Article published online: 2022-01-10

Prevalence of Latent Autoimmune Diabetes in Adult Based on the Presence of GAD 65 Antibodies in North-Eastern Uttar Pradesh, India

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ANAMS (India) 2022;58:17–21.

Abstract

Objective The objective of this paper was (1) to study the prevalence of latent autoimmune diabetes in adult (LADA) in the region of north-eastern Uttar Pradesh, India, based on the positivity for glutamic acid decarboxylase 65 (GAD65) antibodies and (2) to compare the glycemic profile between GAD65-positive and GAD65-negative subjects.

Materials and Methods The subjects were of more than 30 years of age, with either recently diagnosed pre-diabetes/diabetes presenting with the hemoglobin A1c (HbA1c) level of $\geq$5.7% or already diagnosed cases of type 2 diabetes mellitus (T2DM) who had no requirement of insulin therapy for at least 6 months from the time of their diagnosis. All the patients were natives of north-eastern Uttar Pradesh. The GAD65 test was done by the enzyme-linked immunosorbent assay. Further, the glycemic status of GAD-positive and GAD-negative subjects were compared on the basis of fasting blood sugar (FBS), fasting insulin (FI), and homeostatic model assessment for insulin resistance (HOMA-IR). The "unpaired *t*-test" was used to compare and assess the significance of differences between the glycemic profile of GAD65-positive and GAD65-negative subjects using the GraphPad Prism Scientific Software, San Diego, CA, United States. The *p*-value of <0.05 was considered to be significant.

Results A total of 77 patients were included in the study, with the age group ranging from 30 to 75 years (47.81 ± 12.9 years) with the male–female ratio of 1:2.6. The prevalence of LADA was found to be 51.95%. On comparing GAD65-positive and GAD65-negative groups, a higher value of HbA1c levels and FBS were found in the former, whereas FI and HOMA-IR were found to be higher in the latter. On testing for significance of difference, only FI and HbA1c values were significant (*p*-value <0.0001).

Conclusion LADA can no longer be considered a rare type of diabetes mellitus, with the present study showing a high prevalence of LADA in this north eastern region of

Keywords
- type 1.5 diabetes
- slowly evolving immune-mediated diabetes of adult
- double diabetes
- GAD65 antibody
- insulin

published online
January 10, 2022

DOI https://doi.org/10.1055/s-0041-1741063.
ISSN 0379-038X.

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Uttar Pradesh. Identification of adult-onset diabetics accurately as LADA or true T2DM is very crucial for the appropriate treatment, as LADA patients require insulin inevitably and much earlier than true T2DM patients, who can be managed mostly on oral hypoglycemic agents with seldom requirement of insulin.

Introduction

"Latent autoimmune diabetes in adult (LADA)" was introduced in the medical literature by Tuomi et al in 1993.[1] Since then, it has been popularly known as type 1.5 diabetes or double diabetes. Now, it has been renamed as slowly evolving immune-mediated diabetes of adult by World Health Organization (WHO).[2]

LADA is defined by the presence of at least one of the islet cell autoantibodies detected in the serum, associated with adult age at diagnosis (>30 years) and no requirement of insulin at least for 6 months after the initial diagnosis. β cell failure can occur within 5 years of diagnosis, when multiple numbers of autoantibodies are present against islet cells, while in individuals with only glutamic acid decarboxylase antibodies (GADA) or only islet cell antibodies (ICAs), the development of β cell failure occurs after 5 to 12 years from the onset of the disease.[3]

GADA is invariably the persistent autoantibody that is seen in LADA as compared with other autoantibodies; therefore, GADA is the most important test to differentiate LADA from true type 2 diabetes mellitus (T2DM). Moreover, the levels of GADA are said to be inversely proportional to the levels of C-peptide which additionally serves to identify the extent of insulin dependency.[4]

LADA is often clinically misdiagnosed as T2DM because of its presentation in adulthood in contrast to type 1 diabetes mellitus (TIDM) that presents in children and adolescents, although etiopathogenetically it is an autoimmune disease like T1DM, albeit with a little difference.

