Diploma thesis

Associated Autoimmune Diseases in Children and Adolescents with Type 1 Diabetes at the Department of Paediatrics and Adolescent Medicine of the Medical University Graz

submitted by

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OA Dr. in Hildegard Jasser-Nitsche

Graz, 22 August 2018

Affirmation in lieu of an Oath

I hereby formally declare, that I have written the submitted thesis independently and without any illegitimate assistance from third parties. I confirm, that I used no other than the declared sources for the preparation of this academic work. All used sources have been indicated as such and acknowledged by means of complete references in the text.

Graz, 22 August 2018 *Verena Kreiner eh*

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Abstract

Background: Type 1 diabetes (T1D) is the most common chronic metabolic disease in children and adolescents, associated with a variety of additional autoimmune diseases, including autoimmune thyroid disease (AITD), celiac disease (CD), autoimmune gastritis (AG) and Addison's disease (AD). Undiagnosed, these associated diseases can lead to comorbidities such as impaired growth, delayed puberty, poor metabolic control and many more complications. The aim of this study was to assess the prevalence of associated autoimmune disease in T1D patients at the Department of Paediatrics and Adolescent Medicine of the Medical University Graz and whether it changed substantially since 2008, as well as to analyse differences in age at diagnosis of the additional autoimmune disease, gender and the duration of diabetes at the time of diagnosis.

Materials and Methods: A retrospective analysis of 303 children and adolescents (128 females, 175 males) diagnosed with T1D before the age of 18 years, at an average age of 7.1 ± 4.2 years and treated at the Diabetes Outpatient Clinic at the Department of Paediatrics and Adolescent Medicine of the Medical University Graz was performed. Data collected for every patient included gender, date of birth, date of diabetes onset, age at diabetes onset, family history of autoimmune disease and in patients with an additional autoimmune disease, date and age when antibodies were positive.

Results: Of the 303 children and adolescents with T1D, 62 patients (20.5%) developed an additional autoimmune disease. Females (27%) had a significantly higher prevalence of associated autoimmune diseases than males (14%, $P < 0.001$). Since 2008, the prevalence has risen by almost 30%, however without reaching statistical significance ($P = 0.339$). AITD was present in 35 youths (11.6%), with a significant female predominance (24 female, 11 males, $P = 0.028$). 33 patients (10.9%) developed Hashimoto thyroiditis (HT) and two patients (0.6%) had Graves' disease (GD). On average AITD was diagnosed at an age of 9.6 ± 4.2 years and 2.3 ± 2.9 years after T1D onset. CD was diagnosed in 25 children and adolescents (8.3%), showing a significant rise since 2008 (3.2%, $P = 0.035$). Average age at diagnosis of CD was 6.8 ± 4 years and 75% of patients were

diagnosed under the age of 10 years (P = 0.014). Mean diabetes duration was 0.6 ± 1.7 years and no significant gender differences were found. AG was present in 9 patients (3%), with an average age at diagnosis after the onset of puberty (13 ± 2.6 years) and 6.4 ± 4.6 years after T1D onset. No correlation between AG and gender was found.

Conclusion: The high prevalence of associated autoimmune diseases in children and adolescents with T1D, as well as the great variation in average age at diagnosis of the different autoimmune diseases, highlights the importance of routine screening procedures for early recognition and prevention of life-threatening complications.

Zusammenfassung

Hintergrund: Typ-1-Diabetes (T1D) ist eine der häufigsten Stoffwechselerkrankungen im Kindes- und Jugendalter. Weitere Autoimmunerkrankungen, die häufig mit T1D im Zusammenhang stehen, sind Autoimmunthyreopathien (AITD), Zöliakie (CD), Autoimmungastritis (AG) und Morbus Addison. Bleiben diese assoziierten Erkrankungen unerkannt, können Komorbiditäten wie Wachstumsstörungen, verzögerter Pubertätseintritt, schlechte Blutzuckereinstellung und weitere Komplikationen auftreten.

Ziel dieser Studie war es zu ermitteln, wie viele, der an T1D erkrankten Kinder und Jugendlichen, eine weitere Autoimmunerkrankung aufweisen, ob sich deren relativer Anteil verändert, welche dieser Erkrankungen am häufigsten auftreten, in welchem Alter diese diagnostiziert werden, wie lange die Patienten bei deren Diagnose bereits an Diabetes erkrankt sind und ob ein Geschlechtsunterschied besteht. Weiters wurde untersucht, ob und wie sich die Prävalenz der assoziierten Autoimmunerkrankungen seit 2008 verändert hat.

Material und Methoden: Eine retrospektive Analyse von 303 Kindern und Jugendlichen (128 weiblich, 175 männlich), bei denen vor dem 18. Lebensjahr mit einem Durchschnittsalter von 7.1 ± 4.2 Jahren T1D diagnostiziert wurde und die an der Diabetesambulanz der Universitätsklinik für Kinder- und Jugendheilkunde Graz behandelt werden, wurde durchgeführt. Die untersuchten Daten von allen Patienten mit T1D umfassen: Geschlecht, Geburtsdatum, Datum der Diabetesmanifestation und Alter bei Diabetesmanifestation. Zusätzlich wurden bei Patienten mit weiteren Autoimmunerkrankungen Datum und Alter bei der Diagnose von positiven Antikörpern erhoben.

Ergebnisse: 62 (20,5%) der 303 Kinder und Jugendlichen mit T1D entwickelten eine weitere assoziierte Autoimmunerkrankung. Mit 27% hatten weibliche Patienten eine signifikant höhere Prävalenz als männliche (14%, P <0,001). Im Vergleich zu 2008 stieg die Prävalenz um beinahe 30%, jedoch ohne Erreichung statistischer Signifikanz (P = 0,339). AITD wurde bei 35 Kindern und Jugendlichen (11,6%) festgestellt, mit einer deutlichen weiblichen Dominanz (24 weiblich, 11 männlich, P = 0,028). 33 Patienten (10,9%) entwickelten eine Hashimoto-Thyreoiditis (HT) und 2 Patienten (0,6%) einen Morbus Basedow (GD). Im

Durchschnitt wurde eine AITD im Alter von 9,6 ± 4,2 Jahren und 2,2 ± 2,9 Jahre nach T1D Manifestation diagnostiziert. CD wurde bei 25 Kindern und Jugendlichen (8,3%) mit einem Durchschnittsalter bei Diagnose von 6,8 ± 4 Jahren festgestellt. Bei 75% der Kinder und Jugendlichen wurde CD vor dem 11. Lebensjahr diagnostiziert (P = 0,014). Die mittlere Diabetesdauer war 0,6 ± 1,7 Jahre und zeigt keinen signifikanten geschlechtsspezifischen Unterschied. AG wurde bei 9 Patienten (3%) festgestellt. Altersdurchschnitt bei Diagnosestellung war nach Pubertätsbeginn (13 ± 2,6 Jahre) und 6,4 ± 4,6 Jahre nach T1D Manifestation. Zwischen AG und Geschlecht wurde kein Zusammenhang beobachtet.

Schlussfolgerung: Die hohe Prävalenz an Kindern und Jugendlichen, die zusätzlich zu T1D auch andere Autoimmunerkrankungen entwickeln, sowie die großen Altersunterschiede bei der Diagnose dieser weiteren Autoimmunerkrankungen, bekräftigen die Bedeutung regelmäßiger Screeningverfahren.

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Abbreviations

T1D	Type 1 diabetes
ADA	American Diabetes Association
ISPAD	International Society of Paediatric and Adolescent Diabetes
IDF	International Diabetes Federation
GADA	Glutamic acid decarboxylase autoantibody
IA-2A	Islet antigen 2 antibody
ICA	Islet cell autoantibody
IAA	Insulin autoantibody
ZnT8A	Zink transporter 8 autoantibody
HLA	Human leukocyte antigen
CTLA-4	Cytotoxic T lymphocyte-associated antigen-4
PTPN22	Protein tyrosine phosphatase non-receptor type 22
CD	Celiac disease
GFD	Gluten free diet
HbA1C	Haemoglobin A1c
FPG	Fasting plasma glucose
OGTT	Oral glucose tolerance test
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
DKA	Diabetic ketoacidosis
NPH	Neutral protamine Hagedorn
MDI	Multiple daily injections
AITD	Autoimmune thyroid diseases
HT	Hashimoto thyroiditis

GD	Graves’ disease
TSH-R	Thyroid-stimulating hormone-receptor
IFN-g	Interferon gamma
T3	Triiodothyronine
T4	Thyroxine
Tg	Thyroglobulin
TgAb	Thryoglobulin antibody
TPO	Thyroid peroxidase
TPOAb	Thyroid peroxidase antibody
TRAb	Thyroid Stimulating Hormone receptor antibody
SBB	Small bowel biopsy
AGA	Anti-gliadin antbodies
tTG	Anti-tissue transglutaminase antibodies
EMA	Anti-endomysial antibodies
DGP	Anti-deamidated gliadin peptide antibodies
AG	Autoimmune gastritis
IDA	Iron deficiency anaemia
AIF	Antibodies to intrinsic factor
AD	Addison’s disease
UC	Ulcerative colitis
JIA	Juvenile idiopathic rheumatic arthritis

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1 Introduction

1.1 Type 1 Diabetes mellitus

1.1.1 Definition

Type 1 diabetes (T1D) is a chronic disease characterised by persistent hyperglycaemia, resulting from partial or absolute insulin deficiency due to chronic immune-mediated β-cell destruction of the pancreas (1,2).

1.1.2 Classification

The American Diabetes Association (ADA) and the International Society of Paediatric and Adolescent Diabetes (ISPAD) classifies T1D into the two aetiological types, immune-mediated diabetes and idiopathic diabetes (1,3).

1.1.2.1 Immune-mediated diabetes

Patients with immune-mediated diabetes suffer from autoimmune destruction of insulin producing β-cells in the pancreas, causing, at first, a relative insulin deficiency, later absolute insulin deficiency and subsequently can result in ketoacidosis. In 85% to 90% of these individuals specific autoantibodies of the immune destruction are present at manifestation of the disease. The manifestation of this form of diabetes can occur at any age, but predominantly occurs in children and adolescence. Multiple genetic predispositions, as well as environmental factors are known to play a role in immune-mediated diabetes (3).

1.1.2.2 Idiopathic diabetes

Diabetes is classified as idiopathic diabetes when no aetiologies are known, antibodies are absent but the clinical presentation is characteristic for T1D. Patients suffer from varying stages of insulin deficiency and episodic ketoacidosis, however there is no evidence for autoimmunological processes of the β-cells or

HLA-associations. This form of diabetes is strongly inherited and found mainly in Africa and Asia (1,3).

1.1.3 Pathogenesis

T1D is a multifactorial disease, where the exact roles of genetic, environmental and immunological factors are still not fully understood. Currently, it is assumed that environmental factors in genetically susceptible individuals trigger an autoimmune destruction of insulin-producing β cells. CD4+ and CD8+ T cell and macrophage infiltration of the pancreas characterise this process, resulting in the gradual loss of insulin secretion. This takes place over several months or years, during which patients are asymptomatic and euglycemic, as clinical symptoms of T1D only presents after 80% to 90% of β cells have been destroyed. Consequently, serological markers, such as glutamic acid decarboxylase antibodies (GADA), islet antigen 2 antibodies (IA-2A), islet cell antibodies (ICA), insulin autoantibodies (IAA), and zinc transporter 8 autoantibodies (ZnT8A) are often found years before clinical manifestation of T1D (1,4–7).

1.1.3.1 Genetic factors

The percentage of T1D patients with a first- or second-degree family history lies at 10% to 15% however, no simple pattern of inheritance has been identified to date. Nevertheless, the lifetime risk of developing T1D is significantly higher in relatives than in the general population, as shown in Table 1 (1,4).

		Risk of developing T1D
General population		*0.4%*
First-degree relatives		
One parent with T1D	*Father*	*5%*
	Mother	*2.5%*
Both parents with T1D		*20%*
Siblings with T1D	*HLA-identical*	*18%*
	HLA-haplotype identical	*6%*
	HLA-different	*1%*
	Monozygotic twins	*30-50%*

Table 1: Hereditary risk of developing T1D, modified from (8)

Genome-wide association studies have identified more than 60 risk loci on multiple genes for T1D. The most common genes susceptible to T1D are located on chromosome 6 within the major histocompatibility complex (MHC) region, also known as human leucocyte antigen (HLA). HLA genotypes account for 40% to 50% of the genetic predisposition for T1D. Polymorphisms on the insulin gene (Ins-VNTR, IDDM2) on chromosome 11 and the cytotoxic T lymphocyte-associated antigen-4 gene (CTLA-4) on chromosome 2 confer a further 15% of risk. Many more loci such as protein tyrosine phosphatase non-receptor type 22 (PTPN22) gene, AIRE protein (autoimmune regulator) and the gene of the transcription factor FoxP3 haven been associated with T1D (1,4).

Moreover, the HLA, CTLA-4 and PTPN22 have been linked to autoimmune thyroid disease and other autoimmune diseases, supporting the evidence that individuals suffering from T1D often develop a further autoimmune disease (5).

1.1.3.1.1 Human leucocyte antigen

The genes encoding HLA molecules lie within the MHC, which can be divided into three different subgroups:

1) Class I: encoding HLA-A, -B- and -C molecules
2) Class II: encoding HLA-DR, -DQ and -DP molecules
3) Class III: genes for complement components

Class I and class II are transmembrane cell surface glycoproteins, involved in the presentation of self and foreign antigens to T lymphocytes. Class I HLA molecules play a role in antigen recognition by the receptor of cytotoxic T lymphocytes (CD8+), while class II molecules are associated with antigen recognition by T helper lymphocytes (CD4+) (4,9).

Similar to most other autoimmune diseases, class II genes play a more important role than class I genes in T1D. The highest risk haplotypes accounting for approximately 50% of genetic predisposition are HLA-DR3, DQB1*0201 (also known as DR3-DQ2) and HLA-DR4, DQB1*0302 (also known as DR4-DQ8). In European patients with childhood onset of T1D, 90% of patients have one of the above haplotypes and 30% of individuals are DR3/DR4 heterozygous (4,9).

On the other hand, some HLA-alleles such as, the haplotypes HLA-DQB1*0602, -DRB1*0403 and -DPB1*0402, are associated with protection against developing T1D. Approximately 20% of the US population present the haplotype HLA-DQB1*0602 and only 1% of these children developed T1D (4).

1.1.3.2 Environmental factors

The relative low concordance rate of monozygotic twins (30-50%) suggest that, non-genetic factors also play a role in the pathogenesis of T1D. Environmental triggers which have been discussed to initiate pancreatic β-cell destruction include: viruses, dietary factors, growth, toxins, and stressful life events. The exact effect of these factors are still greatly unknown, however further identification and research in this field could contribute to prevention and treatment options (1,2,4).

