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Differential malondialdehyde (MDA) detection in plasma samples of patients with major depressive disorder (MDD): A potential biomarker

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Abstract

Objective: To measure plasma levels of malondialdehyde (MDA), a marker of oxidative stress (OS), in patients with major depressive disorder (MDD) compared with healthy control (HC) subjects in order to determine if it is a possible biomarker of depression.

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Methods: This prospective cross-sectional study enrolled patients with MDD and HC subjects. The plasma levels of MDA were measured using a commercially-available colorimetric assay.
Results: A total of 30 patients with MDD and 20 HC subjects with similar sex, age and body mass index distribution were enrolled in the study. Patients with MDD had significantly higher plasma levels of MDA than the HC subjects. Receiver operating characteristic curve analysis for plasma MDA levels in patients with MDD demonstrated an area under the curve of 0.9767.
Conclusion: The findings of this current study provide further evidence of the role pathophysiological relevance of OS and MDA in MDD. This study provides the basis for the use of MDA as a biomarker for MDD.

Keywords
Major depressive disorder (MDD), oxidative stress, malondialdehyde (MDA), plasma biomarker

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Introduction

Major depressive disorder (MDD) is a complex condition characterized either by depressed mood or anhedonia and a set of vegetative disorders like insomnia, anorexia or fatigue and impaired cognitive function manifested at least for 2 weeks.[1,2] According to the literature, approximately 5% of the global population suffer from MDD, representing approximately 350 million people worldwide with an estimated incidence of 3% per year, although these data may vary across regions.[3] The Global Burden of Diseases, Injuries, and Risk Factors Study ranks MDD in the top ten causes of disability-adjusted life-years for both sexes from teenagers through to elder adults; although the risk of suffering from MDD in women is virtually twice that in men.[4,5] The World Health Organization predicts that by 2030, MDD will be the leading cause of disability worldwide,[6] thereby clarifying the noteworthy impact of this condition.

As the prevalence and consequences of MDD in the world increase, there are still many questions to answer in order to enhance the clinical management of this condition. For example, due to the complex nature of the disease, there are many therapeutic and diagnostic difficulties for this condition, and there are few studies supporting the use of biomarkers as predictive or prognostic factors.[7,8] A more comprehensive understanding of the different pathophysiological mechanisms involved in MDD is crucial for the development of effective therapeutic and diagnostic approaches whilst aiding in the search for appropriate biomarkers. In this context, oxidative stress (OS) is a major aetiopathological agent of MDD.[9] Most biochemical processes in biological organisms are mediated by redox reactions, in which electrons are transferred from a reducing agent to an oxidizing or oxidant agent.[10] Oxidants, also known as free radicals, are products permanently produced in the mitochondria due to cellular respiration, but they also occur in other organelles during their metabolic activities.[11] Antioxidant molecules naturally produced by cells help to reduce the levels of free radicals.[12] OS results from an imbalance between free radicals and antioxidants in favour of the former, leading to a disruption of redox signalling and molecular damage.[13] There is compelling evidence that OS is responsible for direct damage to neurons and glial cells in patients with MDD,

affecting multiple brain regions and negatively influencing different cell pathways and neuroinflammation.[14] Lipid peroxidation is a marker of OS, which consists of the reaction between free radicals and lipids with carbon–carbon double bonds such as polyunsaturated fatty acids (PUFAs), mainly located in the cell membranes.[15] Malondialdehyde (MDA) is a final product of lipid peroxidation and it has been investigated as a relevant marker of psychiatric disorders,[16] including in MDD.[17] However, the reliability of measuring MDA as a marker of OS in these psychiatric conditions is questioned due to the heterogeneity of the available studies and technical limitations.[18] Further studies are required to analyse this relationship in more detail.

The aim of this current study was to measure plasma levels of MDA in patients with MDD in comparison with non-depressed control subjects in order to find a possible application for this molecule as a biomarker of depression.

