Abstract

Congenital disorders of glycosylation (CDG) represent a heterogeneous group of multisystemic metabolic disorders which are caused by defects in biosynthetic pathways of glycoproteins. The screening test for N-glycosylation disorders is the analyses of sialylated isoforms of serum transferrin (Tf) by means of isoelectric focusing (IEF). Two distinct pathological IEF patterns of Tf are observed. A type I pattern is characterized by a decrease of tetra- and an increase of di- and asialotransferrin, whereas a type II pattern shows in addition an increase of tri- and monosialotransferrin. The aims of diploma thesis were: 1) to evaluate reference range for spectrum of sialylated forms of Tf separated by IEF and 2) to perform biochemical and molecular analyses in three patients (P1-P3) with clinical suspicion for CDG. Serum and genomic DNA from three patients with clinical suspicion for CDG and family members of P1 were analysed. Sera from 99 healthy volunteers within the age range of 2-42 years served as a control group. Tf was analysed by IEF with direct immunofixation, SDS-PAGE and Western blot using specific antibody against human Tf (Dako). Profiles of Tf were quantified by AlphaEaseFC software (Alpha Innotech). Data were analysed by software STATISTICA 9.0 (StatSoft). *TF* a *PMM2* genes were analysed by cyclic sequencing ABI PRISM (Applied Biosystems). Reference range for individual Tf isoforms was determined: hexa- (6,3 ± 1,29 %), penta- (18,26 ± 2,23 %), tetra- (55,11 ± 4,05 %), tri- (13,66 ± 2,47 %), di- (6,64 ± 1,90 %), mono- and asialotransferrin (< 1 %). No significant correlations between individual sialotranferrins and age or gender were found. A type I Tf pattern found in P1 was caused by novel heterozygous mutation c.1889A>C in *TF* gene which blocks N-glycosylation of Tf in the position N630. Identical results were found in three family members of P1. In P2, pathological type II Tf pattern was found. No polymorphism was detected in *TF* gene. P2 was classified as CDG II-x. In P3, type I Tf pattern was found. The analyses of *PMM2* gene revealed that P3 is compound heterozygote for mutations c.338C>T and c.422G>A, proving the diagnosis of PMM2-CDG.

key words: *transferrin, isoelectric focusing, CDG, PMM2-CDG, diagnostics*