

Analysis of the association between anxiety and depression levels and oxidative stress levels

 Atakan Savrun¹,  Seyda Tuba Savrun²,  Ali Aygun¹,  Emre Gokcen³,  Yeliz Kasko Arici⁴,  Murat Turkarslan⁵,  Salim Neselioglu⁶,  Ozcan Erel⁶

¹Ordu University, Faculty of Medicine, Department of Emergency Medicine, Ordu, Turkey

²Ordu University Training and Research Hospital, Clinic of Emergency Medicine, Ordu, Turkey

³Bozok University, Faculty of Medicine, Department of Emergency Medicine, Yozgat, Turkey

⁴Ordu University, Faculty of Medicine, Department of Biostatistics and Medikal Informatics Unit, Faculty of Medicine, Ordu, Turkey

⁵Cankiri State Hospital, Clinic of Emergency Medicine, Cankiri, Turkey

⁶Yildirim Beyazit University, Faculty of Medicine, Department of Medical Biochemistry, Ankara, Turkey

Copyright © 2020 by authors and Annals of Medical Research Publishing Inc.

Abstract

Aim: There is a balance between reactive oxygen species (ROS) and antioxidant systems preventing them in the organism. In cases where this balance is impaired, molecular and cellular functions are negatively affected. Thiol-disulfide homeostasis (TDH) is also affected by this imbalance. It has been reported that the balance between oxidative and anti-oxidative systems is disrupted in individuals with psychiatric diseases such as anxiety and depression. Accordingly, it was aimed to determine the level of TDH in the serum of students who took the oral internship exam.

Material and Methods: Volunteer fifth grade students of Medical Faculty, 19 male and 21 female, was included in the study. Of the students included in the study, Beck depression and Beck anxiety, albumin, ischemia-modified albumin (IMA), total thiol (SH + SS), native thiol (SH) and SS levels were measured and SS / SH + SS %, SH / SH + SS % and SS / SH % were calculated before and after the oral internship exam. These data were compared with the association between anxiety and depression level changes before and after the oral internship exam.

Results: It was observed that the anxiety levels of the participants before the oral exam decreased in the period after the oral exam ($p < 0.001$). It was detected that the level of depression of the participants before the oral exam decreased after the oral exam ($p < 0.001$). Changes in IMA and TDH parameters before and after the oral internship exams were examined, but no significant relationship was found ($p > 0.05$).

Conclusion: Oral internship exam of Medical Faculty is a factor increasing anxiety and depression levels of the students. However, no association was found between oral internship exam and TDH parameters.

Keywords: Anxiety; depression; ischemia-modified albumin; native thiol; oral internship exam; total thiol

INTRODUCTION

High energy atoms or molecules with one or more electrons in their outer orbital are called free radicals. Free radicals are called reactive oxygen types (ROS) if they are oxygen-sourced, and reactive nitrogen types (RNS) if they are nitrogen-sourced (1).

There is a balance between ROS and antioxidant systems preventing them in the organism. Disruption of this balance is called oxidative stress. In this situation, molecular and cellular functions are negatively affected (2). Thiol-disulfide homeostasis (TDH) is also found among them (2,3). Thiols are organic compounds containing a sulfhydryl (-SH) group, which has a critical

role in preventing the formation of any oxidative stress in cells. Thiols react with free radicals to protect the cells and tissues (3). Therefore, it has been observed that imbalances in TDH can take a crucial role in the etiology of many diseases (4).

Anxiety and depression are psychiatric disorders with increasing prevalence among the students (5). The examination of the students is also thought among the most important factors increasing the level of anxiety and depression in students (6). It has been reported that the balance between oxidative and anti-oxidative systems is disrupted in individuals with psychiatric diseases such as anxiety and depression (7). Accordingly, it was aimed to determine the level of TDH in the serum of students who

Received: 10.12.2019 Accepted: 24.03.2020 Available online: 24.04.2020

Corresponding Author: OSeyda Tuba Savrun, Ordu University Training and Research Hospital, Clinic of Emergency Medicine, Ordu, Turkey, E-mail: stsavrun@gmail.com

took the oral internship exam. Moreover, the association of anxiety, depression and TDH balance of the students with age, gender and family history was also examined.

