

# Association of HHV-6 and HHV-7 with Diseases of Thyroid Gland



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- ▶ Diseases of the thyroid are manifested by qualitative or quantitative alterations in hormone secretion: insufficient hormone secretion - hypothyroidism or excessive secretion of hormone – hyperthyroidism or thyreotoxicosis.
- ▶ Hashimoto's thyroiditis (*lymphadenoid goiter*) is a common chronic inflammatory disease of the thyroid in which autoimmune factors play a prominent role.

Evidence of the participation of autoimmune factors includes:

- ✓ the lymphocytic infiltration of the gland,
- ✓ the presence of increased concentrations of immunoglobulins and of antibodies against several components of thyroid tissue in the serum.

- ▶ Thyroid gland autoimmune disorders, mostly the autoimmune chronic lymphoid thyroiditis is increasing problem in North part of Europe as well as in Latvia. Unfortunately there is no precise statistics about how many people in Latvia are suffering from thyroid gland disorders.
- ▶ Viral infections are frequently cited as a major environmental factor implicated in subacute thyroiditis and autoimmune thyroid diseases. Viral persistence and inflammation can act synergistically also to induce and sustain autoimmunity:
  - ✓ by unveiling cryptic self-epitopes,
  - ✓ by favouring determinant spreading,
  - ✓ by activating dendritic cells,
  - ✓ by promoting constant priming of new autoreactive T- cells,
  - ✓ by contributing to the efficient generation of effector cells, or restimulating memory T- lymphocytes.

- ▶ Inflammatory and autoimmune processes with unknown etiology are often the background also for disease of thyroid gland.
- ▶ Direct evidence of the presence of viruses or their components in the thyroid gland are available for retroviruses and mumps in subacute thyroiditis, for retroviruses (HTLV-1, HFV, HIV and SV40) in Graves's disease and for HTLV-1, enterovirus, rubella, mumps virus, HSV, EBV and parvovirus in Hashimoto's thyroiditis.
- ▶ During the last few years much attention has been paid to the members of the *Herpesviridae* family demonstrating that they are involved in development of inflammatory and autoimmune diseases.
- ▶ Our work which is in progress is to clarify if there is any association between latent/persistent and active beta-herpesviruses (Human Herpesvirus 6 [HHV-6] and Human Herpesvirus 7 [HHV-7]) infection and diseases of thyroid gland in Latvia.

- The Ethic Committee of the Riga Stradins University (Riga, Latvia) approved the study and informed mutual consent was obtained from a total of 42 subjects (37 females, 5 males, 23 to 77 years old, mean age 50 years) that were consecutively referred to thyroidectomy because of thyroid gland pathology without defining more precise diagnosis before surgery. The exact diagnosis of thyroid gland pathology was established by histological analysis of resected tissue and analysis of thyroid gland hormonal activity.

Groups of patients:

- 1<sup>st</sup> group (n=9): patients with *Struma nodosa III Eutireoticum* without autoimmune process;
- 2<sup>nd</sup> group (n=7): patients with *Struma nodosa III Eutireoticum* with autoimmune process;
- 3<sup>rd</sup> group (n=17): patients with *Struma nodosa III Thyreotoxicum* without autoimmune process;
- 4<sup>th</sup> group (n=5): patients with *Struma nodosa III Thyreotoxicum* with autoimmune process;
- 5<sup>th</sup> group (n=4): patients with papillar cancer of thyroid gland.

