Prevalence of autoimmune comorbidities in newly diagnosed pediatric patients with type 1 diabetes

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PREVALENCE OF AUTOIMMUNE COMORBIDITIES IN NEWLY DIAGNOSED PEDIATRIC PATIENTS WITH TYPE 1 DIABETES

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LIST OF ABBREVIATIONS

T1DM – Type 1 Diabetes Mellitus

DM – Diabetes Mellitus

MODY – Maturity Onset Diabetes of the Young

DKA – Diabetic ketoacidosis

FPG – Fasting Plasma Glucose

OGTT – Oral Glucose Tolerance Test

HbA1c – Hemoglobin A1c

NGSP – National Glycohemoglobin Standardization Program

DCCT – Diabetes Control and Complications Trial

FPG – Fasting Plasma Glucose

HLA – Human Leukocyte Antigen

ICA – Islet Cell Antibodies

ICSA – Islet Cell Surface Antibodies

GADA – Glutamic Acid Decarboxylase Antibodies

IA2 – Islet Antigen 2

IAA – Insulin AutoAntibodies

ZnT8 – Zinc Transporter Isoform 8

CSII – Continuous Subcutaneous Insulin Infusions

CC – Carbohydrate Counting

ICR – Insulin-to-Carbohydrate Ratio

CGM – Continuous Glucose Monitoring

TIR – Time in Range

TBR – Time below Range

TAR – Time above Range

PALS – Paediatric Advanced Life Support

ADA – American Diabetes Association

AITD – Autoimmune Thyroid Disease

CD – Celiac Disease

tTG – Tissue Transglutaminase

EMA – Endomysial Antibodies

DGP – Deamidated Gliadin Peptide

AIT – Autoimmune Thyroiditis

HT – Hashimoto's Thyroiditis

GD – Graves' disease

Anti-TG – Anti-thyroglobulin

Anti-TPO – Anti-thyroid peroxidase

T3 – Triiodothyronine

T4 – Thyroxine

TFT – Thyroid Function Tests

ISPAD – International Society for Pediatric and Adolescent Diabetes

TSH – Thyroid Stimulating Hormone

UNL – Upper Normal Limit

1. INTRODUCTION

1.1. Diabetes Mellitus

1.1.1. Definition

Diabetes mellitus is a heterogeneous metabolic disorder defined by the presence of high blood sugar due to inadequate or absent insulin secretion, impaired insulin action, or a combination of the two. Deficient insulin secretion and/or reduced tissue responses to insulin leads to the consequences of insufficient insulin action on target tissues, ultimately resulting in aberrant glucose, lipid and protein metabolism (1).

Lifelong medical care and self-management education is essential to minimize the risk for acute complications, in addition to preventing and lowering the risk for long-term complications caused by poor glucose control (2).

1.1.2. Classification of Diabetes Mellitus

Diabetes mellitus is a group of diseases with various aetiologies, clinical courses, and evolution; however the common connection is the lack of insulin and the resulting hyperglycaemia (3). The disease is classified by its aetiology into different clinical categories - highlighting type 1 and type 2 diabetes mellitus, gestational diabetes, and other types mainly monogenic diabetes (MODY) or associated with other conditions (4). Type 1 diabetes (also known as insulin-dependent diabetes mellitus or juvenile-onset diabetes) arise from the destruction of pancreatic beta-cells as a consequence of an autoimmune reaction, eventually leading to complete insulin deficiency (3). This type is considered to be accountable for solely 5-10% of people with diabetes, while in the paediatric population T1DM accounts for 85% of cases (3,5). Type 2 diabetes (also known as non-insulin dependent diabetes or adult-onset diabetes) includes those with insulin resistance and a relative insulin deficiency. This form of diabetes comprises 90-95% of all diabetic patients, being the most prevalent type of diabetes globally (4). It is likely to have a multifactorial aetiology, among other things a complex genetic component that is not fully defined; however it is frequently related to an unhealthy lifestyle including excessive body weight and insufficient physical activity. Nevertheless, it is not related to any autoimmune destruction of beta-cells (3).

In some specific situations it may be difficult to set the diagnosis between type 1 or type 2 DM. The diagnosis of T1DM is comprehensive when the patient is a child of less than 10 years who presents with ketoacidosis and elevated islet autoantibodies (6).

Nevertheless, the following characteristics are more likely to be associated with type 2 diabetes: overweight or obesity, age > 10 years, strong family history of type 2 diabetes, high-risk racial or ethnical groups, acanthosis nigricans, or untraceable islet autoantibodies (7). However, due to the epidemic of obesity in the last decades, up to one third of children are overweight or have obesity at the time of diagnosis of T1DM, along with insulin resistance. Nevertheless, these children will present with elevated islet autoantibodies which confirms the diagnosis of T1DM and the requirement for insulin therapy (7).

1.1.3. Diagnostic criteria

The appearance of clinical symptoms in addition to increased blood glucose values are part of the diagnostic criteria of T1DM (8). There are four stages in the development of T1DM (check 1.1.5.1. Stages of T1DM), that can be manifested with various clinical presentations and biochemical results (7). Diverse diagnostic techniques are used for disease confirmation, and in cases of indecisive glucose values, the tests are repeated until there is an established diagnosis (8).

Typical clinical manifestations of diabetes include polydipsia, polyuria, nocturia, enuresis, loss of weight and polyphagia. Specific symptoms that are related to the pediatric population include developmental and growth problems, poor achievement in school, and blurred eyesight. Furthermore, diabetic patients are more prone to all kinds of infections due to the impact of high blood glucose levels that subsequently alter the function of the immune system (8). Overseeing the symptoms or having atypical clinical manifestations of T1DM may lead to misdiagnosis or late diagnosis that increases the risk of developing diabetic ketoacidosis, which subsequently may deteriorate to stupor, coma and eventually death in the cases of unsuccessful therapy (7,8).

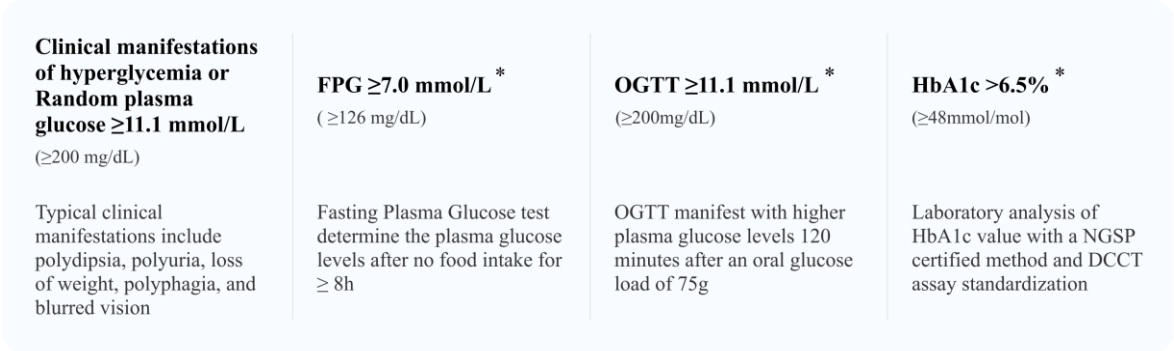
Obtaining a capillary blood specimen and using a portable blood glucose meter is a simple screening tool that can be used if the previously mentioned clinical manifestations of diabetes are present (8). Another simple screening tool is using a urinary dipstick test to check for presence of glucose and ketones in the urine. However, to establish and confirm the diagnosis, a documented plasma glucose assessment is necessary (8). This is provided by using laboratory glucose oxidase estimation instead of a capillary blood glucose measurement (8).

Nevertheless, in some circumstances the diagnosis cannot be done by a single plasma glucose assessment, and therefore fasting plasma glucose (FPG) test and a 2-hour-postprandial glucose measurement, and/or an oral glucose tolerance test (OGTT) might be

necessary to verify the diagnosis (8). The circumstances when this might be needed are when the confirmation of the disease is indecisive or vague. In example, when high blood glucose levels is an incidental discovery and the child is not presenting with any symptoms; if there are only mild or atypical clinical manifestations; or if high blood glucose levels are noticed when the child is having an acute infection, or in the cases of a traumatic, circulatory or any other kind of stressful state that might be transient. Despite all this that is mentioned, an OGTT is rarely used in the clinical setting of diagnosis of T1DM due to the undesirable risk of even higher glucose levels (8).

A HbA1c value ≥6.5% (≥48mmol/mol) can additionally be used for the diagnosis of diabetes, however the test method has to be verified by the National Glycohemoglobin Standardization Program (NGSP) and it has to be standardized or trackable to the Diabetes Control and Complications Trial (DCCT) (4). Nevertheless, it has some insufficient capacity that makes it a suboptimal diagnostic tool (4). Since T1DM patients can progress quickly with dysglycemia, the HbA1c value is less sensitive for diagnosis than the previously mentioned tests (9). Furthermore, it is even less reliable in the situations of patients with hemoglobinopathies, particular types of anemia, or any other state or diseases that act on the physiological red blood cell turnover (4,8). However, HbA1c is an excellent screening tool to use on high-risk individuals, as they usually have a rise in their values as they start to progress in the development of T1DM (8). Additionally, the HbA1c test has pre-analytical superiority to a plasma glucose sample as it can be simply performed and easily stored, including that a fasting state of the patient is not required so the test can be taken throughout the day without patient precautions (4).

The current diagnostic criteria for T1DM are summarized in the figure below (Figure 1) and should include one or several statements for confirmed diagnosis (3):



*Repeated testing should be performed in cases where uncertain hyperglycemia is present

Figure 1. Diagnostic criteria for T1DM (3)

1.1.4. Pathogenesis

Type 1 diabetes mellitus is characterized by cell-mediated autoimmune attack on the pancreatic beta cells, resulting in destruction which eventually leads to complete insulin deficiency (10).