1.1.3.2.1 Viral infections

Several viruses have been linked to the pathogenesis of T1D including enteroviruses (especially coxsackievirus b), rubella, mumps, measles, cytomegalo-virus and retroviruses (10).

Congenital rubella was one of the first viruses strongly linked to T1D. Effective immunization of populations has eradicated rubella however, T1D incidences in these regions are still rising (5,10).

Recent studies have identified enteroviruses as the most probable viral trigger connected to the development of T1D. Two possible mechanisms could be responsible for the damage of beta-cells by enteroviruses:

1. infecting the cells and directly destroying them
2. inducing an autoimmune response against the cells

Studies have supported this first mechanism, as they showed that patients who died of severe system enterovirus infection had damaged islet cells and the enterovirus was present in the beta cells (4,10).

In 1995, Clements et al. published a study showing that, in 27% to 64% of newly diagnosed T1D patients enterovirus RNA was found, compared to 0% to 4% in the control group. Furthermore, the first prospective studies in Finland found enterovirus infections to be more common in siblings who developed T1D than in the control siblings (10–12).

Other studies showed an increased T cell proliferation response to antigens of enteroviruses in prediabetic children as well as children with T1D (10).

Additionally, it is thought that enteroviruses interact with further risk factors, for example, enhancing the immune response to dietary antigens (10).

1.1.3.2.2 Dietary factors

To date, the role of dietary factors in the pathogenesis of T1D is still strongly debated. Early introduction of cow’s milk and gluten, nitrate exposure and vitamin D deficiency have all been associated with the development of T1D (4).

1.1.3.2.2.1 Breastfeeding and cow's milk exposure

Several animal models and clinical studies have been engaged with the role of cow's milk proteins in the development of T1D, however this hypothesis stays highly controversial (10).

Older studies have shown an association between T1D and cow's milk exposure both in infants and older children (>9.2 years). A 1.5 fold increased risk of T1D was found when exposed to cow's milk before the age of three months or weaning before three months (13,14).

Not all reports find such a disease association. A cohort study conducted from 1994 to 2002, found no correlation between T1D and age at initial intake of cow's milk nor breastfeeding duration (15).

Moreover, the most recent prospective study on avoidance of early cow's milk consumption, the TRIGR (Trial to Reduce IDDM in the Genetically at Risk) Randomized Clinical Trial, also showed no association between cows' milk and a reduced T1D risk. In 2159 infants of 15 countries with susceptible HLA genotypes and first-degree relatives with T1D no reduced incidence of T1D was found when weaned to a hydrolysed casein formula compared to a conventional adapted cow's milk formula, suggesting that cow's milk plays no important role in the pathogenesis of T1D (16).

1.1.3.2.2.2 Gluten

It has been known for a long time, that children and adolescents suffering from T1D show a higher incidence of celiac disease (CD) than the healthy population. This correlation can be explained by HLA DR3 and DQ2 association in both diseases (10). Furthermore, T1D antibodies have shown to be gluten-dependant. A prospective study in biopsy-confirmed CD patients observed a disappearance of T1D antibodies after the introduction of gluten free diet (GFD) (10,17).

The timing of introducing cereals and gluten to an infant's diet and its influence on the development of T1D is still controversially discussed. The DAISY (the Diabetes Autoimmunity Study in the Young) Study found an association between an

increase in T1D antibodies and cereal exposure before 4 months of age and after seven months of age. Moreover, the risk of islet autoimmunity was reduced in children still breast-fed during the introduction of cereals. Although no official guidelines exist, the introduction of cereals and other solid food is generally recommended between four and six months by paediatricians in the USA, matching the findings of the DAISY study (15).

1.1.3.2.2.3 Nitrate and nitrite exposure

Nitrate is reduced to nitrite and further into nitrosamines and nitrosamides in the gut. The latter have been linked to the pathogenesis of T1D. Nitrates and nitrites are found in vegetables, meat products and also drinking water. Several studies conducted in previous years have shown an association to the development of T1D however, dose, timing and effect of age remains unclear and unquestionably requires further research (10).

1.1.3.2.2.4 Vitamin D deficiency

The high incidence rates of T1D in northern Europe brought the discussion of an association between vitamin D deficiency and development of T1D. Existing data suggests a lower serum concentration of vitamin D in newly diagnosed T1D patients. Furthermore, a sub study of the EURODIAB (European Community Concerted Action Programme in Diabetes) study showed a decreased risk of developing T1D when vitamin D was supplemented in infancy (6,10).

1.1.3.2.3 Growth

An increase in both weight and height in children and adolescents has been linked to an increased risk of developing T1D. The ‘Accelerator Hypothesis’ assumes that obesity and insulin resistance have a rising role in the development of T1D and contributes to the increasing incidence rates in industrialized countries. Increased growth leads to a higher insulin need and is associated with a more rapid decline

of pancreatic islet function, thereby contributing to a higher risk of developing T1D. In 2000, Hyppönen et al. published a study showing a 50% to 60% increase in T1D risk, when relative weight was increased by a 10%-unit increment in children under the age of three years, and a 20% to 40% increased risk at ages between three and ten years. Further, obesity after the age of three was associated with a more than two-fold risk of developing T1D (10,18).

1.1.3.3 Immunologic factors

The lymphocytic infiltration of pancreatic islets in T1D is an elusive process, of which very little is known to date. Tissues from newly diagnosed T1D for post mortem examination are rare, and with the ethical issue of pancreatic biopsies, only a very small number of studies have been conducted. Many theories on β-cell autoimmunity have been developed including:

- immune response due to molecular mimicry (e.g. β-cells and environmental agents sharing antigenic properties)
- defects in the gene expression on cells of the immune system
- failure in central tolerance (e.g. AIRE gene mutations)
- defects in peripheral tolerance (e.g. abnormal T-cell activation)
- high cytokine levels, activating the caspase cascade leading to apoptosis
- local virus infections, and many more (6).

1.1.4 Epidemiology

T1D is the most common metabolic disease in children and adolescents. It occurs in all age groups, however, compared to other types of diabetes most commonly in children and adolescents. In western countries, T1D accounts for 90% of diabetes cases in children and adolescents, whilst only 5% to 10% of cases over the entire lifespan account for T1D (1,2).

1.1.4.1 Worldwide

The International Diabetes Federation (IDF) estimated that worldwide in 2017, of the 1,94 billion children and adolescents under the age of 15, 586,000 suffered from T1D. This increases to almost double (1,106,200) when the age range is extended to 20 years and 2,54 billion children and adolescents are included (19).

Moreover, there is a strong geographical variation in the number of T1D cases, as shown in Figure 1. Under the age of 20, Europe and North America and the Caribbean account for the most (together 46%) children and adolescents with T1D. Europe, being the highest, has more than one quarter (26%) of the worldwide T1D cases, whilst Africa, being the lowest, only accounts for 5% of cases (19).

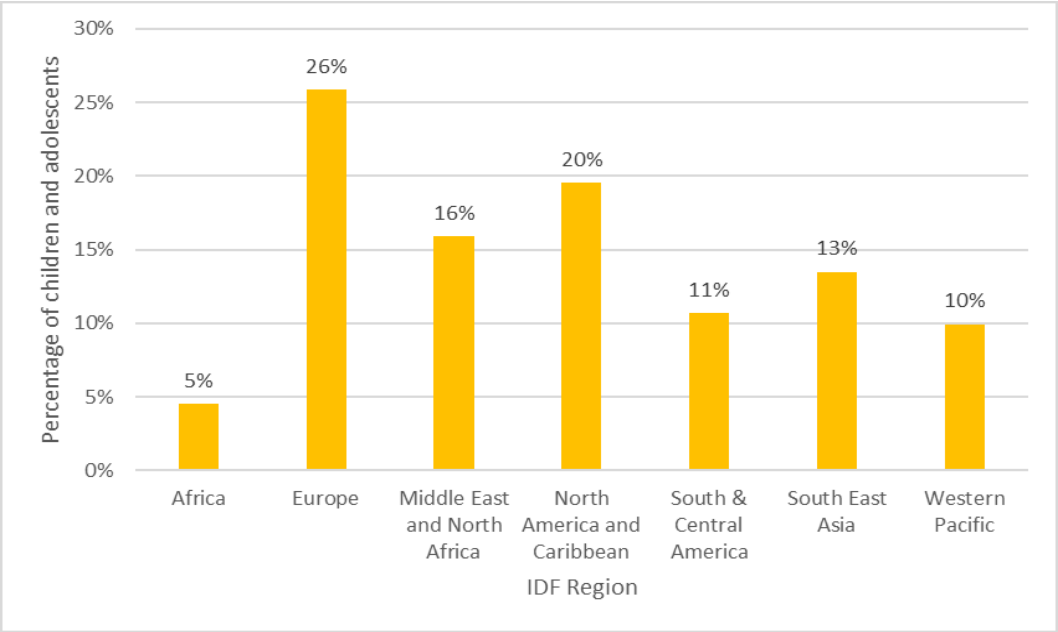

Figure 1: Estimated percentage of children and adolescents (<20 years) with T1D worldwide by IDF region, 2017, modified from (19)

Worldwide 96,100 under the age of 15 and 132,600 under the age of 20 were estimated by the IDF to be newly diagnosed with T1D in 2017. Incidence rates of T1D differ greatly between different regions and countries. Table 1 shows that the highest incidence rates in 2017 were observed in Scandinavian countries, as well as countries in the middle east and north African region. On the other hand, Asian

countries such as Japan, China and Taiwan were shown to have much lower incidence rates (2, 3.1 and 5 per 100,000 population per year respectively) (1,19).

Furthermore, the incidence rate of T1D has steadily increased in the past decades, with a 3% rise of T1D cases per year. This increase is particularly large in young children under the age of five (1,19,20).

Rank	Country	Incidence rates
1	*Finland*	*57.2*
2	*Kuwait*	*44.5*
3	*Sweden*	*39.5*
4	*Saudi Arabia*	*33.5*
5	*Norway*	*29.8*
6	*Algeria*	*26.0*
7	*Morocco*	*26.0*
8	*United Kingdom*	*25.9*
9	*Ireland*	*24.3*
10	*Denmark*	*23.0*

Table 2: T1D (<20 years) incidence rates (per 100,000 population per year) in 2017, modified from the International Diabetes Federation, modified from (19)

Unlike most other autoimmune diseases, which more frequently affect females, the number of children and adolescents suffering from T1D is equal in both genders. However, in regions with a high incidence rate, such a Europe, more males are affected, whilst countries with a lower incidence rate show a female bias (1,20).

1.1.4.2 Austria

The most recent report on Diabetes in Austria by the Ministry of Health and Woman's Affairs showed that 237 children and adolescent under the age of 15 where newly diagnosed with T1D in 2015. This correlates to an incidence rate of 19.2 per 100,000 population per year (21).

Similar to the worldwide data, incidence rates of T1D in Austria also increased over the past years. In 1999 the incidence rate of T1D was 12/100,000 and by 2007 it was 18.4/100, 000. Figure 2 shows this constant rise from 1999 to 2007, which averages to an increase of 0.81/100,000 cases per year. Moreover, the annual increase of T1D cases from 1999 to 2007 averaged to a 4% increase per year, which is also comparable with the worldwide value (22).

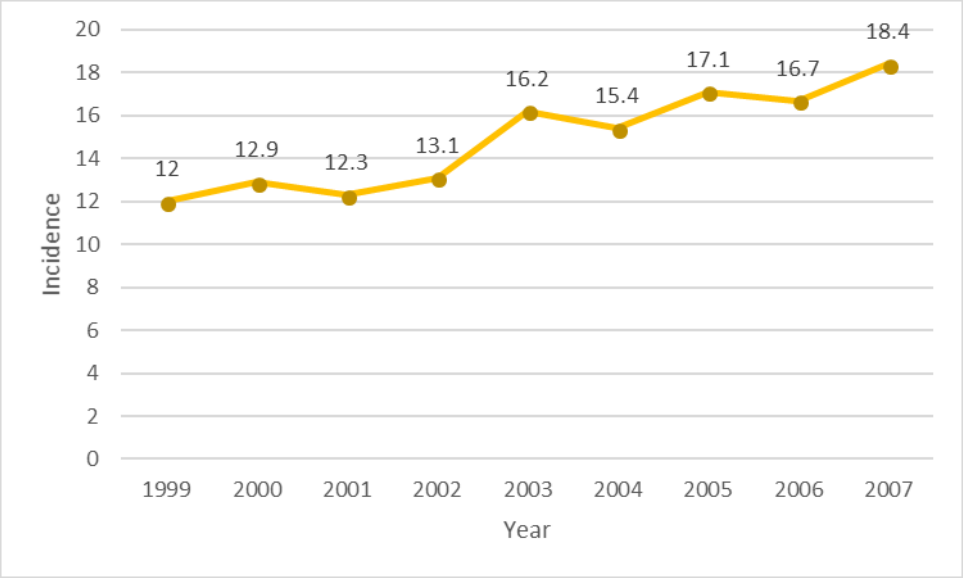

Figure 2: T1D incidence rates (per 100,000 population per year) of children and adolescents (<15 years) in Austria from 1999 till 2007, modified from (22)

1.1.5 Clinical presentation

Polyuria, polydipsia, weight loss, fatigue and poor concentration are typical symptoms at T1D onset. The duration of these symptoms can vary from days to several weeks however, failure to diagnose diabetes increases risk of ketoacidosis (2,23). Clinical presentation of T1D according to urgency of symptoms can been seen in Table 3.

Non-emergency presentations
polyuria, polydipsia
enuresis in a previously toilet-trained child, DD: urinary tract infection
vaginal candidiasis
chronic weight loss or failure to gain weight in a growing child
irritability and decline in school performance
recurring skin infections

Emergency presentations (Diabetic ketoacidosis or hyperosmolar hyperglycaemia)
moderate to severe dehydration
frequent vomiting and abdominal pain, DD: gastroenteritis
continuing polyuria despite dehydration
weight loss (fluid, muscle and fat loss)
flushed cheeks
detection of acetone on breath
hyperventilation (Kussmaul respiration), DD: pneumonia or asthma
disordered sensorium (disoriented, semi-comatose, or rarely comatose)
shock (rapid pulse rate, poor peripheral circulation with peripheral cyanosis)
hypotension (late sign)

Table 3: Clinical presentation of T1D, modified from (23)

1.1.6 Diagnosis

Plasma glucose criteria including: fasting plasma glucose (FPG), 2h-plasma glucose after oral glucose tolerance test (OGTT) and Haemoglobin HbA1c, can be used to diagnose diabetes. Depending on different diagnostic criteria it can be differentiated between preclinical diabetes, prediabetes and manifest diabetes. Additionally, increased levels of glucose and ketones may be found in the urine of T1D patients (8,24).

