control groups in respect of the TAS, TOS, and OSI values ($p > 0.05$ for all). S100B levels of patients diagnosed with GAD were higher than the control group. However, this increase was not statistically significant ($p > 0.05$). A significant difference between the groups was observed in the examination of the 8-OHdG levels ($p = 0.028$) (Table 1).

Discussion

GAD is an extremely commonly seen mental health problem that has a negative effect on quality of life.¹³ The aim of this study was to analyze the serum S100B, 8-OHdG levels and TOS, TAS, and OSI values of patients newly diagnosed with GAD who had not started treatment, to be able to better understand the underlying neurobiological basis of the etiology of GAD. The study results showed that the S100B and 8-OHdG levels of the patients diagnosed with GAD were higher than those of the control group. When the oxidative stress levels were compared between the groups, no relationship was determined. To the best of our knowledge, this is the first study to have evaluated the relationship of S100B with 8-OHdG as a marker of DNA damage, in patients with GAD.

S100B is a glial protein that plays a role in intracellular signal transmission and intercellular communication regulating cell growth and differentiation.¹⁴ It is a protein that shows both harmful and neurotrophic effects depending on the concentration in the brain tissue. Under traumatic brain conditions, an increase in S100B in micromolar concentrations is observed, and this has been associated with a poor outcome.¹⁵ The S100B level in bodily fluids such as blood, cerebral spinal fluid, and saliva is thought to be a reliable marker of active neural distress.⁶ Therefore, it is

Table 1 The Demographic, Clinical, and Laboratory Parameters of the Patients with GAD and the Control Group

Characteristics	GAD (n = 44)	Control (n = 44)	p-value
Gender (F/M) n (%)	26 (40.9%)/18 (59.1%)	26 (40.9%)/18 (59.1%)	1
Age (Years)*	33.70±10.31	31.25±9.12	0.241
BMI (Kg/m ²)*	24.97±4.48	25.40±4.25	0.643
Beck Anxiety Inventory			
Mild (8–15), n (%)	3 (6.8%)	-	
Moderate (16–25), n (%)	20 (45.4%)	-	
Severe (26–65), n (%)	21 (47.8%)	-	
TAS, mM Trolox Eq./L*	1.043 ± 0.209	1.057 ± 0.281	0.794
TOS, μM H ₂ O ₂ Eq./L**	6.61 (3.34–19.13)	6.51 (2.67–121.47)	0.372
OSI, AU**	7.005 (2.53–16.92)	7.17 (1.92–72.84)	0.359
S100B, pg/mL**	171.69 (99.36–593.65)	143.44 (82.19–591.07)	0.116
8-OHdG, ng/mL**	62.05 (26.52–133.39)	54.48 (33.64–118.42)	0.028

Notes: *Mean±standard deviation (SD), **:Median(min-max), Bold values indicate statistical significance at $p < 0.05$.

Abbreviations: BMI, Body mass index; TOS, Total oxidant status; TAS, Total antioxidant status; OSI, Oxidative stress index; 8-OHdG, 8-Hydroxy-2'-deoxyguanosine.

accepted as an important aid in monitoring the course of many diseases, such as neurodegenerative diseases, acute brain damage, and epilepsy.

There is evidence in the literature of a relationship between S100B and the pathogenesis of mood disorders. Studies have shown that serum S100B is higher in mood disorder episodes in major depressive disorder (MDD) than in bipolar disorder.^{8,16} In a study by Wiesmann et al, higher serum S100B levels were reported in schizophrenia patients compared to a healthy control group.¹⁷ Similarly, another study reported that schizophrenia patients with high serum S100B levels showed slow psychopathological improvement following treatment.¹⁸ In another study that examined the potential role of S100B in mood disorders in adolescents and young adults, decreased S100B levels were determined at baseline in all the patients compared to the healthy control subjects. It was stated that depressive and hypo/manic patients had lower S100B levels compared to the control group. No correlation was determined between S100B levels and the severity of depression or hypo/manic symptoms, and it was stated that S100B levels could be affected by patient age and gender, and whether or not there was a family history of psychiatric disorders.¹⁹

In a study investigating serum S100B levels in GAD patients, the serum S100B level was seen to be significantly down-regulated in GAD patients compared to the control group.²⁰ However, in the current study, the S100B levels were found to be higher in GAD patients than in the control group. It was reported in a previous study that this increase emerged as a response to acute environmental stimuli, and caused an increase in behavioral and neural plasticity. However, it has also been reported that chronic mild stress decreases S100B in hippocampal and cerebral spinal fluid.²¹ While S100B can be evaluated as a protective factor in the acute period of anxiety, the effect may decrease in the chronic period.²⁰ It has been concluded that serum S100B levels can show variability depending on disease duration and the level of exposure to chronic stress.

