1. Summary

The first aim of our study was to find out the present occurence of *H. pylori* infection in a group of children with juvenile lymphocytic thyroiditis (JLT) and to compare with the prevalence of this infection in a group of healthy children of the same age.

The group of patients with JLT included 199 children (range 0-18 years). *H. pylori* infection was confirmed in 15 patients (7,5%) by a non-invasive diagnostic test of *H. pylori* antigen positivity in stools by the ELISA test using monoclonal antibodies (Amplified IDEIQA HpStAR ELISA, DakoCytomation, Glostrup, Denmark). This *H.pylori* positive group included 14 girls and 1 boy aged 11,2 ±2,3. 6 children had hypofunction of the thyroid gland, in 3 children was established the atrophic form of the thyroiditis with severe hypothyroidism with presence of myxoedema, the other children suffered from the hypertrophic form of the thyroiditis. 7 subjects underwent gastroscopy which confirmed chronic active gastritis. Only 3 children had gastrointestinal symptoms caused by *H. pylori* infection, the other children were asymptomatic. This finding confirms common information about asymptomatic development of *H. pylori* infection in children in 90% of cases.

The control group included 1545 asymptomatic healthy children in range of 0-15 years of the same geographic region living in different regions (town, village). The prevalence of *H. pylori* infection in healthy children was confirmed in 110 out of 1545 children (7,1%). We found positive correlation with age, but without dependence on sex and the occurence of this infection was significantly more often in children living in low socioeconomic conditions. As specific dependence of the prevalence of *H. pylori* infection on the age in children was confirmed we made a comparison to a control group of healthy children of the same age (range 10-13 years), where the prevalence of *H. pylori* infection reached 8,2% and wasn't different from the prevalence of *H. pylori* infection in group of children with JLT too.

We also confirmed the comparable prevalence of *H. pylori* infection in group of our patients with JLT (7,5%) and in asymptomatic healthy children (7,1%).

The second aim of our study was the analysis of the the group of 231 patients with JLT. The studied criteria were: sex, age, function of the thyroid gland, positivity of autoantibodies at the time of diagnosis, occurence of other autoimmune diseases and the number of siblings with autoimmune diseases.

According to published data we also confirmed the majority of female sex in our group of 231 children with JLT. This fact was also confirmed by the analysis of the family history where only 1,3% parents or grandparents of male sex suffered from autoimmune diseases.

The range of age in our group at the time of diagnoses of JLT confirmed the most frequent development of autoimmune disease during the puberty and adolescence (the range of 11-18 years was in 66%). On the other hand this disease is present in very young pre-school children. We pointed out that this fact mustn't be underestimated.

The positivity of both specific autoantibodies (antiTPO and antiTG) was present in 60,6% patients at the time of diagnoses of JLT. The negativity of both specific autoantibodies was present in 3,5% patients with JLT which doesn't exclude the diagnosis of JLT. Based on our clinical experience with the patients with transient form of congenital hypothyroidism we emphasized the importance of the investigation of blocked antiTSH antibodies especially in female sex patients with the atrophic form of autoimmune thyroiditis in pregnancy. The positivity of these antibodies predict the possibility of blocking the thyroid gland in foetus with development of transient form of congenital hypothyroidism. We can confirm this

diagnosis by measuring TSH in umbilical blood and start therapy with levothyroxine sooner before the result of screening of congenital hypothyroidism. These women must have adequate substitution with levothyroxine because of dependence of foetus on mother's saturation with thyroxine during the pregnancy.

24% of patients with JLT in the group had positive allergic history confirming genetic predisposition to various immunopathological reactions in these individuals. This fact confirms higher risk of development of autoimmune diseases in children with positive history of allergy.

We confirmed high frequency of autoimmune diseases in parent or grandparent of our patients with JLT (44,6%), which confirms genetic disposition to autoimmune immunopathological reaction in such affected families. At the time of JLT diagnosis, 59% of children had already hypofunction of the thyroid gland, 46% of which had severe hypothyroidism with myxoedema , bradycardia, hypotension, growth retardation. In several cases, the affected children were from families with unambiguous genetic disposition to autoimmune diseases. This fact leads to the question of the follow-up of individuals in these affected families due to possible development of autoimmune disease. A late diagnosis of severe primary hypothyroidism in childhood can have permanent aftereffects on the growth and development of the child.