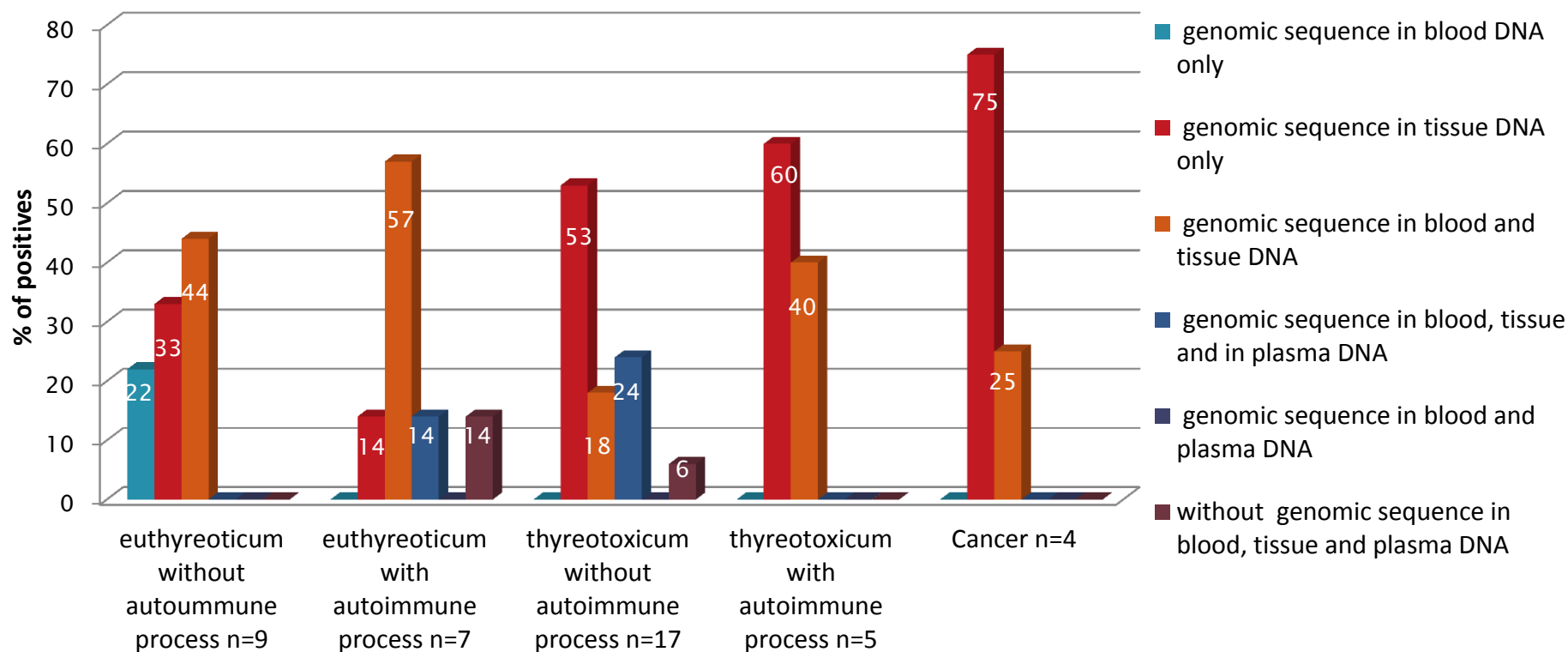


- ▶  $\beta$ -globin PCR was applied in order to check DNA quality. Negative  $\beta$ -globin PCR result for DNA isolated from plasma shows that there is no cell DNA in the sample. Nested polymerase chain reactions (nPCR) with the corresponding primer pairs were used for the detection of HHV-6 and HHV-7 genomic sequences [Bandobashi et al. 1997; Berneman et al., 1992, respectively].
- ▶ HHV-6 viral load was detected using Real-time PCR.

- ▶ The  $\beta$ -herpesviruses' infection in the human population is widely spread throughout the world, including Latvia.
- ▶ Our nPCR data shows that HHV-6 and/or HHV-7 prevalence in thyroid gland tissues is high (95.2% examined thyroid tissue specimens carried genomic sequences of herpesviruses).



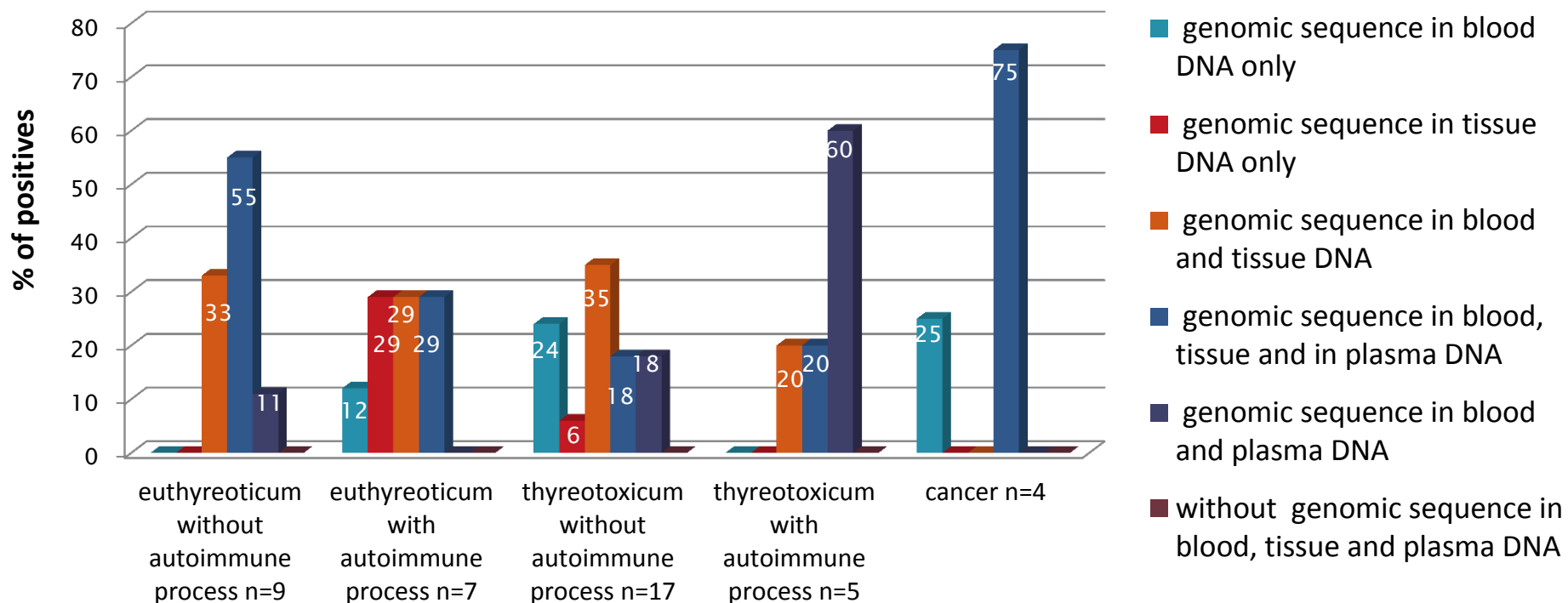
## HHV-6 prevalence in patients with Struma nodosa III euthyreoticum and thyreotoxicum (without and with autoimmune process) or cancer



