Original Article

The seroprevalence of immunoglobulin A transglutaminase in type 1 diabetic patients of South Indian origin

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A B S T R A C T

Context: Celiac disease (CD) is a commonly encountered autoimmune condition in patients with type 1 diabetes (T1D). There is sparse data on the seroprevalence of immunoglobulin A (IgA) transglutaminase (tTG) in T1D patients of South Indian origin. **Aims:** To detect the prevalence of IgA tTG in T1D patients of South Indian origin. To evaluate the relation between the presence of autoimmunity and metabolic control and complications of diabetes. **Materials and Methods:** We conducted a cross-sectional study on 258 T1D patients. All the patients were subjected to biochemical tests and evaluated for microvascular complications. IgA tTG was estimated by ELISA. IgA tTG levels >40 AU/ml was considered positive. **Results:** Of the 258 participants, 12 (4.65%) were found to be positive for IgA tTG antibodies. Distribution of IgA positivity was equal in both sexes. There was a significant negative correlation of IgA tTG positivity with hemoglobin and glycated hemoglobin (HbA1c). **Conclusions:** The seropositivity of CD in South Indian patients with T1D has been observed to be 4.68%. This is much lower compared to studies from North India. This can be explained by both the genetic and dietary factors. The seropositivity correlated negatively with hemoglobin and HbA1c.

Key words: Autoimmunity, celiac, gluten, malabsorption, type 1 diabetes

INTRODUCTION

Celiac disease (CD) is a commonly encountered autoimmune condition in patients with type 1 diabetes (T1D). The prevalence of CD in T1D in various study ranges from 3% to 16%.[1-5] Nearly, 98% of the patients with CD express human leukocyte antigen (HLA)-DQ2 or DQ8 haplotype.[6,7] Around 10–15% of patients with T1D express the highly specific serological markers of celiac CD including autoantibodies to endomysium or tissue transglutaminase (tTG). The majority of the patients are asymptomatic or do not have symptoms severe enough to seek medical attention. The co-occurrence of both diseases can be explained by a shared genetic background and similar trigger mechanisms for the autoimmune processes.[8-11]

The seropositivity of immunoglobulin A (IgA) tTG in T1D patients of North Indian origin has been reported to be 8–22%.[12,13] However, there is sparse data on the seroprevalence of IgA tTG in T1D patients of South Indian origin. We have undertaken this study to detect the prevalence of IgA tTG in T1D patients of South Indian origin and to evaluate the relation between the presence of autoimmunity and metabolic control and complications of diabetes.

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Access this article online

Quick Response Code:

Website:
www.ijem.in

DOI:
10.4103/2230-8210.176359

MATERIALS AND METHODS

The present cross-sectional study was conducted at two centers - Vydehi Institute of Medical sciences and Research Centre, Bengaluru, and Bangalore Diabetes Hospital, Bengaluru. The study was conducted over a period of 2 years, from August 2013 to July 2015. A total of 258 consecutive patients who were diagnosed with diabetes before the age of 18 years, as per the American Diabetes Association criteria, and WHO were classified as T1D based on clinical grounds were recruited for the study.

Exclusion criteria
• Patients with acute illnesses
• Patients with diabetic ketoacidosis
• History of alcohol abuse
• Evidence of pancreatic calcifications
• Patients with other known causes of malabsorption.

A detailed history was taken with emphasis on dietary habits, along with a comprehensive physical examination with emphasis on anthropometry. All patients were subjected to biothesiometry, fundus examination, and biochemical tests which included fasting plasma glucose, glycated hemoglobin (HbA1c), serum calcium, serum albumin, thyroid stimulating hormone, and urine microalbumin estimation. Written informed consent was taken from the parents or from the patient as deemed appropriate, based on the age of the patient. Institutional ethical committee clearance was taken from both the centers.

IgA *t*TG was estimated using commercially available ELISA kits from Diametra Diagnostics, Italy.

Principle of the test
Antitissue *t*TG IgA test is based on the binding of serum or plasma antibodies on the human recombinant tissue *t*TG coated into the microplates. An anti-human IgA horseradish peroxidase conjugate solution recognizes IgA class antibodies bound to the immobilized antigens. A chromogenic substrate solution containing 3,3',5,5'-tetramethylbenzidine is dispensed into the wells. The solution's color changes into yellow. The amount of color is directly proportional to the concentration of IgA antibodies present in the original sample. IgA *t*TG value >40 AU/ml was considered as positive.

Statistical analysis
Data were reported using mean and standard deviation for the continuous variables, number, and percentages for the categorical variables. Independent *t*-test or Mann-Whitney U-test was used to compare the mean values of various clinical parameters between IgA *t*TG positive and negative groups. Spearman's Rank correlation was performed to assess the relationship between variables. Logistic regression analysis was performed to assess the factors associated with IgA positivity. All the analysis were analyzed using SPSS Inc. Released 2009. PASW Statistics for Windows, Version 18.0. Chicago. $P < 5\%$ was considered statistically significant.

