EPIDEMIOLOGICAL, CLINICAL AND GENETIC OBSERVATIONS IN CHILDREN AND YOUTH WITH TYPE 1 DIABETES

PhD Thesis

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INTRODUCTION

1.) The incidence of childhood type 1 diabetes has been increasing for decades (International and Hungarian registries) and type 1 diabetes has now become one of the most common chronic diseases in the Pediatric age group. The incidence in Hungary was 20 new cases per 100,000 children per year in 2011, which translates to a prevalence of 0.16%. In other words, one out of every 600 children under the age of 15 years in Hungary has diabetes. There are large differences in incidence among continents and countries, the variation within Europe is tenfold. There are some reports of regional differences within countries, as well.

2.) In a large proportion of children, diabetes is diagnosed late, in a ketoacidotic state (presentation ketoacidosis), which is the main cause of hospitalisation and early mortality.

3.) There are two major forms of diabetes during pregnancy. Gestational diabetes (GDM) is caused by relative insulin deficiency and develops during pregnancy, pregestational diabetes (PGDM) is the insulin-deficient type 1 diabetes presented before pregnancy. Both forms can be harmful to the fetus and the newborn requires special attention. Despite improvements in the care of pregnant women with diabetes, there are reports of increased prevalence of congenital malformations and disturbances of postnatal adaptation. Systematic studies over the last 15 years are lacking Hungary.

4.) Autoimmune type 1 diabetes presents more and more frequently at a younger age. However, neonatal diabetes and diabetes in young infants are not autoimmune in origin.

5.) It is generally accepted, that type 1 diabetes develops in genetically susceptible individuals as a result of a beta-cell damaging autoimmune process initiated by environmental factors. The genetic background and the pathomechanism, however, is not fully delineated. In some patients, type 1 diabetes can be associated with other organ-specific autoimmune conditions. The clustering of organ-specific autoimmune diseases in the same patient suggests shared common susceptibility pathways that eventually lead to the sequential development of these individual conditions. The clinical and genetic studies of such patients can contribute to our understanding of the etiology and pathomechanism.

6.) The new insulins and novel delivery devices combined with blood glucose monitoring and patient education have considerably improved life expectancy, but glycaemic control is still
far from satisfactory in a large proportion of diabetic children, which increases the risk of micro- and macrovascular complications. Most of these complications develop after decades of diabetes duration and therefore not readily amenable to pediatric investigation. In Hungary, there are no data whatsoever on the prevalence of diabetic complications in young adults with diabetes diagnosed in the pediatric age group.

AIMS

1.) Comparative analysis of incidence and incidence trends (1989-2011) in a western and eastern region of Hungary (county of Baranya and Békés) with similar geographical and population size.

2.) Analysis of patients with diabetic ketoacidosis (DKA) during a five year period (2006-2010) in county Békés.

3.) Study of the prevalence of the disturbances of postnatal adaptation and congenital malformations during a 10 year period (2001-2010) of infants of mothers with gestational and pregestational diabetes.

4.) Case study and genetic investigations in an infant with transient neonatal diabetes.

5.) Phenotypic description, genetic investigation and therapeutic experience in three uncommon cases of autoimmune diabetes triads.

6.) Investigation of co-morbidity, micro- and macrovascular complications in young adults after an average diabetes duration of 20 years in four counties of Southern Hungary.
RESULTS

1.) Comparative incidence of childhood type 1 diabetes in county Baranya and Békés (1989-2011)

Case-material and methods
The database of the National Childhood Diabetes Registry and the hospital records were used. Incidence rate was calculated by standard epidemiological methods.

Results
The yearly incidence during the period 1989-2011 is depicted in Figure 1.

![Figure 1](image_url)

Figure 1. The incidence of childhood (0-15 year) type 1 diabetes (cases per 100,000 per year) in county Baranya and Békés (1989-2011). Mean and 95% confidence intervals (Ba = Baranya, Bé = Békés)

The incidence-rate was similar in both counties in most years. There are differences in some years, but the figures are not statistically significant.

The year to year fluctuation is considerable, but the increasing trend over two decades is obvious, the incidence more than doubled over the last 23 years.
2.) Diabetic ketoacidosis (DKA) (2006-2010)

Case-material and methods
Case histories of children admitted for DKA during 2006 and 2010 were analysed.

