Identification of risk biomarkers for leg ulcers in patients with sickle cell disease using metabolomic analysis in a large cohort

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Introduction: Data from the WHO report that sickle cell disease (SCD) affects around 300,000 people per year, making it one of the most common hereditary diseases worldwide. According to the Ministry of Health, it is estimated that Brazil has a population of approximately 60,000 to 100,000 individuals diagnosed with SCD. Leg ulcers are among the main complications affecting patients with SCD. They are characterized as painful and difficult-to-heal wounds that can arise spontaneously or as a result of local trauma. A comprehensive understanding of the mechanisms contributing to the formation of leg ulcers has not yet been achieved. This pilot project aims to integrate the installation of equipment at our college to promote research related to the metabolic mapping of patients with SCD and their various comorbidities. Material and Method: For untargeted metabolite analysis, we used the UPLC-MS QTof (Waters Co., USA). We included 15 serum samples from individuals divided into three groups: healthy (n=5); SCD patients (n=5); and SCD patients with leg ulcers (n=5). For quality control (QC), we prepared a pool with all conditions (SCD + SCD with ulcers + healthy), using a dilution curve of 100%, 75%, 40%, 25%, 5%, and 0%. To monitor reproducibility among the different groups, we used 3 phenotypic QCs. In the sample preparation, we added 700 µL of acetonitrile (ACN) and 40 µL of MiliQ water to 40 µL of human serum. Subsequently, the solution was centrifuged at 4000g for 10 minutes at 4°C. We injected 1 µL of the supernatant into the HILIC chromatographic column (130Å, 2.1 x 100 mm, 1.7 µm, Waters, USA) with binary gradient elution at a constant flow rate of 0.5 mL/min, with solvents A1 = water containing 0.1% formic acid and B2 = 100% acetonitrile in water containing 0.1% formic acid. The eluted content was automatically inserted into the Q-ToF mass spectrometer (Synapt XS, Waters, USA), operating in positive/negative mode and MSe centroid, in high-energy states (ramp of 20 to 30 V). Spectra were obtained in a range of 50 to 1200 Da. The raw data were analyzed in Progenesis QI software (Waters, USA) for compound identification, using the HMDB database and comparison among groups. Preliminary Results: The preliminary results identified differences between the metabolic profiles of the study groups. Furthermore, principal component analysis (PCA) demonstrated distinct groupings among the metabolic profiles of the phenotypic groups. The chromatograms generated by the extraction duplicates analyses demonstrated consistent reproducibility, also providing a list of 12 potential biomarkers in positive mode and 18 potential biomarkers in negative mode. Among the analyzed metabolites, p-cresol sulfate stood out as a potential biomarker for differentiating individuals with SCD who developed leg ulcers, compared to the other groups in this study. Partial Conclusion: The present study may provide support for the validation of biomarkers for leg ulcers in patients with SCD.

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