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High prevalence of idiopathic (islet-antibody negative) type 1 diabetes among Indian children and adolescents

Islet-antibody negative type 1 diabetes

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Author contributions

VPV: conducted the study, analysed the data, wrote the manuscript

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GZ: conducted the testing, analysed data, reviewed manuscript

KW: conducted and interpreted the assays, reviewed the manuscript

PC: Interpreted the assays, reviewed the manuscript

SP: Conducted the testing, analysed and interpreted data

SA: Interpreted assays, reviewed the manuscript

VB, PD: conducted the study, reviewed the manuscript

Conflict of interest statement

Authors of this manuscript do not have any conflicts of interest

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Key words

Type 1 diabetes, type 1B diabetes, idiopathic type 1 diabetes, islet-antibody, Indian

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Abstract

Objectives: To study the prevalence and clinical characteristics of islet-antibody negative (idiopathic) type 1 diabetes mellitus (T1DM) among Indian children and adolescents at the time of diagnosis of illness.

Methods: In a hospital-based cross-sectional study, we studied 110 patients with T1DM ≤ 18 years of age. This included 61 patients with duration of diabetes ≤ 2 weeks (mean $\pm$ SD age of onset 9.9 ± 4.4 years) and 49 patients with duration 2-12 weeks. Antibodies against GAD65 (GADA), IA-2 (IA-2A) and zinc transporter 8 (ZnT8A), detected by radio-binding assay, were measured in all patients. Insulin auto antibody (IAA) was measured only in subjects with duration ≤ 2 weeks, using a competitive radio-binding assay.

Results: The frequency of GADA, IA-2A, ZnT8A was present in 53%, 34%, 29% respectively, while IAA (measured in 61 patients) was detected in 31%. All four antibodies were absent in 17/61 (28%) patients. The prevalence of islet-antibody negative patients was similar among both sexes and in children with onset below and

greater than 10 years. ZnT8A was the only antibody detected in four patients, and its measurement resulted in 6% reduction in islet-antibody negative patients. Patients with idiopathic T1DM did not differ in their clinical features or fasting plasma C-peptide at onset and after follow-up of 1 year. Compared to idiopathic T1DM, antibody-positive patients had an increased allele frequency of HLA DRB1*0301 [46% vs. 14%, OR=5.10 (CI=1.61-16.16), $p=0.003$].

Conclusion: Nearly 30% of Indian patients were negative for all islet-antibodies at onset of T1DM. Patients with idiopathic T1DM had similar clinical features as antibody-positive subjects.

Introduction

Type 1 diabetes mellitus (T1DM) is a chronic autoimmune disorder resulting from T-cell mediated destruction of pancreatic β -cells¹. Antibodies against different islet-antigens are present in most patients with T1DM and serve as markers of the disease². Most frequently measured are antibodies are those against endogenous insulin (insulin autoantibodies, IAA) and enzymes tyrosine phosphatase (IA-2A), glutamic acid decarboxylase (GADA) and zinc transporter 8 (ZnT8A). In non-Hispanic White (NHW) Caucasian patients, one or more islet-antibodies are present in >90% of the patients at the onset of T1DM^{3,4}, while antibody negative (idiopathic) diabetes is uncommon (5-7%).

A low prevalence of IA-2 and ZnT8 antibodies has been reported in Chinese and Japanese patients with T1DM⁵⁻⁷. Similarly, a low frequency of IA-2A (15-25%) was

found in Indian children with T1DM⁸⁻¹⁴, as compared to patients of European origin (60%-70%)². The frequency of ZnT8A and IAA have not been adequately studied in Indian patients. Only a single study has reported on ZnT8A frequency in Indian children with newly diagnosed T1DM¹¹. Using GADA and IA-2A, we have earlier reported a high frequency (45%) of idiopathic T1DM in Indian children⁸. However, most of these previous studies have certain shortcomings. No study has measured antibodies against all four major islet-antigens and patients have been tested at varying durations of time after diagnosis.

