SAGE Open Medicine
Volume 6: 1–7

DOI: 10.1177/2050312118773953
journals.sagepub.com/home/smo
SAGE

Elevated serum levels of malondialdehyde and cortisol are associated with major depressive disorder: A case-control study

Md Rabiul Islam[1], Md Reazul Islam[1], Imtiaz Ahmed[2], Abdullah Al Moktadir[3], Zabun Nahar[4], Mohammad Safiqul Islam[5], Shelina Fatema Binte Shahid[6], Sheikh Nazrul Islam[7], Md Saiful Islam[1] and Abul Hasnat[1]

Abstract

Objectives: Major depressive disorder is diagnosed on the basis of patient's self-reported experiences, behavior reported by relatives, and a mental status examination, and yet we do not have any reliable biomarker for this. Mood-regulating pathways are affected by oxidative injury to lipids and cortisol is released into the blood due to stimulation of corticotrophin receptors in the adrenal cortex. Here, we aimed to determine serum levels of malondialdehyde and cortisol in major depressive disorder patients and controls.

Methods: We collected blood samples from 247 major depressive disorder patients and 248 controls. Serum levels of malondialdehyde and cortisol were measured by ultraviolet spectrophotometry and enzyme-linked immunosorbent assay kit, respectively.

Results: We found malondialdehyde levels were significantly higher in patients than controls, with mean ± standard deviation at 4.49 ± 1.37 and 2.87 ± 0.82 µmol/L, respectively, $p < 0.001$. Cortisol levels were also found significantly higher in patients than controls, with mean ± SD at 19.22 ± 1.64 and 17.37 ± 1.34 µg/dL, respectively, $p < 0.001$. Significant negative correlation was observed between serum levels of malondialdehyde and cortisol in patients ($r = -0.170$, $p = 0.021$). Receiver operating characteristic analysis showed good diagnostic value for malondialdehyde and cortisol, with the area under the curve at 0.853 and 0.819, respectively.

Conclusion: The present study suggests that increased serum levels of malondialdehyde and cortisol are strongly associated with major depressive disorder. We believe elevations of malondialdehyde and cortisol in serum level arise independently and they could serve as biomarkers for major depressive disorder.

Keywords

Major depressive disorder, serum, malondialdehyde, cortisol, MDD, MDA

Date received: 23 January 2018; accepted: 8 April 2018

Introduction

Major depressive disorder (MDD) is a highly prevalent mental disorder. According to the World Health Organization (WHO) report, depression is predicted to be the second leading disease in the world by 2020.[1] A person's family, work, and personal life are adversely affected by low self-esteem and loss of interest or pleasure in day-to-day activities due to MDD.[2] As depression gives the enormous problems on a person's life, wide-ranging efforts have been placed to divulge the organic mechanisms tangled in MDD.[3] Moreover, diagnosis of depression generally rely on the reports of patients or their relatives, which lead to prejudice and confuse independent explanations for this illness.[4] Therefore, the necessity for biomarkers has become important for many reasons. For example, a trustworthy biomarker can help to diagnose MDD

[1]Department of Clinical Pharmacy and Pharmacology, Faculty of Pharmacy, University of Dhaka, Dhaka, Bangladesh
[2]Department of Pharmaceutical Sciences, School of Health & Life Sciences, North South University, Dhaka, Bangladesh
[3]Department of Pharmacy, Jahangirnagar University, Dhaka, Bangladesh
[4]Department of Pharmacy, University of Asia Pacific, Dhaka, Bangladesh
[5]Department of Pharmacy, Noakhali Science and Technology University, Noakhali, Bangladesh
[6]Department of Psychiatry, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh
[7]Institute of Nutrition and Food Science, University of Dhaka, Dhaka, Bangladesh

Corresponding author:
Md Rabiul Islam, Department of Clinical Pharmacy and Pharmacology, Faculty of Pharmacy, University of Dhaka, Dhaka 1000, Bangladesh.
Email: robi.ayaan@gmail.com

patients precisely as well as it can also be helpful to understand the mechanism of depression. In addition to these, peripheral blood samples are more practical and convenient than tissue or other samples to identify possible biomarkers for any disease.[5]

Interleukins, microRNAs, cytokines, oxidative stress, malondialdehyde (MDA), the hypothalamic–pituitary–adrenal (HPA) axis activities, catabolites of tryptophan, and antioxidant enzyme activities have been analyzed to identify biomarkers of depression.[6–13] MDA and cortisol measurements are comparatively easy and not expensive in the peripheral blood sample among above-mentioned parameters. A recent case-control study revealed that MDA could be a good biomarker candidate for MDD.[8] The HPA axis is the key regulating systems for stress responses that form major pathways for symptoms of depression.[14] Cortisol is released into the blood due to stimulation of corticotrophin receptors in the adrenal cortex.[15] Thus, serum cortisol may be involved in the pathogenesis of depression.

