Journal of Affective Disorders 150 (2013) 829–833

Contents lists available at ScienceDirect

Journal of Affective Disorders

journal homepage: www.elsevier.com/locate/jad

Research report

Reduced PON1 enzymatic activity and increased lipid hydroperoxide levels that point out oxidative stress in generalized anxiety disorder

Mahmut Bulut [a], Salih Selek [b,*], Yasin Bez [a], Ibrahim Fatih Karababa [c], Mehmet Cemal Kaya [a], Mehmet Gunes [a], Ali Emhan [c], Nurten Aksoy [d], Aytekin Sir [a]

[a] Dicle University, Department of Psychiatry, Diyarbakir, Turkey
[b] Istanbul MedeniyetUniversity, Department of Psychiatry, Istanbul 34740, Turkey
[c] Harran University, Department of Psychiatry, Sanliurfa, Turkey
[d] Harran University, Department of Biochemistry, Sanliurfa, Turkey

ARTICLE INFO

Article history:
Received 29 November 2012
Received in revised form
12 March 2013
Accepted 13 March 2013
Available online 24 May 2013

Keywords:
Generalized anxiety disorder
Oxidative stress
Paraoxonase/arylesterase
Lipid hydroperoxide
Diagnostic performance

ABSTRACT

In recent years, there has been an increasing focus on generalized anxiety disorder (GAD) which is one of the most prevalent mental disorders in general population. Psychological, genetic, neurobiological, and neurochemical factors are believed to play role in the etiopathogenesis of GAD. The role of oxidative stress, as a neurochemical cause, in various anxiety disorders has been studied in recent years; however, it has not been thoroughly studied in GAD, yet. In this paper, we aimed to evaluate the serum levels of lipid hydroperoxide (LOOH), paraoxonase, and arylesterase in GAD patients without any co-morbid psychiatric disorders and investigate their diagnostic performance.

Blood samples were collected from 40 GAD patients and 40 healthy control subjects to measure their serum LOOH levels, arylesterase and paraoxonase activities. Obtained results have been compared between groups and receiver operating characteristic (ROC) curve has been drawn for diagnostic performance of measured biochemical markers. Positive and negative predictive values have been estimated where appropriate.

Mean LOOH level of the GAD patients was significantly higher than that of control subjects ($t=-5.49$, $p<0.001$), whereas, mean paraoxonase activity was lower in these patients ($t=3.056$, $p=0.03$). GAD could be predicted for LOOH level over 7.740 μmol/l with 92.5% positive predictive value and 92% negative predictive value.

Increased LOOH level and decreased paraoxonase activity of GAD patients may suggest increased lipid peroxidation and oxidative stress in these patients. LOOH levels may be a state marker for diagnosing GAD.

1. Introduction

Among anxiety disorders, generalized anxiety disorder (GAD) is a common and chronic mental condition with a life time prevalence of 3.3–6.5% that has a high degree of co-morbidity with other anxiety disorders and mood disorders (Grant et al., 2005; Lim et al., 2005; Martin, 2003).

Although several psychological, social, genetic, and biological factors have been suggested to explain the causes of GAD its etiopathogenesis has not been well understood, yet (Ng et al., 2008). Oxidative stress is a biological condition accompanying to mental disorders that has drawn attention in recent years (Ng et al., 2008; Steckert et al., 2010; Wood et al., 2009). Several studies suggested an association between oxidative stress and psychopathology in anxiety disorders including panic disorder (PD) (Herken et al., 2006), obsessive–compulsive disorder (OCD) (Selek et al., 2008), and post-traumatic stress disorder (PTSD) (Tezcan et al., 2003). However, to the best of our knowledge there has been no study published yet investigating the oxidative stress markers in generalized anxiety disorder (GAD). On the other hand, some indirect data about oxidative stress markers in "anxiety", the core symptom of GAD, comes from a few animal studies. They have shown that anxiety conditions are associated with increased levels of free radicals and depleted antioxidants which may lead to the oxidative damage to the brain (Bouayed et al., 2009; Rammal et al., 2008a, b).

Oxidative stress is a biological condition that is characterized by production of excessive amounts of oxidants or decreased levels of antioxidants or both. Excessive amount of free radicals chemically may react with cell membrane lipids, proteins and nucleic acids that consequently have the potential to damage the structure of neuronal cells (Bouayed et al., 2009; Hovatta et al.,

* Corresponding author. Tel.: +90 216 280 33 33.
E-mail addresses: drselek@yahoo.com, drselek@hotmail.com (S. Selek).

0165-0327/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.jad.2013.03.011

2010; Ng et al., 2008). The theory of oxidative stress in mental disorders is based on vulnerability of brain to oxidative damage (Wang and Michaelis, 2010).

Despite growing number of studies related with biological basis of GAD (Greenberg et al., 2012) neither a biological marker nor a diagnostic marker for GAD has been identified yet. In human studies of other anxiety disorders the results of oxidative stress markers are controversial, whereas lipid peroxidation is consistently increased in OCD, social phobia (SP), and PD (Bouayed et al., 2009; Hovatta et al., 2010; Rammal et al., 2008a, 2008b). Due to consistently increased lipid peroxidation in other anxiety disorders we hypothesize that lipid peroxidation may also be increased in GAD. To test this hypothesis, we have investigated human serum paraoxonase (PON1) activity and lipid hydroperoxide levels.