- 2/9 (22.2%) patients from the 1<sup>st</sup> group had HHV-6 genomic sequence in blood DNA only.
- In tissue DNA only 3/9 (33.3%) patients from the 1<sup>st</sup> group, one patient (1/7; 14.3%) from the 2<sup>nd</sup> group, 9/17 (52.9%) patients from the 3<sup>rd</sup> group, 3/5 (60.0%) and 3/4 (75.0%) patients from the 4<sup>th</sup> and the 5<sup>th</sup> groups respectively had HHV-6 DNA genomic sequence.
- Simultaneously in blood and tissue DNAs HHV-6 genomic sequence was detected in all examined groups without statistically significant difference.
- HHV-6 specific sequence in blood, tissue and plasma simultaneously was not revealed in three out of five examined groups.
- HHV-6 viral load was higher in DNA isolated from thyroid gland tissues than from whole blood.



## HHV-7 prevalence in patients with Struma nodosa III euthyreoticum and thyreotoxicum (without and with autoimmune process) or cancer



- HHV-7 genomic sequence in blood DNA only was detected in three from examined five groups.
- Virus specific sequence in tissue DNA only was prevalent in the 2<sup>nd</sup> and the 3<sup>rd</sup> group.
- HHV-7 genomic sequence in blood and tissue DNAs simultaneously was found in all groups except the 5<sup>th</sup>.
- In blood, tissue and plasma DNAs simultaneously HHV-7 genomic sequence more frequently was observed in the 5<sup>th</sup> group.
- Statistically significant difference was not detected between HHV-7 prevalence between different DNAs or groups investigated.

- The data provided are preliminary, more serious work with:
  - ✓ mRNA extraction,
  - ✓ viral load detection,
  - ✓ detection of antiviral antibodies,
  - ✓ detection of TNF- $\alpha$ , IL-6, IL-1 $\beta$  expression level ,
  - ✓ epigenetic analysis of ERp29 and TSHR genes is in progress.
- HHV-6 and HHV-7 genomic sequences, specially in thyroid gland tissue DNAs are common in all examined groups and there is no statistical difference in frequency of HHV-6 and/or HHV-7 between them.
- Fact that in 1<sup>st</sup>, 4<sup>th</sup>, and 5<sup>th</sup> group there was no HHV-6 genomic sequence in blood plasma indicates, that virus is in latent stage, however without mRNA extraction that shows early stage of replication of the virus or at least IgM tests and/or elevated IgG detection we can not be 100% sure about it.

- Without extraction of mRNA from tissue, we can not tell if there is ongoing active HHV-6 and/or HHV-7 infection.
- In our study more frequently we can find HHV-6 genomic sequence (85.7%) in comparison to HHV-7 (57.4%) genomic sequence in thyroid gland tissue.
- It is important to remember that HHV-6 and HHV-7 are lymphotropic viruses and latent/persistent infection of those viruses in healthy population is high.
- In a case of chronic inflammatory process lymphocyte infiltration has been observed and findings of HHV-6 and HHV-7 genomic sequences can be explained by presence of HHV-6 and/or HHV-7 carrying lymphocytes in thyroid gland tissue.

## Preliminary conclusion:

- ▶ Lymphotropic HHV-6 and HHV-7 prevalence in thyroid gland tissues in patients with different thyroid diseases is high. However, it remains to determine whether they are responsible for thyroid diseases or whether they are just bystanders.

To be able to draw final conclusions about possible HHV-6 and/or HHV-7 role in etiopathogenesis of thyroid gland autoimmune and non-autoimmune disorders, more serious research needs to be done.



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