Many researchers consider MDA as a key factor for oxidative stress. MDA is the end product of lipid peroxidation that can be used as a marker for oxidative stress.[16] Reactive oxygen species (ROS) involved in many neuropsychiatric diseases as our brain can be damaged by ROS due to its high metabolic rate.[17] Excess lipid peroxidation occurred due to the increase of ROS in the oxidative process that ultimately causes tissue damage.[18] For these reasons, MDA has got interest, and a number of researches have measured serum MDA levels in depression. Many of these works showed elevated MDA levels in major depression.[8,19]

The HPA axis plays an important role in the maintenance of homeostasis in the face of stress. It is well described that the functional changes of the HPA axis occurred in depression.[20] Furthermore, early life exposure to stress has been identified as a causative factor for HPA dysfunction.[20–22] Central nervous system (CNS) and different tissues get exposure to physiologically active glucocorticoids in depressed patients. Unbound serum cortisol level is correlated with the cerebrospinal fluid (CSF) free cortisol levels. The presence of severe depressive symptoms in MDD patients is due to the extreme CNS exposure to glucocorticoids.[23]

As MDA is a biomarker for oxidative stress and cortisol is a stress hormone, elevation for both of these parameters in serum level increases the risk of depression.[24,25] MDA and cortisol levels have been measured in many studies in depressed patients to draw a deduction for neurobiology but studies targeting the diagnostic values of these parameters are limited. In the present study, we aimed to investigate MDA and cortisol levels in MDD patients and control subjects. After that, our focus was on the diagnostic value of the up-regulated MDA and cortisol. We anticipated decent diagnostic test value of these parameters due to the large study population.

Methods

Study population

It was assumed that exposed controls and alpha risk will be 20% and 5%, respectively. We designed 1:1 matched case-control study to detect an odds ratio of 2 with power 90%.[26] Based on this assumption, the estimated sample size was supposed to be 452 (226 cases and 226 controls). This case-control study enrolled 247 MDD patients and 248 control subjects. The patients were recruited from the Department of Psychiatry, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka, Bangladesh but the control subjects were recruited from different parts of Dhaka city matched by age, gender, and body mass index (BMI) with the patients. Qualified psychiatrists diagnosed all the patients and evaluated controls according to the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-5) using the Structured Clinical Interview for DSM-5 (SCID-5). Diagnoses of the coexistence of other complications were performed by detailed physical and neurological screenings. Patients with comorbid psychiatric disorders and mental retardation were excluded from the study. Additional exclusion criteria were substance abuse or dependency, chronic physical illness or abnormal BMI, and presence of infectious diseases. The study population had not been treated with any medication that could interfere with the serum levels of MDA and cortisol. Also, the study population had no earlier evidence of liver or kidney failure. Pre-designed questionnaires were used to record socio-demographic data. Diverse biographical features (weight, height) and BMI were also measured for the study population.

Blood sample collection

Between 10:00 a.m. and 12:00 p.m. after an overnight fast of 8–10 h, blood samples (5 mL) were collected from the cephalic vein of each participant. The samples were then permitted to clot for 1 h at room temperature. After centrifugation at 3000 r/min for 15 min, serum samples were taken out from the collected blood samples, placed into microtubes and stored at −80°C until analysis.

Quantification of serum MDA and cortisol

The concentration of serum MDA was estimated according to our previously published method using thiobarbituric acid (TBA) reagent and the absorbance of the supernatant was measured spectrophotometrically at 530 nm.[27,28] Serum cortisol was measured using commercially available enzyme-linked immunosorbent assay (ELISA) kit (Diagnostics Biochem Canada Inc.) according to the manufacturer's instructions. The assay was based on competitive binding of antibodies. The ELISA kit consisted of a number of plates with 96 microtiter wells on each plate. All of the microtiter

Table 1. The characteristics of the study population.