While there have been rigorous studies for a long time considering the pathogenesis of T1DM, it is well-known today that there are many factors interplaying in the development of the disease. Genome-wide association studies have found more than 60 genetic variations linked to T1DM (9). Particular combinations of DR and DQ alleles at the Human Leukocyte Antigen (HLA) loci are considered to give out increased or decreased susceptibility for the development of the diseases (7). The HLA locus is contemplated to account for about 50% of the genetic susceptibility (9). Specific haplotypes that show strong linkage of disequilibrium and confining the highest-risk are DRB1*03:01-DQA1*05:01-DQB1*02:01 and DRB1*04-DQA1*03:01-DQB1*03:02 – also expressed as DR3/DR4 or DQ1/DQ8 (7,10). Research has confirmed that there is a 30-fold increased risk of autoimmunity and T1DM in heterozygotes for DR3/DR4 (DQ2/DQ8) (7). Furthermore, other non-HLA risk loci that show the highest contribution to disease development include *INS, PTPN22, CTLA4,* and *IL2RA* genes (11). There is a 15-fold increased estimated risk of developing T1DM if there is a first degree relative who is suffering from the same disease, showing the strong linkage between the genes and the pathogenesis. However, approximately 85% of children who develop diabetes have no family history of the condition (7).

Another significant factor that is part of the pathogenesis of T1DM is the role of the modern environment. It is thought to occur through complex gene-environment interactions (7). Environmental triggers can modulate the immune system which in turn can lead to beta-cell destruction of the pancreas, and thereby finalizing the cascade of developing absolute insulin deficiency (10). Viruses such as enteroviruses, coxsackieviruses, and congenital rubella; as well as environmental toxins (e.g. nitrosamines) or certain types of food (e.g. early exposure in infants to cow’s milk proteins, cereals, or gluten) are considered as potential triggers (10). Feeding the infant breast milk at the time of cereal introduction may be a protective factor for high-risk children. Additionally, omega 3 and vitamin D may have a protective impact; however the role of vitamin D in this matter is still unclear (7).

The pathogenic mechanism involves a complex interactivity between the □-cells of the pancreas, cellular immunity, and humoral immunity (9). The unexpected stimulation of the T-cell-mediated immune system due to the interplay of genetic and environmental triggers begins the cascade of an inflammatory response within the pancreatic cells, in addition to a humoral (B cell) response with creation of autoantibodies (10). These specific autoantibodies are used as serological markers of autoimmune T1DM and include Islet Cell Antibodies (ICA), Islet Cell Surface Antibodies (ICSA), Glutamic Acid Decarboxylase Antibodies (GADA), Islet Antigen 2 (IA2), Insulin Autoantibodies (IAA), and Zinc Transporter Isoform 8 (ZnT8) (8,12). High levels of ICA indicate an increased risk of developing T1DM and can be identified years before the onset of the disease. Furthermore, presence of ICA at the diagnosis of T1DM is anticipated to have a rapid progression of pancreatic □-cell dysfunction (12). Research has proven that IAA and ZnT8 are frequently linked to children under the age of 10, while GAD and IA2 are related to older individuals (8).

Islet autoimmunity and the impaired function of the pancreatic □-cells is a continuum that develops in variable time depending on the patient prior to the clinical manifestations and the diagnosis of T1DM (11). If the diagnosis is set prior to puberty, it is known that in 90% of cases the islet antibodies are present by 5 years of age. Therefore when diagnosis is made in young children, the islet antibodies emerge in the early life (7). Studies have proven that the majority of children and adolescents with two or more islet antibodies will ultimately develop T1DM during the following 15 years, in contrast to only 10% of those that present with a single islet antibody. Nevertheless, antibodies can also emerge in older children and adolescents. Thus individuals that are at high-risk for developing the disease should repeatedly get screened although they don’t present with any antibodies in childhood (7).

1.1.4.1. Stages of Type 1 Diabetes Mellitus

The development of T1DM constitutes a spectrum of phases that occurs prior to the clinical manifestation of disease (11). The progression of developing long-standing T1DM follows the cascade of having a genetic susceptibility and getting exposed to environmental triggers that cause stimulation of the immune system and an immune reaction. The occurrence of two or more islet antibodies (Stage 1), evolves into having abnormal blood glucose values (Stage 2), which subsequently leads to symptomatic T1DM (Stage 3) (7). This knowledge is important as the risk of progressing into symptomatic disease can be

recognized and the rate of disease progression can be anticipated with considerable accuracy (11).

As shown in Figure 2 – type 1 diabetes is distinguished in four stages (7):

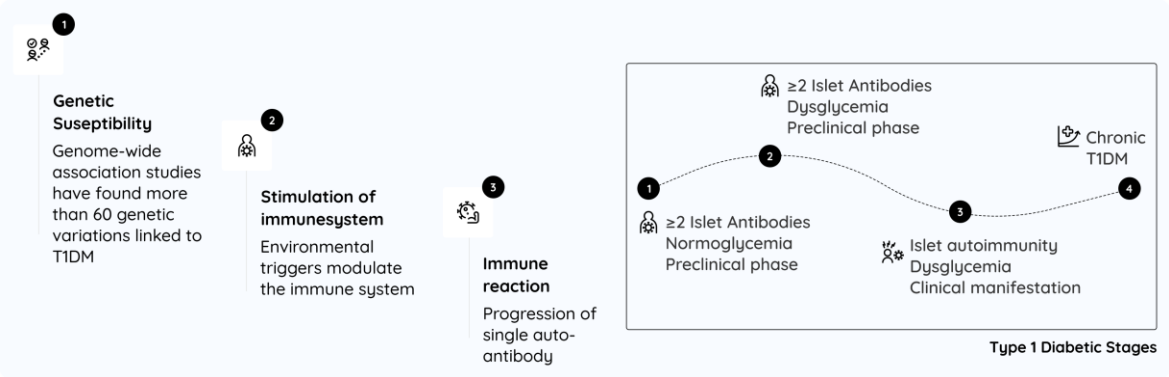


Figure 2. Developmental Stages of Type 1 Diabetes Mellitus (7)

1.1.5. Epidemiology

Due to the multifactorial pathogenesis of T1DM, there is a global variation in the incidence, prevalence and the temporal trends. 5-10% of all worldwide diagnosed diabetics are type 1 (5). From 1990 to 2008, the global incidence of type 1 diabetes has gone up from a rate of 2.8 percent to 4.0 percent per year (13). While considering the incidence of cases per year in children under the age of 15, long-term research statistics has confirmed that the populations of China, other Asian countries, and South America had the lowest occurrence of diagnosed cases ranging from 1-3/100,000 cases per year; about 10-20/100,000 in South European countries and in America; and the highest incidence of 30-60/100,000 cases in Scandinavia annually (14). Finland is the country with the highest incidence of pediatric T1DM in the world, having 52/100,000 cases per year (15). It is believed that this is attributable to the interplay of lifestyle changes, the existing environment, and the susceptible genes (15). Croatia shows regional differences in the incidence rate, being highest in the southern parts (10.91 per 100,000/year) and lowest in central Croatia (8.64 per 100,000/year) (16). Generally, it is supposed that every year, about 96,000 individuals under the age of 15 develop T1DM (8).

T1DM is the most common type of diabetes in children and adolescents, accounting for 85% or more of all diagnosed cases worldwide under the age of 20 (5). The incidence of diagnosing T1DM in adults is lower than in children (5). T1DM correspondingly affects girls and boys in young populations. Nevertheless, it has been reported that there is a slight

male predominance in areas with a high incidence of T1DM (populations of European origin), while areas with a low incidence (populations of non-European origin) have a slight female predominance (5). Additionally, race and ethnicity plays an essential role in the predisposition of diagnosing T1DM. This is proven by the fact that there is a 20-fold higher incidence rate among Caucasians from Europe, in a correspondence to the worldwide occurrence of HLA susceptibility genes. Europe accounts for 26% of the approximate 500,000 young individuals with T1DM globally, while North America and the Caribbean populations account for 22% (8).

1.1.6. Management of Type 1 Diabetes Mellitus

Managing T1DM in the pediatric population, especially in the very young children during their critical developmental years, is a very anxious and complex assignment for the child/adolescent and the parents (17). The repercussions of poor glycemic control during these vulnerable years are significant as they are prone to deleterious cognitive sequelae. Unsatisfactory glucose control may result in a long-term pattern of discouraging consequences that can be difficult to reverse (17). Diabetic management needs a multidisciplinary approach and a care team to arrange education, monitoring and support to the child, parents, school teachers, babysitters and all the people involved in the child's life (18).

1.1.6.1. Nutritional Management

Today there are available advances in treatment and technology of T1DM, nevertheless one of the most important aspects of diabetic care and education is still nutritional management (19). Despite the amount of knowledge that is already established on nutritional needs in children and adolescents, the scientific evidence basis for many elements of diabetic dietary management is still not confined, and it is critical to tailor nutritional treatments and meal plans for the specific individual (18). The nutritional guidance is based on healthy lifestyle guidelines that apply to the whole general population, with the sole difference being the requirement for insulin therapy in the diabetic child (19). Infants and children are very dependent on sufficient daily energy intake and specific nutritional needs of macro- and micronutrients for optimal growth, development and good health (18,19). A daily meal plan should include 50-55% carbohydrates, 30-35% fats, and 10-15% of proteins (19). Restrictive diets of any of the

macronutrients are not recommended as they may cause poor growth, nutrient deficiencies and increased psychosocial burden (18).

Carbohydrates are the key macronutrient that influences the postprandial glycemic reaction, however as mentioned earlier, they are not allowed to be restricted from the diet due to the possible negative impact on growth and development in the pediatric population. Carbohydrate counting (CC) is recommended and implemented in children and adolescents with T1DM as a guide for calculating the amount of insulin needed for the subsequent consumed carbohydrates, following the insulin-to-carbohydrate ratio. Furthermore, CC might be combined with calculations of fats and proteins to more precisely assess the insulin bolus (19).