In view of these deficiencies in earlier reports, we studied the prevalence of islet-antibody negative T1DM in Indian children and adolescents at the time of diagnosis by measuring antibodies against all four major islet-antigens and compared clinical findings of antibody-negative and type 1A patients.

Materials and methods

Study design

We conducted a hospital-based cross-sectional study of children and adolescents with T1DM attending the paediatric endocrinology clinic of Sanjay Gandhi Postgraduate Institute of Medical Science, Lucknow between 2012 to 2016. The hospital is a referral centre for the populous state of Uttar Pradesh in north India. T1DM was diagnosed on the basis of severe hyperglycaemia [blood glucose >16.7 mmol/l (>300 mg/dl)] and insulin requirement from the onset of diabetes. All patients had an onset ≤ 18 years of age. We studied 110 patients with duration <12 weeks (age 10.5 ± 4.8 years, males

n=67, duration 3.4 ± 3.8 weeks). We measured antibodies against GAD, IA-2, and ZnT8 antigens in all patients. Further, in a subset of these (n=61) with duration ≤ 2 weeks, we also measured IAA.

A serum sample for islet-antibodies was collected at presentation. A sample for plasma C-peptide was collected after the blood glucose was controlled. All patients were evaluated at regular intervals and repeat HbA1c and C-peptide were measured after 1 year. The study was approved by the institutional ethics committee and written informed consent was obtained from all patients and/or guardians as appropriate.

Investigations

Islet-antibodies: GADA, IA-2A and ZnT8A were measured by a radio-binding assay using in-vitro transcribed and translated islet antigen labelled with ^{35}S -methionine, as previously described^{3,10,15}. The plasmid containing the ZnT8 dimer containing the carboxy terminals of arginine (R) and tryptophan (W) at residue 325 (ZnT8 COOH R/W dimer) was obtained by the kind permission of University of Colorado, Denver. The assay for ZnT8A had a specificity and sensitivity of 99% and 60% in the Islet Autoantibody Standardization Program (IASP) workshop, 2015. IAA was measured using a radio-binding assay¹⁵. IAA assay had 100% specificity and 28% sensitivity (IASP workshop, 2015). The specificity and sensitivity of GADA was 98% and 74% and IA-2A 99% and 76% respectively (IASP workshop, 2015).

Other assays: C-peptide was measured by immunoradiometric assay (Beckman Coulter, Prague, Czech Republic), IgA-tissue transglutaminase (TTG) antibody by

ELISA (Diesse; Chorus, Siena, Italy), thyroid peroxidase (TPO) antibody by chemiluminescent immunometric assay (Immulite 1000, Siemens, UK) and parietal cell antibodies (PCA) by indirect immunofluorescence using rat stomach as substrate.

HLA analysis: HLA-DR and DQ typing were performed in 51 of 61 patients studied at onset, using SSP kits (AllSet+ Gold, Invitrogen Corp., Madison, WI, USA) as per manufacturer's instructions. It was not feasible in the remainder due to lack of consent for genetic analysis or technical reasons. The positive lanes were visually determined and analysed using UniMatch Software 4.0 (Invitrogen Corp., Madison, WI, USA).

Statistical analysis: Continuous variables were expressed as mean $\pm$ standard deviation or median with inter-quartile range, as appropriate. Continuous variables were compared using unpaired Student's t test or Mann Whitney U test. The chi-square test was used for comparison of categorical data. Data were analysed using the Statistical Package for Social Sciences software (version 19.0, IBM SPSS Statistics). HLA allele frequencies were compared by Fisher's exact test using GraphPad InStat version 3.05 for Windows (GraphPad Software, La Jolla, CA, USA). A two-sided $p < 0.05$ was considered significant.

