Depression and anxiety symptoms are associated with prooxidant-antioxidant balance: a population-based study

Mojtaba Shafiee[1,2*], Mahsa Ahmadnezhad[2,3,*], Maryam Tayefi[4,5,*], Soheil Arekhi[2,6], Hassanali Vatanparast[7], Habibollah Esmaeili[8], Mohsen Moohebati[9], Gordon A. Ferns[10], Naghmeh Mokhber[11], Seyed Rafie Arefhosseini[12,#], Majid Ghayour-Mobarhan[5,#]

Affiliations:

1) Department of Nutrition, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
2) Student Research Committee, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
3) Nutrition Research Center, Department of Community Nutrition, Tabriz University of Medical Sciences, Tabriz, Iran.
4) Clinical Research Unit, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
5) Metabolic Syndrome Research Center, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
6) Evidence Based Medicine Research Group, Mashhad University of Medical Sciences, Mashhad, Iran.
7) College of Pharmacy and Nutrition, University of Saskatchewan, Saskatoon, SK, Canada.
8) Department of Biostatistics & Epidemiology, Faculty of Health, Management & Social Determinants of Health Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.
9) Cardiovascular Research Center, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
10) Brighton & Sussex Medical School, Division of Medical Education, Falmer, Brighton, Sussex BN1 9PH, UK
11) Psychiatry and Behavioral Sciences Research Center, Faculty of Medicine, Mashhad University of Medical Sciences, Iran.
12) Department of Nutritional Biochemistry, Tabriz University of Medical Sciences, Tabriz, Iran.

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[#]**Corresponding Authors:**
Majid Ghayour-Mobarhan MD, Ph.D. Metabolic Syndrome Research Center, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran, Tel: +985138002288, Fax: +985138002287; Email: ghayourm@mums.ac.ir
Seyed Rafie Arefhosseini, MSc, PhD. Department of Nutritional Biochemistry, Tabriz University of Medical Sciences, Tabriz, Iran, Tel: +984113357580, Email: arefhosseini@gmail.com

***** Equally contributed as first author

Abstract

Background: Depression and anxiety are significantly associated with systemic inflammation. Moreover, oxidative stress resulting from a disturbance in the prooxidant-antioxidant balance is linked to inflammation-related conditions. Therefore, depression/anxiety symptoms may also be associated with oxidative stress.

Objective: To examine the association between depression/anxiety symptoms and serum prooxidant-antioxidant balance (PAB) in adults who participated in a large population-based, cross-sectional study.

Methods: Serum PAB values were measured in 7,516 participants (62% females and 38% males) aged 35–65 years, enrolled in a population-based cohort study. Beck Depression and Anxiety Inventories were used to evaluate symptoms of depression and anxiety. Multinomial logistic regression was used to examine the effect of confounders on the status of serum PAB change.

Results: Among men, serum PAB values were increased incrementally from 1.55±0.47 to 1.59±0.47, 1.69±0.38, and 1.68±0.38 in the no or minimal, mild, moderate and severe depression groups, respectively ($P_{trend}<0.001$). Serum PAB values also increased significantly across these four corresponding groups among women [1.70±0.45, 1.73±0.44, 1.75±0.44, and 1.76±0.40, ($P_{trend}=0.005$)]. About anxiety, serum PAB values increased significantly across the four groups in men ($P_{trend}=0.02$) but not in women ($P_{trend}=0.2$). The adjusted odds ratios for serum PAB values among men with severe depression and anxiety symptoms were 1.75 and 1.27, respectively. Moreover, the adjusted odds ratios for serum PAB values among women with severe depression and anxiety symptoms were 1.40 and 1.17, respectively.

Conclusion: Symptoms of depression and anxiety appear to be associated with higher degrees of oxidative stress, expressed by higher serum PAB values.

K**eywords**: Depression; Anxiety; Prooxidant-antioxidant balance; Oxidative stress.