Parameter	Patient group (n = 247)	Control group (n = 248)	*p* value
Age in years, mean ± SD	33.03 ± 10.89	33.55 ± 9.58	0.576[a]
BMI (kg/m^2), mean ± SD	22.82 ± 2.53	23.15 ± 3.01	0.193[a]
Gender, male/female	91/156	102/146	0.407[b]

SD: standard deviation; BMI: body mass index.
$p < 0.05$ (the significant difference between patient and control groups at 95% confidence interval).
[a]*p* values from the t-test.
[b]*p* value from unpaired t-test.

Table 2. Laboratory and clinical outcomes of MDD patients and control subjects.

Parameter	Patient group (n = 247)	Control group (n = 248)	*p* value
	Mean ± SD	Mean ± SD	
Serum MDA (μmol/L)	4.49 ± 1.37	2.87 ± 0.82	< 0.001*
Serum cortisol (μg/dL)	19.22 ± 1.64	17.37 ± 1.34	< 0.001*

MDD: major depressive disorder; SD: standard deviation; MDA: malondialdehyde.
*$p < 0.05$ (significant difference between patient and control groups at 95% confidence interval).

wells were coated with antibodies directed toward an antigenic site on the cortisol molecule. The concentrations of MDA and cortisol were expressed as μmol/L and μgm/dL, respectively.

Statistical analysis

Statistical analysis was performed using SPSS statistical software, version 23.0 (IBM Corp., Armonk, NY). A p value of less than 0.05 was considered to be statistically significant. As the descriptive statistics for normally distributed variables, the mean and standard deviation (SD) error were used. Comparison between cases and controls of analyzed parameters were shown by independent sample t-tests. Pearson's correlation test was used to establish correlations among different study parameters. Serum levels of MDA and cortisol were presented as the mean ± SD. Receiver operating characteristic (ROC) curve was drawn for the identification of cut-off point.

Results

General description of the study groups

The characteristics of the study population have been presented in Table 1. MDD patients and their corresponding controls were alike in terms of age (patients: 33.03 ± 10.89, controls: 33.55 ± 9.58, $p = 0.576$), BMI (patients: 22.82 ± 2.53, controls: 23.15 ± 3.01, $p = 0.193$), and sex (male/female: 91/156, 102/146 patients and controls correspondingly, $p = 0.407$). Female comprised the higher percentage in both patients and control groups (63% and 59%, respectively). BMI values were normal for 84% patients and 78% control subjects.

Biomarker level differences among patients and control subjects

Serum levels of MDA and cortisol for study population were presented in Table 2. We observed MDD patients showed significantly elevated serum levels of MDA than controls, with mean ± SD at 4.49 ± 0.13 and 2.87 ± 0.08 μmol/L, respectively, $p < 0.001$. Cortisol levels were also significantly higher in patients than controls, with mean ± SD at 19.22 ± 0.28 and 17.37 ± 0.40 μg/dL, respectively, $p < 0.001$.

Relation among various research parameters in study population

Pearson's correlation was used to establish correlations among various research parameters in patient and control groups (Table 3). A significant negative correlation was observed between serum levels of MDA and cortisol in depressed patients ($r = -0.170$, $p = 0.021$). Pearson's correlation coefficient suggested that there was no significant correlation between elevated serum levels of MDA and cortisol with the socio-demographic status of the patients ($p > 0.05$). We found significant positive correlations of serum MDA and cortisol level with the number of depressive symptoms present in the patients according to DSM-5 (Figure 1).

Diagnostic performance of investigated biomarkers

The ROC curves of MDA and cortisol were plotted, and the cut-off points for diagnostic measures were determined as 3.40 μmol/L and 17.85 μg/dL, respectively (Figures 2 and 3). The area under the ROC curve (AUC) was 0.853 for MDA

and 0.819 for cortisol, both were significant ($p<0.001$). Higher values were assigned as the disease state. ROC analysis revealed that the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were 80.6%, 70.6%, 73.2%, and 78.5% for MDA, while those were 74.1%, 64.9%, 67.8%, and 71.6% for cortisol, respectively.

Discussion

As per our knowledge, this is the first work to explore the diagnostic potential of serum MDA and cortisol in Bangladeshi depressed patients. The key results of our research are serum levels of MDA and cortisol significantly increased in MDD patients compared with control subjects. Moreover, we found significant diagnostic values for elevated serum levels of MDA (AUC: 0.853, confidence interval (CI): 0.818–0.887, and $p<0.001$) and cortisol (AUC: 0.819, CI: 0.783–0.856, and $p<0.001$).