Although controversies still exist in respect to its pathogenesis and treatment, LADA has become an interesting area of research that has been studied worldwide in the last decade or so.[5,6]

Various epidemiological studies from European populations have reported a prevalence of around 10%.[7] As compared with Caucasians, a lower frequency of LADA has been noted in studies conducted on the Asian population—United Arab Emirates (2.6%), China (5.7%), and Korean population (4.4–5.3%).[8–10] However, one study on the Japanese population has reported a high prevalence of 59%.[11]

A few epidemiological studies have been done from India as well where the prevalence has been reported to vary from 1.5 to 32%.[12–15]

India is referred to as the diabetes capital of the world.[6] Moreover, most of the LADA cases are usually misdiagnosed as T2DM, thus leading to either wrong line of treatment or delay in correct treatment; hence, accurate diagnosis of such cases becomes more imperative. Keeping this in mind, this study was done to estimate the prevalence of LADA among the patients that were undergoing the treatment for impaired glucose tolerance and T2DM at our institute which is a tertiary referral hospital in north-eastern Uttar Pradesh, India.

Materials and Methods

The current study was conducted in the Department of Pathology and Laboratory Medicine in collaboration with the Department of Medicine, over a period of 1 year.

Inclusion Criteria

The subjects were more than 30 years of age, with either recently diagnosed pre-diabetes/diabetes presenting with the hemoglobin A1c (HbA1c) level of ≥5.7% or already diagnosed cases of T2DM who had no requirement of insulin therapy for at least 6 months from the time of their diagnosis. All the patients were natives of north-eastern Uttar Pradesh.

Exclusion Criteria

The exclusion criteria were as follows.

- Patients in whom diabetes mellitus was diagnosed before 30 years of age.
- Patients ≥30 years of age in whom insulin therapy was required within 6 months of the diagnosis made.

Study Design

This was a cross-sectional study. A total of 77 patients of T2DM were studied for GAD65 autoantibodies to estimate the prevalence of LADA. Patients who showed GAD65 positivity were diagnosed as LADA, whereas patients who were GAD65 negative were diagnosed as true T2DM.

Methodology

The GADA test was done by using enzyme-linked immunosorbent assay (ELISA) (Kit by Bioassay Technology Laboratory, Shanghai, China). The reference value of 1.6 ng/mL (corresponding to 5U/mL) was considered as the cut-off level.

Standard curve range was 0.05 to 10 ng/mL; sensitivity was 0.021 to10 ng/mL.

Precision, coefficient of variation (CV [%]) = standard deviation (SD)/mean X 100, intra-assay: CV < 8%, inter-assay: CV <10%.

Other tests simultaneously performed included:

- Fasting insulin (FI) by the chemiluminescent microparticle immunoassay (Architect)

  Reference value: <25 mIU/L.[16]

- Fasting blood sugar (FBS) by the enzymatic glucose oxidase and peroxidase colorimetry

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Table 1 Comparison between GAD65-positive and GAD65-negative subjects

	GAD65 +VE $N = 40$	GAD65–VE $N = 37$	*p-Value*
Mean age (y)	46.4 ± 11.57	49.3 ± 14.4	0.3314
M:F	1:2.3	1:4.2	–
HbA1c (%)	7.47 ± 1.89	4.81 ± 1.33	0.0001[a]
FBS (mg/dL)	114.74 ± 31.34	109.8 ± 18.7	0.4084
FI (μIU/mL)	5.8 ± 4.2	8.3 ± 5.3	0.0241[a]
HOMA-IR	1.88 ± 1.84	2.4 ± 1.7	0.2028

Abbreviations: F, female; FBS, fasting blood sugar; FI, fasting insulin; HbA1c, hemoglobin A1c, HOMA-IR, homeostatic model assessment for insulin resistance, M, male.
[a]Significant.