1. Introduction

Depression and anxiety are two of the most common mental disorders and are significantly associated with systemic inflammation upregulation indicated by an increased production of pro-inflammatory cytokines and oxidative biomarkers (Duivis et al., 2013; Tayefi et al., 2017). The homeostatic buffering mechanisms regulating inflammation and oxidation in healthy individuals become dysregulated in untreated depression (Rawdin et al., 2013). In this regard, we have recently shown that depression and anxiety are associated not only with serum level of hs-CRP, but also with hematological inflammatory markers including white blood cell (WBC) count and red cell distribution width (RDW) (Shafiee et al., 2017). Such a heightened inflammatory state is accompanied by increased oxidative stress due to alterations in prooxidant-antioxidant balance (PAB), increased generation of reactive oxygen species (ROS), lipid peroxidation and decreased anti-oxidant defenses (Kawanishi et al., 2006; Valko et al., 2006).

Oxidative stress has been implicated in a variety of pathological conditions and traumatic events, including CVD, autoimmune diseases, traumatic brain injury, and others (Ehsaei et al., 2015; Heitzer et al., 2001; Sahebari et al., 2015). Oxidative stress is defined as a serious disturbance in the prooxidant-antioxidant balance in favor of the prooxidants, in which elevated levels of intracellular ROS such as hydrogen peroxide (H_2O_2), superoxide radical (O_2^{o-}) or hydroxyl radical (•OH), contribute to tissue damage (Sies, 1997). Prooxidants are derived either from normal metabolic processes or from external sources such as dietary iron, cigarette smoke, and alcohol (Albano, 2006; Stone et al., 2002; van der Vaart et al., 2004). Antioxidants include the three major lipid-soluble antioxidants (e.g. α-tocopherol and vitamin A), water-soluble antioxidants (e.g. vitamin C, urate, polyphenols, and flavonoids) and the enzymatic antioxidants (e.g. catalase, peroxidase, and superoxide dismutase) (Alamdari et al., 2007). Although various methods have been developed to measure either the total oxidants [e.g. total oxidant status assays (Erel, 2005)] or antioxidants [e.g. the ferric reducing ability of plasma (Benzie and Strain, 1996)], these methods are time-consuming and imprecise due to the need for two separate tests to estimate serum prooxidant-antioxidant balance (PAB) (Alamdari et al., 2007). Therefore, we have previously developed a method in which the balance of oxidants and antioxidants can be measured using 3,3´,5,5´-tetramethylbenzidine (TMB) (Alamdari et al., 2008). The serum PAB assay has the ability to measure the prooxidant burden and the antioxidant capacity

simultaneously in one assay to provide a redox index (Alamdari et al., 2008). A high serum PAB value means that the number of oxidants considerably outweigh the number of antioxidants and may reflect a higher oxidative stress state.

Considering the close association between inflammation and oxidative stress, and the fact that depression and anxiety are linked to inflammation (Berk et al., 2013; Duivis et al., 2013), we hypothesized that more severe depression/anxiety symptoms are associated with more oxidative stress reflected by higher values of serum PAB. In this regard, numerous studies have linked increased measures of oxidative stress in depression and anxiety disorders (Hovatta et al., 2010; Yanik et al., 2004). For instance, Sarandol et al. conducted a study on ninety-six patients with a diagnosis of major depressive disorder (MDD) and 54 healthy controls. Plasma malondialdehyde (MDA) and susceptibility of red blood cells (RBC) to oxidation were found to be higher in the MDD group compared with the control group. Moreover, RBC superoxide dismutase (SOD) was significantly elevated in patients with MDD (Sarandol et al., 2007). In another study, the excretion of urinary F2 isoprostane was observed to be significantly higher in patients with depression than in control subjects, even after adjustment for sex, age and body mass index (BMI) (Chung et al., 2013). Irie et al. found a significant positive correlation between depression scores and the 8-hydroxydeoxyguanosine (8-OH-dG) levels in depressed patients. Since 8-OH-dG is a product of oxidative damage to DNA, the authors suggested that clinical depression can be considered as a risk factor for cancer initiation (Irie et al., 2005). Moreover, Yanik and colleagues also investigated the relationship between the potency of oxidative stress and the severity of depression on 21 patients with MDD and 28 healthy controls. The results showed lower total antioxidant potential of plasma (TAOP) and higher oxidative stress index (OSI) in patients with depression than those in control group (Yanik et al., 2004).