Serum MDA levels have been examined in various groups of MDD patients and most of the studies found an increased level of MDA in depression.[29–31] For example, Camkurt et al.[8] engaged 50 patients (drug-naïve, smoking-free, alcohol-free) and 50 control subjects in their study and found the significant elevation of MDA in major depression. Bilici et al.[32] included 30 depressed patients and 32 controls for their study and found the significant elevation of MDA in depression. Meta-analyses summarize current knowledge regarding lipid peroxidation markers in clinical samples of MDD and the effects of antidepressant pharmacotherapy on those markers.[33,34] Likewise, many researches stated elevated MDA levels in depression and a reduction after a successful antidepressant therapy. Mazereeuw et al.[34] reported elevated serum MDA levels in MDD patients and that can be normalized by antidepressants therapy. Galecki et al. studied MDA levels before and after fluoxetine therapy. They recruited 50 MDD patients and 30 control subjects and their conclusion was that fluoxetine therapy significantly lowers MDA levels in depression.[35] In another study, the effects of citalopram and fluoxetine on MDA levels in depression have been evaluated by Khanzode et al. The outcomes of this study also showed significant reduction in MDA levels after antidepressant therapy.[36] Elevated serum MDA levels implicate increased lipid peroxidation products in MDD. Higher levels of MDA and lower levels of antioxidants associate the high degree of oxidative stress in depression.[33] These results suggest that oxidative stress plays a major role in developing depression and that can be alleviated by improving the stressed condition or antidepressant treatment.[34]

Moreover, high level of free serum cortisol is a risk factor for major depression.[37] Hyperactivity of the HPA axis and increased cortisol levels are characteristic of the pathophysiology of MDD.[38] Several studies investigated the serum levels of cortisol in MDD patients and found elevated levels in most of the cases.[39,40] Ahmed et al.[41] investigated

Table 3. Pearson correlation among various research parameters in study population.

Correlation parameters	Patient group (n = 247)		Control group (n = 248)	
	r	*p*	*r*	*p*
Age and cortisol	−0.032	0.666	−0.009	0.928
BMI and cortisol	−0.014	0.845	−0.061	0.546
Education and cortisol	−0.079	0.228	−0.032	0.753
Income and cortisol	−0.051	0.448	−0.096	0.340
Smoking and cortisol	0.024	0.743	−0.176	0.081
Age and MDA	0.072	0.262	0.038	0.551
BMI and MDA	0.101	0.113	−0.118	0.064
Education and MDA	−0.073	0.252	0.086	0.177
Income and MDA	−0.001	0.983	0.078	0.224
Smoking and MDA	−0.108	0.090	−0.067	0.296
MDA and cortisol	−0.170	0.021*	−0.097	0.337

BMI: body mass index; MDA: malondialdehyde; *r*: correlation coefficient.
*p value significance.
Negative values specify opposite correlation. Correlation is significant at p values less than 0.05 (two-tailed).

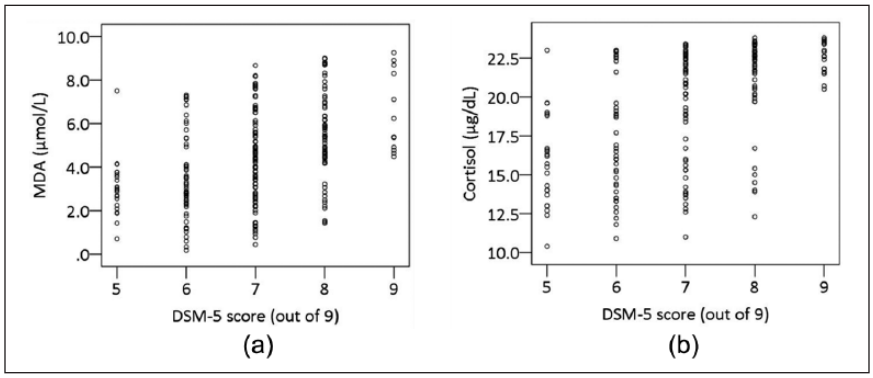

Figure 1. Scatter plot of serum levels of MDA and cortisol in relation to DSM-5 criteria in MDD patients: (a) MDA and (b) cortisol.
DSM-5: Diagnostic and Statistical Manual of Mental Disorders, 5th Edition.

