Original Article

Profile of Auto-antibodies (Disease Related and Other) in Children with Type 1 Diabetes

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Abstract

Background: Type 1 diabetes is associated with several disease-related and other organ-specific autoimmune disorders. Data related to various auto-antibodies in Type 1 diabetes in India is limited. **Materials and Methods:** In this cross sectional study, 92 subjects with T1DM (33 males, 59 females) were evaluated for T1DM related antibodies (autoantibodies to glutamic acid decarboxylase (anti-GAD), autoantibodies to protein tyrosine phosphatise (anti-IA2), anti-islet cell antibody (ICA), insulin autoantibody (IAA), anti-Zinc Transporter(ZnT8) and other organ specific auto antibodies like anti–thyroid peroxidase (anti-TPO), anti-thyroglobulin (TgAb), IgA anti-tissue transglutaminase (IgA anti-tTG), anti-21-hydroxylase, and anti-ovarian antibody (in females). **Results:** Anti-GAD, IA-2, islet cell antibody, insulin autoantibodies (IAA), ZnT8 antibody were present in 79.3%, 32.6%, 61.9%, 63%, and 20.65% subjects, respectively. Only 2.2% patients with Type 1 diabetes were antibody negative. At least one antibody was found in 97.8% and at least two antibodies in 67.3%. The presence of anti-TPO, anti-thyroglobulin, IgA anti-tissue transglutaminase, anti 21-hydroxylase were found in 51%, 25%, 22.8%, and 2.1%, respectively. Anti-ovarian antibody was absent in all females of our study population. The duration of diabetes positively correlated with the number of T1DM specific antibody and also with GAD antibody positivity. Anti TPO positivity correlated with the age of onset of T1DM, but not with the duration of disease or presence of other T1DM specific autoantibody. **Conclusions:** T1DM is associated with a high prevalence of autoantibodies and antibody negative T1DM is rare. The association with other organ specific antibody (especially thyroid and adrenal glands) and celiac disease is also substantial, which reinforces the importance of regular thyroid and celiac disease screening in T1DM subjects. The duration of diabetes positively correlated with number of T1DM specific antibodies.

Keywords: Autoantibodies, prevalence, type 1 diabetes

Introduction

Type 1 diabetes mellitus (T1DM) is an autoimmune disease with selective destruction of the beta cells of the pancreas. Anti-islet cell autoantibodies (ICA) are supportive for the diagnosis of T1DM. The most frequently detected autoantibodies are glutamic acid decarboxylase 65kDa (GAD) autoantibodies, tyrosine phosphatase-associated islet antigen related antibody (islet antigen 2 or IA-2), islet cell autoantibodies (ICA), and zinc transporter (ZnT8) autoantibodies.[1,2] As per western literature, the prevalence of GAD autoantibodies, IA-2 autoantibodies, and ZnT8 autoantibodies are 70%–80%, 60%–70%, and 60%–80%, respectively in children with new onset T1DM.[1] The insulin autoantibodies (IAA) are present in 90% of children who progress to T1DM before the age of 5 years with only 40%–50% of those older than 15 years.[1] Persistently autoantibody negative (PAN), retested at median diabetes diabetes duration of 3.2 yrs is reported to occur in 5% of subjects with TIDM.[3]

Apart from these disease specific autoantibodies, T1DM is also often associated with other autoimmune diseases including autoimmune thyroid disease (AITD), celiac disease, and idiopathic Addison's disease, etc.[2,4,5]Autoimmune markers of these conditions may be present along with T1DM specific

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autoantibodies even prior to clinical onset. Among these, AITD in T1DM is so common that screening for this disease is recommended for every child with TIDM at diagnosis and every 1-2 years thereafter.[2]

Very few studies regarding other organ specific auto antibodies, especially antibody for celiac disease, ovarian antibody and antibody for Addison's disease has been reported. Data regarding the T1DM specific autoantibody profile is also sparse from Indian population.