Results

Among all 110 patients with duration <12 weeks, the prevalence of GADA, IA-2A and ZnT8A was 53%, 34% and 29% respectively (Table 1). Females had a higher frequency of ZnT8A compared to males (42% v/s 21%, $p = 0.03$). ZnT8A positive patients had a higher frequency of DKA at onset (66% v/s 40%, $p=0.02$) and lower fasting plasma C-peptide after 1-year duration [0.07 (0.02-0.15) nmol/l v/s 0.17 (0.08-0.30 nmol/l), $p=0.020$].

Among the 61 patients with a duration ≤ 2 weeks, IAA was detected in 31% of patients. IAA positive patients had lower fasting C-peptide at diagnosis as compared with negative subjects [0.10 (0.04-0.18) nmol/l vs. 0.19 (0.17-0.32) nmol/l], $p=0.03$]. IAA frequency was highest in those with onset <5 years (42%) compared with those between 5-10 (30%) and 10-18 years (28 %), though the differences were not significant. Two or more antibodies were detected in 26 (43%) patients while at least 1 antibody was detected in 44 (72%) patients (Table 2). All four islet-antibodies were absent in 17 (28%) patients. ZnT8A was the only antibody detected in 4 patients; its inclusion reduced the frequency of islet-antibody negative patients from 34% to 28%.

The frequency of idiopathic T1DM was similar in both sexes and among those with onset before and after 10 years of age. Patients with idiopathic T1DM had similar clinical and biochemical parameters at presentation compared with type 1A diabetes (Table 2). The two groups also did not differ in the frequency of other organ-specific autoantibodies. After a follow-up for 1 year, the frequency of DKA, insulin dose

requirement and C-peptide were also similar. Patients with type 1A diabetes had a higher frequency of the allele DRB1*0301 compared with subjects with idiopathic T1DM [34/74 (46%) vs. 4/28, 14% [OR=5.10 (CI=1.61-16.2)]. No other differences in HLA DR or DQ associations were noted.

Discussion

We report that nearly 30% of Indian children and adolescents with T1DM were negative for all four major islet-antibodies. Patients with idiopathic T1DM were clinically indistinguishable from those with type 1A diabetes at diagnosis, or after 1 year of follow-up.

In our study, the frequency of ZnT8A (29%) and IA-2A (34%) were considerably lower than that reported in NHW children and adolescents with T1DM^{2-4,7}. ZnT8A positive patients had increased DKA at the onset and lower fasting C-peptide on follow up at 1 year, which were similar to that observed in Finnish T1DM patients¹⁶. However, the frequency of ZnT8A was higher in females as compared to males, a difference that was not observed in previous studies.

There was a higher prevalence (28%) of islet-antibody negative (idiopathic) T1DM, in comparison to a frequency of 5-10% in children of NHW origin²⁻⁴. While similar studies in other ethnic groups are scarce, idiopathic T1DM has been reported in a frequency of 35% in Chinese patients⁵. In a large multi-ethnic population in UK, idiopathic T1DM was noted in 14% of White children and adults with T1DM vs. 27% in other ethnic groups¹⁷. We noted no clinical differences in patients with idiopathic and type 1A

diabetes, either at onset or after 1 year. In previous reports on NHW children, subjects with idiopathic type 1 diabetes had only few differences compared with those with islet-antibodies^{18,19}.

Idiopathic T1DM in children and adolescents is a heterogeneous entity with numerous variants having been described. For example, a fulminant form of T1DM with sudden onset and frequent history of a viral prodrome has been described in Japanese and Chinese²⁰. In Blacks and Hispanics, an idiopathic T1DM is reported among patients with obesity and acanthosis nigricans presenting with severe hyperglycaemia and DKA²¹. However, patients with idiopathic T1DM in the current study did not resemble either of these variants and were similar to that described in NHW children^{17,18}. The lower frequency of HLA DRB1*03 in our patients with antibody-negative T1DM, as also reported in our earlier study⁸, suggests a difference in the genetic predisposition for type 1A diabetes, but needs further study in a larger cohort. A viral aetiology or MODY gene mutations are also feasible explanations²². Finally, antibodies against hitherto unknown islet-antigens²³ is another possibility. Interestingly, antibodies have been detected against the minor islet antigen SOX13 in 30-70% of Indian patients with T1DM^{13, 24}. A significant presence of other organ-specific antibodies in patients with idiopathic T1DM also provides some support for this hypothesis. While the absence of islet-antibodies did not impact the clinical course in diabetes in this study, it may have relevance in confirmation of diagnosis of T1DM.

