Lipid peroxidation markers in children with anxiety disorders and their diagnostic implications

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Objective: Numerous factors, including genetic, neurobiological, neurochemical, and psychological factors, are thought to be involved in the development of anxiety disorders. The latest findings show that the pathophysiology of anxiety disorders might be associated with oxidative stress and lipid peroxidation; however, no studies have so far investigated lipid peroxidation markers in children with anxiety disorders. Serum levels of lipid hydroperoxide (LOOH) are a reliable marker of lipid peroxidation. Paraoxonase and arylesterase are two enzymes that protect against such peroxidation, and might also be diagnostic markers. In this study, we investigated whether there are associations between anxiety disorders and lipid peroxidation markers in children, and assessed the diagnostic performance of these markers.

Methods: The study group consisted of 37 patients (children and adolescents) with anxiety disorders. A control group, matched for age and gender, was composed of 36 healthy subjects. Venous blood samples were collected, and LOOH levels and paraoxonase and arylesterase activity were measured.

Results: LOOH levels were significantly higher in the anxiety disorders group than in the control group. There were no significant differences in paraoxonase or arylesterase activities between the patient and the control groups.

Discussion: Lipid peroxidation or oxidative damage might play a role in the aetiopathogenesis of anxiety disorders. LOOH may be a potential biological marker for anxiety disorders in children.

Keywords: Anxiety disorders, Children, Lipid peroxidation, Lipid hydroperoxide, Paraoxonase, Arylesterase, Biological marker

Introduction

Anxiety disorders are the most common psychiatric condition in children and adolescents, and they lead to the subsequent development of other psychiatric comorbidities in adulthood. The lifetime prevalence of anxiety disorders is estimated to reach approximately 30%.¹ Males have a higher prevalence rate of the disorder than females.²

Many children experience various types of fears during childhood, some of which are related to their normal development. These anxieties and fears are considered pathological when they become extreme

and persistent.³ Pathological anxiety can be identified as an anticipatory response to internal and external threats. Anxiety disorders at pathological levels can cause disruption to the daily life and social functioning of a person. In addition, anxiety disorders presenting as early onset conditions can be risk factors for the development of depressive and other mood disorders occurring later in life.^{4,5} Some features of anxiety disorders include cognitive (inattention and distractibility, thought blocks, fear of losing control), affective (worry, tension, irritability, despair), behavioural (escape, avoidance, behavioural inhibition), and physical (palpitation, shortness of breath, chest pain, abdominal pain, nausea, vomiting, sweating, chills) symptoms. There are many types of anxiety disorders,

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including separation anxiety disorder, generalized anxiety disorder (GAD), obsessive compulsive disorder (OCD), panic disorder (PD), post-traumatic stress disorder (PTSD), social anxiety disorder (SAD), and specific phobias.⁶

Anxiety disorders are complex conditions, with varied aetiologies. A number of factors, including genetic, neurobiological, neurochemical, psychological, and behavioural factors are thought to be involved in the development of anxiety disorders.⁷ There has been an increase in the number of biological studies of anxiety disorders over the past decade, whereas previously, the psychological, behavioural, and cognitive factors related to anxiety disorders had been the focus of the study. These biological studies have indicated that oxidative damage and lipid peroxidation have a pathophysiological role in anxiety disorders in adult patients, including in PD, OCD, SAD, and GAD.^{8–11} However, to the best of our knowledge, the possible relation between lipid peroxidation and anxiety disorders has not yet been studied in children and adolescents with anxiety disorders.