In this background, the present study was undertaken to determine the prevalence of disease specific autoantibodies (anti-GAD) antibodies, anti-IA2 antibodies, anti-islet cell antibodies, insulin autoantibody (IAA), anti ZnT8 antibodies) and other organ specific autoimmune markers like anti-thyroid peroxidase (anti-TPO) antibodies, antithyroglobulin (anti-TG) antibodies, tissue transglutaminase (tTG-IgA) antibodies), anti-21α hydroxylase antibodies, and anti-ovarian antibodies in subjects with T1DM. We also looked for any association of positive anti-thyroid autoantibody with T1DM specific autoantibodies. This could identify any particular subgroup of subject with T1DM more prone to develop autoimmune hypothyroidism. Apart from these, we also evaluated whether the duration of diabetes is associated with any disease specific antibodies in this population.

Materials and Methods

In this cross sectional study, 92 consecutive subjects with type 1 DM who were on a regular follow-up in the specialty clinic were included. The diagnosis of T1DM was made on the basis of diagnostic criteria for diabetes (American Diabetes Association 2018) coupled with documentation of insulinopenia (on the basis of mixed meal stimulated C-peptide assay (with a cut off of 1.8 ng/ml) when the patient become euglycemic with insulin therapy and following standard protocol) with or without antibody positivity (Anti GAD 65 antibody, IA-2 antibody, ICA antibody, ZnT8 antibody) at presentation/diagnosis.

Blood samples were collected after overnight fasting for the following parameters: Anti-GAD antibody, anti-Islet Cell antibody, anti IA-2 antibody, insulin autoantibody (IAA), anti-Zinc transporter 8 antibodies, anti-TPO antibody, anti-thyroglobulin antibody (anti TG), anti-tissue trans-glutaminase antibody IgA (tTG-IgA), anti 21α hydroxylase antibody, and anti-ovarian antibody (in female subjects only).

Assay methods

The estimation of anti-TPO antibody and anti-thyroglobulin antibody was done by chemiluminescence method using commercially available kits from Siemens Diagnostics (Germany) with Immulite-1000 analyzer. Anti-GAD antibody, anti-islet cell antibody, anti-insulin antibody, insulin autoantibody (IAA), anti-tissue transglutaminase antibody IgA (tTG-IgA), anti 21α hydroxylase antibody, and anti ovarian antibody were measured by ELISA method using kits manufactured by MyBioSource (Southern California, San Diego (USA). ZnT8 antibody was measured by ELISA method using kits manufactured by Cusabio (Houston, Texas, USA).

Autoantibody positivity was considered if the measured level was above the range as mentioned here: Anti–TPO: 35.0 IU/ml, anti-thyroglobulin: 40.0 IU/ml, anti-GAD antibody: 1.0 IU/ml, anti-IA-2:1.0 IU/ml, anti-ICA: 1.0 IU/ml, IAA: 1.0 IU/ml, anti-ovarian antibody: 1.0 IU/ml, anti-21α hydroxylase antibody: 1.0 IU/ml. For anti-tissue transglutaminase antibody the ranges were determined as IgA:<4.0 U/mL (negative), >4.0 U/mL (positive). The presence or absence of above mentioned antibodies were expressed according to reference range provided by the manufacturer and/or the standard range.

For ZnT8 antibody, we recorded the optical density (OD) of all samples and positive and negative controls. According to the protocol, OD sample/OD negative >2.1 was taken as positive and OD sample/OD negative <2.1 was taken as negative. Cut-off for OD values of positive control and negative control for adequacy of test methods was >0.45 and <0.1 respectively.

Statistical analysis

Continuous data are presented as mean ± SD and the results of categorical measurements are expressed in terms of frequency and percentage. All analysis was done by using Microsoft Excel 2007 (Microsoft Inc) and SPSS (Version 21.0).

Written informed consent and child assent was taken from all subjects. The study was approved by the Institutional Ethics Committee of Institute of Post Graduate Medical Education and Research, Kolkata, West Bengal, India.

Results

Baseline characteristics of 92 T1DM subjects are shown in Table 1. Of these 92 subjects 33 (35.9%) were male and 59 (64.1%) were female. The mean age of study population was 14.85 ± 3.99 years.

