Diabetic Autoimmune Markers in Children and Adolescents With Type 2 Diabetes

Eba H. Hathout, MD; Wendy Thomas; Mohamed El-Shahawy, MD, MHA; Fadi Nahab; and John W. Mace, MD

ABSTRACT. Background. There is an increase in the incidence of type 2 diabetes in children and adolescents. Absence of known diabetes autoimmune markers is sometimes required to confirm the diagnosis.

Objective. To identify clinical and autoimmune characteristics of type 2 diabetes in a pediatric population.

Method. We report an analysis of 48 children and adolescents with type 2 diabetes, compared with 39 randomly selected children with type 1 diabetes, diagnosed and followed at the Loma Linda University Pediatric Diabetes Center. Ethnic, familial, seasonal, and autoimmune marker characteristics are outlined. To determine the reliability of antibody testing in confirming the type of diabetes at diagnosis, we studied the incidence of positive islet cell antibodies (ICAs), glutamic acid decarboxylase antibodies (GADs), and insulin autoantibodies (IAAs) at diagnosis in both groups. ICA512, GADs, and IAAs were measured by radioimmunoassay.

Results. The cohort with type 2 diabetes had a similar gender distribution as the group with type 1 diabetes but a significantly higher age at diagnosis. Ethnic background was significantly different between the 2 groups, predominantly Hispanic in type 2 and white in type 1. Body mass index was significantly higher in type 2 diabetes (mean = 31.24 kg/m²). Among the patients with type 2 diabetes, 33% presented in diabetic ketoacidosis, random blood glucose at diagnosis ranged from 11.4 to 22.25 mmol/L (228 – 445 mg/dL), fasting C-peptide levels ranged from 0.89 to 2.7 nmol/L (2.7 – 8.2 ng/mL), and hemoglobin A1C was 10.8% on average. None of these parameters was significantly different from the type 1 diabetes group. Although the incidence of diabetes antibody markers was significantly lower in type 2 versus type 1 diabetes, 8.1% of patients with type 2 diabetes had positive ICAs, 30.3% had positive GADs, and 34.8% had positive IAAs without ever being treated with insulin. In the type 2 diabetes group, none of the Hispanic patients had ICAs. However, there was no significant correlation between any of the diabetes antibodies and obesity, presence of acanthosis nigricans, or family history of diabetes. The frequency of thyroid antibodies was not significantly different from the group with type 1 diabetes. Daily insulin requirements 1 year after diagnosis were significantly lower in type 2 diabetes, ranging from 0 to 1.2 U/kg with a mean of 0.33.


ABBREVIATIONS. ICA, islet cell antibody; GAD, glutamic acid decarboxylase antibody; IAA, insulin autoantibody; BMI, body mass index; DKA, diabetic ketoacidosis; TGA, thyroglobulin antibody; TPOA, thyroid peroxidase antibody; SD, standard deviation.

There has been a dramatic increase in the incidence of type 2 diabetes in children and adolescents during the past decade.² The disproportionate higher prevalence of type 2 diabetes in minority youth in North America has been highlighted in many recent conferences and publications.³ The diagnosis has relied primarily on the clinical criteria of hyperglycemia and the presence of 1 or more of the following: belonging to a high-risk minority population, eg, Mexican American; type 2 diabetes in 1 or both parents; obesity; and signs of insulin resistance, such as acanthosis nigricans.³ Biochemical evidence of endogenous insulin production via basal or stimulated C-peptide levels is sometimes used to confirm the diagnosis.⁴ Presence of any of the β-cell autoimmune markers, islet cell antibodies (ICAs), glutamic acid decarboxylase antibodies (GADs), or insulin autoantibodies (IAAs) was often used to negate or challenge the diagnosis of type 2 diabetes in children and adolescents.³,⁴ However, there are several reports in the adult literature describing and analyzing the incidence of diabetes autoimmune markers in type 2 diabetes in adults.⁵⁻¹¹ The objective of the present study was to outline the ethnic, familial, seasonal, and autoimmune marker characteristics of children and adolescents with type 2 diabetes at the Pediatric Diabetes Center of Loma Linda University.

