Pilot study of untargeted metabolomics in rural workers exposed to the herbicide glyphosate

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Glyphosate is the most widely used herbicide in Brazil and worldwide, as formulations containing this chemical substance are highly effective in controlling weeds, both before and after harvesting agricultural crops. However, despite this benefit, the risks related to acute and chronic exposure still need to be better evaluated, as *in vivo* and *in vitro* toxicity studies demonstrate short- and long-term effects. Nevertheless, data on populations exposed environmentally and occupationally are scarce. Therefore, this study aimed to conduct a pilot investigation of the metabolite profile in agricultural workers living in rural areas of Casimiro de Abreu, Rio de Janeiro (RJ), who are exposed to glyphosate, to guide future research in determining biomarkers of early effects. For the untargeted metabolomics approach, plasma samples were collected from workers (n=20) who reported exposure to commercial formulations containing glyphosate in the questionnaire. Sample preparation included two steps: a protein precipitation, to reduce interferences, and a derivatization, to enhance metabolite detectability and promote stability during gas chromatography-mass spectrometry (GC-MS) analysis. Raw data were processed, and the data matrix was created using the free software R®, version 4.3.1, and the eXtensible Computational Mass Spectrometry (XCMS) Bioconductor® package, version 3.12. Pre-treated data were extracted using the matchedFilter, density, and peakgroups algorithms, and a file was subsequently generated with the metabolites identified by a specific code, in a grouped and aligned manner. Finally, information about the metabolites was statistically normalized using the MetaboAnalyst® platform, version 5.0, and their identification was performed by consulting metabolomics databases. Principal Component Analysis (PCA) and Partial Least Squares Regression – Discriminant Analysis (PLS-DA) multivariate methods were applied. The PCA revealed differences in the metabolic profiles between the group exposed to glyphosate and the non-exposed group, consisting of city residents (n=20). The PLS-DA regression method further modeled and enhanced the differences between the metabolites that distinguish the groups analyzed in this study. The constructed model was validated using a cross-validation test. Additionally, a Volcano Plot was created to compare the absolute changes between the means of the samples in the non-exposed and exposed groups. In this plot, six different molecular features were identified between the groups, including M77T365, which corresponds to the metabolism of N-ribosylhistidine. This metabolite is a marker of histidinemia, a metabolic disorder characterized by an excessive increase in histidine levels in the blood, urine, and cerebrospinal fluid. Thus, the results obtained suggest that the exposed group exhibited a specific pattern of metabolic alterations that can be associated with some pathophysiological disorders. Despite being a pilot study, this one contributes to the advancement of the occupational health area by highlighting the value of metabolomic analysis as a possible tool for biomonitoring and, consequently, for risk assessment of glyphosate exposure.

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