Despite these observations, no studies have evaluated the association between depression/anxiety symptoms and oxidative stress using the serum PAB assay. Therefore, the primary objective of the present study was to examine the cross-sectional association between depression/anxiety symptoms and serum PAB values in a large population-based study.

2. Material and Methods

2.1. Study Population

A total of 7,516 subjects [2,865 (38%) males and 4,651 (62%) females] were derived from a cohort of free living individuals aged 35-65 years from northeastern Iran (Ghayour-Mobarhan et al., 2015). The Mashhad Stroke and Heart Atherosclerotic Disorders (MASHAD) cohort study is a 10-year cohort, which aims to evaluate the impact of various risk factors on the incidence of cardiovascular events among a general urban population (Ghayour-Mobarhan et al., 2015). The first phase of the MASHAD cohort study was started in 2010 and will be completed in 2020. Participants were drawn from three regions in Mashhad using a stratified cluster random sampling technique. Each region was divided into 9 sites centered upon Mashhad Healthcare Center divisions. Once the eligible participants were identified, they were contacted to arrange an appointment for the formal physical examination. Subjects with autoimmune and infectious diseases, pregnant women, and those with a history of stroke or myocardial infarction were not included in our study (Ghayour-Mobarhan et al., 2015). The mean age of men and women were 48.56±8.3 y and 47.48±8.0 y, respectively. All participants gave informed, written consent to contribute in the survey, which was reviewed and approved by the ethics committee of Mashhad University of Medical Sciences (MUMS).

2.2. Data collection and measurements

For all participants, height (cm), weight (kg), and BMI (kg/m^2) were measured based on standard protocols. Body weight was measured to the nearest 0.1 kg with electronic scales, and height was measured to the nearest millimeter with a tape measure. Serum hs-CRP concentration was estimated using an immunoturbidimetric method, with detection limit of 0.06 mg/L (Pars Azmun, Karaj, Iran) (Kazemi-Bajestani et al., 2017). A complete blood cell (CBC) count was also measured for each individual, as described previously (Emamian et al., 2017).

2.3. Chemicals

The chemicals used in this study were including TMB powder (3, 3′, 5, 5′-Tetramethylbenzidine, Fluka), chloramine T trihydrate (Applichem: A4331, Darmstadt, Germany), peroxidase enzyme (Applichem: 230 U/mg, A3791, 0005, Darmstadt, Germany), hydrogen peroxide (30%) (Merck). Molecular biology grade reagents were used and preparations were done in double distilled water.

2.4. PAB assay

As mentioned earlier, the PAB assay is the only test that can measure the balance of oxidants and antioxidants simultaneously in one experiment. PAB values were measured in serum samples using a modified PAB assay we previously developed (Alamdari et al., 2008). In brief, we prepared the standard solutions by mixing varying proportions (0–100%) of 250 μM hydrogen peroxide (H_2O_2) with 3 mM uric acid (in 10 mM NaOH). Next, we dissolved sixty milligrams of TMB powder in 10 mL DMSO. After that, we added 400 μL of the TMB/DMSO to 20 mL of acetate buffer (0.05 M buffer, pH 4.5) in order to prepare TMB cation. Then, 70 μL of fresh chloramine T (100 mM) solution was added to this 20 mL. This was mixed and incubated for 2 hour at room temperature in the dark. Next, 25 U of peroxidase enzyme solution was added to 20 mL of TMB cation solution, dispensed in 1 mL and stored at –20˚C. We added 200 μL of TMB/DMSO to 10 mL of acetate buffer (0.05 M buffer, pH 5.8) to prepare the TMB solution, and the working solution was prepared by mixing 1 mL TMB cation with 10 mL of TMB solution. This solution was also incubated for 2 min at room temperature in the dark and immediately used. Ten microliters (10 μL) of each sample, standard or blank (distilled water) were mixed with 200 μL of working solution in each well of a 96 well plate, which was then incubated in a dark place at 37˚C for 12 min. Once the incubation was finished, 100 μL of 2 N hydrochloric acid (HCL) was added to each well, and the optical density (OD) was measured in an ELISA reader at 450 nm with a reference wavelength of 620 or 570 nm. A standard curve was provided from the values relative to the standard samples. The PAB values are expressed in arbitrary HK unit, which is the percentage of hydrogen peroxide in the standard solution. The values of the unknown samples were then calculated based on the values obtained from the above standard curve.

