Decoding ketamine\'s molecular signature in treatment-resistant depression through bioinformatic analysis

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**Introduction:** Despite the efficacy of major depressive disorder (MDD) treatment using conventional antidepressants associated with psychotherapies, a substantial proportion of individuals remains refractory to these first-line treatments. In this context, treatment-resistant depression (TRD) represents a subset of MDD associated with more severe and chronic symptoms, showed by a higher risk of comorbidities and suicide ideation. Ketamine, traditionally used as an anesthetic, has been showing a rapid-acting antidepressant effect in TRD. Ketamine acts as an NMDA glutamate receptor antagonist, although the mechanism underlying its antidepressant effect remains unknown. Despite representing a promising therapeutic approach to TRD, some individuals remain refractory to ketamine treatment. This study aimed to identify differential proteins related to the ketamine antidepressant response in TRD patients and its associated biological pathways before and after treatment. **Methodology:** The study included 6 male patients with TRD recruited from the Hospital de Clínicas de Porto Alegre. Plasma samples were collected before and after subcutaneous (SC) ketamine treatment (0,05 mg/kg, with adjustments). The effectiveness of ketamine treatment was determined by the Montgomery-Åsberg Depression Rating Scale (MADRS), classifying patients as responders (50% reduction from baseline MADRS score) or non-responders. The proteomics analysis of plasma was conducted by mass spectrometry. Bioinformatic analyses were performed to identify differentially expressed proteins in plasma proteomic data before and after treatment associated with different antidepressant responses, considering significance values of FDR ? 0,4 and |LogFC| >0,06. Each protein was converted to its corresponding gene to perform pathway enrichment analysis (Enrichr software). Python language was used to handle bioinformatics tools and identify significant proteins, genes, and enrichment pathways using the FDR criterion with a significance threshold of $p < 0.05$. **Results:** Patients who responded to ketamine treatment showed a significant reduction in depressive symptoms, with a lower mean score on the MADRS (34.66, SD 1.5) compared to patients who did not respond (40.33, SD 3.78). Proteomic analysis identified 502 proteins, with 18 being differentially expressed with statistical significance. An overlap of 5 genes between responder and non-responder patients before and after treatment was observed. We identified 15 genes with an exclusive differential expression before treatment and 8 after treatment. The association of these genes with inflammatory and cellular signaling processes suggests that ketamine may modulate these processes as part of its antidepressant mechanism of action. Enrichment analysis showed increased pathways related to coagulation, immune complement, and cholesterol before treatment, indicating a possible inflammatory state or altered immune response associated with TRD. Post-treatment observations suggest that ketamine may have specific effects on the metabolism of tyrosine and cholesterol, possibly related to the regulation of neurotransmitters and synaptic plasticity. **Conclusions:** We

identified molecular signatures associated with the TRD condition and the ketamine antidepressant response in TRD and highlighted their underlying biological pathways. These findings may suggest potentially more effective and personalized therapeutic targets to improve TRD clinical management.

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