

## Decreased Serum Levels of Nrf2 and HO-1 in Drug-Naive Patients with Generalized Anxiety Disorder

Yaygın Anksiyete Bozukluğu Olan İlaç Kullanmayan Hastalarda Serum Nrf2 ve HO-1 Düzeylerinin Azalması

Öznur Akil<sup>1</sup>, Mehmet Asoğlu<sup>2</sup>, Adnan Kirit<sup>3</sup>

<sup>1</sup>Başakşehir Çam and Sakura City Hospital, Clinic of Psychiatry, İstanbul, TÜRKİYE

<sup>2</sup>Harran University Faculty of Medicine, Department of Psychiatry, Şanlıurfa, TÜRKİYE

<sup>3</sup>İskenderun State Hospital, Clinic of Medical Biochemistry, Hatay, TÜRKİYE

### Abstract

**Background:** Although many causes such as psychological, social, genetic and biological factors are blamed in generalized anxiety disorder (GAD), its etiopathogenesis is not fully understood. The aim of this study was to examine the serum levels of Nuclear factor-E2-related factor 2 (Nrf2), heme oxygenase 1 (HO-1), and tau proteins in adults diagnosed with generalized anxiety disorder (GAD), and to explore the relationship between these biomarkers and anxiety severity.

**Materials and Methods:** The research involved 44 patients with GAD who had not previously received any medication, along with 44 healthy individuals matched by age and gender. Anxiety severity in the GAD group was assessed using the Beck Anxiety Inventory (BAI). Serum concentrations of Nrf2, HO-1, and tau were measured in a biochemistry laboratory using the enzyme-linked immunosorbent assay (ELISA) method.

**Results:** In individuals diagnosed with GAD, serum levels of Nrf2 and HO-1 were significantly lower than those observed in the control group ( $p=0.023$ ;  $p<0.001$ , respectively). However, no significant difference was detected between the patient and control groups regarding serum tau concentrations ( $p=0.98$ ). According to Spearman correlation analysis, there were no significant correlation between total BAI scores and the levels of Nrf2, HO-1, or tau in the patient group.

**Conclusions:** These findings suggest that the antioxidant defense system is compromised in patients with GAD. Further research assessing Nrf2 and HO-1 is required to clarify their exact contribution to the pathophysiology of GAD.

**Keywords:** Generalized anxiety disorder, Oxidative stress, Nrf2, HO-1, Tau

### Öz

**Amaç:** Yaygın anksiyete bozukluğunda (YAB) psikolojik, sosyal, genetik ve biyolojik faktörler gibi birçok neden suçlansa da etiopatogenezi tam olarak anlaşılamamıştır.

Bu çalışmanın amacı, yaygın anksiyete bozukluğu (YAB) tanısı almış yetişkinlerde serum Nükleer Faktör Eritroid 2 ile ilişkili Faktör 2 (NRF2), Heme oksijenaz 1 (HO-1) ve tau protein düzeylerini incelemek ve bu biyobelirteçlerin anksiyete belirtilerinin şiddetiyle ilişkisinin nasıl olduğunu araştırmaktır.

**Materyal ve metod:** Araştırmaya daha önce herhangi bir ilaç kullanmamış 44 YAB hastası ve yaş ve cinsiyete göre eşleştirilmiş 44 sağlıklı birey dahil edilmiştir. YAB grubunda anksiyete şiddeti Beck Anksiyete Envanteri (BAI) kullanılarak değerlendirilmiştir. Serum Nrf2, HO-1 ve tau konsantrasyonları bir biyokimya laboratuvarında ELISA yöntemi kullanılarak ölçülmüştür.

**Bulgular:** YAB tanısı almış bireylerde serum Nrf2 ve HO-1 düzeyleri, kontrol grubunda gözlenenlerden anlamlı derecede düşüktü (sırasıyla  $p=0,023$ ;  $p<0,001$ ). Ancak, hasta ve kontrol grupları arasında serum tau konsantrasyonları açısından anlamlı bir fark tespit edilmedi ( $p=0,980$ ). Spearman korelasyon analizine göre, hasta grubunda toplam BAI puanları ile Nrf2, HO-1 veya tau düzeyleri arasında anlamlı bir ilişki bulunmadı.