Positive GAD antibody was detected in 73 subjects (79.3%), and islet antigen 2 or IA-2 antibody was present in 30 subjects (32.6%), anti-islet cell antibody (ICA) was present in 57 subjects (61.9%), insulin autoantibody (IAA) in 58 subjects (63%), and anti-ZnT8 in 19 subjects (20.65%). Most importantly, any single antibody was present in 90 subjects (97.8%), and two antibodies were present in 62 subjects (67.3%). However, only 2 subjects (3.2%) were negative for all antibodies. These are shown in Table 2.

The duration of diabetes and number of disease specific antibody had moderate positive correlation (Spearman's ρ=0.44, P = 0.009), but there was no correlation between the age of onset of diabetes and number of disease specific antibodies. The duration of diabetes also correlated with the positivity of GAD antibody (Spearman's ρ=0.44, P = 0.04) and negatively correlated with IAA but it was not statistically significant.

Auto-antibodies specific for thyroid organ such as anti-TPO and anti-TG were discordant. Anti-TPO was present in 48 subjects (51%) and anti-TG was present in 20 subjects (25%). Both the antibodies were present in 18 subjects (19%) and both were absent in 42 subjects (45%), and any one of the antibodies was present in 50 subjects (54%).

Anti-tissue transglutaminase antibody IgA (tTG-IgA) was present in 21 subjects (22.8%) The presence of anti-21α hydroxylase antibody was found in 2 subjects (2.1%). Adrenal function in both was normal. Autoantibody specific for ovary i.e., anti-ovarian antibody was not detected in any of the female participants. The prevalence of other disease specific antibodies is shown in Table 3.

While analyzing the relation between anti TPO with other covariates, we found only the age of onset correlated with anti TPO positivity (Spearman's ρ=0.34, P = 0.048), but no other disease specific antibody had such correlation with anti TPO positivity. However, none of the covariates (age of onset, duration of diabetes, and other type 1 diabetes specific antibody) could predict anti TPO positivity in the binary logistic regression model. Nine subjects were diagnosed hypothyroid and all of them were anti-TPO positive and 15 subjects had subclinical hypothyroidism and were also anti-TPO positive. Twenty-three were anti-TPO positive but were clinically euthyroid.

Table 1: Baseline parameters of study population

Parameter	Values
Sex (Male)	33 (35.9%)
Age (years) (mean±SD)	14±3.99
Age at diagnosis (years) (mean±SD)	8.6±3.55
Duration (years) (mean±SD)	6.3±4
HbA1c (%) (mean±SD)	8.7±1.4
BMI (kg/m^2) (mean±SD)	18.5±2.6

Table 2: Frequency of positivity for disease specific antibody

	Total Subjects (n=92)	Percentage
Anti-GAD antibody	73	79.3
Anti islet antigen-2antibody	30	32.6
Anti islet cell antibody (ICA)	57	61.9
Insulin autoantibody (IAA)	58	63
Anti ZnT8antibody	19	20.65
Single antibody present	90	97.8
Two antibodies present	62	67.3
Three antibodies present	20	21.7
Four antibodies present	3	3.2
All five antibodies present	2	3.2
Antibody negative	2	2.2

Table 3: Frequency of positivity for other autoantibody

	Total Subjects (n=92)	Percentage
Anti TPO antibody	47	51.0
Anti thyroglobulin antibody (ATG)	23	25.0
Anti tissue transglutaminase-IgA antibody	21	22.8
Anti ovarian antibody (Females, n=59)	0	0
Anti 21αhydroxylase antibody	2	2.1

Discussion

We evaluated the prevalence of different type 1 diabetes related autoantibodies and their relationship with the age at onset and duration of diabetes. The prevalence of other autoantibodies affecting other organ systems was also assessed simultaneously. We found that at least one antibody was present in 96.7% of the subjects. It was reported from India by Shivaprasad *et al*. that antibody positivity may reach upto 80% with inclusion of ZnT8 antibody.[6] However, they measured only ZnT8 antibody, GAD antibody, and IA2 antibody. Another Indian study had noted a very high frequency of antibody negativity to the tune of 45% in their recent-onset cohort of T1DM, though they have tested only GAD antibody and IA-2 antibody.[7] On the contrary Hameed *et al*. reported that persistently autoantibody negative (PAN) T1DM occurs in 5% of subjects with TIDM.[3] Our data (antibody negative being only 2.2%) shows that if all the antibodies are tested, then the occurrence of antibody negative type 1 diabetes is low and concordant with the international data.