MATERIAL and METHODS

Study Design

This study is a randomized-controlled trial performed in a Medical Faculty with 700 students. This study was started after getting approval from Ordu University Clinical Researches ethical board (Decision number: 2018/231). Before starting the study, the participants were informed about the study, the informed volunteerism consent form was filled, and the participants' approvals were obtained without being pressured. Volunteer fifth grade students, who were in the same class and who would take the same internship exam in Medical Faculty, were included in the study (There were 45 fifth grade students in total). Medical students not studying in the fifth grade and non-volunteers were excluded from the study. Before starting the study, demographic characteristics, and anxiety and depression levels of the participants were determined. Venous blood samples were also taken from the participants. After the participants took the internship exam lasting about an hour, the anxiety and depression levels of the participants were determined with the same method and blood samples were taken. Beck Anxiety Inventory (BAI) and Beck Depression Inventory (BDI), a universal scale prepared in Likert form, were used to measure the levels of anxiety and depression of the participants. However, the scales were used only for symptomatic examination purposes. The forms were requested to be filled in completely and correctly. Moreover, native thiol (SH), total thiol (TSH), disulphide (SS) and SH / SS parameters were measured before and after the oral internship exam for TDH measurement. The association between TDH parameters, and anxiety and depression levels and oral internship exam was examined.

BDI is a scale which consists of 21 questions and in which the answer of each question is graded between 0 and 3. BDI scores were evaluated as minimal depression between 0-13, mild depression between 14-19, moderate between 20-28 and severe depression between 29-63 (8). BAI is a scale which consists of 21 questions and is prepared in Likert style, and in which the answer of each question is graded between 0 and 3. According to the scores obtained from BAI, the anxiety levels of patients were evaluated as low anxiety in the range of 0-17 points, moderate anxiety between 18-24 points, and high anxiety level in those who scored 25 or more (9).

Biochemical analysis

Before the examination, venous blood samples were taken into the tubes with gel (Becton Dickinson and Company, New Jersey, USA) for biochemical testing studies and EDTA tubes (Becton Dickinson and Company, New Jersey, USA) for TDH testing. After all samples were centrifuged at $1600 \times g$ for 10 minutes, their serum and plasma were separated, and stored at -70°C until working day. Biochemical tests were performed by spectrophotometric methods on AU 2700 auto-analyzer (Beckman Coulter,

Inc, USA).

For thiol measurement, it was evaluated by spectrometry (Roche, Cobas 501, Mannheim, Germany) using "Modified Ellman Method" of Erel et al. (10). Free functional thiol groups (-SH) were formed by breaking disulfide bonds (-S-S) with sodium borohydride (NaBH_4). Unused sodium borohydride residues were removed with formaldehyde. In this way, the reduction of 5.5'-dithiobis- (2-nitrobenzoic) acid (DTNB) and any disulfide bond formed as a result of reaction with DTNB is prevented. As a result of reaction with DTNB, reduced and total native thiol groups were determined. Disulfide parameter is a parameter calculated as half of SH and TSH content. After SH and TSH are determined, SS, SS / SH + SS%, SH / SH + SS% and SS / SH % were calculated (10).

Data Analysis

Statistical analysis was performed using SPSS v26 statistical software (IBM Inc., Chicago, IL, USA). Continuous data were shown as mean \pm SD. Normal distributed variables were compared using paired sample t-test. Non-normal distributed variables were compared using Mann-Whitney U test. Correlation analyzes were used with Pearson and Spearman methods. $p < 0.05$ was considered for statistical significance.

RESULTS

A total of 40 students, 19 (47.5%) males and 21 (52.5%) females, studying in the fifth grade of Medical Faculty were included in the study. The mean age of the participants was detected as 22.2 ± 3.8 . All of the cases were single and had no children. Other demographic characteristics of the cases are given in Table 1.

