PROTEOMIC ANALYSIS OF ATHEROMATOUS PLAQUE BY 2D ELECTROPHORESIS AND BIOINFORMATIC TOOLS

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Cardiovascular diseases, especially atherosclerosis, are included among the conditions with the highest mortality rates in developed countries. It is a pathology caused by the loss of elasticity of arteries due to the formation of an atheromatous plaque mainly composed of lipid molecules, protein, calcium and smooth muscle cells. The study of atheroma’s proteome is a way to identify proteins expressed in these plates aiming to understand more of the disease formation mechanism, and find molecular biomarkers that can be used to diagnosis and monitor the treatment. Some works use the proteome of blood serum or vessels samples to study this disease, this used the plaque itself and aimed to develop an effective methodology of sample preparation for two-dimensional electrophoresis. The atheroma was subjected to total protein extraction by stirring in sodium 20 mM phosphate buffer. After that, the sample’s proteic part was precipitated using 10% TCA/Acetone, resuspended in rehydration buffer, applied on DryStrips from GE Healthcare immobilized pH strips and subjected to isoelectric focusing. The strips are then added to the equilibrium solution in the presence of DTT and then in the presence of Iodocetamide. The second dimension protein separation was performed in 12% polyacrylamide gel in the presence of SDS, and subjected to colloidal Coomassie Brilliant Blue staining. The gel was scanned and the pi and mass spots data were obtained by analysis on the software ImageMaster 7 (GE Healthcare). This data was used for identification of the spots by the TagIdent (Expasy) algorithm. With this tool, lists of atherosclerosis related proteins were obtained, some of them associated to immune process and inflammation. Proteomics is important to identify and understand how these proteins work together in the development of this pathology. The study will continue with the excision, digestion and precise identification of these proteins by mass spectrometry.