Increased psychiatric co-morbidity in GAD patients makes it hard to design a study for understanding the pathophysiology of the disorder. On the other hand, altered levels of oxidative biomarkers in other psychiatric disorders may interfere with the findings. Therefore, studying on pure GAD patients seems to be essential to eliminate these interfering factors.

Human serum paraoxonase (PON1) is a high density lipoprotein (HDL) associated enzyme which exerts paraoxonase and arylesterase activities and protects lipids from peroxidation (Gan et al., 1991). In the deficiency of both enzymes susceptibility to lipid peroxidation is increased (Aslan et al., 2011). Lipid hydroperoxides (LOOH) are the initial products of lipid oxidation process which are derived from reactive oxygen species (ROS) induced peroxidation of unsaturated phospholipids, glycolipids, and cholesterol (Girotti, 1998). There have been only a few studies that investigated PON1 activity and LOOH levels in psychiatric disorders. In a previous study, PON1 activity was shown to be not altered in depressed female patients (Tsuboi et al., 2004) and in another study depressive symptoms were independently correlated with lipid peroxidation in females (Kodydkova et al., 2009). The relationship between PON1 activity, LOOH level, and lipid peroxidation has been shown in Fig. 1.

To investigate the relationship between lipid peroxidation and GAD we have measured LOOH levels and paraoxonase/arylesterase activities in the sera of GAD patients who have no co-morbid any psychiatric or medical conditions and healthy control subjects as well. Additionally, we have analyzed whether these biological markers could be used as diagnostic tools for GAD or not.

2. Material and methods

2.1. Patients and controls

The study was conducted according to the revised version of the Helsinki Declaration and approved by the local ethics committee of Harran University. All subjects were informed about the study protocol and provided their written informed consent. Medical records of the patients were reviewed and demographical and clinical characteristics such as gender, age, and co-morbid conditions were recorded. The patients who had co-morbid axis I or II conditions due to DSM-IV criteria were excluded from the study. Additionally pregnancy, severe systemic diseases, epilepsy, diabetes mellitus, hypertension, drug and alcohol dependence, inadequate blood sampling, severe head injury, vitamin and fish oil use were the exclusion criteria of the study. Forty patients diagnosed as GAD according to DSM-IV diagnostic criteria and 40 healthy volunteer controls were included in the study. Hamilton Anxiety Rating Scale (HAM-A), Clinical global impression-severity of illness (CGI-S) and demographical form prepared by the authors of this study based upon their previous studies were administered to the patients. Possible related factors such as trauma history was also recorded. The presences of clinical findings of the GAD such as irritability, sleeping problems, fatigue etc. were recorded for each patient. The patients were allowed to continue their routine GAD treatments.

2.2. Blood sampling

After 12 h fasting, blood samples were collected during routine laboratory evaluation at 08.00 a.m. Serum samples were collected by centrifugation of obtained blood at 3000 rpm for 10min and they were stored frozen at −80 °C. LOOH levels and paraoxonase/arylesterase activities were measured in serum samples of the study groups.

2.3. Measurement of paraoxonase and arylesterase activity

Paraoxonase and arylesterase activities were measured using paraoxon and phenyl acetate substrates. The rate of paraoxon hydrolysis (diethyl-p-nitrophenylphosphate) 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 M^{-1} cm^{-1}. Paraoxonase activity was expressed as U/l serum. Phenyl acetate was used as a substrate to measure arylesterase activity. Enzyme activity was calculated from the molar absorptivity coefficient of the produced phenol, 1310 M^{-1} cm^{-1}. One unit of arylesterase activity was defined as 1 μmol phenol generated/min under the above conditions and expressed as U/l serum (Eckerson et al., 1983; Haagen and Brock, 1992).

2.4. Measurement of lipid hydroperoxide levels

Serum LOOH levels were measured with a ferrous ionoxidation-xylenol orange (FOX-2) assay. It involves the oxidation of ferrous ion to ferric ion via the effect of various oxidants. The ferric ion is then measured with xylenol orange. The levels of LOOH 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 that appear due to the absence or presence of TPP (Nourooz-Zadeh, 1999).

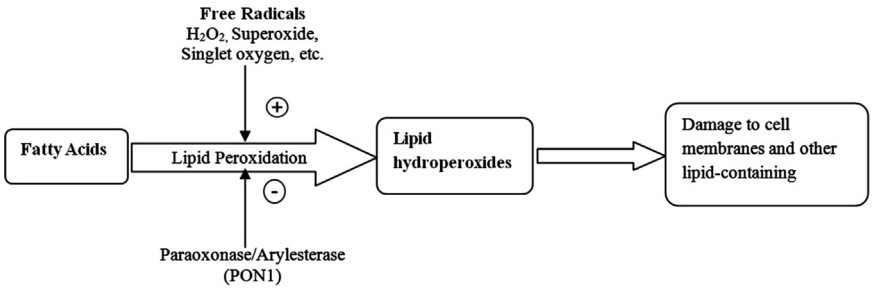

Fig. 1. Effect of paraoxonase/arylesterase on lipid peroxidation.