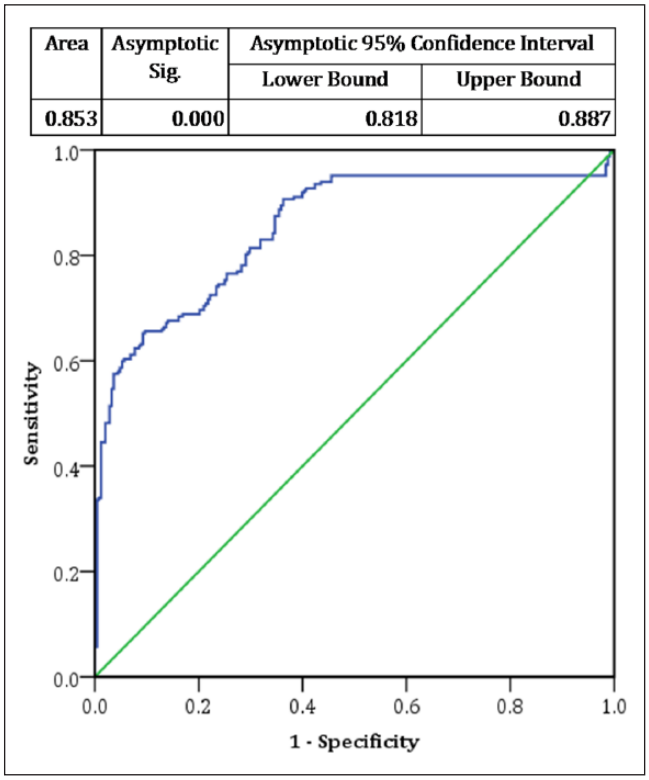

Figure 2. Receiver operating characteristic (ROC) curve for MDA. The cut-off point was detected as 3.40 µmol/L.

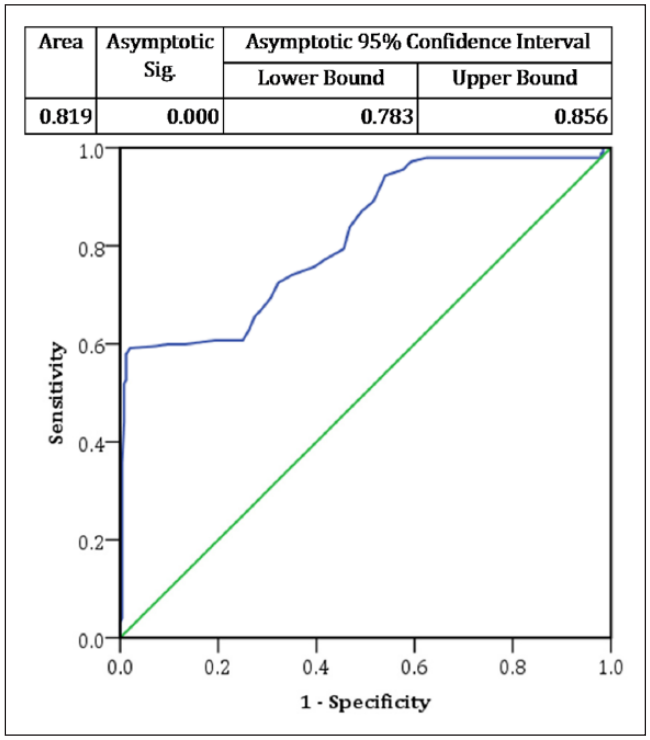

Figure 3. Receiver operating characteristic (ROC) curve for cortisol. The cut-off point was detected as 17.85 µg/dL.

serum cortisol levels in 20 MDD patients and 20 healthy controls and found significantly increased levels of cortisol in depression. Another study by Cubała and Landowski,[42] they recruited 20 treatment-naïve non-late-life MDD patients and in 20 age- and sex-matched healthy controls in their cross-sectional case-control study and found a significantly higher concentration of cortisol in patients as compared to controls. Similarly, many studies showed significant upregulation of serum cortisol in depression and a downregulation after antidepressant therapy. Piwowarska et al. reported increased serum cortisol levels in MDD patients can be normalized by fluoxetine therapy. The study included 21 patients and 24 healthy comparison subjects.[43] In another study, Piwowarska et al. explored serum cortisol levels before and after therapy with clomipramine and they claimed that clomipramine therapy significantly lowers cortisol levels in MDD patients ($p<0.046$). Their study involved 17 MDD patients and 21 control subjects.[38] Unluckily, not any of the above studies enrolled a large number of samples to investigate serum levels of MDA and cortisol in MDD patients. We believe that our study with very large sample size provides more reliable outcome than before.