Lipid peroxidation is a well-known example of oxidative damage to cell membranes, lipoproteins, and other lipid-containing structures. Unsaturated phospholipids, glycolipids, and cholesterol can develop as a result of reactions triggered by free radicals (such as hydroxyl radical, singlet oxygen, and superoxide). Lipid hydroperoxide (LOOH) is the primary product of the oxidation of polyunsaturated fatty acids, and it can be used as a biomarker to detect and quantify early stage lipid peroxidation.¹² The enzymes, paraoxonase and arylesterase, circulate in the bloodstream, and are associated with high-density lipoprotein. These two enzymes protect lipids from peroxidation, and thus, they exhibit antioxidant features. In the brain, oxidative damage can change neurotransmission, neuronal function, and even brain activity,^{13,14} and this condition might predispose the individual to neurodegenerative and neuropsychiatric diseases. The relationships between paraoxonase and arylesterase enzyme activity, oxidative damage, and lipid peroxidation are shown in Fig. 1.¹¹ Lipid

peroxidation has been linked to a variety of neuropsychiatric disorders, including depression, schizophrenia, bipolar mood disorders, attention deficit hyperactivity disorder, and Alzheimer's disease.^{15–18}

In this study, we aimed to investigate whether there are any associations between anxiety disorders and the activity of paraoxonase and arylesterase (which is associated with antioxidative metabolism) or levels of LOOH, an important marker of lipid peroxidation. In addition, we analysed whether these biological markers could be used as diagnostic tools for anxiety disorders.

Methods

Patients and controls

The study included 37 patients with anxiety disorders and 36 healthy volunteer controls from the Child and Adolescent Psychiatry Clinic at the Ankara Pediatric Hematology Oncology Training and Research Hospital. All of the patients were screened for psychiatric disorders using the Schedule for Affective Disorders and Schizophrenia for School-Age Children, Present and Lifetime Version (K-SADS-PL), which is based on the *Diagnostic and Statistical Manual of Mental Disorders*, Fourth Edition (DSM-IV) criteria.¹⁹ We used the Turkish adaptation of this form, created by Gökler *et al.*²⁰ Patients with a comorbid psychiatric, neurological, or genetic disorder were excluded along with patients with a history of chronic systemic diseases, such as endocrinal and allergic diseases. Patients who had previously used psychotropic drugs were excluded as well. The control group consisted of healthy children who were matched for age and gender with the anxiety disorders group. Parents of the subjects were given a complete description of the study, and all provided written informed consent. The study was approved by the ethics committee of the Ankara Pediatric Hematology Oncology Training and Research Hospital.

Blood sampling

Venous blood samples were collected from an antecubital vein of each patient at 09.00 hours after a 12-hour

Figure 1 Effect of paraoxonase and arylesterase enzyme activity on lipid peroxidation.

overnight fast, and a 10 ml sample of venous blood was placed into a biochemistry tube. The biochemistry tubes were centrifuged at 2000 *g* for 15 minutes after an incubation period of 30 minutes. The remaining serum specimens were kept at -20°C until analyses were performed.

Measurement of LOOH levels

Serum LOOH levels were measured with a ferrous ion oxidation–xylenol orange (FOX-2) assay, which involves the oxidation of ferrous ion to ferric ion by various oxidants. The ferric ion is then measured with xylenol orange. The LOOH levels are reduced by the application of triphenyl phosphine (TPP), which is a specific reductant for lipids. LOOH levels can be estimated as the difference in values in the absence or presence of TPP.²¹

Measurement of paraoxonase and arylesterase activity

Paraoxonase and arylesterase activity was measured using paraoxon and phenylacetate substrates.

The rate of paraoxon hydrolysis (diethyl-*p*-nitrophenyl phosphate) was measured by monitoring the increase of absorbance at 412 nm at 37°C . The amount of generated *p*-nitrophenol was calculated from the molar absorptivity coefficient at pH 8, which was 17 000 mol/cm, and paraoxonase activity was expressed as U/l serum.

Phenylacetate was used as a substrate to measure arylesterase activity. Enzyme activity was calculated from the molar absorptivity coefficient of the produced phenol, 1310 mol/cm, with 1 U of arylesterase activity defined as 1 μmol phenol generated per minute under the above conditions, and expressed as U/l serum.^{22,23}

Statistical analysis

SPSS[®] (version 11.5 for Windows; IBM/SPSS, Chicago, IL, USA) was used to analyse the data statistically. With normally distributed and homogeneous variables, the significant differences between the groups were estimated using two-tailed *t*-tests. Bivariate comparisons were examined via Spearman's correlation coefficients, with values corrected for ties. In addition, χ^2 tests were used to evaluate categorical data. Differences were considered significant at $P < 0.05$.