1.1.6.1 Criteria for diagnosis of diabetes

Most T1D cases can be diagnosed from classical symptoms and measurement of blood glucose levels (24). Table 4. shows the criteria, as stated by the ADA, for the diagnosis of manifest diabetes.

Threshold values for diabetes diagnostic

HbA1C	*≥6.5%*
FPG *	*≥ 126 mg/dl (≥7.0 mmol/l)*
OGTT: 2h-plasma glucose**	*≥200 mg/dl (≥11.1 mmol/l)*
Non-fasting	*typical diabetes symptoms or hyperglycaemic crisis and a random plasma glucose ≥ 200 mg/dl (≥11.1 mmol/l)*

*** no caloric intake for at least 8 hours**

**** rarely used in the diagnostic of T1D in children and adolescents, as it is weight-dependant**

Table 4: Diagnostic criteria for diabetes mellitus, modified from (2,3)

1.1.6.2 Criteria for prediabetes

Individuals, with higher glucose levels than considered normal, but not fulfilling the criteria for diabetes (Table 4), fall into an intermediate group. This stage is referred to as prediabetes and is linked to a high risk of developing diabetes in the future. It is associated with an impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) as shown in Table 5. (3)

Threshold values for prediabetes

FPG	*100 – 125 mg/dl (5.6 – 6.9 mmol/l)*	*= IFG*
OGTT: 2h-plasma glucose	*140 – 199 mg/dl (7.8 – 11.0 mmol/l)*	*= IGT*

Table 5: Criteria for increased risk of diabetes, modified from (3)

1.1.6.3 Preclinical diabetes and autoantibodies

β-cell destruction begins long before clinical presentation of T1D however, islet antibodies can be detected months to years before. This stage is known as the preclinical diabetes phase (23). Autoantibodies present in both this preclinical stage and later in manifest T1D are shown in Table 6.

Antibody	**Antigen**	**Prevalence in T1D patients**
ICA	*Islet cells of the pancreas*	*80%*
GADA	*Glutamic acid decarboxylase 65*	*70-80%*
IA-2A	*Tyrosine phosphatase-like insulinoma antigen 2*	*60-70%*
IAA	*Insulin*	*20-90%*
ZnT8A	*Zinc transporter 8*	*70%*

Table 6: Autoantibodies as markers of β-cell autoimmunity and their frequency in T1D, modified from (8,25)

The continuous increase in incidence of T1D has led to the idea of using antibodies as screening methods, to identify individuals at risk of developing diabetes. Close follow-ups in these individuals, may achieve earlier recognition and a less severe course of the disease. Prospective studies in first-degree relatives of individuals with T1D have shown a 62% to 100% risk of developing T1D in 5 to 10 years, when two or more antibodies were present. Moreover, recent studies in the general population showed similar results with an up to 61% 10-year risk of T1D, in the presence of autoantibodies. However, due to a lack of preventative measures for T1D, widespread screening is currently not recommended (24,25).

1.1.7 Therapy

The five cornerstones of T1D therapy include:

- insulin substitution
- diet
- self-monitoring
- exercise
- psychological support

Lifelong therapy with insulin should be started soon after diagnosis of T1D, especially in the presence of ketosis (within 6 hours) to prevent diabetic ketoacidosis (DKA). The healthy pancreas releases basal insulin to maintain glucose level in the fasting state and bolus insulin shortly after meals when glucose levels rise above 80-100 mg/dl. Insulin therapy in T1D aims to replicate this insulin secretion. The dose is dependent on age, weight, daily routines and many other factors and must be individualized for each patient. At onset, typically a dose of 0.5–1.0 U/kg/day of total insulin is given to patients and adjusted according to glycaemic controls (26).

The target HbA1C recommended by the ISPAD is <7.5% and should be measured every three months (27).

1.1.7.1 Short-acting insulin preparations

Regular soluble insulin has been used for many years. Due to self-aggregation into large hexamers, absorption is delayed leading to an onset of action 30 minutes after administration, peak effect at two to four hours and total duration of five to eight hours. Regular insulin can be injected intra-venous; therefore, it is used in the initial therapy when T1D is diagnosed, in management of DKA and during operations (26).

Rapid-acting insulin analogues, available since 1996, include aspart, lispro, and glulisine. Compared to regular insulin, they are absorbed much faster with an onset of action after 10 to 15 minutes of administration, peak effect between 90 and 100 minutes and duration of three to five hours. Nowadays, rapid-acting

insulin analogues are mainly used as bolus insulin therapy after meals and in insulin pump therapy (26).

1.1.7.2 Long-acting insulin preparations

Neutral protamine Hagedorn (NPH) aimed to mimic the basal insulin released by the pancreas and reduce the frequency of insulin injections. Onset of action is two to four hours after administration, peak effect between four and eight hours and duration of action is less than 24 hours, making it suitable for twice daily injections. A disadvantage of NPH therapy is the high variably both inter individual and intra individual. This may be due to inadequate pre-injection mixing of the insulin, which is in suspension (26,28).

Basal insulin analogues used in the treatment of children and adolescents with T1D include glargine, detemir, and degludec. They show much less variation and a more predictable insulin effect than NPH insulin (26,28).

Glargine forms a precipitate after administration and insulin is slowly released from this depot, allowing a 24-hour coverage of basal insulin. The onset of actions begins two to four hours after subcutaneous injection and no peaks occur (26,28).

Detemir has a delayed absorption, as it self-associates into hexamers and reversibly binds to albumin, resulting in a duration of action of up to 24 hours. The onset of action is earlier than that of glargine (one to two hours) but also has no peaks. The application of glargine and detemir is approved from two years of age in Europe (26,28).

Degludec, an ultra-long-acting insulin, forms a soluble depot in subcutaneous tissue, reaching a duration of action up to 42 hours. A three-times-weekly dosing regimen could be possible however, it is not yet approved in Europe (26,28).

1.1.7.3 Insulin therapy regimen

The three main insulin therapy regimens are conventional insulin therapy, basis-bolus therapy and insulin pump therapy (8).

1.1.7.3.1 Conventional insulin therapy

Mixtures of short and long acting insulin containing NPH insulin and regular insulin are injected twice a day. Two-thirds to three-quarters of the daily dose are injected before breakfast and the rest before dinner. The therapy can be optimized by increasing the number of injections to three times a day with a mixture of short and long acting insulin injected in the morning and evening and regular insulin injected at noon. The main drawback of this regimen are the strict scheduled meals to match the insulin peaks. Snacks are often required to lower the unphysiologically high insulin levels between meals and to prevent the development of hypoglycaemia. Due to its inflexible and unphysiological insulin substitution, this method is no longer used in children and adolescents today und and has mostly been replaced by Basis-Bolus regimen or insulin pump therapy (8).

1.1.7.3.2 Basis-bolus regimen

Basis-Bolus regimen aim to mimic the natural secretion of insulin from the pancreatic β-cells. It is administered via multiple daily injections (MDI) with 40% to 60% basal insulin (long-acting) and the rest short-acting insulin (8,28).

Long-acting insulin preparations are administered before bedtime or twice a day (morning and evening), depending on the daily routine of the patient (28).

Short-acting insulin can be injected 30 minutes (regular insulin) to immediately before meals (rapid-acting insulin) and the dose depends on the content of the meal, blood sugar levels pre-prandial and planned physical activity (8,28).

1.1.7.3.3 Insulin pump

The insulin pump, also known as continuous subcutaneous insulin infusion (CSII) is the third therapy regimen used in T1D patients. Only short-acting insulin is used, to achieve a more physiological insulin replacement therapy. Through a self-inserted catheter, a preselected but adjustable basal insulin rate is continuously delivered to the subcutaneous tissue (8,26,28).

Additionally, pre-prandial, depending on the plasma glucose level and the content of the meal, the patient can activate the pump to deliver a bolus of insulin. Modern pumps are connected to a glucose sensor, measuring the tissue glucose level every five minutes, sending an alarm when levels get too high or too low. CSII is often used in infants and toddlers due to their frequent snacking pattern and their requirement for smaller doses of insulin. The insulin used in insulin pumps is mainly rapid-acting insulin analogues such as aspart and lispro and less frequently regular insulin (8,26,28).

1.1.8 Complications

1.1.8.1 Acute complications

Acute and life-threatening complications of T1D include diabetic ketoacidosis (DKA) due to hyperglycaemia at onset or during the course of the disease and severe hypoglycaemia as a side effect of insulin therapy (2).

1.1.8.1.1 Diabetic ketoacidosis

Insulin deficiency together with a counterregulatory increase of catecholamines, glucagon, cortisol and growth hormone, leads to glycogenolysis and gluconeogenesis and results in hyperglycaemia and hyperosmolality. Furthermore, insulin deficiency and increased counterregulatory hormones cause lipolysis and ketogenesis and consequently ketonemia and metabolic acidosis. Osmotic diuresis is a result of hyperglycaemia and hyperketonaemia causing dehydration and loss of electrolytes (29).

Typical symptoms of DKA therefore include:

- dehydration and exsiccosis
- signs of shock (tachycardia, tachypnoea)
- Kussmaul breathing
- smell of acetone in breath
- abdominal symptoms such as pain
- vomiting and nausea
- neurological signs including confusion, drowsiness and loss of consciousness (29).

Commonly, DKA is found in newly diagnosed T1D patients, especially in children under the age of two years. However, it can also occur in patients with known T1D with risk factor such as inconsequent or omitting insulin therapy, failure of the insulin pump, earlier DKA events, gastrointestinal infections with repeated vomiting and psychiatric disorders. Criteria for the diagnosis of DKA, as stated by the ADA, can be seen in Table 7. (29).

Blood glucose		*≥ 200 mg/dl (≥11.0 mmol/l)*
Venous	***pH***	*<7.3 mild*
		<7.2 moderate
		<7.1 severe
	bicarbonate	*<15 mmol/l*
Ketones		*present in blood and urine*

Table 7: Criteria for the diagnosis of DKA, modified from (29)

The main goals of DKA therapy include correction of dehydration, correction of acidosis, reversing ketosis and restoring blood glucose level. This is achieved by substitution of fluids, salts and insulin (29).

1.1.8.1.2 Hypoglycaemia

Abnormally low blood glucose levels are a side effect of insulin therapy in T1D. This can be caused by three main mechanisms including: unregulated and persistent hyperinsulinemia, failure of the counterregulatory hormone glucagon being released and a reduced autonomic response (30). Risk factors for the development of hypoglycaemia include the application of wrong, ill-timed or excessive insulin, decreased exogenous glucose intake by missing meals or fasting and increased glucose utilisation during or after exercise (31).

Hypoglycaemia can be classified as 1) asymptomatic hypoglycaemia (blood glucose <50 mg/dl but no symptoms), 2) symptomatic hypoglycaemia (blood glucose <50 mg/dl and symptoms) and 3) severe hypoglycaemia (symptoms requiring help from another person) (8,31). Symptoms present in hypoglycaemia can be divided into autonomic and central nervous symptoms and are shown in Table 8.

		Symptoms
Autonomic symptoms	***Parasympathetic response***	*hunger, nausea, weakness*
	Sympathetic response	*Nervousness, sweating, tachycardia, tremor*
Central nervous symptoms		*headache, irritability, reduced concentration, confusion, lack of coordination, grimacing, seizures, unconsciousness*

Table 8: Symptoms of hypoglycaemia, modified from (8)

1.1.8.2 Long-term complications

The main long-term complications of T1D diabetes are vascular associated and include microvascular complications such as retinopathy, nephropathy and neuropathy and macrovascular diseases such as atherosclerosis, coronary heart disease and peripheral artery disease. Although vascular complications are rare in children and adolescence, early functional and structural changes may develop

only a few years after diagnosis of the disease. Prevention or delay of these complications may be achieved through strict glycaemic controls, optimising treatment and regular screenings. Possible effects and recommended screening procedures are shown in Table 9. (3,32).

Vascular complication	Outcome	Screening method	Begin and frequency of screening	Possible interventions
Retinopathy	*visual impairment, blindness*	*fundal photography OR mydriatic ophthalmoscopy*	*annually from the age of 10 OR at onset of puberty* AND more frequently if risk features for visual loss are high*	*optimised glycaemic control, laser treatment*
Nephropathy	*microalbuminuria, renal failure, hypertension*	*urinary albumin/creatinine ratio from first-morning urine samples OR albumin excretion rates from timed urine collections*	*annually from the age of 10 OR at onset of puberty**	*optimised glycaemic control, angiotensin converting enzyme inhibitor (ACEI) or angiotensin receptor blockers (ARB)*
Neuropathy	*pain, paraesthesia, foot ulcers, muscle weakness, autonomic dysfunction*	*history and physical examination*	*unclear*	*optimised glycaemic control*
Macrovascular disease	*atherosclerotic, cardiovascular, peripheral arterial and cerebrovascular disease*	*Lipid profile* *Blood pressure (BP)*** *24 h ambulatory blood pressure measurements*	*every 5 years after age of 10 years OR at 2 years of age when family history of hypercholesterolemia or early cardiovascular disease is known* *Annually* *when hypertension** is suspected*	*optimised glycaemic control, statins, ACEI*

*** If the onset of puberty is earlier, screening should be started after a diabetes duration of 2-5 years**

**** systolic blood pressure (SBP) and/or diastolic blood pressure (DBP) >95th percentile for gender, age, and height in more than three measurements**

Table 9: Outcome, screening and interventions for vascular complications, modified from (32)

1.2 Autoimmune thyroid diseases

1.2.1 Definition

Autoimmune thyroid diseases (AITD) are defined by the lymphatic infiltration of the thyroid parenchyma, leading to autoantibody production, tissue damage and consequently an altered function of the gland. They include Hashimoto thyroiditis (HT) and Graves' disease (GD), with a range in clinical presentation, from hypothyroidism to hyperthyroidism, respectively (33–35).

1.2.2 Epidemiology

AITDs can occur at any age being rare in infancy and most common in adolescence. The female/male ratio being 6 to 8:1 shows a strong female predisposition. HT is the most common AITD in children and adolescents followed by GD (34).

Incidence of AITDs has increased over the past years and is due to two main reasons. Firstly, more systematic antibody screenings and an increased awareness of the diseases has led to a rise in the number of patients diagnosed with AITDs. Secondly, a genuine increase of occurrence of the diseases has resulted in a higher incidence (34).

1.2.3 Pathogenesis

Similar to T1D, AITDs have a multifactorial pathogenesis, with susceptibility determined by both genetic and environmental factors. HT and GD have different clinical presentations however, they share aetiological factors, immunological mechanisms and occurrence in members of the same family (35) (36).

AITDs are associated with T- and B-lymphocyte infiltration of the thyroid gland, and subsequent autoreactivity (37,38).