Every parent of a diabetic child should be offered a specialist pediatric dietician with experience in childhood diabetes to get the best suitable nutritional plan for the child and the family. Established routines of regular meal times and everyday activities have been linked to better glycemic control (18).

1.1.6.2. Insulin therapy

The fundamental treatment of T1DM remains the external substitution of insulin to mimic the function of □-cells of the pancreas and thereby sustain the normal physiological processes as in a healthy individual (20,21). The goal is to preserve the basal levels and attain peak levels of insulin during mealtimes. However, there are differences and challenges with external substitution of insulin in comparison to the physiological functions. In a healthy individual, cells generate insulin in the portal system where the liver is the main target, thereby regulating the whole metabolism and processes through feedback systems and an increase or decrease in the insulin quantities depending on the glucose levels. Whereas the fundamental flaws of extrinsic administration of insulin is that it is implemented peripherally, and consequently there is no feedback system to regulate the insulin amount or the glucose levels (21).

Since the recognition of exogenous insulin therapy, the compositions have progressed from refined animal insulins to human insulins that are made by genetically modified organisms to insulin analogues. Subsequently, this led to a more flexible and adjustable therapeutic plan with multiple daily dosages that can result in a more stable glucose control. Rapid- and long-acting insulin analogues have been developed over the last two decades to more accurately imitate the physiological response of healthy □-cells than previous insulin compositions (21).

Although continuous subcutaneous insulin infusion (CSII) pump therapy was established in the late 1970's for treatment of T1DM, it was not generally utilized before the late 1990's in the pediatric population (22). Today, CSII pump therapy has the capacity to configure many alternative basal rates of insulin infusions in the course of different hours of the day. Furthermore, they have the capacity to give insulin boluses at meals in diverse patterns, suiting the unique lifestyles of all patients (23). CSII is providing a more flexible and individualized treatment plan for each child that has various daily routines, nutritional habits, and amount and timing of physical activity (20). Multiple studies have shown that the use of CSII pump therapy in children/adolescents with T1DM lowered their HbA1c values of 0.2-1.1%; decreased the frequencies of hypoglycemic events; while no remarkable elevation in BMI z-score was documented (24).

1.1.6.3. Glucose Monitoring

The procedures of glucose monitoring have evolved from former urine testing, to capillary blood glucose assessment, and nowadays to continuous glucose monitoring (CGM) devices (23). The opportunity to effortlessly assess blood glucose levels at any time of the day has likely been the most significant technological progress in the management of type 1 diabetes (23). An obstacle of capillary blood glucose measurement is that it can solely demonstrate the glucose levels in that certain moment. Therefore, undesirable glycemic events of high or low glucose levels, especially incidents that occur without any symptoms, as well as glucose fluctuations, might not be noticed and thereby stay untreated. This can create unfortunate diabetic emergencies or disappointing HbA1c values that may have long-term consequences (24). Nowadays, advanced CGM devices can be used to combat the limitations of single momentary capillary blood glucose levels. CGM devices quantify subcutaneous interstitial glucose levels at short-term intervals and give out the readings to the patients' scanner or smartphone. This technology generates real-time glucose trends that can be set to alarm when the glucose concentrations surpass the permitted levels (24). Furthermore, a list of core CGM metrics is now structured and well refined for use in the clinical settings. It is established by expert viewpoint from the international consensus group and constitutes recommendations for glycemic target range while utilizing the CGM devices, giving rise to three key CGM measurements – target glucose range (TIR), time below target glucose range (TBR), and time above target glucose range (TAR) (25). TIR represents glucose levels of 3.9-10.0 mmol/L (70-180 mg/dL) (25). The average time spent in different ranges is demonstrated through

percentage (%) of CGM readings throughout the day, and this percentage should be individualized for each diabetic patient. The aim behind this is to have as many CGM readings within TIR while having reduced time in TBR and TAR (25). CGM devices are exceptionally convenient and appreciated in all diabetic age-groups, however specifically for the pediatric population where the young children are not able to express symptoms of low or high blood glucose levels (24).

1.1.6.4. Psychological care

The diagnosis of T1DM is a life-changing event for both the child and the parents. While realizing and dealing with the chronic disease and its possible consequences, it is frequent that the family experiences some emotional and psychological stress (26). It is apparent that children and adolescents with diabetes have a higher rate of anxiety, depression, psychological discomfort, and eating disorders in comparison to those who don't suffer from the disease. Furthermore, those with chronic poor metabolic control, as seen with recurrent events of diabetic ketoacidosis, are more likely to have underlying psychosocial issues or psychiatric disorders (27). As mentioned earlier, management of diabetes should have a multidisciplinary approach available for all patients. This team could include pediatric endocrinologists, nurses, dieticians, as well as psychologists, social workers, and psychiatrists, depending on the clinical state of the child/adolescent. The mental health professionals should interact both with the child and the family, but also with the whole diabetic care team. This creates a synchronized and complete clinical evaluation of the child/adolescent, and thereby all possible obstacles that interfere or complicate the health of the patient can be identified and managed (26,27).

1.1.7. Diabetic Emergencies

Poor glycemic control in the diabetic patient can result in significant acute metabolic consequences. Lack of insulin can result in hyperglycemic emergencies that cause diabetic ketoacidosis (DKA), while hypoglycemia is the outcome of inappropriate insulin dosing or insufficient nutritional intake. These two emergency states are severe consequences of uncontrolled diabetes that must be identified, diagnosed, and treated as soon as possible. DKA and hypoglycemia are related to considerable morbidity and mortality, including significant healthcare costs (28).

1.1.7.1. Diabetic Ketoacidosis

Diabetic ketoacidosis (DKA) is a hyperglycemic emergency that is induced by the lack of insulin which can eventually lead to fatal consequences (29). The patients present with hyperglycemia, metabolic acidosis, and hyperketonemia with subsequent clinical manifestations including dehydration, increased heart rate, increased respiratory rate, deep and rapid breathing identified as Kussmaul breathing, acetone breath smell, nausea, vomiting, stomach pain, blurred vision, disorientation, lethargy, reduced consciousness, and subsequently loss of consciousness as in the state of coma (29,30). The laboratory diagnostic criterion for DKA is summarized in Figure 3 (30).

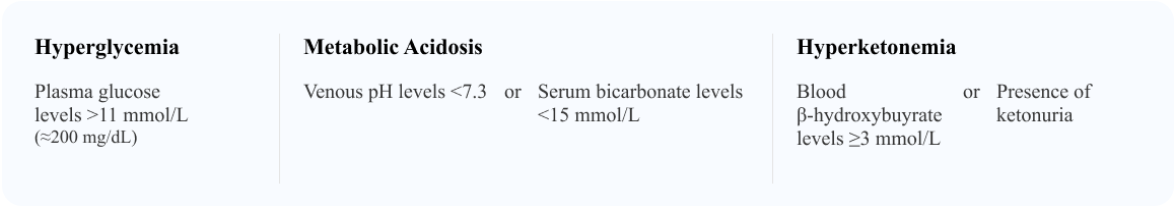


Figure 3. Diagnostic Criteria for DKA (30)

The condition is repeatedly seen in the pediatric population with T1DM, particularly among younger children and frequently accounting as the presenting manifestation of disease development (29,31). The frequency rate of DKA in newly diagnosed T1D children/adolescents ranges from 13-80%, while in children with an established diagnosis the rate is around 5-7% (32,33). The wide rate range of DKA in the newly diagnosed type 1 diabetics is due to the diverse clinical settings, different regions around the world, developmental state of a country, and the economic imbalance (29). A systematic review published in 2012 demonstrated the variation in frequency of DKA in new-onset pediatric T1DM and confirmed the six-fold variation of occurrence rate in different countries around the world (32). In that specific study, countries with the highest frequency of DKA as the presenting manifestation of T1DM in the pediatric population included the United Arab Emirates (80%), Romania, and Saudi Arabia, and with the lowest frequencies manifested in Sweden (14%), Canada, and Finland (32). An updated systematic review from 2018 analyzed and confirmed a substantial connection between the Human Development Index (HDI) and the DKA rate, showing that DKA occurs more frequently in less developed countries (31). Furthermore, the DKA frequency rate is less in countries where the incidence and awareness of T1DM is higher, as in Sweden and Finland that have the highest incidence of T1DM cases in the world (15,32).

Emergency evaluation of DKA patients follows the protocol according to the recommendations for Pediatric Advanced Life Support (PALS). Initial step involves assessment of blood glucose level, blood or urine ketones, blood gases, serum electrolytes, and complete blood count, in addition to the evaluation of dehydration and consciousness level (30). The treatment aims to reestablish the physiological state by enhancing the circulatory volume and tissue perfusion; cautiously correcting the glycemic levels and the subsequent hyperosmolality; correcting the electrolyte imbalance and resolving the ketosis, and ultimately recognizing and treating the comorbidities (34). The treatment of DKA demands frequent clinical and laboratory follow-ups and is dependent on the state of the patient. It includes fluid replacement, insulin therapy, potassium replacement therapy, bicarbonate administration (used in a state of life-threatening hyperkalemia or critical acidosis of venous blood pH <6.9), and phosphate therapy (30).

DKA is a condition that can be prevented with better outpatient treatment and follow-up programs including self-management education to the child/adolescent and the parents (28). Research has proven that patients following self-management education programs have considerably fewer occasions of DKA-events (29). DKA in a child with established T1DM and without other intercurrent illnesses such as infections is frequently linked to psychosocial problems or inadequate insulin management (30). Other circumstances linked to DKA in a child/adolescent with an already established diagnosis include surgery, alcohol abuse, or a malfunctioning insulin-pump (28). Preventative measures include education of the patients on potential adjustments of insulin management during illness or specific situations that require higher insulin dosages; educating the patients on the importance of maintaining adequate hydration in the instances of hyperglycemia due to the subsequent dehydration; enabling patients' home monitoring of blood ketone levels for an early detection of an upcoming state of ketoacidosis (28).