METHODS

The number of patients with type 2 diabetes actively followed at the Pediatric Diabetes Center of Loma Linda University Children’s Hospital at the time of the study was 48. Diagnosis of type 2 diabetes was based on the revised American Diabetes Association criteria¹² of repeated fasting hyperglycemia (plasma glucose: >7 mmol/L; 126 mg/dL) or casual hyperglycemia (plasma glucose: >11.1 mmol/L; 200 mg/dL) in the presence of polyuria, polydipsia, and unexplained weight loss. The diagnosis was further supported by the presence of 2 or more of the following...
factors: Hispanic, Pacific Island or African, native or Asian-American ancestry, history of type 2 diabetes in a first-degree relative, obesity (body mass index [BMI]: >24 kg/m²), and presence of acanthosis nigricans. Evidence of endogenous insulin production by measuring basal and stimulated C-peptide levels in the non-ketotic state was used to confirm the diagnosis. Patients with suspected or genetically documented maturity-onset diabetes of the young or iatrogenic diabetes were given their respective diagnoses. The control group of 39 patients was selected at random (every 10th name in the database given a diagnosis of type 1 diabetes with age of onset of >3 years and current follow-up at Pediatric Diabetes Center of Loma Linda University Children’s Hospital). All patient histories were obtained by members of the Pediatric Diabetes Center at the initial clinic visit. Clinical information was obtained retrospectively by hospital and clinic record review.

Demographic characteristics examined in this study include age at diagnosis, gender, ethnic background, rank among siblings, household size at diagnosis, season of diagnosis, and family history of diabetes of either type. Past medical history also includes mode of delivery and parental ages at birth. Clinical characteristics at the time of diagnosis include symptoms of a viral illness, such as rhinorrhea or cough, BMI, presence of acanthosis nigricans, blood glucose, hemoglobin A1C and C-peptide levels, insulin treatment, and presence of diabetic ketoacidosis (DKA).

IAAs were measured by 125I binding capacity (normal: <5 μU/mL), GAD65 (GAA), and ICA512 were measured by radioimmunoprecipitation assay (normal: <1 U/mL, Endocrine Sciences Laboratories, Calabasas, CA). Thyroglobulin antibodies (TGAs) and thyroid peroxidase antibodies (TPOAs) were measured by immunochemiluminescent assay (Quest Diagnostics at Nichols Institute, San Juan Capistrano, CA).

Data were analyzed in a cross-sectional manner. All data for this study were extracted from pediatric diabetes center charts, which include hospital admission information, onto a structured database form. Data were subsequently entered using Excel, Version 5.0 (Microsoft Co, Seattle, WA), verified by inspection, and checked for erroneous values.

Statistical Analysis
Continuous data were analyzed using Student’s 2-tailed t test. Analysis of categorical variables was accomplished using the χ² test. P values <.05 were assigned statistical significance. All P values are 2-tailed. Age, BMI, hemoglobin A1C, blood glucose, and C-peptide data are presented as mean values ± standard deviation (SD).

RESULTS
Age range at diagnosis was 9.7 to 17.9 years for the group with type 2 diabetes and 3.75 to 17 years for the group with type 1 diabetes (Table 1). Ethnic breakdown in type 1 diabetes was similar to that of the background population in distinction to the group with type 2 diabetes, which proportionately consisted of significantly more Hispanics (P = .001) and fewer whites (P = .011). No particular gender predominance was apparent in either group. There was no significant difference in the ranking of the participants in the family or household size at diagnosis between the 2 groups. Fall was the most common season for diagnosis of type 2 diabetes, compared with winter, which was the predominant season of presentation for the type 1 group (P = .01). There was a significantly higher frequency of type 2 diabetes in first-degree relatives of patients with type 2 diabetes (P = .001). Past medical history did not show a significant difference in the frequency of birth by cesarean section or parental ages at birth between the 2 groups. BMI at diagnosis of type 2 diabetes ranged from 21.90 to 53.08 kg/m² (Table 2). This range was significantly higher than BMI at diagnosis of type 1 diabetes patients (range: 8.40–27.89; P = .0001). Acanthosis nigricans was exclusive to type 2 diabetes, occurring in 37% of patients in that group. The percentage of patients with type 2 diabetes initially treated with insulin was 68.6%. The rest were managed with a combination of diet and exercise, with or without metformin. Although blood glucose, hemoglobin A1C levels, and the percentage of patients presenting in DKA were all lower in type 2 (vs type 1) diabetes, their difference between the 2 groups was not statistically significant.