In HT, interferon gamma (IFN-g) derived from CD4+ T cells enhances expression of MHC class II molecules on thyrocytes, leading to the expansion of autoreactive

T cells. An inflammatory response arises, causing apoptosis of the thyroid cells and subsequently hypothyroidism. Additionally, some patients generate thyroid-stimulating hormone-receptor (TSH-R) blocking antibodies, preventing TSH from binding. The stimulating effect of TSH is ceased, causing atrophy of the thyroid gland, resulting in hypothyroidism (37,38).

On the other hand, in GD, CD4+ T cells specific for the thyroid gland are activated, leading to recruitment of B-cells and generation of antibodies. These antibodies bind to the TSH-R on thyroid follicular cells, mimicking the action of TSH, leading to the stimulation of thyrocyte growth and thyroid hormone production, and consequently resulting in hyperthyroidism (35,37,39).

1.2.3.1 Genetic factors

Genetic factors play an important role in the pathogenesis of AITDs, with some genes being mutual for both GD and HT, indicating a shared genetic susceptibility. These genes include immunoregulatory genes such as HLA, CTLA-4, CD40 and PTPN22 (33).

The HLA class II genes, in particular HLA DR, are best known to be linked with AITDs. In GD, HLA-DR3 is present in up to 55% of patients compared to 30% in the general population. Furthermore, the presence of HLA-DR3 and HLA-DR5 has been associated with a greater risk of developing HT (33).

Another strong susceptibility gene for AITDs is CTLA-4, with polymorphisms in certain alleles of this gene being present in both GD and HT. Additionally, CTLA-4 gene abnormalities have been associated with higher serum levels of thyroid peroxidase (TPO) (33,35).

Thyroid specific genes seem to also play a role in AITDs, most likely interacting with the above loci, influencing phenotype and severity of the diseases (37).

1.2.3.2 Environmental factors

Environmental factors associated with AITDs include iodine excess, infection, stress, selenium deficiency, smoking, exposure to radiation and many more (33,37).

Iodine, which can only be taken in through external sources, is essential for the synthesis of the thyroid hormones triiodothyronine (T3) and thyroxine (T4). However, too high iodine intake can induce autoimmunity by exacerbating lymphocyte infiltration of the thyroid and generating reactive oxygen species which have a toxic effect on thyrocytes. Moreover, rising iodine levels result in an increase of thyroglobulin (Tg) and TPO antibodies (33,37).

Another essential trace element, important for thyroid hormone synthesis and activation, is selenium. It is essential for the function and protection of the gland, as it acts as an antioxidative defence against hydrogen peroxide and its reactive oxygen intermediates, produced by thyroid follicles for hormone synthesis. In adults, a deficiency in selenium is associated with increased levels of hydrogen peroxide consequently leading to a rise in TPO antibodies (35,40).

Studies on the effect of infection, stress and smoking on AITDs show controversial results and further research is needed. (37).

1.2.4 Autoantibodies

Lymphocytic infiltration of the thyroid gland correlates with the presence of thyroid antibodies in serum. These antibodies may be present before clinical onset or laboratory evidence of AITDs. Antibodies found in AITDs are summarized in Table 10 (33).

Antigen	Location	Function	Antibody	Occurrence
Thyroglobulin (Tg)	*part of the follicular colloid*	*play a main role in storage of iodine and thyroid hormone synthesis*	*Thyroglobulin antibodies (TgAb)*	*60-70% of HT patients* *30% of GD patients*
Thyroid peroxidase (TPO)	*on the apical surface of thyrocytes*	*catalyses ionisation and coupling reaction of thyroid hormone synthesis*	*Thyroid Peroxidase antibodies (TPOAb)*	*80-90% of HT patients* *80% of GD patients*
Thyroid Stimulating Hormone receptor (TSH-R)	*on the basal surface of thyroid follicular cells*	*G-protein coupled receptor acting as the main regulator of the thyroid stimulating thyroid hormone synthesis and follicular cell growth*	*Thyroid Stimulating Hormone receptor antibodies (TRAb)*	*90% of GD patients*

Table 10: Autoantibodies associated with AITDs, modified from (33,37)

1.2.5 Hashimoto thyroiditis

1.2.5.1 Clinical presentation

During the progression of HT, symptoms develop very slowly and gradually. In the early phase, the thyroid gland is enlarged due to lymphatic infiltration, leading to the development of a goitre. Over time, thyrocytes get destroyed resulting in atrophy of the organ. With the manifestation of hypothyroidism typical symptoms commence. These include:

- decline in physical and cognitive performance, memory loss and depression
- fatigue
- dry, coarse skin
- dry, brittle hair
- weight gain

- cold intolerance
- constipation
- deep, hoarse voice
- high cholesterol
- slow Achilles tendon reflex
- disturbances in the menstrual cycle, infertility, miscarriage
- myxoedema (8,37,41)

1.2.5.2 Diagnosis

Subclinical hypothyroidism and overt hypothyroidism can be diagnosed by an increased TSH level and the presence of TPO and/or TG antibodies (41). Further laboratory parameters used for the diagnosis are displayed in Table 11.

		Subclinical hypothyroidism	**Overt hypothyroidism**
TSH		↑	↑
T4		*normal*	↓
Antibodies	***TPO***	*present in 80-90% of patients*	
	Tg	*present in 60-70% of patients*	
	Both	*present in 98% of patients*	

Table 11: Diagnostic criteria of HT, modified from (41)

Additionally, ultrasonography of the thyroid gland can be used to detect patients prone to developing hypothyroidism and to confirm the presence of overt hypothyroidism. Due to lymphocytic infiltration of the thyroid normal array of the parenchyma is disrupted, showing a hypoechogenic and inhomogeneous thyroid gland in the ultrasound image. Initially the thyroid gland may be increased in size. Later, when overt hypothyroidism is present, the ultrasound image can show a decreased organ size. When colour duplex sonography is performed, a reduced circulation of the thyroid may be found (41,42).

1.2.5.3 Therapy

Children and adolescents with overt hypothyroidism should receive T4 replacement therapy to minimize symptoms. Table 12 shows the typical dose of levothyroxine for different age groups. After 6-8 weeks of treatment the T4 and TSH level should be measured and, if needed, levothyroxine dosage adapted. Once euthyroidism is reached, thyroid values should be controlled every 6 to 12 months and treatment is generally continued lifelong (43).

Age (years)	Dose (µg/kg/day)
1-5	4-6
6-10	3-4
>11	2-3

Table 12: Recommended Levothyroxine dose in children and adolescents, modified from (43)

One the other hand, treatment of subclinical hypothyroidism in children and adolescents is still very controversial (43). In adults, with risk of overt hypothyroidism, treatment is recommended once serum TSH concentration is >10 mU/L. In children, remission of subclinical hypothyroidism is common, which is why treatment is not recommended whilst no symptoms are present. Instead, regular follow-ups of thyroid function are required (43).

1.2.6 Graves' Disease

1.2.6.1 Clinical presentation

GD is characterized by hyperthyroidism with goitre and endocrine ophthalmopathy. Hyperthyroidism, present in 95% of patients with GD is marked by the following symptoms:

- restlessness, tremor, nervousness, irritability, insomnia, emotional lability
- tachycardia, widened pulse pressure
- weight loss despite increased appetite
- fine, warm, moist skin, thinning of hair
- heat intolerance, sweating
- increased frequency of stools, diarrhoea
- myopathy: weak thigh muscles
- osteoporosis
- impaired glucose tolerance
- menstrual irregularity, infertility (8,44).

Endocrine ophthalmopathy occurs in up to 50% of patients suffering from GD. The main features include exophthalmos, chemosis and extraocular muscle fibrosis. Exophthalmos, causing inability of eyelid closure, can lead to corneal damage and visual loss. Further, impaired extraocular muscle movement, due to fibrosis, results in diplopia and optic neuropathy due to optic nerve compression and can affect colour vision (44,45).

Rarely, patients also experience localized dermopathy, typically including nonpitting oedema over the anterior shins (pretibial myxoedema) and acropachy (44).

1.2.6.2 Diagnosis

Initial screening investigation for GD includes measurement of serum TSH. This is low in both subclinical and overt hyperthyroidism. For further differentiation, the thyroid hormones T4 and T3 must be measured. In case of overt hyperthyroidism these hormones are elevated and in subclinical hyperthyroidism they remain within

reference range. Both T4 and T3 must be determined, as 10% of patients with reduced TSH level have a normal T4 but increased T3 level. This condition is known as T3 toxicosis (42,44,45).

Presence of TRAb in serum can be used to confirm the diagnosis of GD. They are positive in 90% of patients with the disease. TPO and TG antibodies are not specific for GD but may also be elevated (42,44,45).

Ultrasonography of the thyroid may display typical features of GD including enlargement of the organ, hypoechogenicity due to the microfollicular pattern and hyperperfusion (42,44,45).

1.2.6.3 Therapy

Antithyroid drug therapy, with thionamide derivatives, is a possible treatment option for hyperthyroidism. They block important steps in the thyroid hormone synthesis resulting in a decrease of T4 and T3 levels. Available substances for children and adolescents include methimazole and carbimazole. The American Association of Clinical Endocrinologists (AACE) guidelines recommend methimazole as initial therapy as it has the greatest efficacy and fewer side effects. The duration of antithyroid drug therapy is still debated as remission rates vary strongly. The most severe side effect of antithyroid drug therapy is agranulocytosis, however this is very rare (46).

Another, nonsurgical treatment option of GD in children > 5 years of age is radioiodine therapy (44,46).

When patients suffer from a large goitre causing compressive symptoms, failure of antithyroid drug therapy, low radioactive iodine uptake or malignancy is suspected surgery is a possible treatment option in children and adolescents. Total or near-total thyroidectomy is recommended to avoid relapse of hyperthyroidism (46).

1.2.7 Autoimmune thyroid diseases and type 1 diabetes

In children and adolescents with T1D the most common associated autoimmune disease is known to be AITD. The prevalence of AITD in children and adolescents with T1D described by the ISPAD ranges from 3% to 8% and represents a strong predictive factor for the development of hypothyroidism or hyperthyroidism. At diabetes diagnosis approximately 25% of patients show positive thyroid antibodies (47,48).

Risk factors for the development of thyroid antibodies include increasing age, longer diabetes duration and female sex. Children diagnosed with T1D before the age of 5 years developed AITDs after a longer period of time than children and adolescents diagnosed after the age of 5 years. Furthermore, a correlation between pubertal maturation and the production of thyroid antibodies is known, with the disease peaking over the age of 15 years (49,50).

Subclinical hypothyroidism is present in about 3% to 8 % and hyperthyroidism in approximately 0.5% of children and adolescents with T1D. Hyperthyroidism may be a cause of poor glycaemic control, weight loss, tachycardia, agitation and tremor in T1D patients. On the other hand, hypothyroidism shows no significant variation in glycaemic control but can reduce linear growth rate. Both hypothyroidism and hyperthyroidism in T1D is treated the same as in the general population (47,48).

The ISPAD recommends thyroid antibody screening shortly after T1D diagnosis and every two years thereafter. If symptoms occur, growth is impaired or glycaemic control cannot be maintained thyroid antibodies should be measured more frequently. Thyroid function tests may be distorted at T1D diagnosis due to hyperglycaemia or DKA and should therefore be performed once glycaemic control is stable (47,48,50).

1.3 Celiac Disease

1.3.1 Definition

Celiac disease (CD) is an immune-mediated enteropathy induced by gluten proteins. The main storage proteins of gluten, also known as prolamins, associated with CD are gliadin (wheat), hordein (barley) and secalin (rye) (51,52).

It is a systemic disease with clinical presentation varying from silent (mainly undiagnosed), preclinical (latent) to diagnosed (mostly active) CD. The diagnosis is confirmed by a small bowel biopsy (SBB), showing villous atrophy and crypt hyperplasia (51,52).

1.3.2 Epidemiology

The prevalence of CD ranges widely among different countries and regions, due to varying presence of predisposing genes and nutritional practices. In most countries, the prevalence is estimated to be around 1%. The highest paediatric prevalence, being 5.6%, is found in the Saharawi population. In the Asia-Pacific region CD is very rare, with prevalence being as low as 0.05% in the Japanese population. In Germany, the KiGGs Study found a CD prevalence of 0.9% in children and adolescent, with prevalence peaking between the ages of three and six years (51–53).

Incidence rates have shown a great increase worldwide in the past 30 years, however improved availability of serological screening methods with earlier recognition of the disease need to be taken into account (51,52).

There is a slight female predisposition in CD, with the female/male ratio being 2.8:1. Worldwide, the peak in onset of the disease in childhood and adolescence is between nine months and two years of age (33).

1.3.3 Pathogenesis

Indigestion of gluten, due to resistance to the digestive enzymes of the upper gastrointestinal tract, leads to the presence of proline- and glutamine-rich peptides in the lumen of the small intestine. These are believed to be the cause of mucosal immune system stimulation (Figure 3) (33,51,54).

T-cells are activated resulting in an increase in the levels of proinflammatory-mediators such as IFN-g and other cytokines, leading to:
1) active T-cell expansion
2) epithelial damage due to activation of cytotoxic T-cells
3) recruitment of B-cells, producing anti-gliadin and anti-tTG antibodies (33,51,54).