1.1.7.2. Hypoglycemia

Hypoglycemia is an emergency state that is a potential consequence of insulin therapy in diabetic patients. It can manifest with confusion, cephalalgia, weakness, nervousness, difficulty concentrating, drowsiness, dizziness, vision changes, slurred speech, and other autonomic and/or neuroglycopenic symptoms. There are also occurrences of asymptomatic hypoglycemia, and both cases can eventually lead to profound consequences, such as unconsciousness, development of seizures, and death (35). Approximately 1 of 3 patients with T1DM have 2-4 or more hypoglycemic events per

week, while about 5-12% experience severe hypoglycemic events that include unconsciousness and/or development of seizures requiring the assistance of other people to regain euglycemic state (35).

The general definition of hypoglycemia is plasma glucose concentration of less than 3.9 mmol/L (<70 mg/dL). Additionally, by the American Diabetes Association (ADA), it has been elaborated into three classifications of blood glucose levels, defined as "low" – 3.4-3.9 mmol/L (61-70 mg/dL); "very low" – 2.8-3.3 mmol/L (51-60 mg/dL); and "dangerously low" - <2.8 mmol/L (<50 mg/dL) (35).

The clinical state of low blood glucose levels are related to both short- and long-term consequences depending on the severity, duration, and recurrences. Recurrent severe hypoglycemic events are related to arrhythmias, cardiovascular incidence, and inferior brain development including severely affecting the cognitive ability and the brain microanatomy (28,36). On the account of all the potential side effects of hypoglycemia, it is tremendously important to identify, treat promptly, and prevent future cases of severe hypoglycemia (28).

The treatment of low glucose concentrations includes oral or intravenous application of carbohydrates, or parenteral administration of glucagon depending on the state of the child/adolescent (36). 15-20 grams of carbohydrates/glucose can be used in mild cases, while glucagon administration should be utilized in cases of severe hypoglycemia when the patient is unconscious. Constant reassessments of blood glucose levels are required until a euglycemic state of the child/adolescent has been reached (36).

1.1.8. Long-Term Diabetic Complications

DM is well-known to be associated with long-term micro- and macrovascular complications with poor glycemic control (37). An estimation of the glycemic control over the past 2-3 months is achieved through follow-up visits with evaluations of the HbA1c value. Numerous studies have demonstrated a strong relationship between the HbA1c levels and diabetic long-term complications, manifesting the significance of sustaining appropriate glucose levels and HbA1c values (37,38).

The range of recommended HbA1c values for type 1 diabetics is between 6.5% (48 mmol/mol) and 7.5% (58 mmol/mol) depending on various organizations and countries (38). A cohort study that was published in the United Kingdom (2004) demonstrated that the patients with T1DM, who attended the study over 11 years, had a total mean (standard

deviation) HbA1c value of 9.19%. 80% of the attended individuals had an average HbA1c value of >8% (64 mmol/mol), encountering solely 3.4% of patients to attain an average HbA1c value of less than 7% (53 mmol/mol) (39).

Concurrent diseases due to long-term diabetic diagnosis and/or poor glycemic control includes diabetic nephropathy, retinopathy, peripheral and/or autonomic neuropathy, and macrovascular diseases of the cardiovascular, cerebrovascular and/or peripheral vascular systems (40). The most frequent microvascular diabetic complications include visual impairment, kidney disease, and amputations with treatments that solely delay the disease advancement (41).

1.2. Autoimmune Diseases

Autoimmune diseases incorporate a heterogeneous group of more than 100 disorders that have distinctive clinical manifestations, however altogether they have similar pathogenesis of autoimmunity (42). They are defined as either organ-specific as in T1DM, or entailing numerous organs as in systemic lupus erythematosus (42). These disorders present a considerable demand of the healthcare system due to their chronic course, related long-term healthcare costs, in addition to the increasing prevalence of the population (43). Autoimmunity is characterized by the inability to differentiate self-antigens in the human body, thereby stimulating immune responses that disturb the physiological functions in a healthy individual (43).

The overall prevalence of autoimmune diseases in children and adolescents is 5%, with autoimmune thyroid diseases (AITD) being the most common autoimmune disorder (44). It is challenging to express the specific cause of why autoimmunity occurs in some individuals, as the initial stages of autoimmunity are silent and most often asymptomatic until there are clinical manifestations of disease (43). As mentioned earlier in the pathogenesis of T1DM, autoimmune diseases occur due to the interplay of genetics and environmental components (7). The interaction of these factors consequently leads to an inappropriate immune regulation or decreased threshold for the stimulation of lymphocytes, subsequently proceeding to immune activation that reacts to self-antigens in various organ-tissues (43). There are numerous genes involved in having a genetic susceptibility for development of autoimmunity, however the HLA genes are considered to have the strongest susceptibility risk. Nevertheless, it is still not confirmed in what way the HLA genes work to cause autoimmunity (43).

Autoimmunity has a prolonged process of disease development and progression. Regardless of this knowledge, current treatments rather solely treat the end-stages of inflammation and do not address the underlying issues of autoimmunity (43). Nevertheless, research has led to advances in diagnosis and classification of the disorders, moreover improved and innovative treatment options that generate preferable prognosis (42).

It has been widely accepted that autoimmune diseases have a co-occurrence within patients and families (45). Furthermore, studies have provided insight to the fact that the additional autoimmunity has increased and that they provide a consequential comorbidity in pediatric new-onset T1DM (44). It is specifically proven that type 1 diabetics are susceptible to establishing other autoimmune diseases such as AITD (Hashimoto thyroiditis and Graves' diseases, 15-30%), celiac disease (4-9%), autoimmune gastritis (5-10%), and Addison disease (0.5%), among others (44).

1.2.1. Celiac Disease

Celiac disease (CD) is a multisystem, autoimmune, lifelong illness characterized by patchy-villous atrophy in the lining of the small intestine, developing as a result of hypersensitivity to gluten in genetically susceptible individuals (46). CD is particularly recognized due to its increasing prevalence, the extended age range of disease onset, and the wide range of clinical manifestations (47).

Clinical manifestations of CD can be asymptomatic or highly symptomatic (48). Symptomatic CD can manifest with chronic gastrointestinal symptoms such as diarrhea with or without malabsorption; loss of weight; metabolic bone disease; anemia; and general weakness (46). Most individuals with silent disease have hidden symptoms such as decreased bone density; asymptomatic iron or folate deficits; and/or other related autoimmune illnesses that are frequently more clinically significant than the celiac disease itself (46).

As with other autoimmune diseases, CD is strongly related to genetic and environmental components of susceptibility (47). The pathogenesis incorporates the genetic factors – HLA-DQ2 and HLA-DQ8, and the key environmental component that is gluten (46). Gluten is the expression for the group of related prolamins that include complex proteins found in wheat, rye, and barley (47). Research has proven this strong linkage between the genetic factors and development of disease by the fact that more than 90% of individuals with CD are demonstrated to be DQ2 positive, while the majority of the remainder ones are DQ8 positive (47). Either of the two mentioned haplotypes are

required, however they are not enough for the disease progression (46). Nevertheless, research has noted 39 non-HLA genes that are part of the susceptibility of CD development (47). As mentioned earlier, environmental triggers are found to induce the cascade of the inflammatory activation. Some drugs like interferon alpha could trigger the disease in susceptible individuals (48). Other potential environmental factors could include intestinal infections, especially rotaviruses in children or campylobacter infection in adults (47).

Tissue transglutaminase (tTG) is an enzyme released in the course of inflammation and has two important parts in CD – as the selected principal autoantigen for antiendomysial antibodies and as the enzyme that increases the stimulation of the immune reaction to gluten (48). TTG expression and activity are elevated in the mucosa of CD patients and the assessment of IgA-tTG antibodies concentration is used as the first-line screening test due to its high sensitivity and negative predictive value, in addition to that it is a cheaper test than assessment of endomysial antibodies (EMA) (47,48). However, patients that are IgA-deficient don't develop IgA-tTG or IgA-EMA antibodies, thus a false negative screening result is possible. Therefore, IgG-tTG, IgG-EMA and IgG-deamidated gliadin peptide (DGP-IgG) can be evaluated when testing for CD in IgA-deficient individuals (47). A meta-analysis published in 2012 found that DGP-IgG have a pooled sensitivity of 80.1-98.6% (47).

Currently, the disease affects approximately 1% of the world population, and in Europe the highest prevalence is documented in Sweden and Finland (47). CD is more frequently occurring in individuals that have a first-degree relative with the same disease; in individuals with T1DM; in patients with Down's syndrome; or in people with chronic liver disease – specifically primary biliary cirrhosis (46). In the pediatric population with T1DM the prevalence of CD is ranging between 1-16% (49). Other autoimmune disorders occur 10 times more frequently in individuals with CD than in the general population, including T1DM, thyroid disorders, Addison's disease, autoimmune liver disease, among others (46). In a patient that has both an autoimmune illness and CD, the CD has most often a silent manifestation and the other autoimmune disease is generally diagnosed first (46). It is believed that a strict gluten-free diet could improve the management of some related autoimmune diseases (48). Potential reason for the correlations between the developments of these diseases is the similar pathogenesis of genetic predisposition (HLA alleles) and the shared immunological mechanism (46). The involvement of CD as a contributive element for development of other autoimmune diseases is supported by multiple studies. Some studies suggest that the age of diagnosis of CD is associated with

the prevalence of autoimmune disorders, being related to the duration of gluten exposure (50). Furthermore, compared to individuals on a gluten-free diet, patients with CD have an increased prevalence of organ-specific autoantibodies upon diagnosis (51). Eventually, studies have proven that children/adolescents with CD that uphold a gluten-free diet can withdraw diabetes- and thyroid-associated serum antibodies (52).