Table 1. The demographic data of participants

		n	%
Gender	Female	19	48.7
	Male	20	51.3
Marrital status	Single	38	97.4
	Married	1	2.6
Alcohol and tobacco use	No	34	87.2
	Tabacco	3	7.7
	Alcohol+Tabacco	2	5.1
Knowledge of the disease	No	32	82.1
	Heart disease	1	2.6
	DM	2	5.1
	RLS	1	2.6
	DM+Asthma	1	2.6
	Anemia+Migraine	1	2.6
	DM+Panic attack	1	2.6
Drug use	No	30	76.9
	Antidepressant	2	5.1
	Other	7	17.9

DM:Diabetes Mellitus; RLS: Restless legs syndrome

Of the subjects, BAI scoring before the internship exam (Ank1) and BAI scoring after the internship exam (Ank2) were examined. It was observed that the anxiety levels of the participants decreased statistically significantly after the internship exam ($p<0.05$) (Table 2).

Of the subjects, BDI scoring before the internship exam (Dep1) and BDI scoring after the internship exam (Dep2) were examined. It was found that the depression levels of the participants decreased statistically significantly after the internship exam ($p<0.05$) (Table 2).

Table 2. Comparison of the students' BDI and BAI scores in the pre- and post-internship

		Pre			Post			P
		n	%	Mean±SD	n	%	Mean±SD	
BAI	Mild	30	76.9	10.00±7.48 (0-25)	34	87.2	7.44±9.95 (0-42)	0.004**
	Moderate	8	20.5		2	5.1		
	Severe	1	2.6		3	7.7		
BDI	Minimal	33	84.6	8.49±5.89 (0-28)	35	89.7	5.23±5.01 (0-24)	0.0001***
	Mild	4	10.3		3	7.7		
	Moderate	1	2.6		1	2.6		
	Severe	1	2.6		-	-		

BDI: Beck Depression Inventory; BAI: Beck Anxiety Inventory; SD: Standard Deviation

Table 3. Comparison of the students' oxidative stress levels in the pre- and post-internship

Variables	Period	Female (n=20)		Male (n=19)	
		Mean±SD	p	Mean±SD	p
Albumin (mg/dL)	Pre	3.82±0.31	0.171 ^{NS}	4.15±0.27	0.492 ^{NS}
	Post	3.95±0.51		4.06±0.39	
IMA (mg/dL)	Pre	0.95±0.09	0.842 ^{NS}	0.86±0.09	0.252 ^{NS}
	Post	0.95±0.14		0.90±0.11	
Native thiol (µmol/L)	Pre	370.00±36.34	0.952 ^{NS}	403.97±31.86	0.018*
	Post	369.56±44.79		379.99±32.88	
Total thiol (µmol/L)	Pre	407.25±39.62	0.932 ^{NS}	446.91±34.15	0.021*
	Post	407.99±49.80		420.30±35.93	
Disulfide (µmol/L)	Pre	18.62±3.30	0.600 ^{NS}	21.47±3.63	0.376 ^{NS}
	Post	19.22±4.26		20.15±3.07	
Index 1 (%)	Pre	5.04±0.82	0.557 ^{NS}	5.33±0.91	0.958 ^{NS}
	Post	5.20±0.93		5.31±0.74	
Index 2 (%)	Pre	4.57±0.68	0.565 ^{NS}	4.81±0.74	0.966 ^{NS}
	Post	4.70±0.77		4.79±0.60	
Index 3 (%)	Pre	90.86±1.36	0.567 ^{NS}	90.39±1.49	0.972 ^{NS}
	Post	90.60±1.53		90.41±1.21	

IMA: Ischemia Modified Albumin; SD: Standard Deviation

Albumin level before the internship exam (Albumin 1) and albumin level after the internship exam (Albumin 2) were examined separately with respect to gender. The participants' albumin levels increased after the internship ($p>0.05$) (Table 1). IMA level before the internship exam (IMA 1) and IMA level after the internship exam (IMA 2) were examined separately with respect to gender and an increase in IMA level was detected ($p> 0.05$).