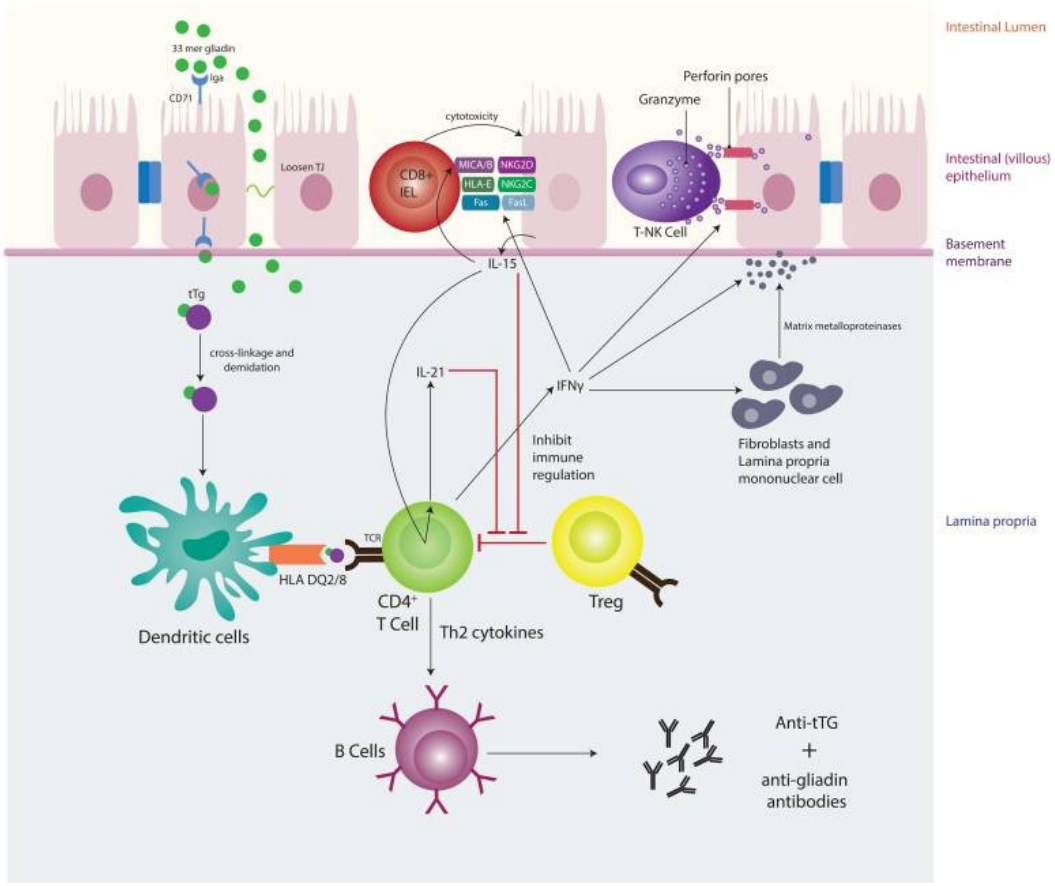

Figure 3: Steps involved in the development of celiac disease (33)

1.3.3.1 Genetic factors

Genetic factors have shown to play a key role in the pathogenesis of CD (Table 13). It is strongly associated with the HLA class II genes HLA- DQ2 and HLA-DQ8. The DQ2 allele is the most common, carried by approximately 95% of CD patients, and the remaining ~5% are DQ8 positive. Less than 1% of CD patients are both HLA-DQ2 and -DQ8 negative. The most prevalent subtypes of DQ2 are DQ2.5 with the alleles DQA1*0501 and DQB1*0201 and DQ2.2 with DQA1*0201 and DQB1*0202. DQ8.1 with the alleles DQA1*0301 and DQB1*0302 is the most frequent subtype of DQ8 (51,54).

	Risk of developing CD
First degree relative	*5-15%*
HLA identical siblings	*~30%*
Monozygotic twins	*83-86%*
Dizygotic twins	*17-20%*

Table 13: Hereditary risk of developing CD, modified from (51,54)

In the USA, it estimated that 25% to 40% of the general population carry the DQ2 and/or DQ8 allele, however only ~4% develop CD. This suggests, other non-HLA genes are also linked to the pathogenesis of CD. These non-HLA genes contribute to development, activation and differentiation of B cells and T cells. They include CTLA-4, CD28, ICOS, MAP3K7 and many more (33,51,54).

1.3.3.2 Environmental factors

Not all children with HLA DQ2 and DQ8 genes consuming gluten develop CD, suggesting environmental factors also contribute to the pathogenesis of CD. A major source of evidence came from the epidemic of CD in Sweden in the mid-1980s, where the incidence of CD in children below 2 years of age increased by a threefold within a few years. Possible causes for this increase were: 1) changing

recommendations on gluten introduction in infants, delaying it from 4 to 6 months and 2) increased gluten content in baby food (51).

Gastrointestinal infection can cause increased mucosal permeability, resulting in more gluten being absorbed by the lamina propria and consequently triggering a stronger immune reaction. Breast-feeding is known to be protective against these infections and by delaying the gluten introduction to 6 months infants were less likely to be breast-fed during gluten introduction, increasing the risk of an immune reaction. In the mid- 1990s Swedish authorities returned to the recommendation of gradual gluten introduction from 4 months of age, quickly decreasing the incidence of CD. This was confirmed by a meta-analysis performed by Akobeng et al., investigating the risk of developing CD, when infants where introduced to gluten whilst being breast-fed versus post-weaning. The results showed a 52% reduced risk of developing CD when breast-fed during gluten introduction. However, it is not known if breast-feeding merely postpones the onset of CD or if it can prevent the disease (51,54).

The amount of gluten, when introduced, can also play a role in development of CD. In Sweden a population-based incident case- referent study followed the epidemic. It found the risk of developing CD was greater when gluten was introduced in large amounts, whilst introduction of small amounts during breast-feeding reduced the risk of CD (51).

Apart from infant feeding practices, a further risk factor of CD includes infections. Several studies have shown a correlation between the number of infection episodes and risk of CD. In particular rotavirus infections have shown a higher risk of CD development in susceptible children and adolescents (51).

1.3.4 Clinical presentation

CD is a systemic disease with gastrointestinal and extraintestinal manifestations (Table 14). Around 40% to 50% of patients have gastrointestinal symptoms such as diarrhoea and abdominal pain. Furthermore, a large group of individuals suffering from CD may be asymptomatic, making diagnosis much more difficult (33,51).

Gastrointestinal manifestations	Extraintestinal manifestations
Diarrhoea	*Aphthous stomatitis*
Abdominal pain	*Dental enamel hypoplasia*
Abdominal distension	*Dermatitis herpetiformis*
Nausea and vomiting	*Decreased bone mineral density, osteoporosis*
Poor appetite	*Arthritis*
Weight loss, Anorexia	*Growth failure, delayed puberty*
Steatorrhea	*Cerebellar ataxia, seizures, depression*
Signs of malnutrition (iron deficiency anaemia, hypoalbuminemia)	*Chronic fatigue*
	Hypertransaminasemia
	Infertility, miscarriage

Table 14: Signs and Symptoms of CD, modified from (33,51)

1.3.5 Diagnosis

Diagnostic criteria for CD include 1) typical symptoms, 2) positive serology, 3) HLA-DQ2 or DQ8 genotypes, 4) small bowel biopsy showing celiac enteropathy 5) positive response to gluten free diet. According to the presence of these criteria different stages of the disease can be identified, as shown in Table 15 (33).

CD	Symptoms	HLA-DQ2 or DQ8 genotype	Antibodies	Histologic abnormalities in SBB
Silent	-	+	+	+
Potential	+/-	+	+	-
Latent	+/-	+	+/-	subtle morphometric abnormalities
Active	+	+	+	+

Table 15: Spectrum of celiac disease and associated diagnostic markers, modified from (51)

33% of patients with potential CD develop villous atrophy in a three-year follow-up, whilst 14.6% lose celiac antibodies. Therefore, if antibodies are positive but SBB is negative, biopsy should be reviewed or repeated after one to two years (33,51).

1.3.5.1 Serological screening

Sensitivity and specificity of serological screenings with autoantibodies have improved over the past years. Children and adolescents with a family history of CD or suffering from other autoimmune diseases, genetic conditions and selective IgA-deficiency should be screened even if asymptomatic, due to the increased risk of developing CD. Antibodies used for screening include IgA and IgG:

- Anti-gliadin antibodies (AGA)
- Anti-tissue transglutaminase antibodies (tTG)
- Anti-endomysial antibodies (EMA)
- Anti-deamidated gliadin peptide antibodies (DGP) (33,51)

Conventional AGA-IgA and IgG have mostly been replaced by newer, more sensitive tests. However, studies have shown an increased sensitivity for children under the age of two, as 17% of CD cases would be missed if AGA screening was omitted (33,51).

EMA, having nearly 100% specificity, has been regarded the gold standard of CD screening. However, due to its high costs the initial screening is mostly done with tTG-IgA, also having a high sensitivity (94%) and specificity (97%). tTG may be elevated in other autoimmune diseases, myocardial damage, tumours, liver disorders and infections suggesting further measurement of IgA EMA to confirm CD. Furthermore, total IgA should be measured to exclude IgA-deficiency (total serum IgA levels less than 0.2 g/L), as these individuals require IgG tests to detect CD. In particular, DPG IgG has been proven suitable in this case. DPG antibodies alone, have shown a lower sensitivity than tTG-IgA, but a combination of tTg-IgA and DPG IgG may allow a more accurate diagnosis of CD. Additionally, diet during CD evaluation must contain adequate gluten, as this affects the sensitivity of serological tests (33,51).

Children and adolescents with uncertain diagnosis can have genetic testing for HLA-DQ2 and -DQ8. If neither DQ2 nor DQ8 haplotypes are present, no further observation is needed as CD is improbable. On the other hand, if individuals possess DQ2 and DQ8 haplotypes, screening and regular follow-ups are recommended (51).

The most recent guidelines by the European Society for Pediatric Gastroenterology and Hepatology and Nutrition (ESPGHAN) suggests at high anti-tTG levels (10 times higher than the upper limit) and presence of typical symptoms of CD, biopsy is no longer needed to confirm the diagnosis. Instead HLA and EMA antibody testing may be used for confirmation. However, this is not applicable for patients suffering from T1D (33,51).

1.3.5.2 Small bowel biopsy

The gold standard for diagnosis of CD remains SBB and histology is classified by the Marsh criteria (Table 16). Marsh 2 and 3 are seen in patients with active CD, and Marsh 1 in patients on a gluten free diet. CD is a patchy disease requiring sampling from different areas of the duodenum, including at least one biopsy from the duodenal bulb and four biopsies from the second and third portion of the

duodenum. As in serological testing, a diet containing gluten is important before performing the biopsy (33,51).

Criteria		Morphology of duodenal mucosal biopsy
Marsh 0		*Normal*
Marsh 1		*≥25 IEL*/100 enterocytes* *Normal architecture*
Marsh 2		*≥25 IEL/100 enterocytes* *Crypt hyperplasia*
Marsh 3	**a**	*Partial villous atrophy*
	b	*Subtotal villous atrophy*
	c	*Total villous atrophy* *>40 IEL/100 enterocytes*
***IEL: intraepithelial lymphocytes**		

Table 16: Marsh Criteria, modified from (8,33)

1.3.6 Therapy

Strict, lifelong gluten-free diet is the main treatment of CD. Patients should no longer eat wheat, rye, barley and their derivatives. Once a gluten-free diet is started symptoms will improve within two to four weeks, with histologic remission taking up to two years. Furthermore, antibodies decrease, with 80% of patients being serologically negative in up to two years (51).

Adherence of gluten is often very challenging for children and adolescents due to limited understanding of the risks, feeling excluded from peers and finding it difficult to resist the temptation, often leading to a poor compliance. Therefore, regular follow-ups evaluating the success of gluten-free diet are required. If antibody levels do not show a decrease after six months, gluten ingestion is still probable and further education on dietary recommendations are needed (51).

1.3.7 Celiac disease and type 1 diabetes

The link between T1D and CD is well known and has led to increased screening frequencies over the past decade. Prevalence of CD in T1D varies from 1% to 10% and in Austria and Germany the Prospective Diabetes Follow-up Registry (DPV) found the prevalence of biopsy proven CD to be 3.2%. Diabetes is diagnosed before CD in about 90% of patients (47,55,56).

Risk factors for the development of CD include presence of other autoimmune diseases, affected first-degree relatives and young age at diabetes onset, in particular under the age of five years. CD is often diagnosed after a short diabetes duration and the majority of cases within 10 years. A female sex bias, which is often reported, was found to be very controversial (47,55).

CD in T1D is often silent and in most cases asymptomatic, but can show a wide spectrum of symptoms including poor linear growth, malnutrition, weight loss even resulting anorexia, delayed puberty, and gastrointestinal symptoms such as chronic diarrhoea and/or constipation, meteorism, pain and malabsorption. Furthermore, an increase in the number of hypoglycaemic episodes and a reduction of insulin requirement may be an indication for undiagnosed CD. GFD is the main treatment of CD and can results in histologic remission, decrease or disappearance of antibodies and reduce the risk of long-term complications. Abdominal symptoms mostly also improve however, unstable glycaemic control and hypoglycaemic episodes may remain (47,50,56).

The ISPAD recommends screening for CD at T1D onset and two and five years thereafter. Furthermore, IgA deficiency should be excluded at diagnosis as these patients requires screening using IgG specific antibody tests. Children and adolescents with first-degree relatives suffering from CD, impaired growth, weight loss, abdominal symptoms, unexplained hypoglycaemia or unstable glycaemic control should be screened more frequently. Additionally, education by paediatric dieticians specialised in diabetes and CD are recommended (47,48,57).

Testing for HLA-DQ2 and HLA-DQ8, has been discussed as a possible screening method for CD, but is still very controversial. The ISPAD does not recommend routine HLA-screening, but instead regular antibody screening and SBB. The high

cost compared to antibody screening and unreliable outcome makes HLA-testing questionable. (47,57,58)

1.4 Autoimmune gastritis

1.4.1 Definition

Autoimmune gastritis (AG) is characterized by autoantibodies against parietal cells and intrinsic factor of the stomach, causing chronic inflammation of the fundus and corpus. To begin the disease is asymptomatic, however with time the chronic inflammation leads to atrophy of the mucosa (chronic atrophic gastritis) and ultimately results in iron deficiency anaemia (IDA) or pernicious anaemia due to vitamin B12 deficiency (59,60).

1.4.2 Epidemiology

Incidence rates for AG are difficult to assess as patients only develop symptoms when the disease progresses to chronic atrophic gastritis. This can take up to 20 to 30 years. Moreover, only 20% to 40% of patients suffering from AG develop IDA and 15% to 25% develop pernicious anaemia. In the general population, the prevalence of AG is 0.3% to 1.98%. Whilst children and adolescents show a relatively low prevalence, it increases with advancing age, reaching a prevalence of 12% in the eighth decade. Unlike the other mentioned autoimmune diseases, AG has no gender predisposition (61–64).

1.4.3 Pathogenesis

In AG, antibodies to parietal cells of the fundus and corpus of the stomach and to their secretory product, intrinsic factor, are present. These antibodies, targeting the enzyme H^+/K^+ ATPase, which is primarily responsible for the acidic pH in the stomach, are cytotoxic to the gastric mucosa. Parietal cell loss leads to an increase in the pH of the stomach and reduction of intrinsic factor. Hypochlorhydria or achlorhydria decreases the iron absorption, by loss of ferric iron reduction and reduced degradation of iron-protein complexes, resulting in iron deficiency. Additionally, reduced gastric acid secretion leads to hypergastrinaemia. Lack of

intrinsic factor causes malabsorption of vitamin B12 and deficiency of vitamin B12 leads to pernicious anaemia (63,65).

Helicobacter pylori has been discussed as an environmental factor playing a role in the initiation of AG. H^+/K^+ ATPase – H. pylori molecular mimicry induces autoreactive T-cells and subsequently gastric autoimmunity. Studies supporting this hypothesis have shown a positive correlation between gastric antibodies and antibodies to H. pylori in patients with AG. Furthermore, eradication of H. pylori has been associated with a decrease in gastric antibodies (60,63).

Genetic factors have also shown to play a role in the pathogenesis of AG. In relatives of patients suffering from pernicious anaemia, PCA and autoimmune gastritis presents in 20% to 30% of individuals. Pernicious anaemia has been linked with HLA-DR2, -DR4 and -DR5 haplotypes, however the evidence is poor (63).

