ABSTRACT

Background: Type 1 *diabetes mellitus* is a multifactorial disease caused by beta cell destruction of Langerhans pancreatic islets. From the genetic aspect the main predisposition lays on HLA class II genes (40 – 50%), molecules of which present exogenous peptides to CD4+ T lymphocytes. Enviromental factors play a crucial role in the etiopathogenesis of T1DM. Through epigenetic regulation (e.g. DNA methylation) the genetic and enviromental factors communicate. The level of methylation in the regulatory regions can significantly affect expression of these genes.

Aims: The aim of the diploma thesis was to define methylation profile of HLA *DQB1* alleles in type 1 *diabetes mellitus* patients and determine their expression.

Methods: The genotyping of HLA class II genes (*HLA-DRB1*, *HLA-DQA1*, *HLA-DQB1)* was performed using sequence specific primers. DNA was treated with sodium bisulfite, regulatory region of *HLA DQB1* was amplified and cloned into *E.coli*, strain DH5α/XL1-Blue. Positive clones were sent for sequencing and results analyzed. RNA was transcribed to cDNA by reverse transcription and the level of expression was analyzed by quantitative PCR.

Results: Statistically significant differences were found in total methylation of *DQB1**0201 and *0302 alleles in the B section of *DQB1* gene. Difference in methylation at single methylation sites was found in *DQB1**0201 and *0302 alleles at position 1894, 2179, 2200, 2302 and 2304 in the exon 2 – intron 2 (B section). Allele *DQB1**0302 was methylated more at position 2179, 2200, 2302 and 2304, however allele *DQB1**0201 was completely methylated at position 1894. Statistically significant differences in expression levels were found between alleles *DQB1* *0202 and *0501(higher *0501 expression), *0301 and *0302 (higher *0301 expression), *DQB1**0302 and *0501 (higher *0501 expression).

Keywords: type 1 *diabetes mellitus*, epigenetic, HLA class II, *HLA DQB1*