RESULTS

The study was carried out from August 2013 to July 2015 and included a total of 258 patients of T1D. The male to female ratio was 1.13 (137 males and 121 females). The mean duration of diabetes was 4.88 years (range: 0.6–18 years). Diabetic retinopathy was present in seven participants and diabetic peripheral neuropathy in one participant. None of the patients had diabetic nephropathy. Concomitant primary hypothyroidism was present in 19 participants, and all of them were on replacement with thyroxine. A positive family history was seen in one participant, where the father had T1D. The baseline anthropometric and biochemical characteristics of the study cohort are shown in Table 1.

Of the 258 participants, 12 (4.65%) were found to be positive for IgA *t*TG antibodies [Figure 1]. Distribution of IgA positivity was equal in both sexes with six positives in each group. None of the positives had symptoms suggestive of CD.

There was a significant negative correlation of IgA *t*TG positivity with hemoglobin [Table 2]. A negative correlation was also seen with HbA1c levels. Comparison between the two groups of IgA positivity and negativity showed patients with positive IgA *t*TG levels had lower hemoglobin (−0.125) and HbA1c (−0.161) [Table 3]. In multistep regression analysis, the same two variables were found to have significant association with IgA *t*TG positivity [Table 4].

Table 1: Baseline parameters of the study group

	Mean±SD
Age (years)	14.33±4.54
Duration of diabetes (years)	4.88±4.64
Height SDS	−0.89±1.08
Weight SDS	−0.78±1.09
Hb (g/dl)	12.75±1.63
FPG (mg/dl)	152.38±30.43
HbA1c (%)	10.12±2.23
Serum creatinine (mg/dl)	0.75±0.17
Urine microalbumin (mg/L)	14.6±2.57
TSH (mIU/L)	3.16±2.03
Serum calcium (mg/dl)	9.04±0.29
Serum albumin (g/dl)	3.87±0.201

SDS: Standard deviation score, Hb: Hemoglobin, HbA1c: Glycated hemoglobin, FPG: Fasting plasma glucose, TSH: Thyroid stimulating hormone, SD: Standard deviation
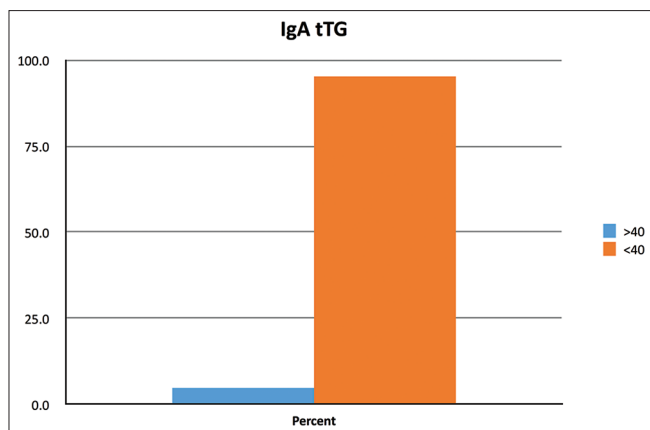
Figure 1: Seropositivity of immunoglobulin A transglutaminase in the study group

DISCUSSION

The present study is among the largest studies done on the prevalence of IgA tTG in patients with T1D in India. A seroprevalence of 4.68% has been seen in this study.

The global prevalence of CD in T1D, based on seroprevalence of IgA tTG, varies from 4.4% to 20.8%,[14-22] with the highest positivity seen in a study from Turkey. In previous studies from India, the positive seroprevalence for IgA tTG in T1D varied from 8.0% to 22%.[23-26] Most of these studies have been reported from North India. A positive seroprevalence of 14.9% was seen in a study by Tandon *et al*.[23] In a study by Bhadada *et al*.,[12] a positive seroprevalence of 11.1% was seen. In a more recent study done by Dayal *et al*.,[13] a positive seroprevalence of 22% was seen. In a study in west India by Joshi and Madvariya,[25] a positive seroprevalence of 15.49% was noticed. In a previous South Indian study by Jacob and Kumar,[26] a positive seroprevalence of 8% was seen using a low tTG cut-off of 7. A multi-center study by Kota *et al*.[27] including patients from the Eastern and Southern parts of India showed a positive seroprevalence of 12%.