2.5. Assessment of depression

To assess depression status, the Beck Depression Inventory (BDI) was used (Dozois et al., 1998). This questionnaire contains 21 items each assessed on a zero (lack of depressive symptoms) to three (severe depressive symptoms) scale. Each item represents a single symptom associated with depression including feelings of hopelessness, feelings of guilt, sadness, crying, fear, loss of appetite, and sleep disturbance over the past 2 weeks (Scogin et al., 1988). A score of 0–13 indicates no or

minimal depression, 14–19 mild depression, 20–28 moderate depression and 29-63 severe depression (Scogin et al., 1988). Ghassemzadeh et al. (2005) have validated the Persian (Farsi) translation of this questionnaire with an acceptable internal consistency (Cronbach's alpha = 0.87) and test-retest reliability (r = 0.74) (Ghassemzadeh et al., 2005).

2.6. Assessment of anxiety

Anxiety symptoms were assessed using Beck Anxiety Inventory (BAI) (Beck et al., 1988). The same as BDI, This questionnaire contains 21 items and each assessed on a zero (lack of anxiety symptoms) to three (severe anxiety symptoms) scale. Thus, the total score of the questionnaire ranges from zero to 63. Scores are classified as the following: 0-7 minimal or no anxiety, 8-15 mild anxiety, 16-25 moderate anxiety and over 26 severe anxiety (Beck et al., 1988). Kaviani and Mousavi showed that the Persian (Farsi) version of BAI has reasonable reliability ($r = 0.83$, $P < 0.001$), validity ($r = 0.72$, $P < 0.001$), and an appropriate internal consistency (Alpha = 0.92) (Kaviani and Mousavi, 2008).

2.7. Statistical analysis

Data analysis was carried out using SPSS-18 software (SPSS Inc., IL, USA). The normality of data was evaluated using Kolmogorov–Smirnov test. Descriptive statistics including mean, frequency, and standard deviation (SD) were determined for all variables and expressed as mean ± SD for normally distributed variables and as median and interquartile range (IQR) for non-normally distributed variables. Chi-square tests were used to compare the qualitative variables. For normally distributed variables, analysis of variance (ANOVA) was performed. Serum PAB was logarithmically transformed (log10) before the analysis to give a normal distribution. The Mann-Whitney U test was used for serum hs-CRP since it was found to be a non-normal variable even after logarithmically transformed. All the analyses were two-sided and p-value <0.05 was considered as significant. Depression and anxiety scores were divided into categories according to their severity and participants in the first group (no or minimal depression or anxiety) were considered as a reference group. Multinomial logistic regression was used to estimate the risk, as approximated by the odds ratio (OR). The odds ratios, with 95% confidence intervals (CI), were obtained using multivariate logistic regression in order to determine the influence of potential confounding factors, e.g. age, current smoking, education level, job status, BMI, WBC count, RDW, and hs-CRP. β-coefficients were calculated using univariate and multivariate linear

regression models to assess the association between depression/anxiety scores and PAB values. We used an interaction term to investigate whether the association between depression symptoms and PAB values differed according to sex. The regression lines were calculated by using simple scatter plots.

3. Results

Among the 7,516 individuals, the average age was 47.9±8.1, with 62% being female. The mean age of premenopausal and menopausal women were 42.6±5.1 and 55.2±5.4, respectively (unshown data). Demographic and biochemical characteristics of the study population are presented in Table 1. Participants were stratified into four groups according to their depression and anxiety scores: "no or minimal", "mild", "moderate", and "severe". As given in Table 1, the number of individuals with no or minimal, mild, moderate and severe depression symptoms were 4671 (62.2%), 1309 (17.4%), 992 (13.2%), and 544 (7.2%), respectively. In addition, the number of subjects with no or minimal, mild, moderate and severe anxiety symptoms were 3606 (48.0%), 2066 (27.5%), 1121 (14.9%), 723 (9.6%), respectively. Regarding literacy level, 13.7% (n=1033) were illiterate, 41.7% (n=3134) had primary education, 33.8% (n=2539) had secondary education and 10.8% (n=810) had tertiary (or higher) education (unshown data). There were no significant differences in age between groups with different categories of depression and anxiety symptoms (p=0.2 and p=0.4, respectively). The proportion of females, current smokers, illiterates, and the unemployed increased significantly across the four groups as shown. Moreover, BMI, WBC count, RDW and hs-CRP also significantly increased with the severity of depression and anxiety symptoms (Table 1).