In addition to psychological and genetic factors in GAD pathogenesis, neurochemical factors are also believed to play a role. In recent years, the role of oxidative stress has been examined as a neurochemical cause of various anxiety disorders. There is evidence in the literature that activated oxidative stress pathways could contribute to MDD, bipolar disorder, and anxiety disorders.²²

In a study that examined the relationship of oxidative stress with disease pathophysiology in children and adolescents diagnosed with anxiety disorder, the TOS and OSI values were found to be significantly higher in the patient group than in the control group, but no significant change was observed in the TAS value.²³ Emhan et al found higher TOS and OSI levels and lower TAS levels in GAD patients.²⁴ However, in the current study, no statistically significant difference was determined between the patient and control groups in respect of the TAS, TOS, and OSI values.

A significant increase was found in the 8-OHdG levels, which are accepted as a sign of DNA damage, in the patient group. It has been suggested in previous studies that DNA damage plays a role in the pathophysiology of schizophrenia and depression and that it is increased in patients with these disorders.^{25,26}

Bulut et al reported that there was an increase in the level of lipid hydroperoxide, which is a biomarker showing lipid peroxidation, in patients with GAD.²⁷ However, it has also been reported that antioxidant enzymes and activity are low in patients with GAD. In this context, it has been determined that patients with GAD have low levels of serum free sulfhydryl and paraoxonase 1.^{27,28} In contrast to previous findings, a large-scale study reported that there were no findings of increased oxidative stress in MDD and anxiety disorders.²⁹ This study has some limitations. Its limitations are the small number of patients and the fact that it is a single-center study.

Conclusion

To the best of our knowledge, this study is the first to have examined the serum S100B level and DNA damage together in patients diagnosed with GAD. The results obtained demonstrated that GAD caused an increase in serum S100B and 8-OHdG levels. It is thought that these biochemical factors could contribute to the etiopathogenesis of GAD. When evaluating the results of this study, it must be taken into consideration that the patients included were those diagnosed with GAD alone and no co-diagnosis, they were not using any psychotropic drugs and/or drugs that could affect the parameters, and that the study participants were medically healthy individuals. There is a need for further studies of larger populations to better understand the effects of S100B on the clinical condition of GAD.

Data Sharing Statement

The data presented in this study may be requested from the corresponding author on reasonable grounds.

Consent

Informed consent was obtained from all individual participants included in the study.

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Disclosure

The authors have no potential conflicts of interest to disclose.