1.4.4 Clinical presentation

Sign and symptoms present in other forms of gastritis, such as stomach pain and development of gastric or duodenal ulcers are not typical in AG. To begin the disease is silent but, with progression of the disease, the development of achlorhydria leads to a delay in gastric emptying, bacterial overgrowth of the small intestine and stomach and an increased tendency of gastrointestinal infections. Furthermore, up to 40% of patients with AG develop IDA and up to 25% of patients present with pernicious anaemia. Symptoms of iron deficiency, vitamin B12 deficiency and anaemia have been summarized in Table 17 (63,65).

Iron deficiency	Vitamin B12 deficiency	Anaemia
Fatigue	*Gastrointestinal malabsorption*	*Shortness of breath*
Restless legs syndrome	*Diarrhoea*	*Pallor*
Brittle nails	*Weight loss*	*Dizziness*
Hair loss	*Atrophic glossitis*	*Tachycardia*
Impaired immune function	*Peripheral neuropathy*	*Light headedness*
Impaired wound healing	*Paraesthesia*	*Decreased cognitive function*
	Weakness	*Reduced exercise performance*
	Ataxia	
	Confusion	
	Impaired memory	
	Frank psychosis	

Table 17: Symptoms of iron deficiency, vitamin B12 deficiency and anaemia, modified from (63,65)

1.4.5 Diagnosis

Serological markers for AG include PCA and antibodies to intrinsic factor (AIF). PCA are present in 60% to 85% and AIF in 30% to 50% of patients suffering from AG. When AG is silent but serological biomarkers are positive, regular follow ups to identify progression to gastric atrophy, IDA and pernicious anaemia are necessary. For example, increased gastrin and reduced pepsinogen levels can be used as markers for gastric atrophy but are not specific to the diagnosis of AG. IDA is defined as hypochromic, microcytic anaemia with reduced serum iron and ferritin concentration and transferrin saturation under 20%. Pernicious anaemia shows macrocytic anaemia and decreased vitamin B12 concentration in peripheral blood (60,63).

The diagnosis of AG requires gastroscopy with at least two biopsies from the antrum and corpus. In early phases of the disease, histological findings from the biopsies show lymphocytic infiltration of the submucosa and lamina propria. As the

disease progresses, parietal and zymogenic cells as well as oxyntic glands reduce in number and intestinal metaplasia becomes visible (63,65).

1.4.6 Therapy

Asymptomatic patients with silent AG do not require therapy however, yearly blood tests assessing iron, vitamin B12 and gastrin levels are necessary. IDA and pernicious anaemia should be treated with supplementation of iron and vitamin B12, respectively. Additionally, in case of an H. pylori infection, triple therapy with a proton pump inhibitor and two antibiotics eradicating the disease can possibly cure early phases of AG (63,66).

1.4.7 Complications

Hypergastrinaemia due to achlorhydria in AG stimulates enterochromaffin-like (ECL) cells. Hyperplasia and dysplasia of ECL cells can evolve into gastric carcinoid tumours, occurring in 4% to 9% of patients with AG. Additionally, patients suffering from AG have a three to six times higher risk of developing gastric adenocarcinoma. Atrophy of the gastric mucosa leading to intestinal metaplasia is considered a precursor for gastric cancer. Therefore, gastroscopic surveillance in AG is an important screening method. Simple hyperplasia or mild dysplasia should be followed up every three to five years, more extensive lesions require more frequent endoscopy and polyps should be removed immediately (63,65).

1.4.8 Autoimmune gastritis and type 1 diabetes

AG is often associated with further autoimmune diseases, with autoimmune thyroid disorders being most common (14% to 30%). Studies showed that 4% to 15% of children and adolescents with T1D additionally suffered from AG (61). However, no correlation between PCA and age at diabetes onset or diabetes duration was found. Furthermore, no gender predisposition was identified in these studies (59,61,63).

It has been suggested diabetes itself plays a role in the development of AG. GADA, an antibody present in T1D, has been identified as an immunological risk factor linked with AG, as it is not only present in the pancreas but also the stomach and the thyroid gland. Additionally, thyroid autoimmunity and the presence of CD antibodies have been associated as risk factors for the development of PCA (57,63) .

The ISPAD does not recommend routine PCA screening. However, when children and adolescents with T1D experience unexplained anaemia or abdominal symptoms PCA should be determined (57).

1.5 Other associated autoimmune diseases

Further autoimmune diseases associated with T1D include Addison's disease (AD), ulcerative colitis (UC), juvenile idiopathic rheumatoid arthritis (JIA) and uveitis (57).

AD is present in up to 2% of T1D patients and suspected when anti-adrenal antibodies (21-hydroxylase antibodies) are present. Low morning cortisol, elevated basal adrenocorticotropic hormone (ACTH) and increased plasma renin confirm the diagnosis. Typical symptoms of AD in children and adolescents with T1D include recurrent hypoglycaemia, reduced insulin requirement, weight loss, hyperpigmentation of the skin, hyponatraemia and hyperkalaemia (8,57).

UC is characterised by inflammation of the mucosa in the colon and shows symptoms such as bloody stools, abdominal pain, impaired growth and weight loss. The main treatment option for UC is corticosteroid therapy, which may affect glycaemic control in T1D patients (8,67).

JIA is the most prevalent non-organ-specific autoimmune disease characterised by symptoms such as joint inflammation, swelling and restricted mobility for at least six weeks (57).

Routine antibody screening for AD, UC and rheumatic diseases are not recommended, instead symptom orientated diagnostic should be conducted (57).

2 Objectives

T1D is one of the most common chronic metabolic diseases in children and adolescents, with a constant rise in incidence rates. It is often associated with further autoimmune diseases, including HT and CD.

The primary objective of this study was to investigate how many children and adolescents with T1D, at the Department of Paediatrics and Adolescent Medicine of the Medical University Graz, developed an additional autoimmune disease.

Secondary objectives of this study are to determine:

- which additional autoimmune diseases is most prevalent
- age at which children and adolescents are diagnosed with a further autoimmune disease
- duration of diabetes at onset of associated autoimmune disorders
- gender differences between the above objectives
- family history of autoimmune diseases
- differences in prevalence of associated autoimmune diseases compared to 2008

3 Materials and methods

3.1 Study design

This study is a retrospective analysis of data extracted from the medical records of patients treated at the Diabetes Outpatient Clinic at the Department of Paediatrics and Adolescent Medicine of the Medical University Graz.

3.2 Study population

The study includes 303 children and adolescents diagnosed with T1D before the age of 18 years and treated at the Diabetes Outpatient Clinic at the Department of Paediatrics and Adolescent Medicine of the Medical University Graz. 128 patients were female and were 175 males. At the time of data collection (October 2017) the age of patients ranged from 2 to 21 years and patients were diagnosed with T1D between the years 2000 and 2017. Average age at diabetes onset was 7.1 ± 4.2 years.

Data from our study was compared to the data collected for a diploma thesis written in 2008. The study in 2008 included 182 children and adolescents with T1D, diagnosed with T1D at an average age of 8 years. 89 patients were female and 83 were male. 30 (16%) of the 182 children and adolescents developed an additional autoimmune disease. Of these 24 (80%) patients developed only AITD, 4 patients (13.3%) developed only CD, and 2 patients (6.7%) developed both AITD and CD.

3.3 Ethical evaluation

The study was conducted in respect to the principles of the World Medical Association's (WMA) Declaration of Helsinki and the study protocol was approved by the ethics committee of the Medical University of Graz (EK-number: 29-495 ex 16/17).

3.4 Data collection

Children and adolescents with T1D treated at the Diabetes Outpatient Clinic receive an annual review of glycaemic control, associated autoimmune diseases and late complications of T1D. Associated autoimmune diseases are evaluated by yearly screenings for thyroid and CD antibodies. PCA were determined as part of a study in 2014 and are currently only measured when AG is suspected.

Data were extracted from the medical records of these patients and anonymised by allocating consecutive identification numbers to patients. Only authorised individuals received access to the original data.

General data collected for every patient included gender, date of birth, date of diabetes onset, age at diabetes onset, family history of autoimmune disorders and presence of an additional autoimmune disease.

Patient with an additional autoimmune disease were examined more closely and the following parameters were collected:

<u>Autoimmune thyroid disease</u>:

- Date and age when thyroid antibodies were positive
    - TgAb and/or TPOAb higher than 100 U/mL
    - TRAb higher than 14 U/mL
- Date of ultrasound confirmation
- Date and age when therapy was started

<u>Celiac disease</u>

- Date and age when antibodies were positive
    - tTG and/or EMA antibodies higher than 10 U/L
    - AGA IgG and IgA higher than 25 U/L
- Date of biopsy and marsh criteria

<u>Autoimmune gastritis</u>

- Date and age when antibodies were positive
    - PCA higher than 15 U/mL

- Date of gastroscopy
- Date of iron deficiency onset
    - normal iron range: 60–180mg/dL
- Date of vitamin B12 deficiency onset
    - normal vitamin B12 range: 180– 1000 pg/mL

3.5 Statistical analysis

The data extracted from the medical records of patients treated at the at the Diabetes Outpatient Clinic was collected in a database in Microsoft Excel version 2015 (Windows). A detailed descriptive analysis was also conducted in Microsoft Excel, by performing calculations and creating charts and graphs.

Significances were calculated using the Fisher's Exact Test of Independence and T-Test in Microsoft Excel and the Chi-Square Test in IBM Statistical Package for Social Sciences (SPSS, version 23.0). *P*-values are given as two-sided probabilities and a significance level of 0.05 was used.

4 Results

For this study, the data of a total of 303 children and adolescents with T1D were collected. The study population was composed of 128 (42.2%) females and 175 (57.8%) males. (Figure 4)

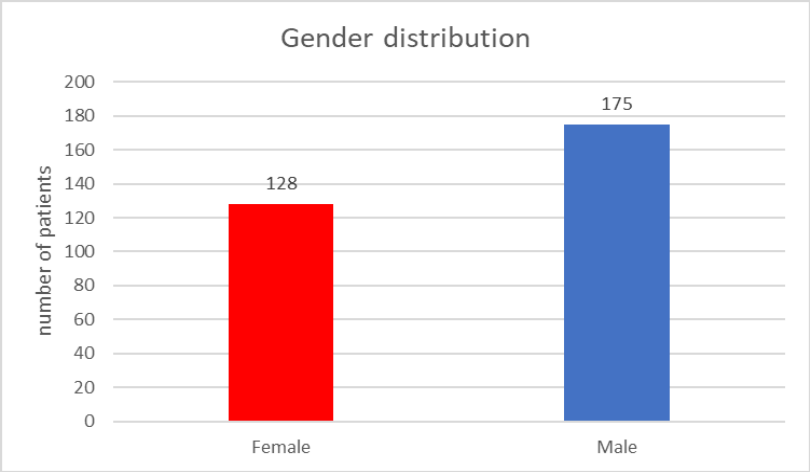

Figure 4: Gender distribution of the study population consisting of 303 patients

The age at onset of T1D ranged from 8 months to 17 years. Peak in age at disease onset was between 3 and 10 years and both male and female patients had a mean age of 7.1 ± 4.2 years at diagnosis. 40% of patients were diagnosed with T1D at five years of age or younger and 60% over the age of five ($P < 0.001$). (Figure 5)

Figure 5: Age distribution of the study population at onset of T1D

4.1 T1D and associated autoimmune diseases

Out of the 303 patients with T1D 62 (20.5%) suffered from an additional autoimmune disease; one associated autoimmune disease was found in 55 (18.2%) patients and two additional autoimmune diseases in 7 (2.3%) patients.

4.1.1 Gender distribution

38 females and 24 males of the study population were found to have an additional autoimmune disease, showing a strong female predominance (P = 0.00086). In the female population 30% developed an additional autoimmune disease, whereas only 14% of the male population had a further autoimmune disease. (Figure 6).

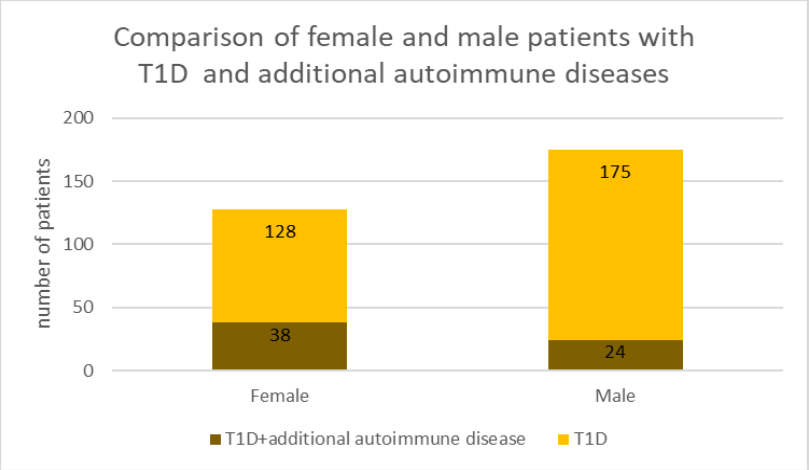

Figure 6: Comparison of 128 female and 175 male patients with T1D and additional autoimmune diseases

4.1.2 Distribution of associated autoimmune diseases

The mean age at onset of an additional autoimmune disease was 9.1 ± 4.2 years and on average 2.2 ± 3.4 years after the diagnosis of T1D. The majority of the 62 patients had an autoimmune thyroid disorder (56%) followed by CD (40%) and AG (15%). Of the patients with AITD, 94% had Hashimoto Thyroiditis and 6% suffered from Graves' Disease. (Figure 7)

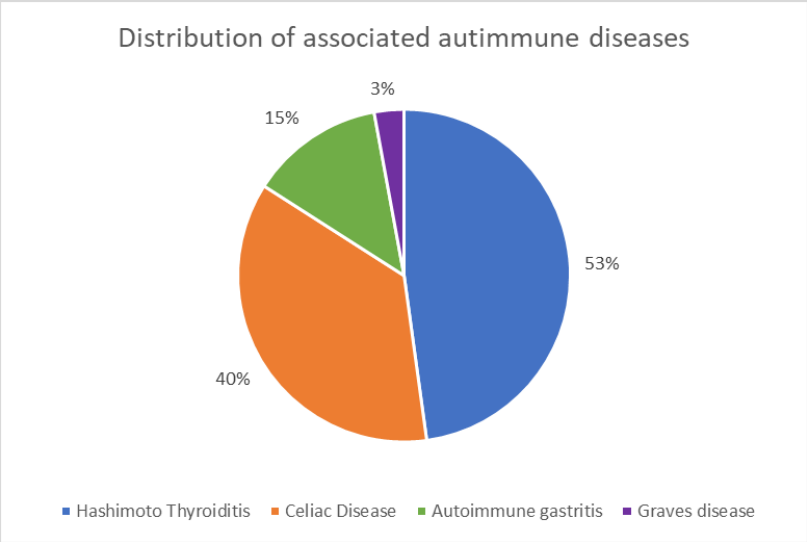

Figure 7: Distribution of associated autoimmune diseases in 62 patients with T1D

4.2 T1D and AITD

AITD was present in 35 (11.6%) of the 303 patients with T1D. The age at onset ranged between 3 and 16 years and on average it was diagnosed at an age of 9.6 ± 4.2 years. The mean diabetes duration until AITD was diagnosed was 2.3 ± 2.9 years. Thyroid autoimmunity was found to be significantly higher in females (24 patients, 68.6%) than in males (11 patients, 31.4%, *P* = 0.028).

