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Evaluation of oxidative and antioxidative parameters in generalized anxiety disorder

Ali Emhan [a], Salih Selek [b,*], Hüseyin Bayazıt [c], İbrahim Fatih Karababa [c], Mahmut Katı [c], Nurten Aksoy [d]

[a] Clinic of Psychiatry, Ministry of Health, Diyarbakır Education and Research Hospital, Diyarbakır, Turkey
[b] Harris County Psychiatric Center, University of Texas Health Science Center at Houston, TX, USA
[c] Department of Psychiatry, Faculty of Medicine, Harran University, Sanliurfa, Turkey
[d] Department of Biochemistry, Faculty of Medicine, Harran University, Sanliurfa, Turkey

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ABSTRACT

Generalized anxiety disorder (GAD) is a prevalent psychiatric disorder. The exact causes of GAD still unknown, in addition to neurochemical and neuroanatomic disorders, genetic and environmental factors are discussed in etiology. In our study we aimed to evaluate the oxidative metabolism's status and investigate the role of oxidative metabolites in GAD. Blood samples were taken from enrolled subjects in appropriate way and total antioxidant status (TAS), total oxidant status (TOS), and oxidative stress index (OSI) were studied in Harran University Biochemistry Labs. Results were compared between groups. The patients' TOS and OSI levels were significantly higher than control group. The patients' TAS levels were significantly lower than controls'. According to our findings, oxidative stress mechanism might have a role in GAD pathophysiology. In the future, total antioxidants may be used as a biologic marker in GAD etiology but more research is needed.

1. Introduction

Generalized anxiety disorder (GAD) is a clinical picture characterized by exaggerated feeling of worry related with various daily events and occurring almost all day long. In addition to anxiety, these patients experience unrest, tiredness, difficulty in concentrating, getting nervous rapidly, muscular straining and insomnia (Pine and McClure, 2005a). The prevalence of GAD is reported to be 3–6% in the general population with being two times more frequent in women than in men; it has been reported that it is the most frequent distress disorder among the patients referred to the primary care institutions (Wittchen et al., 2002).

Although many biological and psychological factors have been considered to play a role in the etiology of GAD, none of these has been found to be a definite cause. In order to make a definite diagnosis, we need a well-documented history and thorough psychological examination as for the other psychiatric disorders (American Psychiatric Association, 2000). Recently, studies have been conducted to investigate the role of oxidative metabolism in the etiology of anxiety disorders (Atmaca, et al., 2004; Ersan et al., 2006; Tezcan et al., 2003).

Oxidants (or pro-oxidants/free radicals) arise from the reactions of degradation involving oxygen as waste molecules. Having some beneficial effects (such as phagocytosis of monocytes and neutrophils), oxidants involve in reactions with many compounds including DNA, protein, and polyunsaturated fats of the cell membrane phospholipids. Among these reactions, particularly those affecting and damaging DNA pave the way for the development of various diseases. The substances called antioxidants diminish the deleterious effects caused by these oxidative products. There is a balance between the oxidants and antioxidants in a living body, and damage occurs when such balance is lost in favor of oxidants, a case called “oxidative stress” in the literature (Haddad, 2004). As described, though several studies have been carried out to investigate the role of oxidative metabolism in the pathophysiology of anxiety disorders, there is no evaluation of the total antioxidant level (TAL), total oxidant level (TOL) or any specific compound (e.g., malondialdehyde, catalase, superoxide dismutase etc.) in the generalized anxiety disorder (Atmaca et al., 2004; Ersan et al., 2006; Tezcan et al., 2003; Wittchen et al., 2002).

Therefore, in this present cross-sectional case-control study, we aimed to determine whether the mechanism of oxidative stress plays a role in the pathophysiology of GAD and examine the diagnostic testing performance of total oxidants and total antioxidants in the etiology of GAD.

* Corresponding author.
E-mail address: drselek@yahoo.com (S. Selek).

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2. Methods

2.1. Study method and subjects

This study included 40 patients, aged between 18 and 65 years, referred consecutively to the Outpatient Clinic of the Medical Faculty of the Harran University, who had had a diagnosis of GAD according to DSM-IV, and gave a written informed consent to participate in the study. The study was approved by the local ethics committee (the Medical Faculty of the Harran University). The patients' previous medical records and follow-up information were reviewed, and the socio-demographic variables including age, gender, comorbidities, the medications used, and the smoking status were recorded. The exclusion criteria of the study were as follows: pregnancy, serious systemic disease, epilepsy, dementia, disorders of alcohol and drug misuse, depression, panic disorders, obsessive compulsive disorder, schizophrenia, bipolar disorder and personality disorders (Axis II pathology), mild to moderate mental retardation, history of serious head trauma, and inadequate sampling. On the day when serum samples were drawn, they were administered a Hamilton Anxiety Rating Scale, Clinical Global Impression, and completed a socio-demographic questionnaire prepared for the study by one psychiatrist (AE).

