

Abstract

Congenital disorders of glycosylation represent (CDG) a group with more than 100 types of metabolic disorders, which are caused by defects in biosynthesis and modification of glycoconjugates. CDG manifest by broad spectrum of clinical symptoms, from disorders of nervous system to disorders of digestion and excretion. Identification of the specific type of CDG is not possible without broad spectrum of biochemical and molecular-genetic methods. The goal of this bachelor thesis was to describe the most often used methods for analysis of glycans, which are components of glycoproteins or glycolipids, in the theoretic part. Isoelectric focusing of selected blood serum glycoproteins, (e.g. transferrin and apolipoprotein C-III) serve as screening methods. Measurement of enzymatic activity, mass spectroscopy, PDO and LLO analysis (protein derived oligosaccharide, lipid-links oligosaccharide) HPLC and CZE (capillary zone electrophoresis and high performance liquid chromatography) are performed in second level. Molecular-genetic methods are used to confirm the final diagnosis by identification of causal mutations in specific gene. The aim of practical part of this bachelor thesis was to analyse the expression of genes OGA (N-acetylglucosaminase) and OGT (N-acetylglucosaminyltransferase). These enzymes take part in the synthesis and hydrolysis of bound N-acetylglucosamine (O-GlcNac) and polypeptidic chain. Disbalance of these two reactions could reflect to various pathological state. Pilot study RNA was isolated by TriReagent from isolated lymphocytes of peripheral blood and gene expression analysis was realised by qPCR (quantitative polymerase chain reaction). This pilot study will be used for study of glycosylation level (O-GlcNac), e.g. in patient who suffer from diabetes mellitus type 2.

Key words: Congenital disorders of glycosylation, isoelectric fokusation, qPCR