The association between depression symptoms and PAB values differed according to sex (β= -0.07, $P_{(interaction)}$ = 0.05). PAB values (log transformed) showed a significant stepwise increase from the "no or minimal" to the "mild", "moderate" and "severe" groups (Table 2). PAB values significantly increased with the severity of depressive symptoms among both men and women (P_{trend}<0.001 and P_{trend}=0.005, respectively). Concerning anxiety, PAB values increased significantly across the four groups among men (P_{trend}=0.02) but not women (P_{trend}=0.2).

In all multivariate analyses, the group who had normal scores for depression, or anxiety, served as a reference group (Table 3). In men, the adjusted odds ratios (95% confidence interval) for serum

PAB values (log transformed) across categories of depression were 1.00, 1.18 (0.92-1.50), 1.92 (1.40-2.63), and 1.75 (1.09-2.79). The adjusted odds ratios (95% confidence interval) for PAB values (log transformed) across categories of anxiety were 1.00, 1.12 (0.92-1.37), 1.36 (1.02-1.82), and 1.27 (0.86-1.88), among men. Furthermore, in women, the adjusted odds ratios (95% confidence interval) for PAB values across categories of depression were 1.00, 1.14 (0.95-1.37), 1.25 (1.03-1.53), and 1.40 (1.08-1.80). Concerning anxiety, these values were 1.00, 1.11 (0.94-1.31), 1.07 (0.88-1.30), and 1.17 (0.93-1.46) (Table 3).

The β-coefficients examining the association of depression/anxiety scores with serum PAB values (log transformed) are presented in Table 4. Examination of the β-coefficients also indicated that depression and anxiety symptoms are associated with serum PAB values, among men [$\beta=2.00$ ($p<0.001$) and $\beta=1.30$ ($p<0.001$), respectively] and women [$\beta=1.07$ ($p=0.001$) and $\beta=0.54$ ($p=0.1$), respectively]. These associations remained significant even after adjustment for age, current smoking habit, education level, job status, BMI, WBC count, RDW, and hs-CRP, especially among men (Table 4). The regression lines of predicted change in serum PAB for change in depression and anxiety scores are illustrated in Figure 1 and 2, respectively.

4. Discussion

Our results suggest that higher depression and anxiety symptoms may be associated with altered oxidative stress status, expressed by higher PAB values. However, this association was stronger for depression than in anxiety and in men than in women.

Some previous studies have reported similar association between depression and increased markers of oxidative stress including MDA (Khanzode et al., 2003; Rybka et al., 2013; Talarowska et al., 2012), isoprostanes (Dimopoulos et al., 2008; Milaneschi et al., 2013; Yager et al., 2010) and 8-OHdG (Forlenza and Miller, 2006; Maes et al., 2009). Higher concentrations of plasma MDA were shown to be associated with the severity of depressive symptoms, not only at the baseline, but also after 8 weeks of antidepressants pharmacotherapy (Talarowska et al., 2012). Rybka et al. also found increased concentrations of MDA and H_2O_2 as well as more DNA damage in patients diagnosed with recurrent depressive disorder, when compared with healthy controls (Rybka et al., 2013). In a

prospective study conducted by Khanzode and colleagues, a significant increase in serum MDA, serum SOD and decrease in plasma ascorbic acid levels was observed in patients with major depression as compared with control participants. However, this trend was significantly reversed after treatment with current antidepressants including citalopram and fluoxetine (Khanzode et al., 2003). As another marker of lipid peroxidation, elevated serum (Yager et al., 2010), plasma (Dimopoulos et al., 2008), and urinary (Milaneschi et al., 2013) concentrations of 8-iso-PGF2α were also observed in depressed individuals compared to a healthy comparison group. However, Milaneschi et al. found this association only in men. Moreover, as a biological marker of oxidative damage to DNA, serum (Forlenza and Miller, 2006) and urinary (Maes et al., 2009) levels of 8-OHdG were also significantly higher in patients suffering from major depression, compared to healthy controls. However, Jorgensen et al. reported that systemic RNA, but not DNA, damage from oxidation, as measured by 8-oxo-7,8-dihydroguanosine (8-oxoGuo) excretion, is positively associated with the severity of depression (Jorgensen et al., 2013). Thus, there is a positive association between depression and markers of oxidative stress; however, there might be an association between depression and antioxidant defense biomarkers, as well.

