resulta em disfunção endotelial, inflamação e vasculopatia. Nesse contexto, a úlcera de perna tem sido associada à hemólise frequente em microvasos que irrigam a região maleolar seguido de danos vasculares e hipóxia tecidual, constituindo um potencial fator etiológico para abertura da úlcera. Os níveis acentuados dos biomarcadores descritos nesse estudo, como LDH, bilirrubina total/indireta, reticulócitos e AST/TGO caracterizam um processo hemolítico evidente em pacientes UP+, enquanto os níveis reduzidos de hemácias, Hb e hematócrito evidenciam a anemia crônica causada pela hemólise frequente, agravando o quadro clínico desses pacientes. Ademais, as úlceras de perna incidiram mais significativamente em pacientes com AF, genótipo caracterizado por taxas hemolíticas mais frequentes: 12 pacientes AF UP+ (p = 0,004). Em virtude do exposto, o presente estudo reforça o caráter hemolítico associado à etiopatogenia das úlceras de perna em pacientes com DF.

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LEVERAGING PLASMA-DERIVED EXOSOMES FOR BIOMARKER DISCOVERY IN SICKLE CELL DISEASE: PREPARATION FOR A LARGE PROSPECTIVE STUDY

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Diverse clinical variability among sickle cell disease (SCD) patients opposes crises prediction, health monitoring and streamlined management. Thus, an unmet need for objective biomarkers prevails. Exosomes are extra-cellular nano-vesicles (50-150 nm), enriched in bioactive lipids, proteins, mRNAs and miRNAs, released by cells. They transport molecular cargo to nearby/distant cells to affect-regulate biological processes. Recent studies by Khalyfa et al. assessed the plasma exosome content, their sources and transcriptomics signature as predictive marker in SCD children with acute chest syndrome. However, the small sample sizes (32 and 33 individuals, respectively) may not capture the clinical variability. Thus, we aim to screen large population (150 patients) with good follow-up available at Regional Blood Center, Ribeirão Preto using omics - proteomics and transcriptomics - of plasma-derived exosomes to identify biomarkers in SCD. However, the grand challenges to this expansive undertaking are: 1) establishing a R3 (reliable, robust and reproducible) exosome extraction protocol, 2) performing high-throughput mass-spectrometry and next generation sequencing, and 3) establishing a reliable multi-level comparative bioinformatics platform to analyze the omics data. Here we present our approach with an international collaborative team to resolve these challenges and preliminary results. Exosomes from plasma from steady state SCD patients and healthy donors were extracted using ultracentrifugation. Exosome characterization involved size-concentration estimate by Nano Tracking Analysis (NTA); western-blot for exosome surface marker CD81, CD63, and CD9, and HSP70 and ALIX as internal controls; shape confirmation by transmission electron microscopy. Interestingly, the size and concentrations were different (n = 4 each): size: 96.05+/-29.71 nm (healthy) and 65.23+/-21.7 nm (sickle), and concentrations: 51.3+/-10.3e9 (healthy) and 107+/-77.5e9 (sickle) particles/ml. Reverse-phase LC-MS/MS was done using an Orbitrap Fusion mass-spectrometer at Butantan Institute. From the bioinformatics pipeline established at the University of Nebraska-Lincoln, we were able to extract expression of ~2000 proteins per sample from 8 SCD, 2 healthy and 1 mast cell culture samples (mast cells, taken as control, excrete exosomes in physiologic state). We identified 25 significantly down-regulated proteins in SCD samples (vs. healthy) using t-test with equal variance ($p < 0.05$)–which include blood proteins, complement proteins and immunoglobins. We identified expression of selected sno-RNAs and miRNAs: RNU 44, miR15a, miR361, miR132, miR16, miR125 and miR181 by qRT-PCR in SCD exosomes. While we are still in process of getting RNA-seq analysis, and the proteomics data are preliminary, these validations are pre-requisites for establishing exosome-based omics-pipeline for biomarker discovery for health monitoring of SCD patients, crises prediction and assessing response-to-therapy.

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MANIFESTAÇÕES NEUROLÓGICAS EM ADULTOS COM DOENÇA FALCIFORME SEM ACIDENTE VASCULAR ENCEFÁLICO ISQUÊMICO ANTERIOR: EXPERIÊNCIA DE SERVIÇO DE REFERÊNCIA

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Objetivo: Identificar pacientes adultos com Doença Falciforme (DF) sem antecedente de acidente vascular encefálico isquêmico (AVEi) que apresentaram manifestações neurológicas graves. **Material e métodos:** Realizada revisão de