The low seroprevalence of IgA tTG in our study population compared to similar studies in other parts of India can be attributed to both genetic and environmental factors. The predominant HLA gene in T1D patients from North India is HLA-DR3, which is strongly associated with the DQ2 haplotype.[28] The HLA DR3-DQ2 haplotype had a strong association with CD.[29] Whereas, there is no significant difference in the prevalence of HLA DR3 in South Indians compared to North Indians, the prevalence of DR3-DQ2 haplotype was found to be significantly lesser in the South Indian population.[30] The current study population comprised predominant ragi or rice eating population, as compared to the predominant wheat

Table 2: Correlation between immunoglobulin A tissue transglutaminase positivity and anthropometric and biochemical variables

	Correlation coefficient#	*P**
Height SDS	0.032	0.611
Weight SDS	0.082	0.191
Hemoglobin	−0.125	0.045
FPG	0.079	0.206
HbA1c	−0.161	0.01
Serum calcium	−0.51	0.412
Serum albumin	0.090	0.14
Urine microalbumin	−0.024	0.706
TSH	−0.005	0.930

#Spearman's Rho, *P<0.05 is considered significant. SDS: Standard deviation score, Hb: Hemoglobin, FPG: Fasting plasma glucose, HbA1c: Glycated hemoglobin, TSH: Thyroid stimulating hormone

Table 3: Comparison of parameters between immunoglobulin A tissue transglutaminase positive (>40) group and negative (<40) group#

	IgA tTG positive	IgA tTG negative	Z-score	*P**
Height SDS	−1.03±0.94	−0.82±0.96	−0.733	0.464
Weight SDS	−0.88±1.09	−0.78±1.10	−0.331	0.741
Hb (g/dl)	11.39±1.22	12.81±1.62	−1.418	0.003
FPG (mg/dl)	158.17±24.76	152.1±30.70	6.065	0.50
HbA1c (%)	8.87±1.59	10.19±2.26	−1.32	0.045
Urine microalbumin (mg/L)	15.17±1.85	14.57±2.6	0.59	0.436
TSH (mIU/L)	2.52±1.30	3.19±5.69	−0.67	0.682
Serum calcium (mg/dl)	8.92±0.23	9.05±0.29	−0.13	0.132
Serum albumin (g/dl)	3.85±0.08	3.87±0.20	−0.059	0.642

#Mann–Whitney's U-test was used for nonparametric data, and independent *t*-test was used for parametric data. *P<0.05 is considered significant. Hb: Hemoglobin, HbA1c: Glycated hemoglobin, FPG: Fasting plasma glucose, SDS: Standard deviation score, TSH: Thyroid stimulating hormone, tTG: Tissue transglutaminase, IgA: Immunoglobulin A

Table 4: Multi step regression analysis indicating predictors of positive IgA tissue transglutaminase levels (>40)

	B	SE	*P*
Height SDS	−0.242	0.332	0.466
Weight SDS	−0.115	0.256	0.652
Hb	−0.538	0.248	0.030
HbA1c	−0.302	0.147	0.040
Constant	−8.152	7.728	0.291

*P<0.05 is considered significant. SE: Standard error, SDS: Standard deviation score, Hb: Hemoglobin, HbA1c: Glycated hemoglobin, IgA: Immunoglobulin A

eating population in the previous studies from other parts of India. This can explain the lower prevalence of seropositivity for CD in our study. None of the participants in our study had any symptoms suggestive of CD. Routine screening for CD in patients with T1D has been recommended at the time of diagnosis and every 1–2 years thereafter by ISPAD.[31] Considering the low seroprevalence of IgA tTG positivity in our study, the utility of the recommendation in South Indian T1D patients may need to be explored further.

A positive seroprevalence of IgA tTG antibodies was associated with low levels of hemoglobin. Previous studies have reported a prevalence of anemia in 12–69% of cases of CD.[32] It is mainly due to iron deficiency and is typically refractory to oral iron treatment.[33] Other causes of anemia include folate and B12 deficiency.

The relationship between glycemic status and CD in T1D is complicated. On one hand, the erratic absorption of glucose can lead to periods of high glycemic variability. This can manifest as an increased HbA1c.[34] On the other hand, patients are typically prone to recurrent hypoglycemic episodes caused by decreased absorption of carbohydrates.[35] The finding of low HbA1c levels with seropositivity can be explained on this basis. Similar findings have been observed in previous studies which have shown increased prevalence of hypoglycemia in T1D patients with CD compared to those without CD.[36]

The strengths of our study include the large sample size and a study population that had belonged to the same ethnic background having similar dietary habits. A higher cut-off for IgA tTG was taken in our study for improving the specificity. The absence of concomitant IgA measurements and the unwillingness of IgA tTG positive participants to undergo duodenal biopsy are the drawbacks of our study.

CONCLUSION

The seropositivity of CD in South Indian patients with T1D has been observed to be 4.68%. This is much lower compared to studies from North India. This can be explained by both genetic and dietary factors. The seropositivity correlated negatively with hemoglobin and HbA1c.

Conflicts of interest
There are no conflicts of interest.

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