**Sonuç:** Bu bulgular, YAB hastalarında antioksidan savunma sisteminin zayıfladığını göstermektedir. YAB patofizyolojisine tam katkılarını netleştirmek için Nrf2 ve HO-1'i değerlendiren daha fazla araştırmaya ihtiyaç vardır.

**Anahtar Kelimeler:** Yaygın anksiyete bozukluğu, Oksidatif stres, Nrf2, HO-1, Tau

**Corresponding Author:** Öznur Akil, Başakşehir Çam and Sakura City Hospital, Clinic of Psychiatry, İstanbul, TÜRKİYE

**E-mail:** droznurakil@gmail.com / **ORCID ID:** 0000-0002-6103-0823

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## Introduction

Generalized anxiety disorder (GAD) affects 0.8%-6.4% of the global population and is characterized by persistent anxiety, irritability, and physical symptoms such as muscle tension and sleep disturbances, often leading to a chronic decline in quality of life (1,2). Although its multifactorial etiopathogenesis remains not fully understood, growing research highlights the role of disrupted oxidative metabolism in psychiatric conditions such as depression, attention-deficit/hyperactivity disorder (ADHD), and schizophrenia (3-5). While oxidative stress is increasingly thought to influence the neurobiological mechanisms of anxiety disorders, empirical evidence in this specific area remains scarce (3,6,7).

Nuclear factor erythroid 2-related factor 2 (Nrf2) is recognized as the main transcription factor involved in redox sensing, plays a critical role in regulating oxidative stress. (8). Microarray analyses have identified numerous genes regulated by Nrf2. Among these, the glutamate-cysteine ligase catalytic subunit (GCLC), NAD(P)H quinone oxidoreductase 1 (NQO1), and heme oxygenase-1 (HO-1) are directly regulated by Nrf2 (9). It has been reported that Nrf2 plays a crucial role in protecting the brain from oxidative stress. Studies have demonstrated that elevated Nrf2 expression in astrocytes can protect neurons from oxidative damage (8). Recent evidence suggests that the Nrf2 pathway does not only function as an antioxidant switch but also acts as a key regulator of neuroinflammation in anxiety disorders. Systematic reviews have highlighted that the impairment of Nrf2-mediated signaling is closely associated with the increased levels of pro-inflammatory cytokines, which are thought to be part of the core pathophysiology of GAD (10). Additionally, HO-1 activity has been identified as a key component of the cellular defense mechanism against oxidative stress. Conversely, suppression of this gene has been linked to apoptotic cell death, contributing to oxidant-induced cytotoxicity (11). The involvement of HO-1 in GAD may reflect a complex compensatory mechanism. While HO-1 serves a protective role against oxidative injury, its dysregulation has been linked to ferroptosis-a form of iron-dependent cell death-which is increasingly recognized as a factor in the neurobiology of emotional disorders (12).

Tau protein is mainly expressed in the central and peripheral nervous systems. It plays an important role in stabilising axonal microtubules in central neurons and maintaining axonal transport (13-16). Studies suggest that serum tau protein levels are a reliable biomarker candidate in many neurological diseases, especially Alzheimer's disease (17,18). Beyond its well-known role in neurodegeneration, tau protein has recently been implicated in stress-induced synaptic plasticity deficits.

Contemporary studies have indicated that chronic stress can trigger tau hyperphosphorylation, potentially contributing to the persistence of anxiety-like behaviors and cognitive rigidity seen in GAD patients (19). Recent findings in 2024 suggest that Tau protein alterations are not merely passive markers of damage but are actively involved in the remodeling of amygdala circuits under chronic stress. Increased levels of phosphorylated Tau (P-tau) have been linked to the disruption of synaptic plasticity, which may explain the persistence of hyperarousal and anxiety symptoms in patients with GAD (20). A 2023 study focusing on peripheral biomarkers has demonstrated that elevated serum total tau levels correlate with the clinical severity of anxiety and depressive symptoms (21). These findings support the hypothesis that subclinical axonal injury or transport dysfunction, reflected by serum tau fluctuations, may be a contributing factor to the pathophysiology of chronic anxiety disorders.