The co-occurrence of T1DM with CD was already assumed in 1954. Since then, it has become generally accepted that the prevalence of CD is higher in type 1 diabetics. Recent studies have confirmed that the prevalence of CD in T1DM patients ranges from 4.4-12.4%, in contrast to the 1% prevalence of CD in the general population (53). Once again, this is believed to occur due to the common genetic foundation, and therefore many hospitals are now screening their diabetic patients for CD, as it often has a silent manifestation (46,53).

1.2.2. Autoimmune thyroiditis

The most prevalent coexisting autoimmune illness in children and adolescents with T1DM is autoimmune thyroiditis (AIT) (49). It is 2-4 times more common in T1DM patients than in the general population, presenting most frequently as Hashimoto's thyroiditis (HT) and less frequently as Graves' disease (GD) (49). Overall thyroid antibody positivity is recognized in about 2.9-4.6% of the general pediatric population (49). While in the pediatric population with T1DM, the prevalence of positive thyroid antibodies are ranging between 14-25% of individuals, showing the substantial difference in frequency between healthy individuals and children/adolescents with T1DM (54). In a large cohort study of 25,759 individuals with T1DM, AITD manifested in 20% of the participants, where 19% had HT, and only 1% had GD (55).

As with previously mentioned autoimmune disorders, the etiology of AITD is multifactorial and is dependent on the interplay of genetic, environmental and constitutional elements (56). In individuals with T1DM the appearance of the HLA alleles DQA1*0301, DQB1*0301, and DQB1*0201 are associated with development of Graves' disease, while the appearance of HLA DQA1*0501 is linked with Hashimoto thyroiditis (49). Environmental triggers include high iodine intake, possibly stress, and infections like congenital rubella being specifically linked to HT (56). Furthermore, a strong correlation has been confirmed between thyroid antibody positivity and the female gender. In contrast to males, female adolescents with T1DM have three times higher risk to establish positive thyroid autoantibodies (49). Research has proven that sex hormones like estrogen and

androgens can influence and alternate the immune system. Overall, androgens appear to suppress the immunological activity, whereas estrogen appears to have a stronger effect on immune cells and promote immune activity (57).

AITD is identified by the formation of autoantibodies against the thyroid gland, lymphocytic infiltration, and consequently the progression of various stages of thyroid malfunction (49). Antibodies are formed against thyroglobulin (anti-TG) and against thyroid peroxidase (anti-TPO) that causes functional damage of the thyroid gland. These thyroid gland proteins are responsible and have a crucial part in iodination and development of the thyroid hormones – triiodothyronine (T3) and thyroxine (T4) (49). Anti-TG and anti-TPO antibodies are present only in 17-25% of type 1 diabetics at the time of diagnosis, while the majority manifest with the antibodies 2.5-3 years after the T1DM diagnosis (49). This knowledge supports the current guidelines issued by the ADA that recommend screening the pediatric patients with T1DM for anti-TPO, anti-TG, and thyroid function tests (TFT) shortly after the diagnosis (54). While the International Society for Pediatric and Adolescent Diabetes (ISPAD) guidelines recommend screening new-onset type 1 diabetic patients of thyroid stimulating hormone (TSH) and anti-TPO, and subsequently screening every second year in symptomless children/adolescents without goiter or in the absence of thyroid autoantibodies (54).

Subsequently following the abnormal thyroid function either hyperthyroidism as in GD develops or hypothyroidism as in HT (54). Hyperthyroidism presents with signs and symptoms of increased metabolism, such as increased heart rate, weight loss, fatigue, heat intolerance, diaphoresis, increase stool frequency, nervousness, restlessness, and tremor while hypothyroidism manifest with symptoms as bradycardia, weight gain, fatigue, lethargy, intolerance to cold, loss of hair, constipation, growth retardation, and dry coarse skin (56). Apparent clinical signs of thyroid diseases are uncommon in T1DM patients since TSH and antibodies are routinely tested (49).

Clinical manifestations and supporting laboratory tests are used to diagnose AITD. Depending on disease state and type, the patients may present as euthyroid, hypothyroid or hyperthyroid (56). As mentioned earlier, the simplest way to establish the existence and etiology is to measure circulating autoantibodies. Since 98% of patients are positive for either anti-TPO or anti-TG antibodies in the case of AITD, a negative test for both antibodies essentially rules out the diagnosis (56). In the diagnosis of autoimmune hypothyroidism, anti-TPO is more specific and sensitive than anti-TG. The golden standard for diagnosing HT is an elevated TSH along with anti-TPO antibodies (56).

Treatment for patients with autoimmune hypothyroidism includes T4 supplementation. Thyroid autoantibodies can stay elevated or drop with T4 therapy, and goiter size in HT decreases approximately a third over a two-year period (56). Treatment for Graves' disease with anti-thyroid medications (carbimazole, methimazole, propylthiouracil) causes a decrease in thyroid stimulating antibodies and other thyroid antibodies. The severity of thyroiditis as well as other immunologic alterations is also decreasing (56). Other useful medications include long-acting beta-blockers (propranolol or atenolol). Furthermore, thyroid cells can be gradually destroyed by radioiodine, which can be used as initial therapy or to cure relapses following a trial of anti-thyroid medications (56).

2. OBJECTIVES

The main aims of this study are:

1. Determine levels of antibodies against thyroglobulin (anti-TG) and thyroid peroxidase (anti-TPO) in newly diagnosed T1DM children and adolescents.
2. Determine levels of tTG-IgA and DGP-IgG in newly diagnosed T1DM children and adolescents.

Hypothesis of this study are:

1. Increased prevalence of antibodies against thyroglobulin (anti-TG) and thyroid peroxidase (anti-TPO) in newly diagnosed children and adolescents with T1DM.
2. Increased prevalence of tTG-IgA and DGP-IgG in newly diagnosed children and adolescents with T1DM.

3. MATERIALS AND METHODS

3.1. Subjects

In this retrospective cross-sectional study 110 newly diagnosed T1DM patients were enrolled, which were admitted to the Division of Pediatric Endocrinology at the University Hospital of Split in the period from January 2016 to December 2019. This study was approved by the Ethical Committee of University Hospital of Split and was performed in accordance with the Declaration of Helsinki.

3.2. Clinical and biochemical characteristics of study participants

A detailed clinical examination and medical history were taken from each subject, and we were particularly focused on the early signs of T1DM pediatric population: polyuria, nocturia, polydipsia, polyphagia, and weight loss (8). Body height and weight were measured using a stadiometer and an electronic scale (both from Seca, Hamburg, Germany), and BMI was determined by multiplying the patient's body weight in kilograms by the square of his or her body height in meters. The concentrations of insulin, c-peptide, anti-Tg, anti-TPO, TSH, T4 and T3 were determined using an electrochemiluminescence immunoassay (COBAS e601, Roche Diagnostics GmbH, Mannheim, Germany), and the concentrations of plasma hemoglobin A1c were determined using high-performance liquid chromatography (HumaNex A1c, HUMAN, Wiesbaden, Germany). Additionally, tTG-IgA and DGP-IgG were measured using a chemiluminescence immunoassay (IDS-iSYS, IDS, Tyne & Wear, UK). The concentrations of glucose were measured using routine laboratory methods (ARCHITECT ci16200; Abbott, Chicago, Illinois). Furthermore, levels of pH and bicarbonates were determined using potentiometer (ABL800 FLEX; Brønshøj, Denmark), while concentrations of ß-hydroxybutyrate were measured on point of care devices (FreeStyle Optium Neo; Chicago, Illinois).

3.3. Definitions

The ISPAD definition of diabetic ketoacidosis incorporate the biochemical criteria for diagnosis that include hyperglycemia of >11 mmol/L (200 mg/dL); venous pH levels <7.3 or serum bicarbonate <15 mmol/L; and an abnormal increase of ketones in blood or urine (30). Ketonemia represents a level of blood ß-hydroxybutyrate concentration ≥3 mmol/L, while urine ketones are typically ≥2+ „moderate or large“ positive (30).

Autoantibodies to □-cell antigens, such as ICA, IAA, and GADA occur before clinical appearance of diabetes develops, and are therefore widely employed as preclinical

disease indicators (58). Elevated levels of islet autoantibodies verify the diagnosis of T1DM and the requirement for insulin therapy (7).

The definition of positive antibodies for celiac disease in paediatric patients includes tTG-IgA with levels ≥10 U/mL and/or DPG-IgG ≥10 U/mL (59). Furthermore, positive antibodies for autoimmune thyroiditis were defined as anti-TG ≥38 U/mL and/or anti-TPO ≥13 U/mL.

3.4. Statistical analysis

Prism 9 for Mac OS x (version 9.1.0.; GraphPad, La Jolla, CA, USA) was used for statistical analysis. Data were tested for normal distributions using the Kolmogorov-Smirnov test. Continuous variables are presented as the mean value ± SD, whereas categorical variables are reported as whole numbers (N) and percentages (%). Comparison of parameters was done by Student t-test and Chi-squared. Statistical significance was defined as $P < 0.05$.

4. RESULTS

The study group consisted of 110 subjects, where 65 subjects were male (59.1%) and 45 subjects were female (40.9%). Average age of the participants was 9.39 ± 4.48 years, while other anthropometric characteristics are presented in Table 1.