Results
Epidemiological data:
Presentation ketoacidosis
During the five year period, 49 new cases of diabetes was diagnosed and 12 children presented with DKA, which corresponds to a prevalence of 25 %.

Ketoacidosis in insulin-treated children
There were 27 episodes of DKA between 2006 and 2010 in insulin-treated patients in our care. Considering the total number of cases in our clinic, this translates to a yearly frequency of 5.1 %.

Management of DKA:
Both groups (presentation DKA versus insulin-treated group, respectively) demonstrated gross hyperglycaemia (mean blood glucose 33.9 ± 9.8 mmol/l and 27.8 ± 6.5 mmol/l and severe ketoacidosis (pH 7.06 ± 0.13 and 7.12 ± 0.13).
The children were treated according to the protocol of our intensive care unit and for the analysis of treatment, the biochemical parameters of the two groups were combined.
Blood glucose decreased with a rate of 0.95 mmol/hour during the first 12 hours and with a rate of 0.45 mmol/l thereafter. The mean pH increased from 7.12 to 7.29 over the first 12 hours and reached the normal range at the end of the first day of treatment.

3.) Infants of diabetic mothers. Anthropometry, fetal and neonatal complications

Methods
Hospital documentation of infants of diabetic mothers admitted in the neonatal unit between 2001 and 2010 were retrospectively analysed.

Results
During the 10 year period, 398 of the 11731 pregnancies (3.4%) were complicated with diabetes. 375 mothers (94.3%) had gestational and 23 (5.7%) had pregestational diabetes. 32% of the infants were transferred to the neonatal unit.
Neonatal anthropometry (Figure 2)

Neonatal macrosomia (birth weight > 90 centile) was observed in one fifth of the infants.

![Pie charts showing birth weight, length, and head circumference centiles](image)

**Figure 2.** Main anthropological characteristics

Neonatal complications (Figure 3)

![Bar chart showing various clinical symptoms and their occurrence in patients](image)

**Figure 3.** Disturbancies of postnatal adaptation
Hypoglycaemia (blood glucose < 2.6 mmol/l) developed in 39% of newborns, in the majority of the cases within the first 8 hours. Hypoglycaemia was symptomatic in 55% of the infants. Hypocalcaemia was observed in 17%, hyperviscosity in 23%, hyperbilirubinaemia in 32%, respiratory distress syndrome and/or transient tachypnoe in 22% and cardiac complications in 13% of the cases. 10% of the babies suffered from birth injuries.

**Congenital malformations (Table 1)**

Congenital anomalies were seen in 17% of the cases, severe malformations were present in 5 (4%) infants: ventricular septal defect requiring surgery (3 cases), bilateral cheilognathopalatoschisis (1 case), VACTERL association (1 case).

<table>
<thead>
<tr>
<th>Table 1. Congenital malformations</th>
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</thead>
<tbody>
<tr>
<td><strong>congenital malformations</strong></td>
</tr>
<tr>
<td><strong>cardiovascular</strong></td>
</tr>
<tr>
<td>atrial septal defect</td>
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<tr>
<td>ventricular septal defect</td>
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<tr>
<td><strong>renal</strong></td>
</tr>
<tr>
<td>Multicystic kidney</td>
</tr>
<tr>
<td>Pyelectasy</td>
</tr>
<tr>
<td>Duplex kidney</td>
</tr>
<tr>
<td><strong>orofacial clefts</strong></td>
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<tr>
<td>Cheilognathopalatoschisis</td>
</tr>
<tr>
<td><strong>skeletal</strong></td>
</tr>
<tr>
<td>Polydactyly.</td>
</tr>
<tr>
<td><strong>multiplex</strong></td>
</tr>
<tr>
<td>VACTERL</td>
</tr>
<tr>
<td><strong>syndrome</strong></td>
</tr>
<tr>
<td>Klippel-Feil</td>
</tr>
<tr>
<td><strong>other</strong></td>
</tr>
<tr>
<td>Hygroma of the neck</td>
</tr>
</tbody>
</table>
4.) 6q24-associated transient neonatal diabetes (TNDM) with unbalanced chromosome-translocation

Transient neonatal diabetes in a young infant was associated with a dysmorphic phenotype (pes planovalgus, joint contractures and progeria-like appearance) and congenital abnormalities (dilated renal pelvis, persistent foramen ovale, atrial and ventricular septal defect). The karyotype showed an unbalanced chromosome anomaly disorder, a paternally inherited duplication of the 6q chromosome region \((46,XX,\text{der}(13)t(6;13)(q24;q?)\). Based on the available published data it is likely, that this chromosome anomaly has caused this complex phenotype.