4.2.1 Hashimoto thyroiditis

33 patients (10.9%) showed positive thyroid antibodies, with 22 being female (66.7%) and 11 being male (33.3%, *P* = 0.056). (Figure 8)

Furthermore, 13 children and adolescents (4%) of the study population suffered from overt hypothyroidism. Patients diagnosed with T1D at age five years or younger had a prevalence of HT of 10% and when diagnosed over the age of five prevalence increased to 12%, although this did not reach statistical significance (*P* = 0.851).

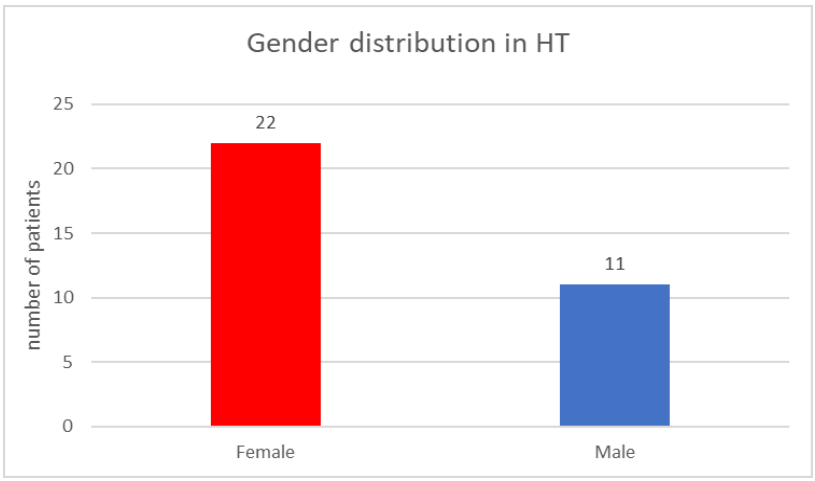

Figure 8: Gender distribution of 33 patients with T1D and HT

4.2.1.1 Age at onset of HT

The age of HT onset peaked between 5 and 13 years with a mean age at diagnosis of 9.7 ± 4.3 years. The youngest patient diagnosed with HT was female and three years of age and the oldest patient, also female, was 16 years of age. There was no significant difference in mean age at diagnosis between female (9.9 ± 3.7 years) and male patients (9.4 ±3.7 years, *P* = 0.7). (Figure 9)

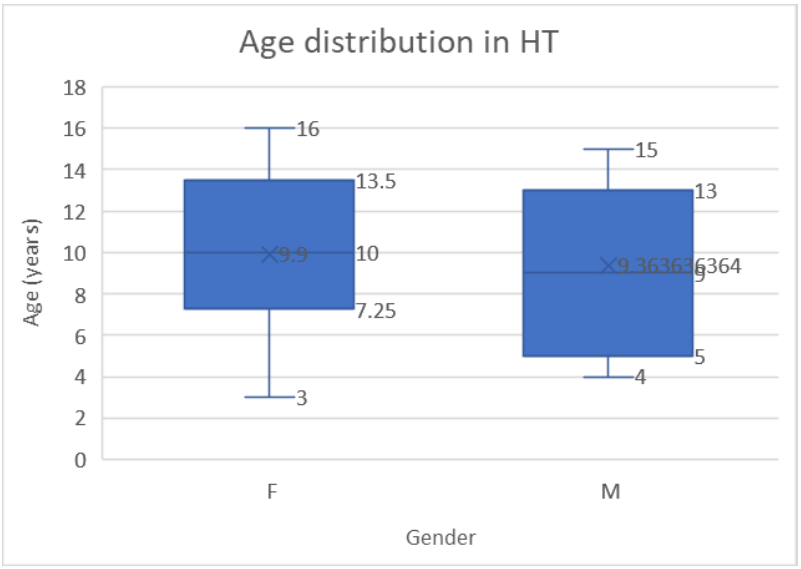

Figure 9: Age distribution at onset of HT in males and females with T1D

4.2.1.2 Diabetes duration

When HT additionally developed, the average age at T1D onset was 7.6 ± 4.5 years. The duration from T1D onset to diagnosis of positive thyroid antibodies ranged from 0 to 9 years with a mean time of 2.2 ± 2.9 years. No significant difference between male and female diabetes duration was found ($P = 0.85$).

Children, under the age of five, on average developed positive antibodies 4.5 ± 3.3 years after T1D onset, whereas patients older than five years had a mean diabetes duration of 0.8 ± 1.5 years ($P = 0.00019$). HT was never diagnosed before T1D, however 15 patients presented with positive thyroid antibodies at diabetes diagnosis.

4.2.1.3 Ultrasound confirmation

26 (73%) of the 33 patients with elevated thyroid antibodies underwent ultrasound examination of the thyroid gland, confirming HT. In 11 patients this was conducted in the first year after diagnosis and on average it was carried within two years after the detection of positive thyroid antibodies.

4.2.1.4 Age at therapy begin

Therapy was started with Euthyrox 25 µg to 125 µg or Thyrex 25 µg when patients developed overt hypothyroidism (reduced T4). Out of the 33 patients with positive thyroid antibodies, 13 (4%) receive treatment. Average begin was 1.8 ± 1.9 years after the diagnosis of positive thyroid antibodies, with one patient receiving therapy directly after diagnosis and four patients within the first year. 50% of the female patients required therapy and 18% of the male patients, however no statistical significance was observed ($P = 0.132$).

4.2.2 Graves' Disease

GD was present in 2 patients (0.6%) with T1D, both being female. Average age at onset of T1D was 6.5 ± 4.9 years and duration until GD was diagnosed was 2 years. The mean age at which thyroid antibodies were detected was 8.5 ± 4.9 years. In the course of the disease one patient required therapy and the second patient switched to HT.

4.3 T1D and CD

The second most prevalent additional autoimmune is CD, with 25 children and adolescents (8.3%) showing positive CD antibodies. There was a slight female predisposition, with 14 patients (56%) being female and 11 patients (44%) being male, but without reaching statistical significance (*P* = 0.549). (Figure 10)

10% of patients diagnosed with T1D at five years or younger developed CD, whilst this was only 7% when T1D was diagnosed after the age of five years, however this observation was not statistically significant (*P* = 0.398).

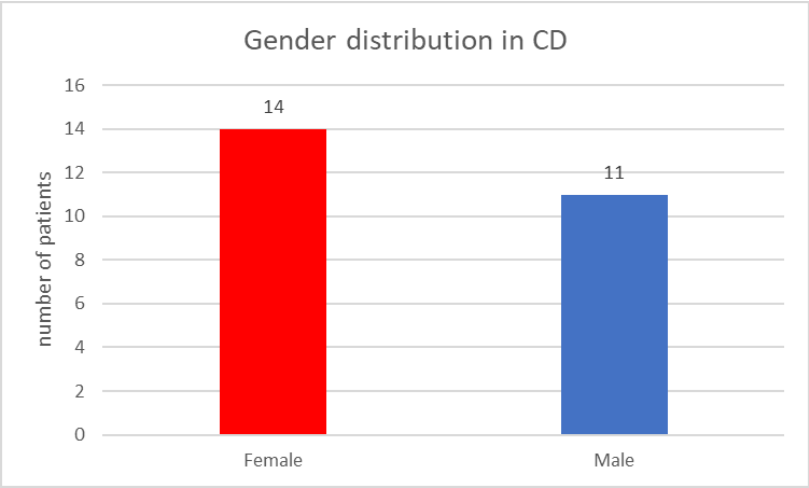

Figure 10: Gender distribution of 25 patients with T1D and CD

3 children and adolescents (1%) with T1D only showed positive CD antibodies while 22 patients (7.3%) had proven CD. In 18 of these patients CD was confirmed by biopsy, three patients refused biopsy and one patient was diagnosed at a different hospital.

4.3.1 Age at onset of CD

The average age at diagnosis of CD antibodies was 6.8 ± 4 years and no significant gender difference was observed (*P* = 0.718). Age at disease onset peaked between 3 and 10 years in female patients and between 2 and 8 years in males. The youngest patient with positive antibodies was aged two years and male. The oldest patient at onset of CD was 16 years of age and female. A positive association was found between young age and development of positive CD antibodies, as 75% of the patients were diagnosed with CD before the age of 10 years (*P* = 0.014). (Figure 11)

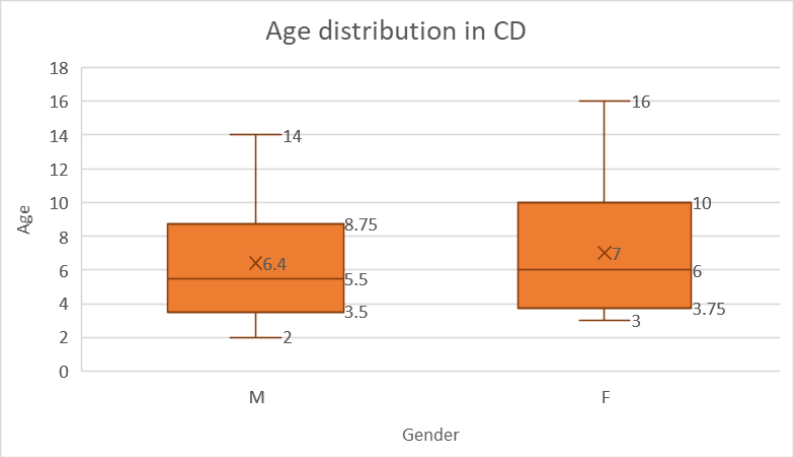

Figure 11: Age distribution at onset of CD in males and females with T1D

4.3.2 Diabetes duration

The mean age at diabetes onset in patients who also developed positive CD antibodies was 6.4 ± 4.4 years. This was lower in the male population (6.4 ± 3.9 years) than in the female population (7 ± 4 years), but did not reach statistical significance (P = 0.532).

50% of the patients were diagnosed with T1D at age five years or younger and 91% of these also developed CD antibodies before or at the age of five. One patient was diagnosed with CD before T1D, 15 patients (63%) of the population had positive antibodies at the same time as T1D onset and 8 patients (33%) developed CD after T1D was diagnosed. Males (7 patients, 70%) showed positive CD antibodies at T1D onset more often than females (8 patients, 57%), without statistical significance (P = 0.678).

The average duration from diabetes onset to CD diagnosis was 0.6 ± 1.7 years and the maximum was 4 years. Females generally had a shorter duration (0.4 ± 1.8 years) between T1D onset and detection of CD antibodies than males (0.9 ± 1.7 years), but statistical significance was not reached (P = 0.464).

4.3.3 Biopsy and Marsh Criteria

18 patients with elevated CD antibodies underwent SBB, 3 patients refused biopsy and one patient was diagnosed at a different hospital. In 17 patients this was done within the first year after diagnosis and in one patient the following year. Approximately 90% of this population showed Marsh criteria stage 3, confirming the diagnosis of CD. Around half of the patients presented with Marsh 3c, corresponding to severe histological changes. (Table 18)

Marsh criteria		Number of patients	Percentage of patients
Marsh 1		*1*	*6%*
Marsh 2-3		*1*	*6%*
Marsh 3		*16*	*89%*
	a	*3*	*17%*
	a-b	*2*	*11%*
	b	*2*	*11%*
	c	*9*	*50%*

Table 18: Number and percentage of patients with Marsh criteria 1-3 in biopsy proven CD

4.4 T1D and AG

9 patients were diagnosed with AG, representing 3% of the total study population. 6 of these patients (66.7%) were female and 3 patients (33.3%) were male (*P* = 0.317). (Figure 12)

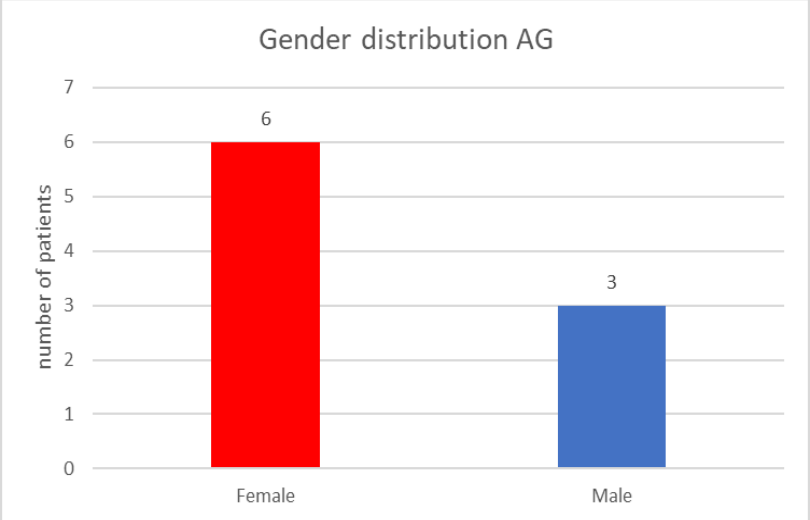

Figure 12: Gender distribution of 9 patients with T1D and AG

4.4.1 Age at onset of AG

Unlike CD and HT, AG was diagnosed after the onset of puberty with an average age of 13 ± 2.6 years. Most patients showed positive PCA after the age of 10 years, and the youngest patient was 9 years of age and the oldest 18 years of age. Generally female patients (12.3 ± 2.2 years) were diagnosed at an earlier age than male patients (15.7 ± 2.1 years, *P* = 0.053). (Figure 13)

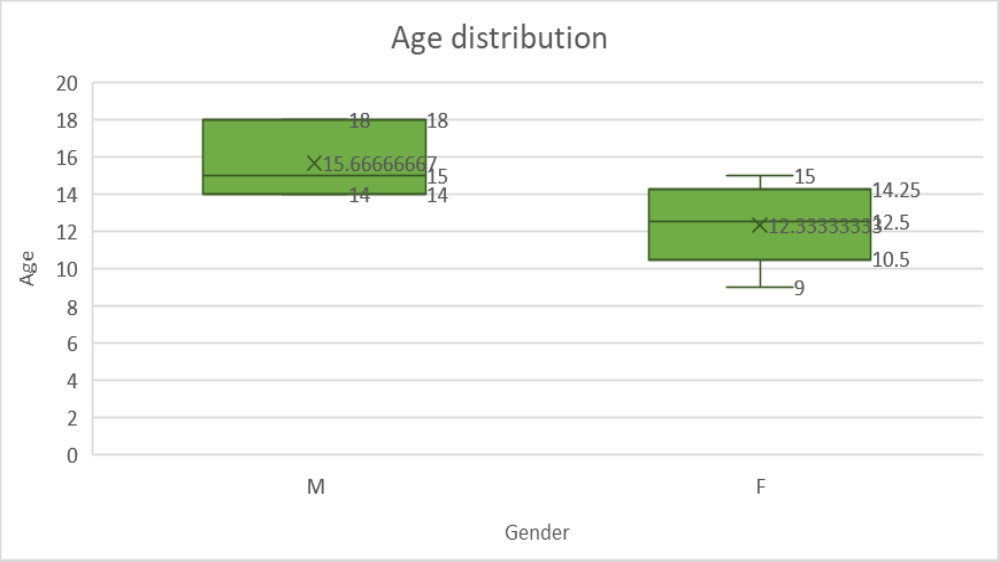

Figure 13: Age distribution at onset of AG in males and females with T1D

4.4.2 Diabetes duration

All patients who developed AG were aged two years or older at T1D onset and on average they were 7 ± 3.7 years old. Female patients were slightly older at T1D diagnosis than male patients (7.2 ± 3.6 years vs 6.7 ± 4.7 years, respectively), however without statistical significance (*P* = 0.863). Duration from T1D onset to detection of positive PCA ranged from 1 to 15 years, with a mean time of 6.4 ± 4.6 years. The duration between T1D onset and AG had a mean time of 9 ± 6.6 years in males and 5 ± 3.4 years in females, but did not reach statistical significance (*P* = 0.269). AG was never diagnosed before the onset of T1D.

