Additional Verification of Active Human Herpesvirus-6 Infection in AIT Patients' Thyroid Gland Tissues Using Electron Microscopy

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Introduction. Human herpesvirus-6 (HHV-6) is an ubiquitous ß-herpesvirus that possesses immunomodulating properties and has effectively colonized the vast majority of the human population approaching 100 % in seroprevalence. It is spread during early childhood and establishes a persistent lifelong relationship with its host. Although the primary targets for HHV-6 replication are $CD4^+$ and $CD8^+$ T lymphocytes, HHV-6 sequences have been found in different solid organs, including the thyroid gland. HHV-6 has been associated with several autoimmune diseases and recent studies show possible involvement of herpesviruses in development of autoimmune thyroid disease (AIT).

Aim, Materials and Methods. The aim of this study is to obtain additional evidence of active human herpesvirus-6 infection in AIT patients' thyroid gland tissues.

In this study were enrolled three AIT patients with confirmed active HHV-6 infection. One female age of 41 with clinical diagnosis *Struma nodosa III euthyreoticum*, male age of 45 with clinical diagnosis *Struma nodosa III-IV thyreotoxicum* and female age of 28 with clinical diagnosis *Struma nodosa IV thyreotoxicum*.

Molecular methods: PCR, nPCR, RT-PCR, Real Time PCR, Restriction analysis. Electron microscopy with DURCUPAN material fixation method.

Results. All three AIT patient thyroid gland tissue samples were positive for HHV-6 U79/80, U51 and U12 mRNA revealing active HHV-6 infection.

HHV-6 sequences were found only in patients' thyroid gland tissue samples, none of it was found in peripheral blood samples. In all three patients' blood DNA samples the viral load was under the detection limit of the kit used, while in all HHV-6 positive tissue samples viral load was detected (1306, 802 and 5923 viral copies/10^6 cells).

In the thyroid gland tissues of all three patients was accounted and ultrastucturally were observed viral-like particles and tegument-like materials of infected cells present in the cytoplasm, some in the cytosol in the Golgi apparatus or in structures resembling multivesicular bodies consistent with morphological features of HHV-

6 predominantly with intracellular distribution in the follicular subset of thyroid cells, rarely in the extracellular compartments. All morphological findings related with HHV6 infection were considered from 100 nm in their size (average size 130 nm).

Restriction analysis with endonuclease incubation showed HHV-6 B in all tested samples.

Conclusions. The first obtained results demonstrate possible HHV-6 virus-like particles in thyroid gland tissue, which could be an additional evidence for active HHV-6 infection AIT patients' samples; however, further investigation is required to confirm the HHV-6 virus particle presence.