Before the internship exam, the levels of SH, TSH, SS, Index1 (SS / SH), Index2 (SS / TSH) and Index 3 (SH / TSH) were examined and no significant changes were detected ($p>0.05$) (Table 3).

DISCUSSION

In this study, the association between the anxiety level, the depression level and TDH of the fifth grade students of Medical Faculty and the oral internship exam was examined. According to the literature data reached, no study was found to evaluate TDH, SS and SH levels among the students of Medical Faculty who took the oral internship exam. Therefore, it was thought that this study was the first study in this field.

Exam is a method used to measure or evaluate knowledge and skills (11). It is thought that the psychological effects of the exam start from the university entrance exam and continue throughout the university education. In the studies conducted, it is stated that the exam has a negative effect on the students and approximately 30% of the students experience exam anxiety (13). There are studies in the literature reporting that exams affect the students' psychological conditions, like anxiety and depression, negatively (14,15). Similarly, it was reported in the studies performed on the students that exam anxiety caused negative effects such as depression, substance abuse and behavioral disorders (16,17). In our study, similar to the literature, it was found that the exam statistically significantly increased anxiety and depression levels of the students ($p<0.001$) (Table 2). We believe that this situation can be attributed to the students' thinking that there is an association between their exam success and their future expectations, their failure may decrease their acceptance in their social environment and therefore they will lose confidence in themselves.

It was reported that oxidative stress due to an increase in ROS or an imbalance in antioxidant level, which is a protective mechanism, plays a role in the etiopathogenesis of many diseases in the organism (18). It has been stated that the antioxidant system is activated in order to prevent this situation when ROS is increased, and SH in the organism is affected and transformed into SS (4). Plasma thiols have pro-oxidant or anti-oxidant effects in physiological conditions. Thiols show an anti-oxidant or pro-oxidant effect depending on the physiological condition or the oxidative stress status of the organism (19,20). Antioxidants (methionine, N-acetyl cysteine, taurine, homocysteine, etc.) containing thiol groups are thought to play an active role in conditions like anxiety and

depression. It was reported in a study that oxidative stress increased in patients with panic attacks (10). Similarly, it is known that the level of oxidative stress increases in disease situations such as Parkinson (11). In another study conducted on patients with schizophrenia, it was observed that thiol and native thiol levels were lower in patients than in the control group (12). In the study realized on the patients with major depression by Baykan et al., it was presented that serum native thiol levels were found to be significantly higher in the patient group than in the control group, while serum disulfide levels were found to be significantly lower (13). It was demonstrated in another study performed on the patients with generalized anxiety disorder that total thiol and native thiol levels of the patients were higher than the control group (14). In our study, the anxiety and depression levels of the participants were higher before the oral internship exam when compared to that after the oral internship exam ($p<0.05$). However, there was no significant association between this change and TDH ($p>0.05$). We believe that the level of anxiety and depression decreases after the oral internship exam, which takes about two hours, but oxidative stress parameters may not have changed enough to be measured in the blood during this decrease period.

CONCLUSION

The internship exam of Medical Faculty is a factor that increases the students' level of anxiety and depression. However, it cannot be said that oral internship exams have an effect on oxidative stress. Therefore, we consider that more participants should be included in the study and the time interval, when a change would be possibly observed, should be longer.

Competing interests: The authors declare that they have no competing interest.

Financial Disclosure: There are no financial supports.

Ethical approval: This study was approved from Ordu University Clinical Researches Ethical Board (Decision number:2018/231) and written informed consent for all matters was obtained.