Finding diagnostic markers of psychiatric illness is an interesting area of research. In many earlier studies, peripheral biomarkers were planned for different psychiatric diseases. Fındıklı et al.[44] demonstrated that serum levels of G protein-coupled estrogen receptor 1 (GPER1) had a prognostic value for the existence of anxiety and GPER1 receptor action can be an applicant marker for generalized anxiety disorder (GAD). Bulut et al.[45] examined paraoxonase action in patients with GAD, and the AUC value was 0.980. Camkurt et al.[8] indicated an AUC value of 1.0 for MDA in MDD patients. Moreover, Güneş et al.[46] identified that prolidase was a decent marker for schizophrenia (AUC: 1.0). Selek et al.[47] also stated very good diagnostic performance for both prolidase (AUC: 0.989) and catalase (AUC: 0.989). Now, a rising body of evidence occurs concerning the diagnostic performance of many peripheral biomarkers in mental illness. From this point of view, our study found that MDA and cortisol levels had significant diagnostic values for major depression (AUC: 0.853 and 0.819, $p<0.001$). We do not make an interpretation from this; MDA and cortisol are unique diagnostic indicators for MDD. Nevertheless, as we clarified above, amplified MDA and cortisol look like a dependable result for MDD in most of the patients. By adding our findings to the earlier information, we prudently infer that MDA and cortisol levels could be the applicant markers for MDD. We recommend further works in the similar field should emphasis on greater and more similar samples to detect whether MDA and cortisol could be indicators for depression. The limitations of this study are the lack of food intake data of study population and the single measurement of cortisol.

Conclusion

The current study explored that MDD patients have increased serum levels of MDA and cortisol than the control subjects. Moreover, it was observed that the diagnostic values of both MDA and cortisol were significant. Serum levels of MDA and cortisol may be the candidate biomarkers for major depression. ROC analysis confirmed the high diagnostic performance of MDA and cortisol in depression. These outcomes should be treated as preliminary and need to be established by further studies examining the diagnostic performance of MDA and cortisol for MDD.

Acknowledgements

The authors are grateful to all the staff and physicians at the Department of Psychiatry, BSMMU, for their technical and administrative support. The authors are also thankful for the laboratory support provided by Institute of Nutrition and Food Science (INFS), University of Dhaka, Dhaka-1000, Bangladesh.

Consent to publish

All study participants or their primary caregivers acknowledged that anonymous data would be published in journal articles.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical approval

The study was approved by the ethical review committee at the Department of Psychiatry, BSMMU. All data were collected from the Department of Psychiatry, BSMMU, Dhaka, Bangladesh. All investigations were conducted according to the principles expressed in the Declaration of Helsinki.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

Informed consent

All the study participants were well briefed about the objective of the study and they signed informed consent. The written consent of the related was obtained from the primary care-giver if independent thinking capacity of any MDD patient was suspected.