Patients and methods

Patients and study protocol

This prospective cross-sectional study enrolled consecutive patients with MDD from the Department of Psychiatry and Medical Psychology, University Clinic of Navarra, Pamplona, Spain and from the Department of Medicine and Medical Specialities, Hospital Universitario Principe de Asturias, Alcala de Henares, Spain between February 2017 and March 2019. Healthy control (HC) subjects with similar sex, age and body mass index (BMI) distribution were recruited from the local population of Alcalá de Henares, Madrid. The inclusion criteria were as follows: (i) confirmed diagnosis of MDD by a psychiatric specialist, either single or recurrent, following the Diagnostic and Statistical Manual of Mental Disorders criteria, Fifth Edition (DSM-V);[19] (ii) a minimum 14 points score on the 17-item Hamilton Rating Scale for Depression (HRSD);[20] (iii) aged 18–65 years. The exclusion criteria were as follows: (i) acute infection in the last 3 months or chronic bacterial/viral infection; (ii) presence of a cardiovascular disease, including hypertension and ischaemic heart disease; (iii) presence of an endocrine or metabolic disease, including diabetes mellitus and hypercholesterolaemia, or a BMI > 30 kg/m^2; (iv) the use of immunomodulatory drugs or steroids in the last 3 months; (v) presence of an autoimmune disease; (vi) presence of a haematopoietic, lung, hepatic or renal disorder; (vii) a history of prior malignancy; (viii) immunodeficiency or malnutrition; (ix) pregnancy or lactation; (x) the presence of a concomitant psychiatric illness, assessed with the MINI International Neuropsychiatric Interview.[21] The patients with refractory MDD included in the study were not homogenous in terms of their use of psychotropic drugs. Axis 1 or 2 psychiatric disorders according to the DSM-V criteria were excluded in all of the healthy control subjects through the application of The Structured Clinical Interview for DSM-5 (SCID-5).

This study was reviewed and approved by the Ethics Committee of the University Clinic of Navarra (no. 2017.199) and the Ethics Committee of the Hospital Universitario Principe de Asturias (no. LIB 06/2015). Written informed consent was obtained from all participants involved in the study.

MDA determination

Blood samples were obtained from all participants via standard venipuncture using an established aseptic technique. Samples were obtained at the time of the evaluation. After collection, the blood samples were centrifuged and the plasma was isolated, aliquoted and stored at -80°C until further analysis.

A colorimetric lipid peroxidation assay kit (ab118970; Abcam®, Cambridge, UK) was used to measure plasma MDA levels according to the manufacturer's instructions. The MDA that is present in the plasma sample (10 µl) reacts with thiobarbituric acid (TBA) to generate an MDA-TBA adduct that is quantified colorimetrically. This assay detects MDA levels as low as 0.1 mol/well. For the colorimetric assay, a 0.1 mol/l MDA standard was prepared and serial dilutions were made for the standard curve. The MDA-TBA product generated by the kit was measured immediately in a microplate reader (Victor 2 multifunction device; Wallac, Victoria, Australia) at an optical density of 532 nm.

Statistical analyses

All statistical analyses were performed using GraphPad Prism 5.1 (Graphpad Software Inc., San Diego, CA, USA). Data are presented as mean ± SD or *n* of patients (%). Data were compared using Mann–Whitney *U*-test. Receiver operating characteristic curve analysis was undertaken for MDA. The data for plasma MDA levels are presented as median with the interquartile range (IQR). A P-value < 0.05 was considered statistically significant.

Results

This study enrolled 30 patients with MDD and 20 HC subjects with similar sex, age and BMI distribution (Table 1). There were no significant differences between the patients with MDD and the HC subjects with regard the characteristics studied, except for the employment status ($P < 0.01$). Seventeen patients (56.7%) with MDD had suffered at least one previous MDD episode. In the MDD group, the mean ± SD duration of

Table 1. Demographic and clinical characteristics of patients with major depressive disorder (MDD) and healthy control (HC) subjects that were enrolled in a study to determine if the plasma levels of malondialdehyde (MDA) are a biomarker of depression.

Characteristic	Patients with MDD $n = 30$	HC subjects $n = 20$
Age, years	43.26 ± 13.14	40.45 ± 12.46
Age range, years	27–53	25–53
Sex, female	19 (63.3%)	12 (60.0%)
College degree	16 (53.3%)	12 (60.0%)
Currently employed	13 (43.3%)	18 (90.0%)*
Family history of depression	17 (56.7%)	8 (40.0%)
Family history of other psychiatric disorders	22 (73.3%)	11 (55.5%)
Body mass index, kg/m^2	26.74 ± 5.41	25.5 ± 5.36
Alcohol drinker		
Never	7 (23.3%)	3 (15.0%)
Occasionally	20 (66.7%)	16 (80.0%)
Everyday	3 (10.0%)	1 (5.0%)
Cigarette smoker		
Never	12 (40.0%)	6 (30.0%)
Occasionally	8 (26.7%)	11 (55.0%)
Everyday	10 (33.3%)	3 (15.0%)

Data presented as mean ± SD or *n* of patients (%).