There are inconsistent results regarding the association of depression with antioxidant defense biomarkers such as SOD and glutathione. While some authors observed decreased RBC SOD activity (Rybka et al., 2013) and lower serum SOD levels in patients diagnosed with recurrent depressive disorder (Stefanescu and Ciobica, 2012), others reported increased RBC SOD in depressed patients (Galecki et al., 2009; Sarandol et al., 2007). Similarly, RBC glutathione peroxidase (GPx) activity has been reported to be higher (Bilici et al., 2001), lower (Kodydkova et al., 2009), or the same (Galecki et al., 2009) in depressed individuals when compared to healthy controls. For example, Stefanescu et al. reported a significant decrease of both SOD and GPx specific activities in the serum of patients with MDD, as compared to the control group. Furthermore, the results showed that recurrent depressive patients have significantly lower SOD and GPx specific activities, when compared with first episode group (Stefanescu and Ciobica, 2012). In another study, depressed patients had significantly higher levels of copper-zinc SOD and catalase (CAT), as compared to control subjects, and there were no significant differences in activity levels of GPx between groups (Galecki et al., 2009). Bilici and colleagues also found higher antioxidative enzyme activities in depressed patients in comparison to

healthy controls, which was significantly decreased to normal levels following three months of treatment with selective serotonin reuptake inhibitors (SSRIs) (Bilici et al., 2001). Therefore, depression is also associated with disturbed antioxidant defense biomarkers.

Furthermore, a limited number of studies have also reported a significant positive association between severity of depression and serum oxidative stress index (OSI), defined as the ratio of total oxidant status (TOS) to total antioxidant capacity (TAC) (Cumurcu et al., 2009; Gupta et al., 2016; Yanik et al., 2004). Cumurcu et al. conducted a study on 55 MDD patients and 40 healthy controls and found significantly higher OSI in the MDD group compared with those of the controls. Furthermore, the results showed a significant positive correlation between the serum OSI and the severity of the disease (Cumurcu et al., 2009). In another study, OSI was determined in 101 cases with MDD along with 106 age and sex matched controls. Similarly, a significant increase in OSI was reported in patients with MDD when compared to healthy controls, which was associated with the severity of the disease (Gupta et al., 2016). Yanik et al. observed a significantly higher OSI values in MDD patients than those of controls as well as a significant positive correlation between OSI values and Hamilton Depression Rating Scale (HDRS) (Yanik et al., 2004). Thus, considering our results, and the findings of others, we conclude that depression is associated with a disturbance in the balance between pro-oxidants and antioxidants.

There are also several reports of disturbed oxidative status among patients with anxiety disorders such as panic disorder (Kuloglu et al., 2002), social phobia (Atmaca et al., 2008), and obsessive compulsive disorder (Ozdemir et al., 2009). A study conducted on twenty patients diagnosed with panic disorder and twenty healthy controls showed a significantly higher SOD, GPx and MDA levels in the patients group compared to normal control group (Kuloglu et al., 2002). Compared to the control group, patients diagnosed with social phobia have also showed significantly higher MDA, SOD, GPx and CAT levels. Furthermore, a positive correlation was reported between Liebowitz Social Anxiety Scale (LSAC) scores and MDA, SOD, GPx and CAT levels (Atmaca et al., 2008). In another study, the levels of MDA and SOD were reported to be significantly higher in patients with obsessive-compulsive disorder than age-and sex-matched controls. However, in comparison to healthy subjects,

the activities of GPx and CAT were significantly lower in patients (Ozdemir et al., 2009). Therefore, depression and anxiety are both associated with an altered oxidative stress status.