Results

In the anxiety disorders group ($n = 37$), the age of the participants was 11.2 ± 3.1 years (mean $\pm$ SD; range 6–16 years), while that of the control group was 11.1 ± 2.6 years (range 6–16 years). In the anxiety disorders group, 14 of the patients were male and 23 were female; in the control group, 17 of the participants were male and 19 were female. There was no difference

Table 1 Demographic data and subtypes of anxiety of the study participants

	Anxiety disorders (<i>n</i> = 37)	Healthy controls (<i>n</i> = 36)	Comparison
Sex, <i>n</i> (%)			
Male	14 (38)	17 (47)	$P = 0.41$, $\chi^2 = 0.658$
Female	23 (62)	19 (53)	
Age, years, mean $\pm$ SD	11.2 ± 3.1	11.1 ± 2.6	$P = 0.84$, $t = 0.193$
Age range, years	6–16	6–16	–
Anxiety subtypes, <i>n</i> (%)			
Generalized anxiety disorder	25 (67)	–	–
Obsessive compulsive disorder	5 (14)	–	–
Separation anxiety disorder	4 (11)	–	–
Social anxiety disorder	2 (5)	–	–
Panic disorder	1 (3)	–	–

Table 2 Serum levels of parameters in study subjects

Parameters	Anxiety disorders (<i>n</i> = 37)	Healthy controls (<i>n</i> = 36)	<i>P</i> value
Lipid hydroperoxide ($\mu\text{mol/l}$)	2.4 ± 0.6	2.0 ± 0.6	$<0.05^*$
Paraoxonase (U/l)	180.4 ± 79.5	165.7 ± 77.9	>0.05
Arylesterase (U/l)	227.8 ± 43.1	221.9 ± 54.5	>0.05

Data are presented as mean $\pm$ SD.

*Significant.

in mean age ($t = 0.193$, $P = 0.84$) or gender ($\chi^2 = 0.658$, $P = 0.41$) distribution between the groups. The demographic data and subtypes of anxiety disorders are summarized in Table 1.

LOOH levels were significantly higher in the anxiety disorders group than in the control group ($P = 0.015$, $t = 2.5$). There were no significant differences in paraoxonase ($P = 0.427$, $t = 0.8$) or arylesterase ($P = 0.612$, $t = 0.5$) activity between the patient and the control groups (Table 2).

Age was not correlated with LOOH levels or with paraoxonase and arylesterase activity in patients with anxiety disorders, and there were no significant differences between male and female patients ($P > 0.05$).

Discussion

This is the first study to investigate serum lipid peroxidation markers in children with anxiety disorders. We investigated the serum LOOH levels in child and adolescent patients with anxiety disorders, and found that serum LOOH levels were significantly higher in patients with anxiety disorders than in the normal healthy control subjects. The most studied oxidant marker in adult patients with anxiety disorders is malondialdehyde (MDA). LOOH and MDA are the

products of the oxidation of polyunsaturated fatty acids. Many research groups have reported elevated MDA levels in patients with OCD, PD, or SAD.^{24,25,10} Similarly, another research study reported elevated LOOH levels in patients with GAD.¹¹

Lipid peroxidation is a degenerative process that affects cell membranes and other lipid-containing structures under conditions of oxidative stress.^{12,13} Oxidative stress is a biological condition that is characterized by the production of excessive amounts of oxidants, decreased levels of antioxidants, or both.²⁶ The human brain, as the most metabolically active tissue in the body, produces a comparatively large amount of free radicals. In addition, the brain is rich in the precise fatty acid composition needed for oxidation, and has relatively modest antioxidant defences.²⁷ Consequently, the brain is highly vulnerable to oxidative stress,²⁷ and this condition might predispose certain individuals to neurodegenerative and neuropsychiatric diseases. Structural imaging studies have indicated bilaterally decreased hippocampus volumes in anxiety disorders.^{28,29} We suggest that oxidative metabolism may contribute to the formation of anxiety disorders by affecting neuronal structure.