The strengths of the study include a well- defined cohort studied at diagnosis of the illness and a follow-up duration of at least 1 year in all patients. In addition, the assays

for all islet-antibodies were validated in recent proficiency workshops. The main limitations of our study were a relatively small sample size and the lack of HLA genotyping in a small proportion of patients with recent -onset diabetes.

In conclusion, nearly one-thirds of Indian children and adolescents with T1DM were negative for all islet-antibodies at onset. However, their clinical features and β -cell reserve were similar to patients with type 1A diabetes. Further studies to delineate the clinical characteristics and aetiology of idiopathic T1DM are required.

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Table 1**Prevalence of islet-antibodies at diagnosis in children and adolescents with T1DM**

Antibody	N (%)	95 % CI
GADA ^a	58 (53)	42.8-61.8
IA-2A ^a	37 (34)	24.5-42.7
ZnT8A ^a	32 (29)	20.9-37.3
IAA ^b	19 (31)	19.7- 42.6
GADA and/or IA-2A	38 (62)	49.2-73.8
GADA and/or ZnT8A	33 (54)	41-65.6
GAD and/or IAA	35 (57)	44.3-68.9
Any antibody	44 (72)	60.7- 83.6
No antibody	17 (28)	16.4-39.3

^a Measured in all 110 patients^b IAA measured in 61/110 patients with duration ≤ 2 weeks; all combination of antibodies is for 61 patients

IAA – Insulin antibody, GADA – glutamic acid decarboxylase antibody, IA-2A – insulinoma associated protein, ZnT8A- zinc transporter 8 antibody.

Table 2

Comparison of children and adolescents with islet-antibody positive and negative (idiopathic) type 1 diabetes of duration <2 weeks

Parameter	All patients (n=61)	Type 1A diabetes (n= 44)	Idiopathic type 1 diabetes (n=17)
Age at onset (years)	9.9±4.4	9.8±4.2	10±5.2
Duration of symptoms before diagnosis (weeks)	2.0 (0.5-3.5)	2.0 (0.6-3.4)	2.0 (0-5)
History of viral prodrome n (%)	19 (31)	11 (25)	8 (47)
Sex (Male: Female)	39:22	27:17	12:5
Plasma glucose at diagnosis (mmol/l)	25.1±5.0	25.2±5.1	24.8±4.8
DKA at diagnosis	28 (46)	22 (50)	6 (35)
BMI (kg/m ²)	15.0±2.3	15.1±2.5	14.9±1.8
HbA1c at diagnosis (%)	12.4±2.8	12.2 ± 2.9	12.9 ± 2.7
Fasting C-peptide at diagnosis (nmol/l)	0.23±0.19 (n=34)	0.20±0.15 (n=24)	0.31±0.22 (n=10)
Fasting C-peptide after 1 year (nmol/l)	142±135 (n=38)	119±126 (n=28)	205±142 (n=10)
TPO antibody	14 (23)	12 (27)	2 (12)
TTG antibody	11 (18)	7 (16)	4 (23)
PCA (n=54)	6 (11)	5/39 (13)	1/15 (6)
DRB1*03:01 allele		34/74 (46)	4/28 (14) *

Mean $\pm$ SD or median (inter quartile range) whichever is appropriate. DKA- Diabetic ketoacidosis, TTG – tissue transglutaminase, TPO – thyroid peroxidase, BMI –body mass index, PCA- parietal cell antibody

*Allele frequency $P=0.003$

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