ORCID iD

Md Rabiul Islam https://orcid.org/0000-0003-2820-3144

References

1. Mental Health: New Understanding, New Hope. The world health report 2001. http://www.who.int/whr/2001/en/whr01_en.pdf?ua=1 (accessed 14 May 2017).
2. Wakefield JC, Schmitz MF, First MB, et al. Extending the bereavement exclusion for major depression to other losses: evidence from the National Comorbidity Survey. *Arch Gen Psychiatry* 2007; 64(4): 433–440.
3. Hsu KJ, Young-Wolff KC, Kendler KS, et al. Neuropsychological deficits in major depression reflect genetic/familial risk more than clinical history: a monozygotic discordant twin-pair study. *Psychiatry Res* 2014; 215(1): 87–94.
4. Breslau N. Depressive symptoms, major depression, and generalized anxiety: a comparison of self-reports on CES-D and results from diagnostic interviews. *Psychiatry Res* 1985; 15(3): 219–229.
5. Colburn WA, DeGruttola VG, DeMets DL, et al. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clin Pharmacol Ther* 2001; 69(3): 89–95.
6. Andreazza AC, Frey BN, Erdtmann B, et al. DNA damage in bipolar disorder. *Psychiatry Res* 2007; 153(1): 27–32.
7. Camkurt MA, Acar S, Coşkun S, et al. Comparison of plasma MicroRNA levels in drug naive, first episode depressed patients and healthy controls. *J Psychiatr Res* 2015; 69: 67–71.
8. Camkurt MA, Fındıklı E, İzci F, et al. Evaluation of malondialdehyde, superoxide dismutase and catalase activity and their diagnostic value in drug naïve, first episode, non-smoker major depression patients and healthy controls. *Psychiatry Res* 2016; 238: 81–85.
9. Maes M, Yirmyia R, Noraberg J, et al. The inflammatory & neurodegenerative (I&ND) hypothesis of depression: leads for future research and new drug developments in depression. *Metab Brain Dis* 2009; 24(1): 27–53.
10. Michel TM, Frangou S, Thiemeyer D, et al. Evidence for oxidative stress in the frontal cortex in patients with recurrent depressive disorder—a postmortem study. *Psychiatry Res* 2007; 151(1–2): 145–150.
11. Ruiz-Litago F, Seco J, Echevarría E, et al. Adaptive response in the antioxidant defence system in the course and outcome in first-episode schizophrenia patients: a 12-months follow-up study. *Psychiatry Res* 2012; 200: 218–222.
12. Steiger A and Kimura M. Wake and sleep EEG provide biomarkers in depression. *J Psychiatr Res* 2010; 44(4): 242–252.
13. Vreeburg SA, Hoogendijk WJ, van Pelt J, et al. Major depressive disorder and hypothalamic-pituitary-adrenal axis activity: results from a large cohort study. *Arch Gen Psychiatry* 2009; 66(6): 617–626.
14. Leibowitz A, Boyko M, Shapira Y, et al. Blood glutamate scavenging: insight into neuroprotection. *Int J Mol Sci* 2012; 13: 10041–10066.
15. Stephens MA, Mahon PB, McCaul ME, et al. Hypothalamic-pituitary-adrenal axis response to acute psychosocial stress: effects of biological sex and circulating sex hormones. *Psychoneuroendocrinology* 2016; 66: 47–55.
16. Bulut M, Selek S, Gergerlioglu HS, et al. Malondialdehyde levels in adult attention-deficit hyperactivity disorder. *J Psychiatry Neurosci* 2007; 32(6): 435–438.
17. Maes M, Galecki P, Chang YS, et al. A review on the oxidative and nitrosative stress (O&NS) pathways in major depression and their possible contribution to the (neuro)degenerative processes in that illness. *Prog Neuropsychopharmacol Biol Psychiatry* 2011; 35(3): 676–692.
18. Herken H, Uz E, Ozyurt H, et al. Evidence that the activities of erythrocyte free radical scavenging enzymes and the products