The other finding of this study was that there were no significant differences in paraoxonase and arylesterase activity between the patient and the control groups. The relation between anxiety disorders and the activity levels of a great number of antioxidant enzymes has previously been studied. In a recent study, paraoxonase enzyme activity was found to be lower in adults with GAD, although no significant difference in arylesterase activity was found.¹¹ So far, the most studied antioxidant enzymes in psychiatric disorders are superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT). In one study, SOD, GSH-Px, and CAT enzyme levels were found to be significantly higher in patients with SAD.¹⁰ In a different study, higher SOD and GSH-Px activity levels were reported in patients with PD.²⁵ However, a third study, on PTSD, did not find any significant changes in SOD, GSH-Px, or CAT activity.³⁰

The restlessness, irritability, over-excitement, and worrying present in anxiety disorders can cause stress in daily living. Stress challenges an organism's homeostatic mechanisms.³¹ Recent studies have shown a relation between excessive production of toxic oxygen metabolites and psychological stress. In the human and animal studies, psychological stress was found to be related to induction of oxidative damage.^{32–34} Aschbacher *et al.*³⁴ reported recently that exposure to chronic stress promotes oxidative damage, causing ceaseless activation of the hypothalamic–pituitary–adrenal axis. In a study in animals, high anxiety levels significantly increased oxidative stress.³²

Similarly, it has been shown that oxidative stress could trigger anxiety-related behaviour in mice.³⁵ In addition, oxidative stress has been shown to decline with treatment in cases of anxiety disorder.^{8,36} All of these results suggest that oxidative stress can play a role in the aetiopathogenesis of anxiety disorders.

In the present study, there was no significant correlation between measured LOOH levels and antioxidant enzymes and patient demographics, such as gender and age. These results suggest that increased lipid peroxidation in children with anxiety disorders might be related to the disorder itself.

Until recently, there has been no method of determining any specific 'diagnostic marker' for anxiety disorders. Bulut *et al.*¹¹ reported recently that 'products of lipid oxidation' (which would include LOOH), might be used as a diagnostic factor for GAD. It has long been known that lipid peroxidation reactions are induced by oxidative stress.³⁷ In addition, oxidative damage has been shown to be related to the psychopathology of anxiety disorders.^{10,24,25} Our results also suggest that increased serum LOOH levels might have diagnostic value for children and adolescents with anxiety disorders.

The most prominent limitation of this study is the relatively small sample size, which may not represent all types of anxiety disorders. In addition, owing to the heterogeneity of the anxiety diagnosis in the participants, we could not evaluate the correlation between disease severity and serum lipid peroxidation markers. However, exclusion of any comorbid psychiatric disorders in the anxiety samples increased the reliability of our study.

Lipid peroxidation in the neurons could induce brain inflammation. Animal studies have shown that oxidative stress and inflammation in the brain, adrenal glands, and systemic circulation may play a critical role in the development of anxiety disorders such as PTSD.³⁸ In future studies, simultaneous measurement of lipid peroxidation and inflammation would be beneficial in assessing the cumulative effect of lipid peroxidation in the aetiology of anxiety disorders. In the present study, lipid peroxidation markers were assessed in patients who were not receiving treatment for anxiety disorders. Therefore, there is a need for further evaluation of changes in serum lipid peroxidation markers in the treatment of children with anxiety disorders.

Conclusion

Remarkably high levels of LOOH, along with no statistical changes in paraoxonase and arylesterase activity, suggest an oxidative imbalance in paediatric patients with anxiety disorders. Therefore, lipid peroxidation or oxidative damage might have a role in the aetiopathogenesis of anxiety disorders, and

LOOH might be a potential biological marker for anxiety disorders in children. Studies with larger samples are necessary to increase our knowledge about the role of lipid peroxidation in children with anxiety disorders.

Acknowledgement

We thank the children who participated as volunteers in this study, as well as their families.

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