The inconclusive nature of these findings prompted us to explore the possible association between anxiety disorders and these biomarkers. Given the high prevalence and adverse outcomes of GAD, elucidating its underlying mechanisms is crucial. The Nrf2/HO-1 pathway serves as a master regulator of the antioxidant response, whereas Tau protein is essential for maintaining axonal transport and structural integrity in neurons. Recent evidence suggests a critical intersection between these systems; impaired Nrf2 signaling and the resulting oxidative stress can trigger tau hyperphosphorylation, leading to synaptic plasticity deficits that may underlie the persistence of anxiety-like behaviors (19,20). Furthermore, elevated serum Tau levels have been linked to clinical severity in emotional disorders, potentially reflecting subclinical neuroaxonal injury (21). Therefore, evaluating these biomarkers simultaneously in the same patient cohort provides a comprehensive perspective on whether antioxidant defense failure (Nrf2/HO-1) is associated with subclinical neuroaxonal changes (Tau) in the pathophysiology of GAD. Therefore, the present study aimed to determine whether serum Nrf2, HO-1, and tau levels differ between patients with GAD and healthy controls, as well as to assess their relationship with GAD severity. To the best of our knowledge, this is the first investigation to examine serum Nrf2, HO-1, and tau levels in individuals with GAD.

## Materials and Methods

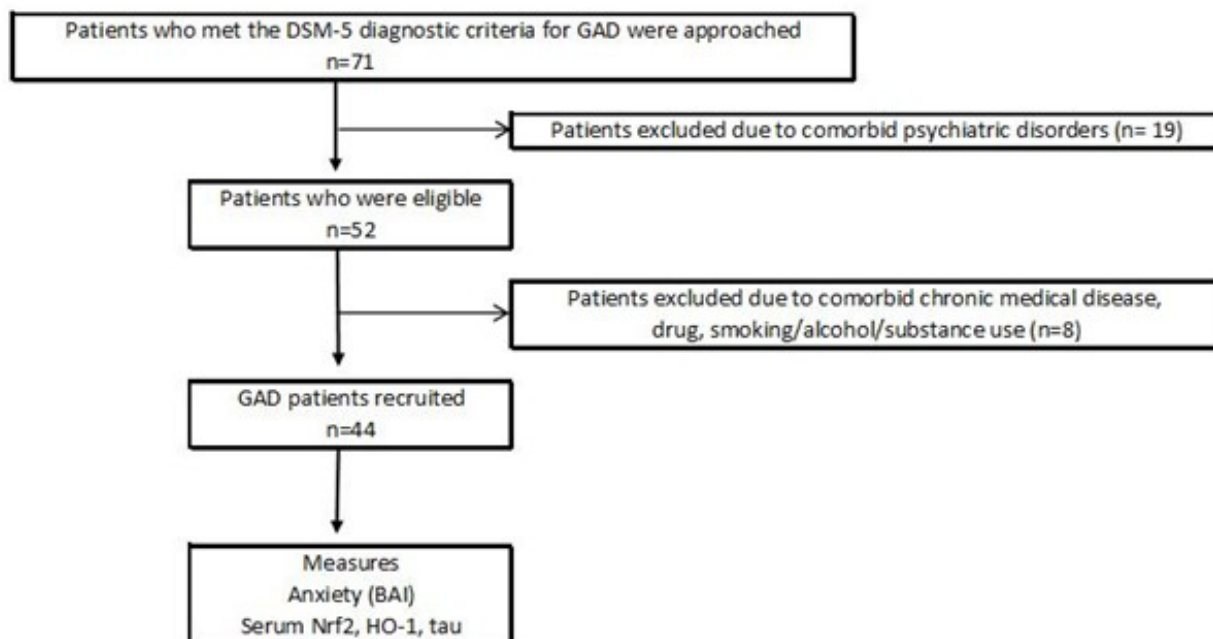
### Subjects

This research was conducted as a cross-sectional case-control study. It included 44 individuals (26 females and 18 males, aged

18-55 years) who were diagnosed with generalized anxiety disorder (GAD) according to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) criteria and attended the Psychiatry Outpatient Clinic of Harran University Faculty of Medicine Hospital between October 2018 and April 2019. The inclusion criteria for the patient group were the absence of known chronic medical disease, medication, smoking, alcohol use, or substance use. Patients with additional psychiatric comorbidities, known chronic medical disease, pregnancy, history of severe head injury, medication, vitamin supplementation use, and insufficient blood samples were excluded from the study. The healthy control group of this study consisted of 44 patients (27 female, 17 male; 18-55 years of age)

who were selected from among the relatives of the patients and the staff working in the hospital, who did not have psychiatric or organic diseases, did not use drugs, did not use cigarettes/alcohol/substances, and were matched with the patient group and variables such as age, gender, body mass index (BMI) (Figure 1). BMI groups were defined based on the World Health Organization (WHO) criteria: underweight ( $<18.5$  kg/m<sup>2</sup>), normal (18.5-24.9 kg/m<sup>2</sup>), overweight (25.0-29.9 kg/m<sup>2</sup>), and obese ( $\geq 30$  kg/m<sup>2</sup>).