According to published data, in our group we confirmed the most frequent occurence of autoimmune diseases of the thyroid gland from the spectrum of autoimmune diseases. The prevalence of chronic lymphocytic thyroiditis in families of our patients was 72% and the prevalence of Graves-Basedow disease was 15,5%. The prevalence of autoimmune diseases in siblings of our patients with JLT was present in 7,4% of cases and the most frequent was JLT too.

The associated autoimmune disease was present in 15,2% of patients in our group. In 5,5% of the cases, another autoimmune disease of non-endocrine organs was first diagnosed, and then after several years was established the diagnosis of JLT with hypofunction of the thyroid gland. We recommend consequent searching out JLT in patients with other autoimmune disease and the follow-up of the development of organ specific autoantibodies in patients with diagnosis of more autoimmune diseases in order to make an early diagnosis of pre-clinical period of serious endocrinopathy such as autoimmune adrenalitis or type 1 diabetes mellitus.

The third aim of our study was the determination of immunoreactivity in patients with JLT *H. pylori* positive, negative and in a healthy control group *H. pylori* positive and negative. To our best knowledge so far, nobody has been interested in *H. pylori* infection and present autoimmune thyroiditis regarding a possible stimulating role of the microbe and its lipopolysaccharide (LPS) on immunocompetent peripheral blood mononuclear cells (PBMC). *H. pylori* can be one of the possible triggers of thyroid autoimmunity. The hypothesis is a possible cross reactivity between H. pylori antigens and thyroid gland antigens, it means „molecular mimicry“ phenomenon, when originally antiinfectious immunity becomes autoimmunity. These common antigens seem to be Lewis antigens x,y which are expressed on beta-chain of gastric proton pump, on gastric mucin, in epithelial cells of the thyroid gland, in pancreas and similar antigens are expressed on *H. pylori* surface as part of lipopolysaccharide.

After isolation of PBMC from the peripheral blood of the studied inviduals by Ficoll density gradient centrifugation, their immunoreactivity was analysed by determination of basal cytokine and chemokine production and after stimulation with *H.pylori* and its lipopolysaccharide (Lewis antigens) using the method of protein microarray. We analysed the production of 23 cytokine and chemokine. We examined 53 patients with JLT (20 patients *H.pylori* positive, 33 patients *H.pylori* negative) of average age of 15 years (range 7-26

years) and a control group of 40 healthy girls (20 girls *H. pylori* positive, 20 girls *H. pylori* negative) from the same socio-economical group. The immunoreactivity to *H.pylori* and to its LPS differed between studied groups.

The individulas with positive *H. pylori* infection (JLT *H. pylori* positive group and *H. pylori* positive healthy control group) reacted by massive chemokine response to *H.pylori* lysate and to its LPS after stimulation blood mononuclear cells. JLT *H. pylori* positive patients produce upon stimulation proinflammatory cytokines, mainly IL-6.

H. pylori negative healthy controls had the lowest basal cytokine and chemokine production, but in comparison to JLT *H. pylori* positive and negative individulas significantly increased production of IFN-γ (Th1 cytokine) and regulatory cytokine TGF-β too (Th3 cytokine), which suppresses production of Th1 cytokines. JLT

H. pylori negative patients had a higher production of TGF-β to LPS stimulation in comparison to *H.pylori* positive healthy controls, as well as in comparison to JLT *H.pylori* positive patients. The production of IL -10 as another regulatory Th3 cytokine was in *H.pylori* negative healthy controls rather decreased, as well as the production of IL-5.

In patients with *H.pylori* infection, it means in patients JLT *H. pylori* positive and *H.pylori* positive healthy controls was observed a lower production of regulatory cytokines, which can be explained either by exhaustion of their immunocompetent cells or by negative influence of *H.pylori* infection.

These tests confirmed significant influence of *H.pylori* infection on immunocompetent cells, especially in individuals with autoimmune disposition and consequently with present immune dysbalance. An increasing incidence of autoimmune diseases including juvenile lymphocytic thyroiditis requires both complex care of these patients, together with searching out possible associated autoimmune diseases and searching out possible triggers of this immunopathological reaction. As a result of our findings we recommend in patients with autoimmune thyroiditis active searching out *H.pylori* infection and, in case of positivity, eliminating this infection.