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PHD THESIS

MOLECULAR MARKERS FOR DIAGNOSTIC AND TREATMENT OF MAJOR DEPRESSIVE DISORDER

A B S T R A C T

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A B S T R A C T

Of all the types of psychiatric pathologies there is a particular concern for depression, having an increasing incidence among the general population. An estimated 3.8% of the general population suffers from depression, including 5.7% of people over 60 and 5% of adults (4% of men and 6% of women) (1). Women are about 50% more likely than men to suffer from depression. More than 10% of pregnant women and those who have recently given birth suffer from depression globally (2). Each year, approximately 700,000 people commit suicide and it is the fourth leading cause of death in 15-29 years old (1).

Depressive disorder (depression, as it is known) is characterized by a persistent sadness or depressed mood, loss of pleasure (anhedonia) or enthusiasm in activities for a considerable period of time, fatigue, lack of energy, diminished functionality. Depression is different from ordinary mood swings and everyday feelings, having a major impact on all facets of life, including social interactions and global functioning (3).

Patients with major depression are 40% to 60% more likely than the average population to die prematurely (4), either from suicide (5) or from physical health problems that are often ignored (neoplastic pathology, diseases cardiovascular (6), diabetes, HIV infection, etc.).

The underlying causes of mental illness, specifically depressive disorder, are often unknown. Findings from many different fields can be incorporated into theories. Mental disorders are usually identified by a person's behavior, feelings, perceptions, or thoughts. This can be linked to specific brain areas or activities, often in a social context. When making a diagnosis, in addition to the biological side, societal norms, cultural, religious beliefs and practices must be taken into account (7).

In addition, this type of pathology lacks adequate methods of specific diagnosis and curative, effective treatment, often leading to recurrent episodes of the disease that significantly reduce the quality of life of these patients and considerably shorten it (8).

Due to the increased incidence of depressive pathology and the high risk to public health, research in this field is currently being conducted intensively,

representing an important research topic at the global level. Depression and self-harm/suicide are among the priority conditions covered by WHO programs (1).

Current diagnostic methods are based on the clinical interview, which guides the diagnostic criteria, based on two main classification systems: DSM-5 (7) and ICD-10 (3). Inventories and questionnaires, laboratory tests and imaging investigations are also used, but there are no specific diagnostic methods.

In this context, I chose to study this disease as part of my doctoral research, in the hope that I will be able to make a contribution that will be included in the vast and international archive of medical research for major depressive disorder and that the data obtained could be corroborated with the global results , bringing this important problem even closer to the solution, to increase the quality of life of these patients, to establish a clear diagnosis, to prevent and reduce the occurrence of complications, to reduce the overall level of morbidity and mortality caused by major depression.

In this sense, we tried to identify new diagnostic and treatment biomarkers from blood compartments from patients previously diagnosed with major depressive disorder, using in all experimental parts of this project also samples from individuals without major depression or other psychiatric disorders, which we performed the same basic research analyses, being useful for comparison (as controls).

The analyzes were carried out in a multidisciplinary manner, in the sense that they were not limited to the use of single technologies, and the molecular species investigated did not belong to single classes of molecules, but species originating from genetic material (especially non-coding) were studied such as microRNA, which is not involved in protein translation, by real-time polymerase chain reaction (RT-PCR) and microarray techniques, as well as all metabolites (of all classes - amino acids, phospholipids, amines, etc.) of plasma, exosomes and plasma without exosomes, by modern techniques of high performance liquid chromatography coupled with mass spectrometry (UHPLC/MS). The obtained results were corroborated in parallel with those from the existing specialized literature.

The research work began with a literature review with the aim of critically and systematically reviewing the differences in miRNA levels of MDD patients compared to healthy controls at baseline, before and after antidepressant treatment, as well as their potential diagnostic and therapeutic relevance in the context of the development of new biomarkers. The literature review corroborated the existing data in the literature and statistically confirmed that miRs have a satisfactory diagnostic accuracy

in differentiating patients with depressive disorder from healthy individuals. Also, miRs showed statistically significant differences before and after antidepressant treatment, all of which lead to better characterization and reliable diagnosis of MDD, which could open new horizons in the field of neuropsychiatry (9).

The second study aimed to investigate the distribution of miR-26a, miR-30c, miR-93, miR-101 and miR-494 between intracellular and extracellular blood compartments that were differentially expressed and distributed in different biological fluids, such as plasma, serum, PNBC, exosomes, plasma without exosomes, in patients diagnosed with MDD compared to healthy controls, both before and after antidepressant treatment. Their relative abundance was assessed in plasma, white blood cells (peripheral blood mononuclear cells (PBMC), plasma-derived exosomes and exosomes-free plasma (EDP) of patients with depressive disorder and in the same blood compartments of healthy controls, and before and after antidepressant treatment of patients diagnosed with depressive disorder. These results were compared with existing databases as well as other published articles on the subject in order to evaluate miRNAs that can be classified as possible biomarkers for early detection of depressive disorder (10).

In the third study, ultra-high-performance liquid chromatography coupled with electrospray ionization-quadrupole-time-of-flight-mass spectrometry [UHPLC-QTOF-[ESI+]-MS] was used to perform metabolomic profiling untargeted plasma samples from patients with depressive disorder before and after treatment with escitalopram, as well as from plasma samples from healthy volunteers, to evaluate their potential function as diagnostic and therapeutic biomarkers for depressive disorder. Metabolomics is an emerging technique for the simultaneous discovery of circulating metabolites with significantly altered levels in depressive disorder. In this regard, we observed a decrease in different classes of molecules such as amino acids (tryptophan, proline), amines (spermine), lysophosphatidylcholine and acetylcarnitine, in the samples of patients diagnosed with depression compared to healthy controls (11).

All techniques used in this research project (RT-PCR, UHPLC-QTOF-[ESI+]-MS) fall under the category of modern diagnostic techniques, which are not only intended to be minimally invasive for the patient (requires only the collection of biological samples), but also to remove the universal dogma "one size fits all" through the personalized analysis of the genetic and metabolic profile, for each

individual patient, which can open new horizons towards a deeper understanding of the diagnosis and treatment of major depressive disorder.

This PhD thesis demonstrates that research in a multidisciplinary manner, using various techniques and targeting molecules from various biochemical classes, with the aim of discovering specific biomarkers for depressive disorder, represents a step forward towards modern, personalized and evidence-based medicine. The investigation of the genetic material in the circulating biological fluid and the different metabolites could help to deepen the understanding of the mechanisms of the depressive disorder and could also represent new precision biomarkers for early and specific diagnosis, which could then lead to a targeted treatment of this disease.

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