Reference value according to American Diabetes Association:

Normal: <100 mg/dL, pre-diabetes: 100 to 125 mg/dL, and diabetes: ≥126 mg/dL[9]

and

- Homeostatic model assessment for insulin resistance (HOMA-IR), which was calculated by the formula—FI (μIU/mL) × FBS (mg/dL) /405.

Interpretation <1 = very sensitive to insulin; >1.9 = early insulin resistance; >2.9 = significant insulin resistance.[17]

Sample collection: 5 mL of venous blood was taken—3 mL in a plain vial for FI and GAD65 assay and 2 mL in fluoride vial for FBS.

All the data were expressed as mean ± SD. The **"**unpaired *t*-test**"** was used to compare and assess the significance of differences between GAD65-positive and GAD65-negative subjects using the GraphPad Prism Scientific Software, San Diego, CA, United States. The *p*-value of <0.05 was considered to be significant.

Results

A total of 77 patients were included in the study, the age group ranging from 30 to 75 years (47.81 ± 12.9 years). The majority of the subjects were females (i.e., 56/77) and minority were males (i.e., 21/77) with the male–female ratio (M: F) of 1: 2.6. The mean age among female patients was 41.3 ± 11.2 years and among male patients was 53.3 ± 15.7 years.

Forty out of seventy-seven patients showed positivity for the GAD65 antibody (51.95%), while the remaining 37 were found to be negative for the GAD65 antibody (48.05%). Among the 40 GAD65 antibody-positive patients, 28 were females and 12 were male patients (M:F= 1:2.3) . In the GAD65 negative group, 30 out of 37 were females and seven were males (M:F= 1:4.2).

On comparing the GAD65 positive group with the GAD65 negative group, the significance of difference for FI and HbA1c values was seen (*p*-value <0.0001) (►**Table 1**), whereas FBS and HOMA-IR were not found to be significantly different between the two groups.

Discussion

Nowadays LADA is gaining significant interest among clinicians and researchers because of its overlapping features with both T1DM andT2DM. Like T1DM, LADA is also an autoimmune disease leading to pancreatic β cells failure resulting in initially inadequate and ultimately no insulin production. However, it differs from T1DM in not requiring insulin at least 6 months after the diagnosis as the process of autoimmune destruction of β cell is comparatively slower. Another difference is that T1DM shows characteristic clustering of autoantibodies, whereas LADA mainly shows positivity for GADA/GAD65 antibodies.[4,5,18]

LADA is usually misdiagnosed as T2DM because of presentation /onset at higher age group (>30 years) and ultimate development of insulin resistance, but LADA differs from classical T2DM with respect to lower body mass index, better lipid profile, worse glycemic profile, lesser insulin resistance, lower insulin levels, and earlier requirement of insulin [18]

Genetically too, LADA shows overlapping features with T1DM and T2DM. Alike T1DM, it is closely linked to HLA gene complexes that increase the risk of T1DM and is also seen to be associated with PTPN22, INS, and SH2B3. Alike T2DM, it shows association with transcription factor 7- like 2 and zinc finger MIZ type1 aberrations.[5,19–22]

The prevalence of LADA observed all over the world varies, may be because of heterogeneity of the disease. Studies reported on Indian populations too have shown unduly varied results ranging from very low to fairly high prevalence.[12–15]

We attempted to study the prevalence of LADA in North Indian population, particularly in this region of north-eastern Uttar Pradesh. Diagnosis of LADA was based on the positivity for anti-GAD65 antibody. The comparison of glycemic profiles between GAD65-positive and GAD65-negative subjects was performed.

