Cytoplasmic concentration of cholesterol in patients with Niemann-Pick type C: comparison with normal subjects

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Niemann-Pick C disease (DNPC) is a neurodegenerative sphingolipidosis caused by lysosomal accumulation of non-esterified cholesterol in various tissues. The Filipin test, which detects cholesterol in the cytoplasm of DNPC fibroblasts, is considered the gold standard for diagnosis, but it is a subjective analysis, and has often inconclusive results. The aim of this study was to quantify the fluorescence in the Filipin test of fibroblasts from healthy control subjects and patients with classic and variant type of DNPC in order to combine a new tool to the traditional diagnosis. Fibroblasts of normal subjects and classic and variant DNPC patients were cultured in Ham’s F-10 medium with 20% FBS at 37 °C. The medium was changed every 2 days. After confluency, the cultures were trypsinized and 10,000 cells were transferred to 24 well plates (D-MEM medium with 5% LPDS). After 72 hours, was added 0.73ug/100uL LDL into each well and the cells were incubated for 24 hours. The coverslips were removed from the plate, fixed and stained. The slides were visualized with a fluorescence microscope and the number of pixels measured by the CellM program. The results indicated a significant increase (p <0.032 - one-way ANOVA) in the amount of cholesterol in individuals DNPC classic and variant compared to controls. We didn’t observe differences between classic and variant phenotype. These findings indicate that this may be a useful tool for the diagnosis of DNPC, however, the variant DNPC phenotype still needs a molecular analysis to distinguish of the classical type.