*$P < 0.01$; data were compared between the two groups using Mann–Whitney *U*-test; no significant between-group differences for the other parameters ($P \geq 0.05$).

their depressive episode before recruitment was 16.12 ± 2.85 weeks. At the time of the study, the mean $\pm$ SD HRSD score was 15.95 ± 1.25 in the MDD group. Three of 30 (10.0%) patients with MDD presented with psychotic (delusional) symptoms during the current episode. All 30 patients with MDD received pharmacological treatment: 30 (100.0%) received antidepressants, 28 (93.3%) received anxiolytics or hypnotics, five (16.7%) received mood stabilizers and 10 (33.3%) received atypical antipsychotics. Of these, 28 patients (93.3%) received combination pharmacotherapy; 19 of 30 patients (63.3%) received two different types of medication and nine of 30 patients (30.0%) received at least three different types of medication. None of the patients received electroconvulsive therapy.

Patients with MDD had significantly higher plasma levels of MDA than the HC subjects ($P < 0.0001$) (Figure 1). The median (IQR) plasma MDA levels were 26721 (10236–45039) μmol/l in the patients with MDD compared with 6904 (1245–17456) μmol/l in the HC subjects.

Receiver operating characteristic curve analysis for plasma MDA levels in patients with MDD demonstrated an area under the curve of 0.9767 (Figure 2).

Discussion

This current study demonstrated significantly higher plasma levels of MDA in patients with MDD compared with HC subjects. These current findings agree with previous studies that have suggested that OS is a critical pathophysiological mechanism of MDD and MDA can be readily measured in the plasma of patients with depression.[22,23]

Previous studies have aimed to find the most suitable marker of OS in patients with MDD.[24] Different plasma components have been studied, including enzymatic and non-enzymatic antioxidants, free radicals and the products of oxidative damage.[22] Decreased antioxidant levels and augmented free radicals and products of oxidative damage have been detected in patients with MDD and shown to be directly correlated with disease severity and number of episodes of depression.[24,25] The presence of MDA in the plasma is evidence of lipid peroxidation and oxidative damage.[11] MDA is formed

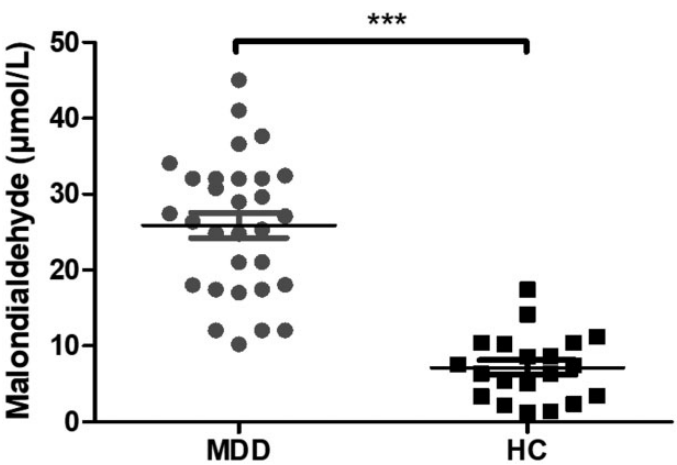

Figure 1. Plasma malondialdehyde levels in patients with major depressive disorder (MDD) and healthy control (HC) subjects. The central black horizontal line for each group is the median. ***$P < 0.0001$; Mann–Whitney *U*-test. The colour version of this figure is available at: http://imr.sagepub.com.