Among patients with type 2 diabetes, 30.3% had
positive GADs (>1.0 U/mL), 34.8% had positive IAs (>5 μU/mL), and 8.1% had positive ICAs (>1.0 U/mL; Table 2). Positive ICAs were inversely correlated with Hispanic ancestry. Otherwise, there was no significant correlation among any of the 3 antibodies and race or presence of obesity, acanthosis, or a family history of diabetes of either type. ICA and GAD frequencies in type 2 patients were significantly lower than in type 1 diabetes patients (positive frequency range: 71%–76%; P < .01). However, the frequency of positive TGAs and TPOAs was not significantly different between the 2 groups. Subsequent clinical correlation showed that 3 of the type 2 diabetes patients with positive thyroid antibodies had concomitant hypothyroidism at the time of the study, whereas none of the type 1 group had evidence thereof.

Treatment initiation and modification decisions for patients with type 2 diabetes in this study were made by 2 pediatric endocrinologists. One year after diagnosis, 50% of patients with type 2 diabetes were not on insulin therapy and were controlled with diet and exercise alone or in combination with metformin. Retrospective analysis showed that 47% of these patients had a first-degree relative with type 2 diabetes, 36% had acanthosis nigricans, 89% had BMI >24 kg/m², and 27% had DKA at diagnosis. In addition, 18.75% of these positive ICA patients had positive GADs (Table 3). 37.5% had positive IAAs, and 0.0% had positive ICAs. The majority of patients with type 2 diabetes not requiring insulin 1 year after diagnosis stopped insulin up to the time of the present review, conducted 3 to 5 years after their diagnosis. The remaining patients were either diagnosed within 2 years of this review or lost to follow-up.

Addition of, or transition to, insulin therapy was based on lack of glycemic improvement (hemoglobin A₁C remains or rises to >8%) despite maximal attempts at diet and exercise and a trial of metformin up to 2 g per day. In patients treated with insulin 1 year after diagnosis, weight-adjusted daily insulin requirements were significantly lower than in patients with type 1 diabetes (P = .025), ranging from 0 to 1.2 U/kg with a mean of 0.33. All type 2 patients with positive ICA titers were on insulin therapy 1 year after diagnosis.

DISCUSSION

Type 2 diabetes in children and adolescents underwent a >10-fold increase over the past decade. This increase has been primarily attributed to a problem of childhood obesity, which has reached epidemic proportions. Genetic, ethnic, and environmental risk factors have also been considered. This, together with a potentially long asymptomatic period for type 2 diabetes, raise serious concerns for long-term microvascular and macrovascular complications.

Demographic characteristics of adolescents with type 2 diabetes in our study are similar to those reported previously by others in terms of predominance of Hispanic (Mexican American) ancestry, and association with obesity, acanthosis nigricans, and family history. However, given that the ethnic breakdown in our control group was similar to that of the background population, our study did not show the significantly higher incidence of type 2 diabetes in black adolescents described by others. Of interest is the 1 patient with type 2 diabetes with a family history of type 1 diabetes in a first-degree relative. That patient is a black female, diagnosed at 13 years old, with BMI of 41.6 kg/m², significant acanthosis, normal C-peptide, and negative diabetes and thyroid antibodies. Verification of the presumed type 1 diabetes in that relative was not possible.

The increase in BMI in type 2 versus type 1 diabetes might have been exaggerated by the fact that all our patients with type 2 diabetes were pubertal at the time of the study. The trend shown in this study for type 2 diabetes to be diagnosed in the fall might be the consequence of the summer vacation, a period of relative excess eating and decreased activity for many adolescents. In contrast, it might have been a coincidental finding brought to medical attention by requirements of a school physical examination.

The diagnosis of type 2 diabetes relies heavily on clinical criteria, such as Hispanic or black ancestry, positive family history, obesity, evidence of insulin resistance, and absent or milder-than-expected plasma glucose fluctuations, ketosis, or hypoglycemic reactions to insulin. Measurable fasting or stimulated C-peptide levels, reflecting endogenous pancreatic insulin secretion, are not exclusively diagnostic of type 2 diabetes, because they can theoretically be present in the honeymoon phase of type 1 diabetes. However, absence of β-cell autoimmune markers (ICA, GAD, and IAA without previous insulin therapy) has been a diagnostic prerequisite for type 2 diabetes in most pediatric cases, particularly pertaining to trials of noninsulin therapeutic modalities.