Ethical approval for this study was obtained from the by Harran University Faculty of Medicine Clinical Research Ethics Committee (approval no: 10, date: October 04, 2018) and written informed consent was obtained from all participants.



**Figure 1.** Recruit flow chart

### Measures

The socio-demographic questionnaire created by the researchers consists of questions such as age, gender, marital status, educational status, occupation, duration of illness, family history of psychiatric disorders.

Beck Anxiety Inventory (BAI) is self-reporting questionnaire constructed to measure somatic symptoms of anxiety (22). This inventory consists of 21 items and is scored between 0-3. In BAI,

it is questioned how much the feeling of distress has disturbed the patient in the last week with the questions asked. The score range obtained in the scale is 0-63 (23). The clinical severity of anxiety was evaluated according to the BAI total score. In the BAI, total scores of 0-9 reflect minimal levels of anxiety; scores of 10-18 indicate mild anxiety; scores of 19-29 represent moderate anxiety; and scores of 30-63 correspond to severe anxiety.

### Blood Samples

A total of 5 mL of venous blood was collected from the antecubital vein of each participant in both the patient and control groups following a fasting period of at least eight hours. The collected samples were transferred into gel-containing biochemistry tubes and promptly centrifuged at 4000 rpm for 10 minutes at 4°C to obtain the serum samples. The separated serum was divided into two equal aliquots, placed in Eppendorf tubes, and stored at -80°C until further analysis. Serum levels of Nrf2, HO-1, and tau were determined in the biochemistry laboratory using the Enzyme-Linked Immunosorbent Assay (ELISA) technique.

### Human Nrf2 (NFE2L2), Human HO-1, and Human Tau Measurements

Human Nrf2 (NFE2L2) concentrations were determined using an ELISA kit (FineTest, catalog no: CSB-EL015752HU), and the results were reported in ng/mL. Human HO-1 levels were analyzed with an ELISA kit (Fine Test, Catalog No: CSB-E08266h) and expressed as ng/mL. Similarly, human tau levels were assessed using a Fine Test ELISA kit (Catalog No: CSB-E12011h) and presented in pg/mL.

### Statistical Analysis

Statistical analyses were performed using SPSS software, Version 23.0 (SPSS Inc., Chicago, IL, USA). The Pearson chi-square test was used to compare categorical variables. The Shapiro-Wilk test was used to evaluate the normality of the data distribution. As the data did not exhibit normal distribution, non-parametric

statistical methods were used instead. The Mann-Whitney U test was used to compare two independent groups, and the strength and direction of associations between variables was determined using Spearman's correlation coefficient. A p-value below 0.05 was considered statistically significant.

## Results

### Sociodemographic Characteristics of GAD and Control Groups

Forty-four patients with GAD (59.1% female) and 44 healthy controls matched for age and gender (61.4% female) were included in the study. The median age of the GAD group was 32.5 (25.0 - 41.0) years, while the median age of the control group was 30.5 (24.0 - 38.0) years. There were no statistically significant differences between the groups regarding age or sex ( $p = 0.392$  and  $p = 0.827$ ). BMI distribution was also similar between the groups ( $p=0.580$ ). Considering the marital status, 37 (84.1%) of the patients were married, 7 (15.9%) were single, 27 (61.4%) of the control group were married and 17 (38.6%) were single. 7 (15.9%) of the patients had no education, 22 (50%) of them were primary school graduates, 5 (11.4%) of them were secondary school graduates, 4 (9.1%) of them were high school graduates, 6 (13.6%) of them were college or university graduates. While primary school graduates were more in the patient group (50%), high school graduates were more in the control group (45.5%). Detailed sociodemographic characteristics of the participants are illustrated in Table 1.

