P-073 - ALPHA-GLUCOSIDASE ENZYMATIC ASSAY USING NATURAL SUBSTRATE. OUR PATHWAY TO A MORE RELIABLE TEST FOR POMPE DISEASE DIAGNOSIS.

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Acid alpha-glucosidase deficiency is the main cause of Pompe disease. This disorder caused by homozygous or compound heterozygous mutations in the GAA gene, generates a phenotypic expression characterized by proximal muscle weakness, respiratory failure and altered CK levels. Different approaches have been done to get an accurate diagnostic test. The preferred method for diagnosis of the disease is confirmation through biochemical assays showing absent or decreased enzyme activity in at least two samples. Here we show the results obtained in our laboratory in the search for a reliable enzymatic test to diagnose this disease. **METHODS:** Enzymatic assays were carried out using 4-methylumbelliferyl-Alpha-D-glucopyranoside (4-MUG) as substrate and acarbose as inhibitor. Abnormal values for inhibition percentage and iso-enzymes relation were considered probable cases. Five to 10 ml total blood was extracted from these patients to isolate leucocytes for confirmation. A similar technique using 4-MUG and acarbose was completed with these samples. Patients with consistent alterations in inhibition and isoenzymes relation where confirmed as positive cases. A method using glycogen as substrate was developed later to obtain specific enzyme activity levels. **RESULTS:** A total of 13133 DBS samples referred from high-risk patients screened throughout the country were analyzed in a period of 14 years (2005-2018). Abnormal values in 930 samples were found. Isolated leucocytes were obtained and analyzed from these patients obtaining 69 altered values. Later, with the new glycogen assay available patients were called back and 50 samples could be recollected and analyzed with this technique. Low enzymatic levels (0.00 – 0.24 mmol/mgprotein/hour) were confirmed for all them. These results showed significant difference with reference controls. (Reference range values: 0.38 -2.93 mean 0.91). **CONCLUSION:** Enzymatic assay using glycogen as substrate and inhibition with acarbose proved to be a trustworthy test for Pompe disease diagnosis.