of lipid peroxidation are increased in different forms of schizophrenia. *Mol Psychiatry* 2001; 6(1): 66–73.
19. Lopresti AL, Maker GL, Hood SD, et al. A review of peripheral biomarkers in major depression: the potential of inflammatory and oxidative stress biomarkers. *Prog Neuropsychopharmacol Biol Psychiatry* 2014; 48: 102–111.
20. Yehuda R, Giller EL, Southwick SM, et al. Hypothalamic-pituitary-adrenal dysfunction in posttraumatic stress disorder. *Biol Psychiatry* 1991; 30(10): 1031–1048.
21. Heim C, Newport DJ, Bonsall R, et al. Altered pituitary-adrenal axis responses to provocative challenge tests in adult survivors of childhood abuse. *Am J Psychiatry* 2001; 158: 575–581.
22. van Bodegom M, Homberg JR and Henckens MJAG. Modulation of the hypothalamic-pituitary-adrenal axis by early life stress exposure. *Front Cell Neurosci* 2017; 11: 87.
23. Zhang HY, Zhao YN, Wang ZL, et al. Chronic corticosterone exposure reduces hippocampal glycogen level and induces depression-like behavior in mice. *J Zhejiang Univ Sci B* 2015; 16(1): 62–69.
24. Herbert J. Cortisol and depression: three questions for psychiatry. *Psychol Med* 2013; 43(3): 449–469.
25. Khoubnasabjafari M, Ansarin K and Jouyban A. Reliability of malondialdehyde as a biomarker of oxidative stress in psychological disorders. *Bioimpacts* 2015; 5(3): 123–127.
26. Lemeshow S, Hosmer DW Jr, Klar J, et al. *Adequacy of sample size in health studies*. Chichester: John Wiley & Sons, 1990, p. 19.
27. Nahar Z, Sarwar MS, Islam MS, et al. Determination of serum antioxidant vitamins, glutathione and MDA levels in panic disorder patients. *Drug Res (Stuttg)* 2013; 63(8): 424–428.
28. Sarwar MS, Sarkar RC, Bhowmick R, et al. Effect of socioeconomic status and estimation of lipid peroxidation and antioxidant in preeclamptic pregnant women: a case-control study. *Hypertens Pregnancy* 2015; 34(1): 125–135.
29. Han C, Lim YH and Hong YC. The association between oxidative stress and depressive symptom scores in elderly population: a repeated panel study. *J Prev Med Public Health* 2016; 49(5): 260–274.
30. Rybka J, Kędziora-Kornatowska K, Banaś-Leżańska P, et al. Interplay between the pro-oxidant and antioxidant systems and proinflammatory cytokine levels, in relation to iron metabolism and the erythron in depression. *Free Radic Biol Med* 2013; 63: 187–194.
31. Sarandol A, Sarandol E, Eker SS, et al. Major depressive disorder is accompanied with oxidative stress: short-term antidepressant treatment does not alter oxidative-antioxidative systems. *Hum Psychopharmacol* 2007; 22(2): 67–73.
32. Bilici M, Efe H, Köroğlu MA, et al. Antioxidative enzyme activities and lipid peroxidation in major depression: alterations by antidepressant treatments. *J Affect Disord* 2001; 64(1): 43–51.
33. Bajpai A, Verma AK, Srivastava M, et al. Oxidative stress and major depression. *J Clin Diagn Res* 2014; 8(12): CC04–CC07.
34. Mazereeuw G, Herrmann N, Andreazza AC, et al. A meta-analysis of lipid peroxidation markers in major depression. *Neuropsychiatr Dis Treat* 2015; 11: 2479–2491.
35. Gałecki P, Szemraj J, Bieńkiewicz M, et al. Lipid peroxidation and antioxidant protection in patients during acute depressive episodes and in remission after fluoxetine treatment. *Pharmacol Rep* 2009; 61(3): 436–447.
36. Khanzode SD, Dakhale GN, Khanzode SS, et al. Oxidative damage and major depression: the potential antioxidant action of selective serotonin re-uptake inhibitors. *Redox Rep* 2003; 8(6): 365–370.
37. Zvěřová M, Fišar Z, Jirák R, et al. Plasma cortisol in Alzheimer's disease with or without depressive symptoms. *Med Sci Monit* 2013; 19: 681–689.
38. Piwowarska J, Wrzosek M, Radziwoń-Zaleska M, et al. Serum cortisol concentration in patients with major depression after treatment with clomipramine. *Pharmacol Rep* 2009; 61: 604–611.
39. Afsar B. The relationship of serum cortisol levels with depression, cognitive function and sleep disorders in chronic kidney disease and hemodialysis patients. *Psychiatr Q* 2014; 85(4): 479–486.
40. Gillespie CF and Nemeroff CB. Hypercortisolemia and depression. *Psychosom Med* 2005; 67(Suppl 1): S26–S28.
41. Ahmed S, Moussa F, Moustafa A, et al. Cortisol level in depressed patients and its relation with suicidal risk and anhedonia. *Egypt J Neurol Psychiatry Neurosurg* 2016; 53(4): 193–199.
42. Cubała WJ and Landowski J. C-reactive protein and cortisol in drug-naïve patients with short-illness-duration first episode major depressive disorder: possible role of cortisol immunomodulatory action at early stage of the disease. *J Affect Disord* 2014; 152–154: 534–537.
43. Piwowarska J, Chimiak A, Matsumoto H, et al. Serum cortisol concentration in patients with major depression after treatment with fluoxetine. *Psychiatry Res* 2012; 198: 407–411.
44. Fındıklı E, Camkurt MA, Karaaslan MF, et al. Serum levels of G protein-coupled estrogen receptor 1 (GPER1) in drug-naive patients with generalized anxiety disorder. *Psychiatry Res* 2016; 244: 312–316.
45. Bulut M, Selek S, Bez Y, et al. Reduced PON1 enzymatic activity and increased lipid hydroperoxide levels that point out oxidative stress in generalized anxiety disorder. *J Affect Disord* 2013; 150(3): 829–833.
46. Güneş M, Bulut M, Demir S, et al. Diagnostic performance of increased prolidase activity in schizophrenia. *Neurosci Lett* 2016; 613: 36–40.
47. Selek S, Altindag A, Saracoglu G, et al. Oxidative markers of Myeloperoxidase and Catalase and their diagnostic performance in bipolar disorder. *J Affect Disord* 2015; 181: 92–95.