**Table 1.** Demographics characteristics of patients and healthy controls

| Characteristic                  |            | GAD group (n=44)   | Control group (n=44) | P     |
|---------------------------------|------------|--------------------|----------------------|-------|
| Age (years), Median (Q1-Q3)     |            | 32.5 (25.0 - 41.0) | 30.5 (24.0 - 38.0)   | 0.392 |
| Sex                             | Female     | 26 (59.1)          | 27 (61.4)            | 0.827 |
|                                 | Male       | 18 (40.9)          | 17 (38.6)            |       |
| BMI (kg/m <sup>2</sup> ), n (%) | Weak       | 0 (0)              | 1 (2.3)              | 0.580 |
|                                 | Normal     | 24 (54.5)          | 19 (43.2)            |       |
|                                 | Overweight | 15 (34.1)          | 18 (40.9)            |       |
|                                 | Obese      | 5 (11.4)           | 6 (13.6)             |       |

GAD: Generalized anxiety disorder, BMI: Body mass index

Continuous variables (Age) are presented as Median (25<sup>th</sup>-75<sup>th</sup> percentiles) and compared using the Mann-Whitney U test. Categorical variables (Sex, BMI) are presented as n (%) and compared using the Chi-square test. Statistical significance was defined as  $p<0.05$

### Clinical Features of the Group with GAD

Patients were evaluated based on previous treatment history, disease duration, and anxiety severity. It was observed that 28 (63.6%) patients had no previous treatment history. According to the Beck Anxiety Inventory, anxiety severity was mild in 3 (6.8%) patients, moderate in 20 (45.4%) patients, and severe in 21 (47.8%) patients. Regarding the duration of illness, symptoms had been present for 6-12 months in 13 (29.5%) patients, and for 1 year or more in 31 (70.5%) patients (Table 2).

**Table 2.** A summary of clinical results of the GAD patients

| Clinical parameter                                                                                                              | n  | %    |
|---------------------------------------------------------------------------------------------------------------------------------|----|------|
| <b>Previous treatment history</b>                                                                                               |    |      |
| Yes                                                                                                                             | 16 | 36.4 |
| No                                                                                                                              | 28 | 63.6 |
| <b>Disease duration</b>                                                                                                         |    |      |
| 6-12 months                                                                                                                     | 13 | 29.5 |
| 1 year and over                                                                                                                 | 31 | 70.5 |
| <b>Anxiety severity</b>                                                                                                         |    |      |
| Mild                                                                                                                            | 3  | 6.8  |
| Moderate                                                                                                                        | 20 | 45.4 |
| Severe                                                                                                                          | 21 | 47.8 |
| Note: Data are presented as descriptive statistics using frequencies (n) and percentages (%), GAD: Generalized anxiety disorder |    |      |

Serum Nrf2 levels were compared between the GAD and control groups, revealing a significant difference. The patient group had significantly lower serum Nrf2 levels than the control group ( $p=0.023$ ). Similarly, when serum HO-1 levels were analyzed, a statistically significant difference was also observed between the two groups, with the GAD group showing markedly lower HO-1 levels ( $p<0.001$ ).

The GAD group showed lower serum tau levels, but this difference was not statistically significant when compared to the control group ( $p>0.05$ ) (Table 3).

**Table 3.** Laboratory results of patients and healthy controls

| Parameter                                                                                                                                                                                                                                                                                                                               | GAD group (n=44) Median (Q1-Q3) | Control group (n=44) Median (Q1-Q3) | p-value | z-value |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------|-------------------------------------|---------|---------|
| Nrf2 (ng/ml)                                                                                                                                                                                                                                                                                                                            | 15.4 (12.2-19.8)                | 148.5 (131.0-165.0)                 | 0.023   | -2.27   |
| HO-1 (ng/ml)                                                                                                                                                                                                                                                                                                                            | 8.6 (6.1-11.4)                  | 52.3 (45.0-62.0)                    | <0.001  | -4.06   |
| Tau (pg/ml)                                                                                                                                                                                                                                                                                                                             | 76.0 (52.0-98.0)                | 74.5 (55.0-95.0)                    | 0.980   | -0.03   |
| Note: Since the laboratory parameters and anxiety scores did not meet the assumptions of normality, they are presented as Median and Interquartile Range (IQR; 25 <sup>th</sup> -75 <sup>th</sup> percentiles). Intergroup comparisons were performed using the Mann-Whitney U test. Statistical significance was defined as $p<0.05$ . |                                 |                                     |         |         |

No significant correlations were observed between GAD severity, as measured by BAI scores, and serum levels of Nrf2, HO-1, or tau ( $r=0.09$ ,  $p=0.56$ ;  $r=-0.04$ ,  $p=0.78$ ;  $r=-0.05$ ,  $p=0.77$ , respectively). Furthermore, there was no statistically significant relationship observed between how long the disease had lasted and the levels of Nrf2, HO-1 or tau in the serum ( $p>0.05$ ).