Table 1. Baseline anthropometric characteristics of the subjects enrolled in the study

Parameter	All subjects (N=110)
Sex – N (%)	
Male	65 (59.1)
Female	45 (40.9)
Age (yr)	9.39 ± 4.48
Height (cm)	140 ± 28.03
Weight (kg)	35.86 ± 18.3
BMI (kg/m^2)	16.86 ± 3.09
BMI z score	- 0.29 ± 1.62

BMI, body mass index.

Additionally, 35 (31.8 %) subjects were presented with signs of ketoacidosis, with average glucose levels 27 ± 12.63 mmol/l. Furthermore, the average HbA1c value of the participants was 11.39 ± 2.35, while other biochemical characteristics are presented in Table 2.

Table 2. Biochemical characteristics of the subjects enrolled in the study

Parameter	All subjects (N=110)
Glucose (mmol/l)	27 ± 12.63
Hemoglobin A1c (%)	11.39 ± 2.35
Insulin (mIU/l)	5.37 ± 5.75
C-peptide (nmol/l)	0.32 ± 0.27
pH	7.33 ± 0.13
Bicarbonate (mmol/l)	17.4 ± 7.31
ß-hydroxybutyrate (mmol/l)	3.49 ±2.65
Ketoacidosis	35 (31.8)

The most frequent first symptom of T1DM in our pediatric population was polyuria (92.7 %), while the least common first symptom was polyphagia that was present in only 25 subjects (22.7 %), as seen in Table 3.

Table 3. Clinical characteristics of the subjects enrolled in the study

Parameter	All subjects (N=110)
Polyuria	102 (92.7)
Nocturia	94 (85.5)
Polydipsia	95 (86.4)
Polyphagia	25 (22.7)
Weight loss	65 (59)

The cumulative prevalence of positive antibodies for celiac disease was 10.9 % in newly diagnosed patients with T1DM. The additional distribution by year is presented in Figure 4.

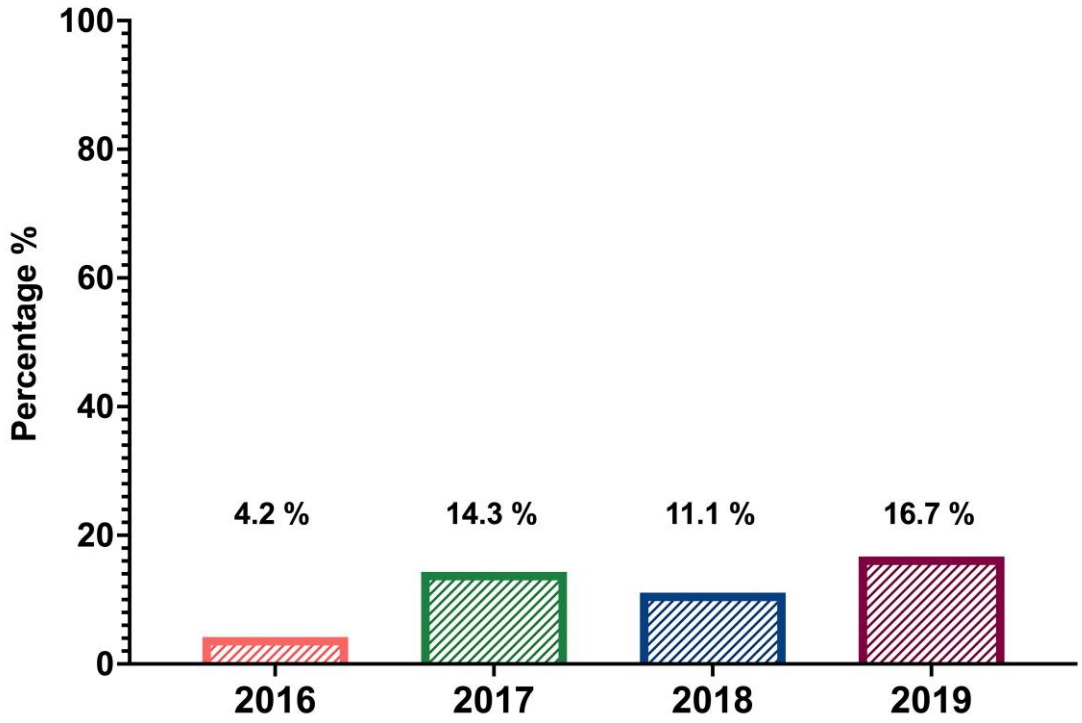


Figure 4. Prevalence of antibodies for celiac disease in subjects enrolled in the study

The cumulative prevalence of positive antibodies for autoimmune thyroiditis was 11.8 % in newly diagnosed patients with T1DM. The additional distribution by year is presented in Figure 5.

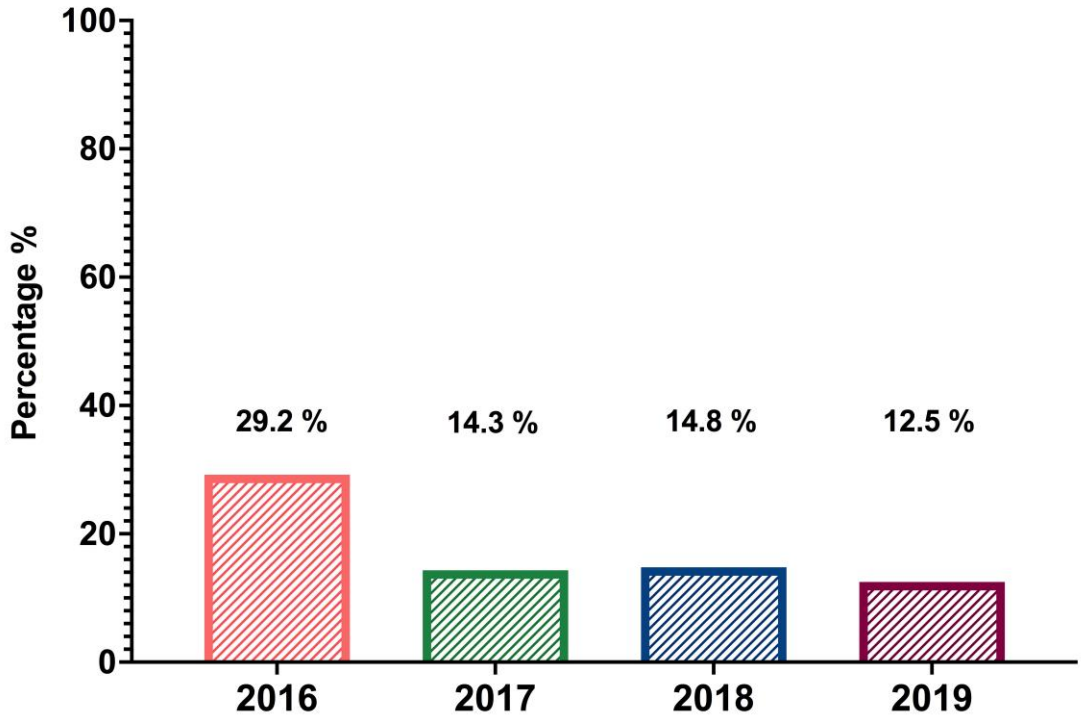


Figure 5. Prevalence of antibodies for autoimmune thyroiditis in subjects enrolled in study

Subjects with additional autoimmune disease are statistically significantly older compared with subjects without additional autoimmune disease (11.3 ± 4.03 vs. 8.64 ± 4.44 yr, P=0.004). Table 4 compares additional biochemical markers between subjects with additional autoimmune disease and subjects without additional autoimmune disease.

Table 4. Anthropometric and biochemical characteristics after stratification in subgroups with and without additional autoimmune diseases

Parameter	**With additional autoimmune disease (N=31)**	**Without additional autoimmune disease (N=79)**	**P***
Age (yr)	11.3 ± 4.03	8.64 ± 4.44	0.004
BMI z score	-0.32 ± 1.13	-0.27 ± 1.78	0.881
Glucose (mmol/L)	27.79 ± 12.58	26.69 ± 12.72	0.685
Hemoglobin A1c (%)	11.98 ± 2.44	11.16 ± 2.28	0.098
pH	7.33 ± 0.12	7.34 ± 0.13	0.797
Bicarbonate (mmol/L)	17.27 ± 7.55	17.45 ± 7.26	0.909
ß-hydroxybutyrate (mmol/L)	0.24 ± 0.17	0.38 ± 0.35	0.016
TSH (mIU/L)	3.18 ± 3.84	2.21 ± 1.42	0.056
T4 (nmol/L)	99.6 ± 27.86	102.9 ± 74.69	0.812
T3 (nmol/L)	1.19 ± 0.45	1.31 ± 0.61	0.346
Anti-TG (U/ml)	43.05 ± 134.2	0.87 ± 2.36	0.006
Anti-TPO (U/ml)	176.9 ± 318.2	1.84 ± 2.11	<0.001
tTG IgA (U/ml)	44.81 ± 72.8	2.02 ± 1.48	<0.001
DGP IgG (U/ml)	24.02 ± 45.24	1.19 ± 2.18	<0.001

BMI, body mass index; TSH, thyroid stimulating hormone; T4, thyroxine; T3, triiodothyronine; Anti-TG, anti-thyroglobulin; Anti-TPO, anti-thyroid peroxidase; tTG, tissue transglutaminase; DGP, deamidated gliadin peptide.