We found that the association between depression/anxiety symptoms and serum PAB values were stronger among men than in women. We have previously shown that the relationship between depression/anxiety symptoms and inflammatory markers such as hs-CRP, WBC count, and RDW is stronger among men than in women (Shafiee et al., 2017; Tabatabaeizadeh et al., 2018; Tayefi et al., 2017). This weaker association in women may be partly explained by antioxidant (Sugioka et al., 1987) and anti-inflammatory (Vegeto et al., 2008) properties of estrogen in premenopausal women. Depression symptoms reduced in women with postpartum depression who had estradiol deficiency by treatment with 17β-estradiol (Ahokas et al., 2001). The antioxidant action has been considered as a potential mechanism to explain the complex neuroprotective effect of estrogens (Prokai-Tatrai et al., 2008). Moreover, in their review of the literature, Vegeto et al. concluded that estrogen anti-inflammatory action plays a prominent role in protecting the central nervous system against neurotoxic stimuli (Vegeto et al., 2008). Thus, we assume that the antioxidant and anti-inflammatory properties of estrogen may alleviate the heightened oxidative and inflammatory state in premenopausal women with depression and anxiety.

The present study is the first to examine the association of depression/anxiety symptoms with serum PAB values, as a marker of oxidative stress. The strengths of our study include a large population-based sample, using validated self-administered questionnaires and using a sex-stratified analysis. We also controlled for potential confounders such as age, current smoking habit, education level, job status, BMI, WBC count, RDW, and hs-CRP. We also acknowledge some limitations in our study, including: (a) the possibility of misclassification of depression/anxiety due to the use of self-administered tools instead of more accurate face-to-face interviews, and (b) the fact that we have measured both depression/anxiety symptoms and PAB values at baseline and we cannot draw any conclusions about causality; whether the oxidative stress preceded the depression/anxiety or vice-versa. However, the MASHAD study is a longitudinal cohort and will be continued for at least a decade. We therefore intend to analyze the relationship between aggravation of depression/anxiety and baseline PAB values.

In conclusion, this study showed a positive association between depression/anxiety symptoms and PAB values, which may be an indicator of higher oxidative stress in patients with depression and anxiety. However, this association was much stronger in depression than in anxiety and in men than in women. However, further studies are needed to clarify the direction of the relationship between depression/anxiety and oxidative stress.

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Table Legends

Table 1. Values are expressed as mean±SD for variables with normal distribution, and median and interquartile range for hs-CRP as a non-normally distributed variable. Categorical data are expressed as number (percentage). BMI: body mass index; WBC: white blood cell; RDW: red cell distribution width; hs-CRP: high sensitivity C-reactive protein. *P<0.05; **P<0.01; ***P<0.001 (refer to overall chi-square, ANOVA and Mann-Whitney tests p-values).

Table 2. Values are expressed as mean±SD.

Table 3. Odds ratios with 95% confidence intervals (95% CI) obtained from multiple logistic regression test adjusted for potential confounders (i.e. age, current smoking habit, education level, job status, BMI, WBC count, RDW, and hs-CRP).

Table 4. β-coefficients obtained from linear regressions both crude and adjusted for potential confounders (i.e. age, current smoking habit, education level, job status, BMI, WBC count, RDW, and hs-CRP).

Table 1. Demographic and biochemical characteristics of individuals in groups of Depression and Anxiety.