The control group consisted of 40 healthy volunteers. These volunteers' socio-demographic variables including age and gender as well as the smoking status were also recorded. Inadequate sampling was determined as an exclusion criterion.

Blood samples were drawn from the antecubital vein following 12 h fasting. Thereafter, blood samples were poured into tubes, centrifuged at 3000 rpm for 5 min to separate plasma, and stored on ice to be processed within 6 h at most. The separated plasmas were stored at $-80\,^{\circ}C$ for determining total antioxidant level (TAL) and total oxidant level (TOL). The plasma TAL and TOL levels were measured to calculate the oxidative stress index (OSI) in the Biochemistry Laboratory of the Harran University. The patients were gender-matched with the control group.

2.2. Data collection tools

2.2.1. Socio-demographic form

Each patient was applied a routine follow-up and adverse effect scale and a socio-demographic form which has been prepared for recording demographics, height, body weight, smoking status and the laboratory results. Data from the patients were screened and recorded.

2.2.2. Hamilton anxiety rating scale (HAM-A)

On the day of serum sampling, this scale was administered to all subjects participated in the study by an investigator (AE) in the Department. Used for determining the severity of anxiety and the distribution of symptoms, this scale comprises a total of 14 questions interrogating both somatic and psychic symptoms and compassing sub-dimensions. A five-point Likert-type measurement is provided. A total score is obtained by summing the points of items. The validity and reliability of its Turkish version has been conducted by Yazıcı et al. (1998).

2.2.3. Clinical global impression scale (CGI)

CGI is a 3-item scale to evaluate the severity of disease, the improvement, and therapeutic efficacy index. The therapeutic efficacy index has two subsets as therapeutic effect and adverse effect (Guy, 1976). CGI was applied to assess the severity of comorbidities when blood samples were taken. For remission, the comorbidities having a CGI severity subscore ≤ 2 were considered as in remission.

2.3. Measurement and estimation of variables

2.3.1. Total antioxidant level (TAL) measurement

The total antioxidant status of the plasma was measured using a novel automated colorimetric measurement method for TAS developed by Erel (2004). In this method the hydroxyl radical, the most potent biological radical, is produced by the Fenton reaction, and reacts with the colorless substrate O-dianisidine to produce the dianisyl radical, which is bright yellowish-brown in color. Upon the addition of a plasma sample, the oxidative reactions initiated by the hydroxyl radicals present in the re-action mix are suppressed by the antioxidant components of the plasma, preventing the color change and thereby providing an effective measure of the total antioxidant status of the plasma. The assay results are expressed as mmol Trolox Eq/L, and the precision of this assay is excellent, being lower than 3% (Cao and Prior, 1998).

2.3.2. Total oxidant level (TOL) measurement

The total oxidant status of the plasma was measured using a novel automated colorimetric measurement method for TOS developed by Erel, (2005). In this method oxidants present in the sample oxidize the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction is enhanced by glycerol molecules, which are abundantly present in the reaction medium. The ferric ion makes a colored complex with xylenol orange in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide and the results are expressed in terms of micromolar hydrogen peroxide equivalent per liter (μmol H2O2 Eq/L).

2.3.3. Oxidative stress index (OSI) measurement

Oxidative stress index (OSI) was calculated as the total oxidant level (TAL)/total antioxidant level (TOL) ratio (Kosecik et al., 2005).

2.4. Statistical assessment of data

Statistical analyses were performed using “SPSS 15.0 for Windows” software. Following the descriptive statistics, a Q–Q diagram was graphed in order to assess the distribution of the parameters investigated. A t-test was used for the paired group comparison of the data showing normal distribution, and Chi-square test for comparing the ratios. Spearman's correlation test was used for correlation analysis. A P value of < 0.05 was accepted as significant.

3. Results

The demographical data of the patients and control subjects are given in Table 1. Ages, gender, duration of illness were similar among groups ($p > 0.05$, for all comparison). (Table 1).

TAS, TOS and OSI levels in patients and controls were summarized in Table 2. TAS, TOS and OSI levels were statistically higher in GAD patients compared to healthy controls. ($P > 0.05$ for all comparison).

Table 1
Sociodemographic and clinical characteristics of the patients and controls.

	Patients	Controls	P value
Age ($mean \pm SD$)	42.48 ($\pm$ 12.03)	41.5 ($\pm$ 12.67)	0.993
Gender: female/male (n)	26/14	26/14	
Duration of illness (years): median	5 (1–30)		

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