Atakan Savrun ORCID: 0000-0001-7468-4159

Seyda Tuba Savrun ORCID: 0000-0002-6512-2987

Ali Aygun ORCID: 0000-0002-5190-1445

Emre Gokcen ORCID: 0000-0002-6018-6105

Yeliz Kasko Arici ORCID: 0000-0001-6820-0381

Murat Turkarslan ORCID: 0000-0002-4884-9543

Salim Neselioglu ORCID: 0000-0002-0974-5717

Ozcan Erel ORCID: 0000-0002-2996-3236

REFERENCES

1. Karabulut H, Gulay MS. Free Radicals. Mehmet Akif Ersoy University. Health Sciences Institute 2016;4:50-9.
2. Fang YZ, Yang S, Wu G. Free radicals, antioxidants, and nutrition. Nutrition 2002;18:872-9.

3. Erel O, Neselioglu S. A novel and automated assay for thiol/disulphide homeostasis. *Clinical Biochemistry* 2014;47:326-32.
4. Turkyilmaz E, Yildirim M, Cendek BD, et al. Evaluation of oxidative stress markers and intra-extracellular antioxidant activities in patients with endometriosis. *Eur J Obstet Gynecol Reprod Biol* 2016;199:164-8.
5. M Tokdemir, OP Ors, I Perçinel, et al. Approach to Depressive Patient: A Case Report. *Smyrna Tıp Dergisi* 2018;50-3.
6. Ergin A , Uzun SU, Topaloglu S. Future Occupational Anxiety and Trait Anxiety Levels of 5th And 6th Year Medical Students at Pamukkale University and Their Contributing Factors. *Medical J Mugla Sitki Kocman University* 2016;3:16-2.
7. Maddock C, Pariante CM. How does stress affect you? An overview of stress, immunity, depression and disease. *Epidemiol Psychiatr Soc* 2001;10:153-62.
8. Evaluation of Infertile Women with Ovulation Induction and Intrauterine Insemination Therapy by Beck Depression Scale (Thesis) Dr. Serkan Sagol Edirne 2015.
9. Savrun A, Bozkurt S, Okumus M, et al. Determination of Anxiety Levels by Beck Anxiety Inventory in Emergency Medicine Residents Before and After Night Duties. *J Clin Anal Med* 2017;8:155-9.
10. Erel O, Neselioglu S. A novel and automated assay for thiol/disulphide homeostasis. *Clinical Biochemistry* 2014;47:326-32.
11. Zeynep K, Ozlem T. The Effect of Coping with Test Anxiety Study Based Creative Drama on Gifted Students' Test Anxiety Prof. Afyon Kocatepe University *J Social Sciences* 2019;21:97-107.
12. Erozkun A. Exam anxiety and coping behaviors of university students. *SBE* 2004;12:13-38.
13. Lufi D, Okasha S. & Cohen A. Anxiety: A Distinction and Some Initial Data", *Psychological Reports* 2004;20: 975-78.
14. Steinmayr R, Crede J, McElvany N, & Wirthwein L. Subjective well-being, test anxiety, academic achievement: Testing for reciprocal effects. *Frontiers in Psychology* 2016;6:1-13.
15. Lewinsohn PM, Shankman SA, Gau JM, et al. Prevalence and co-morbidity of subthreshold psychiatric conditions. *Psychological Med* 2004;34 (: 613-22.
16. Nelson JM, Gregg N. Depression and anxiety among transient adolescents and university students with ADHD, dyslexia, or concomitant ADHD / dyslexia. *J Attention Disorders* 2012;16:244-54.
17. Alansari BM. The relationship between depression and anxiety among undergraduate students in eighteen Arab countries: an intercultural study. *Social Behavior and Personality: an international journal*. 2005;33: 503-12.
18. Trotta E, Bortolotti S, Fugazzotto G, et al. Familial vitamin E deficiency: Multi organ complications support the adverse role of oxidative stress. *Nutrition*. 2018;63:57-60.
19. Korkmaz V, Kurdoglu Z, Alisik M, et al. Impairment of thiol-disulfide homeostasis in preeclampsia. *J MaternFetalNeonatalMed*. 2016;29:3848-53.
20. Atmaca G. Antioxidant effects of sulfur-containing amino acids. *Yonsei Med J* 2004;45:776-88.