5.) Autoimmune organ-specific triads

Three uncommon pediatric cases are presented, in which type 1 diabetes was associated with two other organ-specific autoimmune/allergic conditions. The clinical phenotype, the sequential development of the individual diseases, the therapy and finally some molecular genetic studies are discussed.

a.) Type 1 diabetes, Hashimoto’s thyroiditis and rheumatoid arthritis

The clinical phenotype of an unusual triad is described with sequential development of organ-specific autoimmune diseases starting with type1 diabetes at age of 2 yr followed by thyroid autoimmunity and finally by juvenile rheumatoid arthritis. The patient had a hitherto unpublished genotype of the HLA class II genes /HLA DRB1*0401-DQA1*03-DQB1*0301/(DR14)-DQB1*0503/, which is reported to be a protective genotype in the Hungarian population. The child was a heterozygote (AG) for the \(\text{CTLA4}\) gene and a heterozygote (CT) for the \(\text{PTPN22}\) gene.

b.) Type 1 diabetes, hypothyroidism and Addison’s disease

Two cases are described in whom first type 1 diabetes developed, which was followed by autoimmune thyroiditis (hypothyroidism) and adrenal insufficiency, prompting the diagnosis of autoimmune polyendocrine syndrome type 2 (APS2) \((\text{Table 2})\). Retrospectively, Addison’s
disease could have been suspected by the symptoms, the frequent hypoglycemia and unexplained decrease in insulin requirement in case 1, in whom diagnosis was made during an Addisonian crisis. Increased skin pigmentation suggested diagnostic tests in case 2, who remained well under hormone replacement therapy for years, but suddenly died under unknown circumstances, possibly due to an Addisonian crisis.

Table 2. Patient data

<table>
<thead>
<tr>
<th></th>
<th>1. patient</th>
<th>2. patient</th>
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<tbody>
<tr>
<td>age at the time of diabetes diagnosis</td>
<td>2 year</td>
<td>3 year</td>
</tr>
<tr>
<td>age at the time of thyroid autoimmunity diagnosis</td>
<td>8 year</td>
<td>11 year</td>
</tr>
<tr>
<td>age at the time of Addison’s disease diagnosis</td>
<td>8 year</td>
<td>15 year</td>
</tr>
<tr>
<td>symptoms</td>
<td>fatigue, susceptibility to hypoglycaemia, low insulin need</td>
<td>skin pigmentation, low insulin need</td>
</tr>
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</table>

c.) Type 1 diabetes, insulin allergy and Crohn’s disease

A 14-year-old boy with type 1 diabetes mellitus developed progressive allergic skin reactions to insulin. Similar skin reactions developed after using other available brands of insulin. He had eosinophilia and high total IgE level. Inhalative and food specific IgE were negative. The Prick skin test, the Hungarian standard intradermal test, the intradermal skin tests with Novo allergy kits and the LTT were all negative. In an attempt to desensitize the patient, we introduced a rapid acting insulin analogue regimen consisting of five small premeal bolus injections of insulin aspart. This resulted in some, but still unsatisfactory improvement, which prompted us to start treatment with continuous subcutaneous insulin administration using a portable pump. The small basal infusion rate served as a kind of desensitization and the allergic skin reactions gradually disappeared. Unexpectedly, a few weeks later, he developed periprostal abscess. The subsequent gastrointestinal and immunological investigations suggested inflammatory bowel disease, possibly Crohn’s disease and appropriate treatment was started.
d.) **Overview of autoimmune organ-specific triads**

*Table 3* summarises the clinical and epidemiological characteristics of the triads. Diabetes (with one exception) manifested early in life predating the other autoimmune diseases with years.

The second condition was autoimmune hypothyrosis in three cases of the triads. Finally, Addison’s disease developed as a third condition coinciding with the manifestation of hypothyroidism in one child and many years later in another one.