4.4.3 Gastroscopy

Two patients underwent gastroscopy within the first year after detection of positive PCA. Both of these patients were female. One of these patients showed histologically proven AG. In the second patient a combination of A- and B-Gastritis was observed.

4.4.4 Iron and vitamin B12 deficiency

Iron deficiency was found in 4 patients (44%) prior to detection of positive PCA. All of these patients were female. Vitamin B12 deficiency was not present in any of the patients with AG.

4.5 T1D and other autoimmune diseases

None of the children and adolescents in the study population had Addison's disease. 1 patient (0.3%) had ulcerative colitis and 3 patients (1%) suffered from rheumatic diseases. Two of these patients had juvenile idiopathic rheumatic arthritis and one patient had uveitis.

4.6 T1D and two associated autoimmune diseases

7 patients (2.3%) out of the study population developed two additional autoimmune diseases, six female and one male. All patients were diagnosed with an AITD, being HT in six out of the seven cases. Four patients developed CD as a third autoimmune disease and three patients were diagnosed with AG.

T1D onset was under the age of 10 years in all patients, with a mean age of 5.4 years. One patient was first diagnosed with T1D and HT and developed AG five years later. Four patients were diagnosed with T1D followed by both additional autoimmune diseases at the same time. The time between these onsets varied from two to 10 years. Finally, two patients were diagnosed with all three

autoimmune diseases at diabetes onset, one at the age of 4 years and the other at the age of 9 years.

4.7 Family history

37 patients (12%) of the study population had a positive family history of T1D in first degree relatives. 15 patients (7%) had fathers, 8 patients (3%) had mothers and 15 patients (5%) had siblings with T1D. In one patient both parents suffered from T1D. 26 male patients (15%) had relatives with T1D. In the female population this was much lower, with only 11 patients (8%) having relatives with T1D, but this did not reach statistical significance ($P = 0.119$). The mean age at diabetes onset did not vary from the average onset in the remaining population.

Four patients had mothers with HT and three of these also developed the disease. CD was found in five relatives of the study population and two of these patients also developed CD. One patient, who suffered from all three autoimmune diseases, had a mother with HT and CD.

4.8 Prevalence of autoimmune diseases in 2008 compared to 2017

Compared to the data collected for the diploma thesis in 2008, the prevalence rate of associated autoimmune diseases in children and adolescents with T1D increased from 16% to 20.5% in 2017, however without statistical significance ($P = 0.339$). Prevalence of AITDs decreased by 2.6% from 14.2% in 2008 to 11.6% in 2017, also not reaching statistical significance ($P = 0.398$). On the other hand, prevalence of CD showed a significant rise from 3.2% in 2008 to 8.3% in 2017 ($P = 0.035$). (Table 19)

Autoimmune disease		Prevalence		*P* value
		2008	**2017**	
All associated autoimmune diseases		*16%*	*20.5%*	*0.339*
AITD		*14.2%*	*11.6%*	*0.398*
	HT	*14.2%*	*10.9%*	*0.315*
	GD	*0%*	*0.6%*	*0.53*
CD		*3.2%*	*8.3%*	*0.035*
AITD and CD		*1.1%*	*1.3%*	*1*

Table 19: Comparison of prevalence rates of associated autoimmune diseases in 2008 (68) and 2017

5 Discussion

T1D is the most common chronic metabolic disease in children and adolescents, with around 80,000 new cases worldwide annually. It is associated with a variety of comorbidities, including an increased prevalence of additional autoimmune diseases such as AITD, CD and AG. These diseases may lead to impaired growth, delayed puberty, malnutrition, poor metabolic glucose control and many other complications, not only in the overt form of the diseases but also in asymptomatic patients. Early recognition of associated autoimmune diseases, by antibody screening, therefore plays an important but still debated role in the prevention of morbidity in children and adolescents with T1D (1,50,69).

5.1 Prevalence of associated autoimmune diseases

In our study population of 303 children and adolescents, diagnosed with T1D and treated at the Diabetes Outpatient Clinic at the Department of Paediatrics and Adolescent Medicine of the Medical University Graz, an overall prevalence rate of positive thyroid, CD and parietal cell antibodies of 20.5% was found. This is 2 to 6-fold higher than the prevalence in the general population (69). A similar study conducted at the Medical University of Graz in 2008 found additional autoimmune diseases in 16% of patients with T1D, equating to an almost 30% increase in associated autoimmune diseases in the past nine years, however not reaching statistical significance (68).

Although more male children and adolescents were diagnosed with T1D, a significant female sex bias was observed in the development of associated autoimmune diseases. Prevalence of an additional autoimmune disease in female patients was 27%, whilst only 14% in male patients, which is consistent with the results of previous studies (49,50,55,64,70,71).

5.2 Autoimmune thyroid disease

With a prevalence of 11.6%, AITD was the most common autoimmune disease in T1D patients. Compared to the reported prevalence rates of 3% to 8% in literature, the number of T1D patients with positive thyroid antibodies in our study is slightly higher (57). Additionally, a clinical distinction between HT and GD can be made, with prevalence rates of 10.9% and 0.6% respectively. Previous literature describes slightly higher prevalence rates of HT, whilst GD lies within the European range of 0.2% to 3% (64,71). Overt hypothyroidism was found in 4% of our study population, which is comparable to prior reports showing occurrence rates of 4% to 18% (64).

Controversial to most literature, the prevalence rate has declined by almost 3 percentage points from 14.2% to 11.6%, since the previous study at the Medical University of Graz in 2008 (34,68). A similar decline in prevalence of thyroid antibodies was found in a multicentre survey published in 2008, investigating children and adolescents with T1D treated at paediatric departments of universities, general hospitals and paediatric practices in Germany and Austria. This study also showed a decrease in prevalence rate of positive thyroid antibodies from 21% in 1995 to 14% in 2006 (50). This may be explained by the large increase in young children diagnosed with T1D but AITD onset remaining at an older age (50).

The mean age at T1D onset in patients with positive thyroid antibodies was 7.5 ± 4.5 years, marginally lower than observed in previous studies (50). On average positive thyroid antibodies were diagnosed 2.3 years (± 2.9 years) after the onset of T1D at a mean age of 9.6 ± 4.2 years. Moreover, a positive correlation between increasing age at T1D onset and the development of positive thyroid antibodies was found. Children and adolescents diagnosed with T1D at age five years or younger had a significantly longer duration (4.3 ± 3.3 years) between T1D onset and presence of positive thyroid antibodies than patients diagnosed with T1D over the age of five years (0.8 ± 1.5 years). A study conducted by Riquetto et al. also found this longer time to develop HT in younger children with T1D compared to older patients (70). Furthermore, similar to previous reports, AITD was never diagnosed before T1D and prevalence increased with increasing age (64).

Overt hypothyroidism was observed in 39% of patients with positive thyroid antibodies, which corresponds to 41% found by Riquetto et al. (70). These patients, on average, received treatment 1.8 years after the diagnosis of positive thyroid antibodies. Earlier begin of treatment, when thyroid antibodies are positive but the thyroid gland shows euthyroid function, is not recommended (50).

As shown in previous literature, a significant higher number of female patients were diagnosed with AITDs than male patients (49,50,71). More than two thirds of the children and adolescents with AITD were girls, suggesting that female gender is a risk factor for the development of positive thyroid antibodies. No difference was found between average age at onset of AITD and average diabetes duration in female and male patients.

5.3 Celiac disease

CD was found to be the second most common associated autoimmune disease, with a prevalence of 8.3%. This corresponds to a 7 to 8-fold higher prevalence than in the general population and is comparable to previous studies, showing prevalence rates between 0.6% and 16.4% (50,64,72). Additionally, similar to earlier research in this field, our study observed a significant rise in prevalence of CD, from 3.2% in 2008 to 8.3% in 2017 (50,68).

Unlike AITD, CD was diagnosed at a younger age. Age at onset of CD peaked between 3 and 10 years with an average of 6.8 ± 4 years. Moreover, 75% of patients were diagnosed with CD before the age of 10 years showing a strong association between young age and the development of CD. This confirms findings of existing literature and may be explained by the shared risk factor of early gluten exposure, triggering both T1D and CD (50,55).

Furthermore, patients suffering from CD also had a younger age at T1D onset than patients diagnosed with AITD. Our study found a mean age at onset of 6.4 ± 4.4 years, comparable to the ages found by a multicentre study, published by Craig et al. (55). This study, comparing the prevalence and clinical characteristics of CD in T1D patients across three continents observed ages at diabetes onset ranging from 6 to 7.3 years, with an average age of 7.15 years in Europe (55).

Children and adolescents developed CD on average 0.6 ± 1.7 years after the onset of T1D. 4% of patients were diagnosed with CD before T1D, 65% within the first year after diabetes diagnosis and 30% after one year. Although not statistically significant, patients diagnosed with T1D ≤ 5 years of age had a prevalence of CD of 10%, whilst patients over the age of five had a prevalence of 7%. This is consistent with existing data, confirming the high prevalence at young age, short diabetes duration and typical diagnosis after T1D onset. Furthermore, it emphasises the importance of antibody screening at T1D onset and gives rise to the question, whether younger patients should be screened more frequently (49,55,64).

More females than males were diagnosed with CD, but without reaching statistical significance. This is also controversial in literature, with studies showing either no correlation, female predominance or even male sex bias (50,55,59). Moreover, no correlation between gender and age at CD diagnosis, age at T1D onset and diabetes duration was found.

5.4 Autoimmune gastritis

The prevalence of AG in our study was 3%, which is 2 to 3-fold higher than in the general population. Due to the limited number of studies examining T1D and AG in children and adolescents, rather than in adults, prevalence rates of PCA have shown many controversies. A cross-sectional study by Fröhlich-Reiterer et al. found a prevalence of 5.29% and a review by Krzewska et al. observed positive PCA in 5.3 to 7.2% of children and adolescents with T1D (59,63,64). Unlike the yearly thyroid and CD antibody screening of children and adolescents with T1D, PCA was only determined once as part of a study conducted in 2014, which may explain the low prevalence in our study.

Contrasting to CD, AG was diagnosed at a much older age and mostly after the onset of puberty, with a mean age of 13 ± 2.6 years. This confirms findings of previous studies, conducted not only in children and adolescents but also in adults, showing a strong correlation between increasing age and prevalence of positive PCA (59).

Patients suffering from AG had mean age at T1D onset of 7 ± 3.7 years and showed the longest duration until the development of positive antibodies. Similar to existing literature, AG on average developed 6.4 ± 4.6 years after the diagnosis of T1D (61). Interestingly, a difference in diabetes duration between female and male patients was found and is consistent with previous studies (61). Mean time between T1D onset and diagnosis of AG was 9 ± 6.6 years in males and only 5 ± 3.4 years in females, however this did not reach statistical significance.

Although also not statistically significant, more female patients were diagnosed with AG than male patients and this observation was confirmed by previous studies (59,61). No significant gender difference was found in age at diagnosis of AG and age at T1D onset in patients with positive PCA.

5.5 Addison's Disease

Unlike existing data, none of the children and adolescents in the present study were diagnosed with Addison's disease. The rare occurrence of this disease, with prevalence rates of 0.3% to 1.6% in literature, may be a reason for this (49,69,73).

5.6 Two or more associated autoimmune diseases

A further observation of our study, was the prevalence of developing two or more associated autoimmune diseases, which was found to be 2.3%. This is equivalent to a 1.3% increase since 2008, however PCA where not determined in the previous study (68). Jing et al. found comparable results observing a prevalence of 4.3% in patients aged younger than 13 years (69). Further comparisons were difficult, as our study is one of the few studies investigating the prevalence of several autoimmune diseases in children and adolescents under the age of 18.

5.7 Limitations and future studies

A limitation of this study may be its retrospective design. It relied on the results of regular antibody screening, conducted at the Diabetes Outpatient Clinic at the Department of Paediatrics and Adolescent Medicine of the Medical University Graz, which is dependent on the compliance of patients. If annual reviews were missed, the associated autoimmune diseases may not have been recognised or diagnosed at a much later stage, leading to an underestimation of prevalence rate. Furthermore, the documentation of family history of additional autoimmune diseases was not consistent in all patients, resulting in an underestimation of prevalence of positive family history.

Future studies should therefore have a prospective design. Additionally, investigation of possible risk factors associated with the development of these associated autoimmune diseases in T1D patients may be of value. Risk factors to be reviewed could include poor metabolic control, genetic factors and environmental factors such as diet and exercise.

6 Conclusion

In this study, we examined the prevalence of additional autoimmune diseases in 303 children and adolescents with T1D. The high prevalence of 20.5% confirms the need for regular antibody screenings during follow-up of diabetes patients. AITD was found to be the most prevalent autoimmune disease, followed by CD and AG. Moreover, a strong female predominance was observed. The average age at diagnosis also varied greatly amongst the different autoimmune diseases, highlighting the importance of screening procedures.

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