References

- DeMartini J, Patel G, Fancher TL. Generalized anxiety disorder. *Ann Intern Med.* 2019;170(7):ITC49–ITC64. doi:10.7326/AITC201904020
- American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders. 5th Leid.* American Psychiatric Publishing; 2013.
- Daitch C. Cognitive Behavioral Therapy, Mindfulness, and Hypnosis as Treatment Methods for Generalized Anxiety Disorder. *Am J Clin Hypn.* 2018;61(1):57–69. doi:10.1080/00029157.2018.1458594
- Özdemir N, Şahin ŞK, Elboğa G, Altındağ A. Yaygın Anksiyete Bozukluğunda Bilişsel Çarpıtmaların Tedaviye Uyuma Etkisi. *Alpha Psychiatry.* 2020;21(4):380–386.
- Stein DJ, Vasconcelos MF, Albrechet-Souza L, Ceresér KMM, De Almeida RMM. Microglial over-activation by social defeat stress contributes to anxiety-and depressive-like behaviors. *Front Behav Neurosci.* 2017;11:207. doi:10.3389/fnbeh.2017.00207
- Michetti F, D'Ambrosi N, Toesca A, et al. The S100B story: from biomarker to active factor in neural injury. *J Neurochem.* 2019;148(2):168–187. doi:10.1111/jnc.14574
- Donato R, Cannon BR, Sorci G, et al. Functions of S100 Proteins. *Curr Mol Med.* 2013;13(1):24–57. doi:10.2174/156652413804486214
- Schroeter M, Sacher J, Steiner J, Schoenknecht P, Mueller K. Serum S100B Represents a New Biomarker for Mood Disorders. *Curr Drug Targets.* 2013;14(11):1237–1248. doi:10.2174/13894501113149990014
- Salim S. Oxidative stress and the central nervous system. *J Pharmacol Exp Ther.* 2017;360(1):201–205. doi:10.1124/jpet.116.237503
- Kruk J, Aboul-Enein HY, Kladna A, Bowser JE. Oxidative stress in biological systems and its relation with pathophysiological functions: the effect of physical activity on cellular redox homeostasis. *Free Radic Res.* 2019;53(5):497–521. doi:10.1080/10715762.2019.1612059
- Graille M, Wild P, Sauvain JJ, Hemmendinger M, Canu IG, Hopf NB. Urinary 8-OHdG as a biomarker for oxidative stress: a systematic literature review and meta-analysis. *Int J Mol Sci.* 2020;21(11):3743. doi:10.3390/ijms21113743
- Ulusoy M, Şahin N, Erkmen H. Turkish version of Beck Anxiety Inventory: psychometric properties. *J Cogn Psychother.* 1996;1(12):163–172.
- Gündüz A, Gündoğmuş İ, Sertçelik S, et al. Validity and reliability of the Turkish version of generalized anxiety disorder scale-revised. *Psychiatry Invest.* 2021;18(10):949–957. doi:10.30773/pi.2021.0174
- Yardan T, Erenler AK, Baydin A, Aydın K, Cokluk C. Usefulness of S100B protein in neurological disorders. *J Pak Med Assoc.* 2011;61(3):276–281.
- Arolt V, Peters M, Erfurth A, et al. S100B and response to treatment in major depression: a pilot study. *Eur Neuropsychopharmacol.* 2003;13(4):235–239. doi:10.1016/S0924-977X(03)00016-6
- Schroeter ML, Steiner J. Elevated serum levels of the glial marker protein S100B are not specific for schizophrenia or mood disorders. *Mol Psychiatry.* 2009;14(3):235–237. doi:10.1038/mp.2008.85
- Wiesmann M, Wandinger KP, Missler U, et al. Elevated plasma levels of S-100b protein in schizophrenic patients. *Biol Psychiatry.* 1999;45(11):1508–1511. doi:10.1016/S0006-3223(98)00217-0
- Rothermundt M, Missler U, Arolt V, et al. Increased S100B blood levels in unmedicated and treated schizophrenic patients are correlated with negative symptomatology. *Mol Psychiatry.* 2001;6(4):445–449. doi:10.1038/sj.mp.4000889
- Rajewska-Rager A, Dmitrak-Weglarz M, Kapelski P, et al. Longitudinal assessment of S100B serum levels and clinical factors in youth patients with mood disorders. *Sci Rep.* 2021;11(1):1–9. doi:10.1038/s41598-021-91577-6
- Shen Z, Cui L, Mou S, et al. Combining S100B and Cytokines as Neuro-Inflammatory Biomarkers for Diagnosing Generalized Anxiety Disorder: a Proof-of-Concept Study Based on Machine Learning. *Front Psychiatry.* 2022;13:881241. doi:10.3389/fpsy.2022.881241
- Buschert J, Hohoff C, Touma C, et al. S100B overexpression increases behavioral and neural plasticity in response to the social environment during adolescence. *J Psychiatr Res.* 2013;47(11):1791–1799. doi:10.1016/j.jpsychires.2013.08.001
- Maes M, Bonifacio KL, Morelli NR, et al. Generalized Anxiety Disorder (GAD) and Comorbid Major Depression with GAD Are Characterized by Enhanced Nitro-oxidative Stress, Increased Lipid Peroxidation, and Lowered Lipid-Associated Antioxidant Defenses. *Neurotox Res.* 2018;34(3):489–510. doi:10.1007/s12640-018-9906-2
- Guney E, Fatih Ceylan M, Tektas A, et al. Oxidative stress in children and adolescents with anxiety disorders. *J Affect Disord.* 2014;156:62–66. doi:10.1016/j.jad.2013.11.016
- Emhan A, Selek S, Bayazit H, Karababa IF, Kati M, Aksoy N. Evaluation of oxidative and antioxidative parameters in generalized anxiety disorder. *Psychiatry Res.* 2015;230(3):806–810. doi:10.1016/j.psychres.2015.11.001
- Nishioka N, Arnold SE. Evidence for Oxidative DNA Damage in the Hippocampus of Elderly Patients with Chronic Schizophrenia. *Am J Geriatr Psychiatry.* 2004;12(2):167–175. doi:10.1097/00019442-200403000-00008

26. Forlenza MJ, Miller GE. Increased Serum Levels of 8-Hydroxy-2'-Deoxyguanosine in Clinical Depression. *Psychosom Med*. 2006;68(1):1–7. doi:10.1097/01.psy.0000195780.37277.2a
27. Bulut M, Selek S, Bez Y, et al. Reduced PON1 enzymatic activity and increased lipid hydroperoxide levels that point out oxidative stress in generalized anxiety disorder. *J Affect Disord*. 2013;150(3):829–833. doi:10.1016/j.jad.2013.03.011
28. Kaya MC, Bez Y, Karababa IF, et al. Decreased serum sulphhydryl levels as a sign of increased oxidative stress in generalized anxiety disorder. *Psychiatry Invest*. 2013;10(3):281–285. doi:10.4306/pi.2013.10.3.281
29. Black CN, Bot M, Scheffer PG, Penninx BWJH. Oxidative stress in major depressive and anxiety disorders, and the association with antidepressant use; results from a large adult cohort. *Psychol Med*. 2017;47(5):936–948. doi:10.1017/S0033291716002828

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