**Table 3.** Autoimmune organ-specific triads  
(the age at manifestation in brackets)

<table>
<thead>
<tr>
<th></th>
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<th>1. disease</th>
<th>2. disease</th>
<th>3. disease</th>
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<td>girl</td>
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<td>Thyreoiditis (11)</td>
<td>Rheumatoid arthritis (15)</td>
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<td>2a</td>
<td>boy</td>
<td>Type 1 diabetes (2)</td>
<td>Thyreoiditis (7)</td>
<td>Addison’s disease (7)</td>
</tr>
<tr>
<td>2b</td>
<td>girl</td>
<td>Type 1 diabetes (3)</td>
<td>Thyreoiditis (11)</td>
<td>Addison’s disease (15)</td>
</tr>
<tr>
<td>3</td>
<td>boy</td>
<td>Type 1 diabetes (16)</td>
<td>Insulin allergy (16)</td>
<td>Crohn’s disease (17)</td>
</tr>
</tbody>
</table>

6.) **Autoimmune comorbidity and microvascular complications in childhood-onset type 1 diabetes after 20 years of diabetes duration.**

**A questionnaire study**

**Methods**

We assessed the prevalence of autoimmune co-morbidities and that of micro- and macrovascular complications after 20 years of diabetes duration using postal questionnaire.

**Result**

6.3% of the patients had celiac disease. Diabetes was diagnosed at a significantly earlier age in patients with diabetes and celiac disease as compared to those without celiac disease (*Figure 4*). Thyroid autoimmunity was reported in 7.6% of cases. They were significantly older with longer duration of diabetes. However the age at onset of diabetes was similar (*Figure 5*).
Every fifth patients reported retinopathy, one sixth of patients was treated for hypertension. Neuropathy was found 3.4 % and kidney disease in 4.8 % of the cases (Table 4).
Apart from retinopathy and hypertension, the prevalence of microvascular complications was relatively low. Considering the limitations of questionnaire studies, laboratory screening is warranted to assess the true prevalence of comorbidities and complications.

### SUMMARY/NEW OBSERVATIONS

1.) The incidence of childhood type 1 diabetes was found to be similar in a south west and in a south east county of Hungary. It was concluded, that for a statistically meaningful comparison of regional differences in incidence in Hungary, geographical units larger than counties are to be considered and due to the year to year fluctuation of incidence, several decades of observation are required for reliable trend-analysis.

2.) The prevalence of diabetic ketoacidosis (DKA) during a five year observation period was described.
   a.) 25% of the newly diagnosed children presented with DKA. This high proportion (which is similar to reports in the literature) is of public health significance and calls for actions. We need to get the educational message (the most frequent symptoms of diabetes) through to health care professionals, teachers and parents to reduce ketoacidosis at presentation.
   b.) We have shown – as the first population-based study in Hungary – that annually 5.1% of insulin-treated diabetic children required hospitalisation for the treatment of DKA.

3.) The fetal and neonatal consequences of maternal diabetes were analysed in a large retrospective study (the first of its kind in Hungary in the first decade of the 21st century) in a county hospital. Our observations – confirming the data from other countries – emphasizes that
despite modern diabetes management, there is still a higher incidence of fetal macrosomia and adverse neonatal outcomes and a higher rate of severe congenital malformations.

4.) Our case-report and genetic studies of transient neonatal diabetes extended the clinical phenotype of the disorder suggesting its divergence from other monogenic forms of transient neonatal diabetes. Transient neonatal diabetes is a relatively new entity; most of the cases published have not yet reached adult age. Therefore, the clinical and laboratory follow-up of every single case can be contributory to the better description of this phenotype.

5.) a.) We performed genetic studies of three polymorphisms (HLA, CTLA4 and PTPN 22) in a unique autoimmune organ-specific triad, type 1 diabetes, autoimmune hypothyroidism and rheumatoid arthritis. These genetic studies suggest that diabetes can develop in early life in patients carrying a protective HLA genotype and two minor genetic factors conferring susceptibility to diabetes and other organ-specific autoimmune diseases.

b.) With the analysis of two cases of diabetes, thyroid autoimmunity and Addison’s disease, we suggested, that in diabetes associated with thyroid autoimmunity, the risk of Addison’s disease is increased, and regular screening (cortisol, ACTH and adrenal antibodies) is warranted to prevent the development of life-threatening full-blown adrenal insufficiency.

c.) We described a hitherto unpublished pediatric triad of type 1 diabetes, insulin allergy and Crohn’s disease and confirmed the potential of insulin pump treatment in the process of desensitisation.

6.) In a population-based questionnaire-study (the first of its kind in Hungary) we have estimated the prevalence of diabetes comorbidity, microvascular complications and hypertension after 20 years of diabetes duration in a cohort of patients diagnosed in childhood.