	Depression severity score				**Anxiety severity score**			
	No or minimal N=4671	**Mild** N=1309	**Moderate** N=992	**Severe** N=544	**No or minimal** N=3606	**Mild** N=2066	**Moderate** N=1121	**Severe** N=723
Sex (Female) n(%)	2645 (56.6)	885 (67.6)	698 (70.4)	423 (77.8)***	1923 (53.3)	1347 (65.2)	817 (72.9)	564 (78.0)***
Current smoking n(%)	895 (19.2)	298 (22.8)	249 (25.1)	172 (31.6)***	681 (18.9)	455 (22.0)	266 (23.7)	212 (29.3)***
Illiterate n(%)	597 (12.8)	197 (15.0)	145 (14.6)	94 (17.2)***	455 (12.6)	273 (13.2)	178 (15.8)	127 (17.5)***
Unemployed n(%)	2388 (51.1)	780 (59.6)	624 (63.0)	401 (74.0)***	1728 (48.0)	1200 (58.1)	746 (66.7)	517 (71.5)***
Age (year)	47.7±8.1	48.3±7.9	47.9±8.4	48.0±8.0	47.9±8.1	47.8±8.1	48.1±8.2	47.6±8.0
BMI (kg/m^2)	27.6±4.6	28.2±4.6	28.2±5.0	28.7±5.1***	27.4±4.5	27.9±4.6	28.6±5.0	28.7±5.0***
WBC ($10^9/L$)	6.0±1.4	6.1±1.6	6.1±1.6	6.2±1.7**	6.0±1.4	6.0±1.6	6.0±1.5	6.2±1.6*
RDW (fl)	41.5±3.2	41.7±2.9	41.8±3.0	42.2±3.2***	41.4±3.2	41.7±3.1	41.7±3.0	42.0±3.2***
Serum hs-CRP (mg/L)	1.47 (0.94-3.05)	1.69 (0.98-3.63)	1.69 (1.00-3.91)	1.79 (1.02-4.40)***	1.42 (0.93-2.90)	1.65 (0.99-3.61)	1.65 (1.01-3.70)	1.85 (1.04-4.45)***

Table 2. Pro-oxidant-antioxidant balance (PAB) in groups of Depression and Anxiety.

		Depression severity score				ANOVA		Anxiety severity score				ANOVA	
		No or minimal N=4671	**Mild** N=1309	**Moderate** N=992	**Severe** N=544	**F**	**p-value**	**No or minimal** N=3606	**Mild** N=2066	**Moderate** N=1121	**Severe** N=723	**F**	**p-value**
Log PAB	**Males**	1.55±0.47	1.59±0.47	1.69±0.38	1.68±0.38***	9.6	<0.001	1.56±0.47	1.59±0.45	1.63±0.43	1.63±0.43*	3.2	0.02
	Females	1.70±0.45	1.73±0.44	1.75±0.44	1.76±0.40**	4.3	0.005	1.70±0.45	1.73±0.44	1.72±0.44	1.73±0.44	1.4	0.2

Table 3. Adjusted odds ratios of having mild, moderate or severe depression or anxiety symptoms associated with pro-oxidant-antioxidant balance (PAB) among men and women.

		Depression severity score														
		Reference group and mildly affected group					Reference group and moderately affected group					Reference group and severely affected group				
		β	**Wald**	**Sig.**	**OR**	**CI**	**β**	**Wald**	**Sig.**	**OR**	**CI**	**β**	**Wald**	**Sig.**	**OR**	**CI**
Log PAB (Adjusted)	Males	0.16	1.75	0.2	1.18	0.92-1.50	0.65	16.3	<0.001	1.92	1.40-2.63	0.56	5.50	0.02	1.75	1.09-2.79
	Females	0.13	2.20	0.1	1.14	0.95-1.37	0.22	4.96	0.02	1.25	1.03-1.53	0.33	6.78	0.009	1.40	1.08-1.80
		Anxiety severity score														
		Reference group and mildly affected group					Reference group and moderately affected group					Reference group and severely affected group				
		β	**Wald**	**Sig.**	**OR**	**CI**	**β**	**Wald**	**Sig.**	**OR**	**CI**	**β**	**Wald**	**Sig.**	**OR**	**CI**
Log PAB (Adjusted)	Males	0.11	1.30	0.2	1.12	0.92-1.37	0.31	4.36	0.04	1.36	1.02-1.82	0.24	1.55	0.2	1.27	0.86-1.88
	Females	0.10	1.67	0.2	1.11	0.94-1.31	0.07	0.53	0.4	1.07	0.88-1.30	0.15	1.86	0.1	1.17	0.93-1.46

Table 4. β-coefficients examining the association of depression/anxiety scores with pro-oxidant-antioxidant balance (PAB).

		Depression score			Anxiety score		
		β	**t**	**Sig.**	**β**	**t**	**Sig.**
Log PAB (Crude)	Males	2.00	5.5	<0.001	1.30	3.8	<0.001
	Females	1.07	3.2	0.001	0.54	1.5	0.1
Log PAB (Adjusted)	Males	1.60	4.4	<0.001	1.04	3.0	0.003
	Females	0.94	2.8	0.005	0.45	1.3	0.2