*Groups comparison were performed using the t test

5. DISCUSSION

In this retrospective cross-sectional study we determined the levels of antibodies that are specific for celiac disease and autoimmune thyroiditis in 110 newly diagnosed T1DM children and adolescents that were admitted to the University Hospital of Split from 2016 to 2019. The results from this cross-sectional study indicated that the cumulative prevalence of positive tTG-IgA and/or DGP-IgG antibodies specific for celiac disease was 10.9% in newly diagnosed patients with T1DM. Furthermore, the study identified that 11.8% of newly diagnosed patients with T1DM had positive anti-TG and/or anti-TPO antibodies specific for autoimmune thyroiditis. In comparison to a single-center-study from Poland that was published in *Frontiers in Endocrinology* in 2020, they identified 13.39 % of their participants to have thyroid autoimmunity, which is similar to our results of 11.8 % of newly diagnosed T1DM children/adolescents (44). In the same study, they recognized that 7.7% of newly diagnosed type 1 diabetics have celiac autoimmunity, which is slightly less than 10.9% that our results indicated (44). However, the conclusion of these results and comparisons is that general specific autoimmunity, other than diabetes antibodies, among new-onset T1D patients is proven to be a common phenomenon, which may have a crucial part for predicting the possible clinical manifestation of autoimmune diseases like AITD and CD upon the diagnosis of T1DM. Furthermore, these results show the importance of screening for other autoimmune diseases in newly diagnosed T1D patients.

In our study, 79 subjects (71.8%) were without an additional autoimmune disease at the time of diagnosis of T1DM, while 31 subjects (28.2%) were with an additional autoimmune disease at the time of diagnosis of T1DM. Comparing this to the study that was published in *Frontiers in Endocrinology* in 2020, the general specific autoimmunity (other than diabetes antibodies) at T1DM diagnosis remained steady during the study period from 25% in 2010 up to 23.6% in 2018, which is similar to our study results that had the prevalence of 28.2% of children/adolescents with an additional autoimmune disease upon the diagnosis of T1DM (44). Furthermore, another study published in *Diabetes Care* in 2011 showed that 32.6% of patients with T1DM were positive for at least one additional organ-specific autoantibody in newly diagnosed T1DM patients, again matching with our study results (60). This is relevant and important knowledge for the clinical management of type 1 diabetic patients as additional autoimmunity constitutes a consequential comorbidity, moreover it may provide insight into the aetiology of these autoimmune diseases.

Another significant recognition in our study was that subjects with an additional autoimmune disease were statistically significantly older compared with subjects without

additional autoimmune disease. This recognition is supported by an observational study published in *World Journal of Diabetes* in 2020 where their results demonstrated that compared to patients with isolated T1DM; those with T1DM and an additional autoimmune disease were older in age (61).

Furthermore, our study concluded that the most common primary symptom of T1DM in our paediatric population were polyuria (92.7%), followed by polydipsia (86.5%) and nocturia (85.5%). Weight loss occurred in 65 patients (59%), while polyphagia presented in only 25 individuals (22.7%), accounting to being the rarest first symptom in T1D paediatric patients. Additionally, 35 children (31.8%) presented with ketoacidosis at the time of diagnosing T1DM. This is in high accordance with a study published in *Pediatrics* in 2014 that had stable prevalence of DKA in newly diagnosed T1D patients (30.2% in 2002-2003, 29.1% in 2004-2005, and 31.1% in 2008-2010) (62).

There are some limitations to this study. First, the cross-sectional methodology used in this study excludes longitudinal follow-up of our subjects, which could possibly affect the antibody levels. Secondly, the patients in this study were from a single-center Division of Paediatric Endocrinology, Department of Paediatrics, University Hospital of Split, which can influence the generalizability of the results.

6. CONCLUSION

This study has confirmed that new-onset T1DM in children and adolescents can present with non-islet, organ-specific autoantibodies. These results emphasize the importance of screening for CD and AITD in newly diagnosed T1DM individuals. Furthermore, this study suggested that individuals with T1DM and an additional autoimmune disease are generally older than those with isolated T1DM.

7. REFERENCES

1. Alberti KGMM, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: Diagnosis and classification of diabetes mellitus. Provisional report of a WHO consultation. Diabet Med. 1998;15:539–53.

2. American Diabetes Association. Standards of medical care in diabetes-2014. Diabetes Care. 2014;37:S14–80.

3. American Diabetes Association. Diagnosis and Classification of Diabetes Mellitus. Diabetes Care. 2014;37:S81–90.

4. American Diabetes Association. 2. Classification and diagnosis of diabetes. Diabetes Care. 2015;38:S8–16.

5. Maahs DM, West NA, Lawrence JM, Mayer-Davis EJ. Epidemiology of type 1 diabetes. Endocrinol Metab Clin North Am. 2010;39:481–97.

6. Tuomi T, Santoro N, Caprio S, Cai M, Weng J, Groop L. The many faces of diabetes: A disease with increasing heterogeneity. Lancet. 2014;383:1084–94.

7. Couper JJ, Haller MJ, Greenbaum CJ, Ziegler AG, Wherrett DK, Knip M, et al. ISPAD Clinical Practice Consensus Guidelines 2018: Stages of type 1 diabetes in children and adolescents. Pediatr Diabetes. 2018;19:20–7.

8. Mayer-Davis EJ, Kahkoska AR, Jefferies C, Dabelea D, Balde N, Gong CX, et al. ISPAD Clinical Practice Consensus Guidelines 2018: Definition, epidemiology, and classification of diabetes in children and adolescents. Pediatr Diabetes. 2018;19:7–19.

9. Dimeglio LA, Evans-Molina C, Oram RA. Type 1 diabetes. Lancet. 2018;391:2449–62.

10. Daneman D. Type 1 diabetes. Lancet. 2006;367:847–58.

11. Insel RA, Dunne JL, Atkinson MA, Chiang JL, Dabelea D, Gottlieb PA, et al. Staging presymptomatic type 1 diabetes: A scientific statement of jdrf, the endocrine society, and the American diabetes association. Diabetes Care. 2015;38:1964–74.

12. Samuelsson U, Ludvigsson J, Sundkvist G. Islet cell antibodies (ICA), insulin autoantibodies (IAA), islet cell surface antibodies (ICSA) and C-peptide in 1031

school children in a population with a high background incidence of IDDM. Diabetes Res Clin Pract. 1994;26:155–62.

13. Dabelea D, Mayer-Davis EJ, Saydah S, Imperatore G, Linder B, Divers J, et al. Prevalence of Type 1 and Type 2 Diabetes Among Children and Adolescents. JAMA. 2014;311:1778–86.

14. Norris JM, Johnson RK, Stene LC. Type 1 diabetes—early life origins and changing epidemiology. Lancet Diabetes Endocrinol. 2020;8:226–38.

15. Knip M. Type 1 diabetes in Finland: past, present, and future. Lancet Diabetes Endocrinol. 2021;9:259–60.

16. Stipančić G, Sabolić LLG, Šepec MP, Radica A, Skrabić V, Severinski S, et al. Regional differences in incidence and clinical presentation of type 1 diabetes in children aged under 15 years in Croatia. Croat Med J. 2012;53:141–8.

17. Pierce JS, Kozikowski C, Lee JM, Wysocki T. Type 1 diabetes in very young children: a model of parent and child influences on management and outcomes. Pediatr Diabetes. 2015;18:17–25.

18. Smart CE, Annan F, Higgins LA, Jelleryd E, Lopez M, Acerini CL. ISPAD Clinical Practice Consensus Guidelines 2018: Nutritional management in children and adolescents with diabetes. Pediatr Diabetes. 2018;19:136–54.

19. Tascini G, Berioli MG, Cerquiglini L, Santi E, Mancini G, Rogari F, et al. Carbohydrate counting in children and adolescents with type 1 diabetes. Nutrients. 2018;10:109.

20. Danne T, Phillip M, Buckingham BA, Jarosz-Chobot P, Saboo B, Urakami T, et al. ISPAD Clinical Practice Consensus Guidelines 2018: Insulin treatment in children and adolescents with diabetes. Pediatr Diabetes. 2018;19:115–35.

21. Mathieu C, Gillard P, Benhalima K. Insulin analogues in type 1 diabetes mellitus: Getting better all the time. Nat Rev Endocrinol. 2017;13:385–99.

22. Sherr J, Tamborlane W V. Past, present, and future of insulin pump therapy: Better shot at diabetes control. Mt Sinai J Med. 2008;75:352–61.

23. Beck RW, Bergenstal RM, Laffel LM, Pickup JC. Advances in technology for management of type 1 diabetes. Lancet. 2019;394:1265–73.

24. Sherr JL, Tauschmann M, Battelino T, de Bock M, Forlenza G, Roman R, et al. ISPAD Clinical Practice Consensus Guidelines 2018: Diabetes technologies. Pediatr Diabetes. 2018;19:302–25.

25. Battelino T, Danne T, Bergenstal RM, Amiel SA, Beck R, Biester T, et al. Clinical Targets for Continuous Glucose Monitoring Data Interpretation: Recommendations From the International Consensus on Time in Range. Diabetes Care. 2019;42:1593–603.

26. Frank MR. Psychological issues in the care of children and adolescents with type 1 diabetes. Paediatr Child Health. 2005;10:18–20.

27. Delamater AM, de Wit M, McDarby V, Malik JA, Hilliard ME, Northam E, et al. ISPAD Clinical Practice Consensus Guidelines 2018: Psychological care of children and adolescents with type 1 diabetes. Pediatr Diabetes. 2018;19:237–49.

28. Umpierrez G, Korytkowski M. Diabetic emergencies-ketoacidosis, hyperglycaemic hyperosmolar state and hypoglycaemia. Nat Rev Endocrinol. 2016;12:222–32.

29. Ehrmann D, Kulzer B, Roos T, Haak T, Al-Khatib M, Hermanns N. Risk factors and prevention strategies for diabetic ketoacidosis in people with established type 1 diabetes. Lancet Diabetes Endocrinol. 2020;8:436–46.

30. Wolfsdorf JI, Glaser N, Agus M, Fritsch M, Hanas R, Rewers A, et al. ISPAD Clinical Practice Consensus Guidelines 2018: Diabetic ketoacidosis and the hyperglycemic hyperosmolar state. Pediatr Diabetes. 2018;19:155–77.