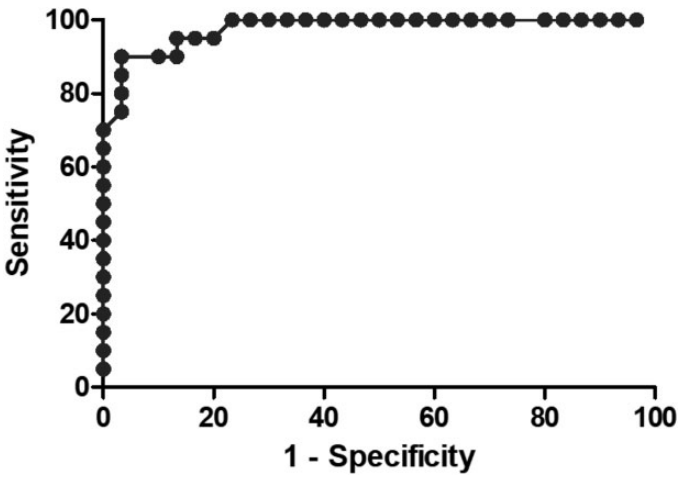

Figure 2. Receiver operating characteristic curve analysis for plasma malondialdehyde levels in patients with major depressive disorder demonstrated an area under the curve of 0.9767.

through a reaction of free radicals with PUFAs like omega 3 and omega 6 PUFAs.[15] Both fatty acids, omega 3 and omega 6, are crucial components of cell membranes.[26] The omega 6/omega 3 ratio appears to be a pivotal marker of health and disease.[26] A reduced omega 6/omega 3 ratio (< 4:1) is a favourable marker whereas a high ratio is associated with negative outcomes.[26] In the brain, a healthy omega-6/omega 3 ratio is critical for mediating serotoninergic transmission and influencing the inflammatory response.[27] Importantly, altered neurotransmission and neuroinflammation are two of the main drivers involved in the pathophysiology of MDD and patients with MDD show a high ratio of omega 6/omega 3.[28,29] As a consequence, omega 3 supplementation is being studied as a nutraceutical product against MDD, which acts by reducing the production of MDA and protecting depressed patients from OS damage.[30,31] Conversely, elevated levels of MDA lead to an increased accumulation of reactive oxygen species and reactive nitrogen species in neurons, mitochondrial dysfunction and an abnormal activation of multiple cell signalling pathways.[32] OS is closely related to many inflammatory pathways, exerting synergic damage.[11] In patients with MDD, both processes are responsible for enhanced cell death, reduced neurogenesis and neuroplasticity, increased autoimmune responses and neurodegeneration, thereby contributing with the progression of depression.[33] Previous research has described MDA as the most important mutagenic product of lipid peroxidation.[34] Therefore, increased levels of MDA may be related to accelerated aging of the brain and systemic tissues.[35] Overall, there is considerable evidence that MDA contributes to the pathophysiology of MDD through multiple mechanisms; and an improvement of depressive symptoms and antidepressant therapy is associated with a reduced levels of serum MDA in those patients.[24]

Despite the relevance and applications of measuring plasma MDA as a marker of oxidative damage, some methodological approaches might not be sensitive enough and they may be disturbed by interference from related species or by an overestimation from analysis conditions.[36] In this current study, a commercial colorimetric kit was used that was based on the reaction

between MDA and TBA. The TBA assay is the most commonly used method for providing MDA levels in plasma and other biological samples.[18] This method is cheap, consistent and reproducible.[37] This current study has provided additional insight into the pathogenesis and evaluation of plasma markers in patients with MDD. Previous research from this research team demonstrated that patients with MDD exhibited an enhanced bacterial translocation, blunted regulatory T cell expansion, altered monocytes, an expanded CD4+ T cell population and dysregulated cytokines compared with non-depressed subjects.[38,39] Elevated plasma MDA levels should be considered as a major marker of OS damage, which may be caused all of the systemic alterations described previously. Further studies and analysis of additional markers of lipid peroxidation and OS will increase the understanding of the role OS in patients with MDD, which might result in the development of novel medications.

In conclusion, this current study has demonstrated a significant increase in plasma MDA levels in patients with MDD compared with HC subjects. Measuring this marker in the plasma of individuals suspected of having MDD might aid in the clinical diagnostic of this psychiatric disorder. The findings from this current study support the relevant role of OS in the pathogenesis of MDD, while encouraging further research in this field. This study provides the basis for the use of MDA as a biomarker for MDD.

Declaration of conflicting interest

The authors declare that there are no conflicts of interest.

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