Anti-GAD antibody was found to be the commonest (79.3%) followed by IAA (63%), and ZnT8 antibody was found to be positive in only 20.65% in our population. Data from international literature shows the prevalence of GAD autoantibodies, IA-2 autoantibodies, ZnT8 autoantibodies, and IAA to be in the range of 70%–80%, 60%–70%, 60%–80%, and 40%–90%, respectively in children with new onset T1DM.[1] Studies done earlier from this country had shown that GAD antibody is present in 64.7%, IA2 antibody in 19.3%, and ZnT8 antibody is present in 31.8%.[6] This is in clear contrast to our study where we had a much higher positivity for GAD antibody and IAA. This was also concordant with the western data. However, we found a lower ZnT8 antibody positivity. As reported by Wenzlau JM, *et al*. the ZnT8 antibody positivity decreases significantly (from 57.38% at 1-2 years of duration of disease to 51.2% at 2-3 years of duration of the disease).[8] We had a mean duration of 6.3 ± 4 years and this could explain the lower positivity rate of ZnT8 antibody in our cohort.

The presence of one or more antibodies is usually required to have a positive immunological diagnosis of type 1 diabetes. We found that 67.3% were positive for at least two antibodies in our cohort. Among the combination of dual antibodies, GAD + IAA was positive in 48%, GAD + IA2 was positive in 34.40%, GAD + ICA was positive in 28%.

The increasing number of disease specific antibody had moderate positive correlation with the duration of diabetes, but there was no correlation with the ages of onset of diabetes. The positivity of GAD antibody also correlated with the

duration of diabetes and IAA was negatively correlated, which not statistically significant. Although, thyroid dysfunction is reported to be high in type 1 diabetes,[9] we could not find any positive correlation of anti-TPO antibody with any other disease specific antibody. However, increasing age of onset but not the duration of disease correlated with anti-TPO antibody positivity.

Autoantibodies directed against other organ systems are common in T1DM. Studies published from this country are sparse and that too lacks data for organs beyond thyroid.[10] Autoantibodies against thyroid are the commonest as was noted in earlier studies.[11,12] Anti-TPO antibody is much commoner compared to the anti-thyroglobulin antibody.

Anti-ovarian antibody has been tested in various situations including autoimmune variety of premature ovarian failure. However, this has not been commonly tested especially in T1DM. We have tested anti-ovarian antibody in female subjects with T1DM and found to have no one with positive results.

The prevalence of Addison's disease in T1DM is about 0.5%[13] and studies from this country earlier had noted a prevalence of 1.3%.[12] We have found that the prevalence of 21α hydroxylase antibody is 2.1%, which is consonant with the observation from an earlier study from this country.[12]

Another common association of T1DM with autoimmune disease is celiac disease. Studies done earlier from this country had shown a 34.1% prevalence of celiac disease associated anti-tissue transglutaminase IgA antibody in subjects with T1DM.[14] Another study from the country had shown a prevalence of 11.1% of celiac disease associated autoantibody in T1DM.[15] We appreciate that, before declaring a patient to be negative for celiac antibody by IgA, anti-tTG, total IgA deficiency must be excluded. However, for positive cases this assumption is not valid. We found a prevalence of 22.8% positivity of anti-tissue transglutaminase IgA antibody in our population. Although this is a limitation of this measurement, it may actually under-estimate the true prevalence but will not overestimate it. However, celiac disease was clinically manifested in only one patient.

Conclusion

Our study has shown T1DM is associated with a high prevalence of autoantibodies and antibody negative T1DM is rare in our population. The association with other organ specific antibody (especially thyroid and adrenal glands) and celiac disease is substantial. Screening of other organ specific antibody e.g. anti-TPO antibody, anti-thyroglobulin antibody, anti-tissue trans-glutaminase antibody IgA (tTG IgA), and anti-21α hydroxylase antibody may also be useful in this population at a risk for other auto immune disease.

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Conflicts of interest

There are no conflicts of interest.

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