A high prevalence of LADA (51.95%) was found in our study similar to the study done by Takagi et al in Japan in 2018, who also reported a high prevalence of LADA (59%). However, this was quite high when compared with most of the studies done in India as well as other parts of Asia, where

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till date, the maximum prevalence has been reported to be 32%. [12–15]

The discrepancy seen may be because of:

- A small sample size (77 patients) in this study as compared with a few of the studies where the research was done on larger sample sizes.[13,23–25]
- In the current study, we solely relied on GAD65 measurement to determine the prevalence of LADA patients. Testing for other ICAs was not done. However, some of the studies done elsewhere also considered a co-positivity of IA-2/ICA and/or ZnT8 autoantibodies along with GADA for the detection of LADA. This may have resulted in the comparatively lower positive prevalence reported in those studies.[8,26,27]
- Being an institutional-based study, only small population of selective subjects with altered HbA1c levels was enrolled. This may have resulted in a spuriously higher prevalence seen in our study as compared with the community-based studies done in India as well as elsewhere on considerably large sample sizes.[12,28]
- GADA estimation method—In this study, we used ELISA. Although most of the recently done studies too have used ELISA, but the variation in sensitivity and detection range from one kit to another is a well-known fact. Also, a few other studies quoted used other methods for GAD65 estimation like radio-binding assays, etc.[26,28]
- Ethnicity—Studies reporting the prevalence of LADA on the Indian population show a wide range of prevalence. Sachan et al in their study done in north India have reported the prevalence of 1.5%; Brahmkshatriya et al in Western India have reported the prevalence of 5%; Unnikrishnan et al and Guntaka et al in South India have reported the prevalence of 25.3 and 32% respectively. These marked differences seen may be quite possible due to different ethnicity based on different lifestyles, dietary habits, environmental factors, and genotypic and phenotypic characteristics.[12–15,29]

The prevalence of LADA in our patients was highest among the age group of 30 to 50 years (57.41%). With progression in age, it was observed that the LADA prevalence subsequently went down. This is in concordance with the study done by Kumar and de Leiva, who also documented a higher prevalence in the individuals below 40 years of age (13.9%) compared with elderly patients.[5]

On comparing the glycemic profile between the two groups, significantly higher values of HbA1c were found in GAD65-positive group, whereas significantly higher values of FI were seen in GAD65-negative group. This was similar to the study done by Zaharieva et al, where they have reported a significant difference in HbA1c and FBS values between LADA and true T2DM.[30] Carlsson et al too showed FBS and HbA1c levels to be on the higher side in GAD65-positive subjects and HOMA-IR levels to be more elevated in true T2DM than LADA subjects though no significant differences were found.[18]

In a slight contrast to these studies, Desai et al reported FBS, HbA1c, and HOMA-IR all to be on the higher side in LADA patients and all of these showed significant differences statistically between LADA and true T2DM.[31]

Conclusion

The high prevalence of LADA found in the diabetic patients in this study conducted in north-eastern Uttar Pradesh proves that LADA can no longer be considered a rare type of DM.

It also emphasizes the importance of identifying adult-onset diabetics accurately as LADA or as true T2DM from the therapeutic point of view since LADA patients require insulin therapy inevitably and much early as compared with true T2DM, who can be managed mostly on oral hypoglycemic agents with seldom requirement of insulin.

This study had its drawbacks too.

- Small sample size, being an institutional study done in a period of just 12 months and only on subjects showing HbA1c levels of $\geq$5.7%.
- The study design was cross-sectional; hence, no follow-up was done to see whether the patients diagnosed as LADA and true T2DM actually required different treatment regimes.
- Sole reliance on GAD65 antibodies, in classifying the subjects as LADA and T2DM. This is because a few studies have reported that approximately 5% of T2DM cases may show variable positivity for islet cell autoantibodies.[32]

We recommend further studies on larger cohorts using various study designs and research protocols for a better understanding of this clinically tricky entity that may guide the physicians in correct diagnosis and better management of these patients.

Funding
None.

Conflict of Interest
None declared.

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