### Discussion

The aim of the present study was to examine serum levels of Nrf2, HO-1, and tau in adults diagnosed with generalized anxiety disorder (GAD) and to explore potential associations between these biomarkers and the severity of the condition. According to the available literature, no previous studies have specifically assessed serum Nrf2, HO-1, and tau concentrations in individuals with GAD. Our findings demonstrated that serum Nrf2 and HO-1 levels were reduced in adults with GAD. However, there was no statistically significant difference in serum tau levels between the GAD and healthy control groups. Furthermore, no significant correlations were identified between the severity of GAD symptoms and these biochemical parameters.

Although existing research on this topic remains limited and somewhat inconsistent, alterations in oxidative stress parameters may be implicated in GAD. For instance, Emhan et al. (24) and Ercan et al. (25) reported that individuals with GAD exhibited significantly higher total oxidant status and oxidative stress index compared to healthy controls. Moreover, Emhan et al. (24) observed reduced total antioxidant status in GAD patients, suggesting a potential role of oxidative stress in the disorder. Additionally, Fındıklı et al. (26) reported significantly elevated serum Malondialdehyde (MDA) levels and reduced SOD and CAT levels in GAD patients compared with controls, indicating increased oxidative processes and diminished antioxidant defenses (25).

Nrf2, recognized as a key endogenous antioxidant regulator within the brain, activates the transcription of antioxidant enzymes such as HO-1. Beyond its antioxidant role, Nrf2 also participates in modulating inflammatory responses, neurotransmission, and neuronal plasticity (26,27). Recent animal studies further suggest that activation of the Nrf2/HO-1 signaling pathway can restore cellular homeostasis and redox balance, thereby preventing oxidative stress-related disorders, including anxiety (28,29). Sağlam et al. (30) stated that anxiety levels can be reduced by regulating Nrf2 levels in their study with rodent models. In their study, Li et al. (28) demonstrated that activating the Nrf2/HO-1 pathway reduced anxiety-like behaviours in rodents.

In this current study, patients suffering from GAD had serum levels of Nrf2 and HO-1 that were significantly lower than those observed in the healthy control group. This result, consistent with previous information, shows that the antioxidant system is impaired in patients with GAD, regardless of disease duration and severity. Moreover, there may be a close relationship between the Nrf2/HO-1 pathway and GAD.

In our study, the most striking finding was the dramatic reduction in serum Nrf2 and HO-1 levels in patients with GAD compared to healthy controls ( $p=0.023$  and  $p<0.001$ , respectively). Theoretical frameworks identify the Nrf2/HO-1 pathway as the 'master rheostat' of cellular antioxidant response; while healthy individuals maintain high levels of these proteins to neutralize routine oxidative stress, our GAD cohort exhibited a profound depletion of this defensive shield. The significantly higher levels of Nrf2 and HO-1 in the healthy control group reflect a functional state of redox homeostasis that is evidently lost in GAD patients. This severe downregulation suggests that the pathophysiology of GAD is not merely characterized by increased oxidants, but by a fundamental failure of the brain's internal protective machinery. When compared to the robust antioxidant profile of healthy controls, the compromised Nrf2/HO-1 levels in patients indicate that GAD creates a state of 'biochemical vulnerability,' where the neural environment is left unprotected against oxidative insult. This theoretical contrast provides a strong argument that pharmacological or lifestyle interventions aimed at restoring Nrf2/HO-1 levels to those observed in our healthy control group could be a key strategy in preventing the progression of the disorder.