Evidence of β-cell autoimmunity in adults with type 2 diabetes, however, has been well documented in the literature. GAD positivity has been reported in 3.8% to 16.1% of patients with type 2 diabetes. ICA prevalence in type 2 diabetes was reported as 1.6% in 1 study and 4.8% in another. Moreover, some reports suggested that ICA positivity in type 2 diabetes predicted future insulin requirement and that GAD positivity correlated with a younger age of diagnosis, lower C-peptide at diagnosis, and increased probability of insulin requirement. No differences in BMI or serum C-peptide levels could be found between GAD-positive and GAD-negative type 2 patients at diagnosis. Of interest is 1 study showing that fasting C-peptide levels in ICA-positive patients with type 2 diabetes were similar to patients with type 1 diabetes, 3 years after diagnosis.

In the present study, the higher incidence of β-cell autoimmunity in type 2 diabetes patients alert us to an increasing problem. As described by others, the trend is not explained by an increase in BMI or by other risk factors for type 2 diabetes.

**TABLE 3.** Diabetes Autoimmune Markers in Noninsulin-Requiring Patients With Type 2 Diabetes

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Percentage With Positive Titer</th>
<th>Percentage With Negative Titer</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAD</td>
<td>18.75</td>
<td>81.25</td>
</tr>
<tr>
<td>IAA</td>
<td>37.5</td>
<td>62.5</td>
</tr>
<tr>
<td>ICA</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

http://www.pediatrics.org/cgi/content/full/107/6/e102
antibodies in adolescents with type 2 diabetes (Table 2) supports the previously reported correlation of positive diabetes antibodies with younger age at diagnosis.8 Our data also suggest that ICA antibody positivity at diagnosis could be a predictor of future insulin need.17,18

The possibility that positive diabetes antibodies in adolescents with nonsulin-requiring diabetes may represent a form of early-onset latent autoimmune diabetes similar to that described in adults (latent autoimmune diabetes in adults)21,22 needs to be considered. This form of diabetes, however, was not reported to be specifically associated with obesity or Hispanic ancestry. Long-term data on insulin requirements can shed more light on this area. There is also the possibility that some type 2 patients, particularly those requiring insulin, are actually obese patients with type 1 diabetes. Studying genetic determinants specific to either type of diabetes, eg, human leukocyte antigen-DQ/DR subtypes, might be needed in the future for typing of diabetes in such patients.23 The fact that autoimmunity should not exclude the possibility of type 2 diabetes in adolescents is supported by the presence of positive thyroid (TPOA and TGA) antibodies and hypothyroidism in type 2 patients, which was detected in 3 patients in this series. Because the focus of this study was the typing (and not the diagnosis) of diabetes in children, antibody levels in healthy controls were not included.

Data regarding insulin treatment in type 2 adolescents, particularly at the time of diagnosis, should be interpreted with caution. Many patients had been misdiagnosed as having type 1 diabetes based on either age or DKA at presentation. In addition, lack of a Food and Drug Administration-approved stepwise approach to the treatment of type 2 diabetes in children often facilitated the use of insulin as initial therapy. Moreover, social and cultural factors interfering with compliance with nonsulin regimens, particularly in the adolescent age group, frequently lead to use of insulin in nonsulin-requiring patients.

Another entity not addressed in this study consists of patients who are both insulin-deficient and insulin-resistant.24 This is exemplified by obese type 1 adolescent patients, with onset of diabetes in early childhood, evidence of compliance in a supervised setting, and daily insulin requirements exceeding 2 U/kg. Whether those patients, who have both types of diabetes, could be categorized and treated as such merits additional exploration.

The above data reflect the broad spectrum of pancreatic and peripheral abnormalities that could lead to adolescent diabetes. Our study and many studies in the adult literature suggest that the presence of diabetes autoimmune markers should not be used to exclude the diagnosis of type 2 diabetes in children and adolescents. Larger prospective pediatric diabetes studies are needed to confirm the validity of this conclusion. Acceptance of this concept in scientific and research settings could open the door to trials of a broader range of therapeutic modalities for a group of children and adolescents who were previously labeled insulin-dependent for life.

REFERENCES

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