31. Große J, Hornstein H, Manuwald U, Kugler J, Glauche I, Rothe U. Incidence of Diabetic Ketoacidosis of New-Onset Type 1 Diabetes in Children and Adolescents in Different Countries Correlates with Human Development Index (HDI): An Updated Systematic Review, Meta-Analysis, and Meta-Regression. Horm Metab Res. 2018;50:209–22.

32. Usher-Smith JA, Thompson M, Ercole A, Walter FM. Variation between countries in the frequency of diabetic ketoacidosis at first presentation of type 1 diabetes in

children: A systematic review. Diabetologia. 2012;55:2878–94.

33. Maahs DM, Hermann JM, Holman N, Foster NC, Kapellen TM, Allgrove J, et al. Rates of diabetic ketoacidosis: International comparison with 49,859 pediatric patients with type 1 diabetes from England, Wales, the U.S., Austria, and Germany. Diabetes Care. 2015;38:1876–82.

34. Nyenwe EA, Kitabchi AE. The evolution of diabetic ketoacidosis: An update of its etiology, pathogenesis and management. Metabolism. 2016;65:507–21.

35. Driscoll KA, Raymond J, Naranjo D, Patton SR. Fear of Hypoglycemia in Children and Adolescents and Their Parents with Type 1 Diabetes. Curr Diab Rep. 2016;16:77.

36. McGill DE, Levitsky LL. Management of Hypoglycemia in Children and Adolescents with Type 1 Diabetes Mellitus. Curr Diab Rep. 2016;16:88.

37. Nordwall M, Abrahamsson M, Dhir M, Fredrikson M, Ludvigsson J, Arnqvist HJ. Impact of HbA1c, followed from onset of type 1 diabetes, on the development of severe retinopathy and nephropathy: The viss study (vascular diabetic complications in Southeast Sweden). Diabetes Care. 2015;38:308–15.

38. Lind M, Pivodic A, Svensson AM, Ólafsdóttir AF, Wedel H, Ludvigsson J. HbA 1c level as a risk factor for retinopathy and nephropathy in children and adults with type 1 diabetes: Swedish population based cohort study. BMJ. 2019;366:4894.

39. Saunders SA, Wallymahmed M, MacFarlane IA. Glycaemic control in a type 1 diabetes clinic for younger adults. QJM. 2004;97:575–80.

40. Donaghue KC, Marcovecchio ML, Wadwa RP, Chew EY, Wong TY, Calliari LE, et al. ISPAD Clinical Practice Consensus Guidelines 2018: Microvascular and macrovascular complications in children and adolescents. Pediatr Diabetes. 2018;19:262–74.

41. Forbes JM, Cooper ME. Mechanisms of diabetic complications. Physiol Rev. 2013;93:137–88.

42. Wang L, Wang FS, Gershwin ME. Human autoimmune diseases: A comprehensive update. JIM. 2015;278:369–95.

43. Rosenblum MD, Remedios KA, Abbas AK. Mechanisms of human autoimmunity. J Clin Invest. 2015;125:2228–33.

44. Głowińska-Olszewska B, Szabłowski M, Panas P, Żołądek K, Jamiołkowska-Sztabkowska M, Milewska AJ, et al. Increasing Co-occurrence of Additional Autoimmune Disorders at Diabetes Type 1 Onset Among Children and Adolescents Diagnosed in Years 2010–2018—Single-Center Study. Front Endocrinol (Lausanne). 2020;11:476.

45. Somers EC, Thomas SL, Smeeth L, Hall AJ. Autoimmune diseases co-occurring within individuals and within families: A systematic review. Epidemiology. 2006;17:202–17.

46. Green PH, Jabri B. Coeliac disease. Lancet. 2003;362:383–91.

47. Lebwohl B, Sanders DS, Green PHR. Coeliac disease. Lancet. 2018;391:70–81.

48. Di Sabatino A, Corazza GR. Coeliac disease. Lancet. 2009;373:1480–93.

49. Kakleas K, Soldatou A, Karachaliou F, Karavanaki K. Associated autoimmune diseases in children and adolescents with type 1 diabetes mellitus (T1DM). Autoimmun Rev. 2015;14:781–97.

50. Ventura A, Magazzu G, Greco L. Duration of exposure to gluten and risk for autoimmune disorders in patients with celiac disease. Gastroenterology. 1999;117:297–303.

51. Toscano V, Conti FG, Anastasi E, Mariani P, Tiberti C, Poggi M, et al. Importance of Gluten in The Induction of Endocrine Autoantibodies and Organ Dysfunction in Adolescent Celiac Patients. Am J Gastroenterol. 2000;95:1742–8.

52. Ventura A, Neri E, Ughi C, Leopaldi A, Città A, Not T. Gluten-dependent diabetes-related and thyroid-related autoantibodies in patients with celiac disease. J Pediatr. 2000;137:263–5.

53. Pall H, Newhook L, Aaron H, Curtis J, Randell E. Young Age at Diagnosis of Type 1 Diabetes Is Associated with the Development of Celiac Disease—Associated Antibodies in Children Living in Newfoundland and Labrador, Canada. Children. 2015;2:403–11.

54. Kochummen E, Marwa A, Umpaichitra V, Perez-Colon S, Chin VL. Screening for autoimmune thyroiditis and celiac disease in minority children with type 1 diabetes. J Pediatr Endocrinol Metab. 2018;31:879–85.

55. Hughes JW, Riddlesworth TD, DiMeglio LA, Miller KM, Rickels MR, McGill JB. Autoimmune diseases in children and adults with type 1 diabetes from the T1D exchange clinic registry. J Clin Endocrinol Metab. 2016;101:4931–7.

56. Swain M, Swain T, Mohanty BK. Autoimmune thyroid disorders—An update. Indian J Clin Biochem. 2005;20:9–17.

57. Tanriverdi F, Silveira G, Maccoll GS, Bouloux PMG. The hypothalamic-pituitary-gonadal axis: immune function and autoimmunity [Internet]. J Endocrinol. 2003;176:293-304.

58. Korhonen S, Knip MM, Kulmala P, Savola K, Åkerblom HK, Knip M. Autoantibodies to GAD, IA-2 and insulin in ICA-positive first-degree relatives of children with type 1 diabetes: a comparison between parents and siblings. Diabetes Metab Res Rev. 2002;18:43–8.

59. Husby S, Koletzko S, Korponay-Szabó IR, Mearin ML, Phillips A, Shamir R, et al. European Society for Pediatric Gastroenterology, Hepatology, and Nutrition Guidelines for the Diagnosis of Coeliac Disease. J Pediatr Gastroenterol Nutr. 2012;54:136–60.

60. Triolo TM, Armstrong TK, McFann K, Yu L, Rewers MJ, Klingensmith GJ, et al. Additional Autoimmune Disease Found in 33% of Patients at Type 1 Diabetes Onset. Diabetes Care. 2011;34:1211–3.

61. Frommer L, Kahaly GJ. Type 1 diabetes and associated autoimmune diseases. World J Diabetes. 2020;11:527–39.

62. Dabelea D, Rewers A, Stafford JM, Standiford DA, Lawrence JM, Saydah S, et al. Trends in the prevalence of ketoacidosis at diabetes diagnosis: The search for diabetes in youth study. Pediatrics. 2014;133:e938–45.

8. SUMMARY

Objectives: The aim of this study was to determine levels of antibodies for celiac disease and autoimmune thyroiditis in newly diagnosed type 1 diabetes mellitus (T1DM) children and adolescents.

Patients and methods: This study enrolled 110 newly T1DM pediatric patients in the period from January 2016 to December 2019. Evaluation included physical examination, medical history, anthropometric measurement and blood sample.

Results: The cumulative prevalence of positive antibodies for celiac disease was 10.9 %, while prevalence of positive antibodies for autoimmune thyroiditis was 11.8 % in newly diagnosed patients with T1DM. Furthermore, subjects with additional autoimmune disease were statistically significantly older compared with subjects without additional autoimmune disease (11.3 ± 4.03 vs. 8.64 ± 4.44, P=0.004).

Conclusion: This study has confirmed that new-onset T1DM in children and adolescents can present with non-islet, organ-specific autoantibodies. These results emphasize the importance of screening for celiac disease and autoimmune thyroiditis in newly diagnosed T1DM individuals.

9. CROATIAN SUMMARY

Naslov: Prevalencija autoimunih komorbiditeta u novootkrivenih pedijatrijskih bolesnika s šećernom bolesti tipa 1

Ciljevi: Cilj ovog istraživanja bio je utvrditi razine antitijela na celijakiju i autoimuni tireoiditis kod novootkrivene djece i adolescenata s šećernom bolesti tipa 1 (ŠBT1).

Materijali i metode: U ovu je studiju uključeno 110 novootkrivenih pedijatrijskih bolesnika s ŠBT1 u razdoblju od siječnja 2016. do prosinca 2019. Procjena je uključivala fizikalni pregled, povijest bolesti, antropometrijsko mjerenje i uzorak krvi.

Rezultati: Kumulativna prevalencija pozitivnih antitijela na celijakiju bila je 10,9%, dok je prevalencija pozitivnih antitijela na autoimuni tireoiditis bila 11,8% u novootkrivenih bolesnika s ŠBT1. Nadalje, ispitanici s dodatnom autoimunom bolešću bili su statistički značajno stariji u usporedbi s ispitanicima bez dodatne autoimune bolesti (11,3 ± 4,03 naspram 8,64 ± 4,44, P = 0,004).

Zaključci: Ova je studija potvrdila da kod pacijenata s noovotkrivenom ŠBT1 mogu biti prisutan s auto-antitijelima koja nisu specifična za beta stanice, već su specifična za druge organske sustave. Navedeni rezultati ukazuju na važnost probira na celijakiju i autoimuni tiroiditis kod novootkrivenih bolesnika s ŠBT1.

10. CURRICULUM VITAE

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