The brain is particularly susceptible to oxidative damage and has a relatively modest antioxidant defense (31). High oxidative stress and proinflammatory cytokines in the brain can deregulate neuronal plasticity and neurogenesis, which can ultimately result in psychiatric disorders (32). The tau protein plays a role in regulating the assembly and disassembly of axonal microtubules. It has been stated that tau protein is associated with the severity of neurodegeneration and reflects neurofibrillary pathological changes (33). Tau protein has been considered as a potential marker of neuroaxonal damage in several studies (13-17).

The elevated prevalence of anxiety symptoms among individuals with Alzheimer's disease, relative to their cognitively healthy counterparts, has drawn increasing attention to the potential link between neurodegeneration and anxiety (34). Nevertheless, studies specifically investigating the association between tau protein and anxiety symptoms remain limited. In a rodent study, Li et al. (27) demonstrated that tau aggravates stress responses and anxiety-related behaviors by inhibiting the proliferation and

maturation of neurons within the ventral dentate gyrus of the hippocampus, suggesting that tau may represent a potential molecular target for the treatment of stress-induced anxiety-like behaviors (28). Similarly, another experimental study reported that tau hyperphosphorylation contributed to both cognitive deficits and anxiety symptoms (34). Conversely, several investigations have failed to identify a significant relationship between tau, anxiety, or neurodegeneration in cognitively intact individuals (35). In the present study, no statistically significant difference in serum tau levels was observed between the groups, a finding consistent with certain prior reports. To the best of our knowledge, this is the first study to assess serum tau protein levels in patients diagnosed with GAD. Further research is warranted to elucidate the potential relationship between GAD and tau protein.

The rationale for investigating Nrf2, HO-1, and Tau protein together lies in their interconnected roles within the neurobiological 'defense-damage' balance. While Nrf2 and HO-1 represent the primary cellular shield against oxidative insult, Tau protein acts as a sensitive indicator of stress-induced structural remodeling in brain regions such as the amygdala (20). Although our findings did not show a statistically significant difference in serum Tau levels between groups, the concurrent analysis of these markers remains crucial. It allows for the differentiation between early-stage antioxidant depletion and overt neuroaxonal injury, suggesting that in GAD, cellular defense mechanisms may be compromised before significant structural protein alterations occur (21). From a clinical and theoretical perspective, the preservation of serum Tau levels despite impaired antioxidant defenses may point to a 'therapeutic window.' This suggests that GAD, at this stage, may be primarily a functional disorder rather than a structural neurodegenerative condition, and early intervention targeting the Nrf2/HO-1 pathway could potentially prevent future axonal damage and cognitive decline associated with chronic anxiety. As the first study to examine this triad in GAD, this integrated approach is vital for elucidating whether oxidative imbalance precedes or parallels neuroaxonal dysfunction.

### Study Limitations

This study, however, has several limitations. Firstly, the relatively small sample size limits the generalizability of the findings; thus, replication in larger cohorts is recommended. Second, the analysis was limited to serum samples, as cerebrospinal fluid samples were not obtained. Another limitation pertains to the inability to measure phosphorylated tau using the ELISA kit employed in this study. Additionally, due to its cross-

sectional design, it was not possible to examine the effects of antidepressant treatment on oxidative stress in patients with GAD. Longitudinal and interventional studies are therefore required to clarify these effects. Nonetheless, the inclusion of GAD participants without comorbid psychiatric disorders and without exposure to psychotropic medications, antioxidant agents, tobacco, or alcohol constitutes a methodological strength, particularly considering the high rate of psychiatric comorbidity in this population.

## Conclusion

In conclusion, these findings may indicate that decreased Nrf2 and HO-1 serum levels in patients with GAD are insufficient in antioxidant defense. The causal relationship between anxiety disorders and oxidative imbalance is unclear. Future studies involving Nrf2 and HO-1 measurement are needed to elucidate the precise nature of anxiety disorders, namely their role in the pathophysiology of GAD.

**Ethical Approval:** Ethical approval for this study was obtained from the by Harran University Faculty of Medicine Clinical Research Ethics Committee (approval no: 10, date: October 04, 2018).

**Author Contributions:**

Concept: Ö.A., M.A., A.K.

Design: Ö.A., M.A., A.K.

Data Collection or Processing: Ö.A., M.A., A.K.

Analysis or Interpretation: Ö.A., M.A., A.K.

Literature Search: Ö.A., M.A., A.K.

Writing: E.U., E.S., O.T., A.A., O.T.

Critical Review: Ö.A., M.A., A.K.

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