# Auto-antibody profile in young diabetic adults

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**ABSTRACT**

**BACKGROUND**
Diabetes Mellitus (DM) is increasingly affecting young adults. Overlapping clinical features has been a great problem in diagnosing the subtype of diabetes . This cross-sectional study was conducted to determine the antibody profile and C-peptide levels in young adults with diabetes and its utility in diagnosing the subtype of diabetes.

**METHODS**
In this cross sectional study, blood samples from 53 diabetic patients between 15-40 years were collected and tested for Glutamic Acid Decarboxylase antibody (GAD), Insulinoma Antibody 2 (IA2), Islet Cell Antibody (ICA) and fasting C peptide levels. All antibodies were measured using ELISA kits and C peptide by ECLIA in Cobas 8000 auto-analyzer. Statistical analysis was done using the IBM SPSS version 20.0 software. Chi square test and Kruskal - Wallis tests were used to find the statistical significance of difference for categorical and continuous variables, respectively.

**RESULTS**
The study subjects were divided into Type 1 diabetes (28.3%), Type 2 diabetes (32.1%) and other types of diabetes (39.6%). In Type 1 diabetes the percentage of GAD, IA2 and ICA antibodies were 66.7%, 40%, 46.7% and in other types of diabetes 19%, 4.8%, 4.8%, respectively. Only ICA antibody (5.9%) was seen in Type 2 diabetes. Mean C peptide was lowest in Type 1 (0.61 ± 0.91) and highest in Type 2 diabetes (3.9 ± 2).

**CONCLUSIONS**
Auto antibody profile in different types of diagnosis was described. Auto-antibody profile and C peptide levels along with history and clinical features aids to diagnose the type of DM in the young.

**KEYWORDS**
Diabetes of the young, Auto antibody profile, Glutamic Acid Decarboxylase antibody (GAD), Insulinoma Antibody 2 (IA2), Islet Cell Antibody (ICA)

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## BACKGROUND

Diabetes mellitus, one of the fastest growing health challenges of the 21st century, has a major impact on the lives and well-being of individuals, families, and societies worldwide. Characterized by hyperglycemia, this group of metabolic disorder result from defects in insulin secretion, insulin action, or both.

According to data given by International Diabetes Federation (IDF), the prevalence of diabetes has risen significantly worldwide and also in China, India, and other Asian countries. This global increase may be due to population growth, ageing, urbanization and changes in lifestyle. The proportion of undiagnosed diabetes in adults is also very high (66.8%) in developing countries.[1]

Distinguishing the type of diabetes in patients, especially in the young age, is challenging due to overlapping features. Early diagnosis and classification is clinically important and has implications for prognosis and management. This could be a possible viable method to minimize complications and death.

A β- cell centric approach could help in classifying the type of diabetes. The autoimmune process, which proceeds to damage the beta cells, is marked by circulating auto-antibodies. Auto-antibodies against a variety of β- cell components including Glutamic Acid Decarboxylase (GAD65), Insulinoma Antigen-2 (IA-2), Islet Cell Antigen (ICA) are supposed to reflect the beta cell damage. These antibodies may even appear in the preclinical phase of diabetes.

Different auto- antibodies, or their combinations, could reflect different pathogenic pathways leading to a beta cell destruction of variable intensity.

Previous studies have reported a heterogeneous distribution of auto- antibodies in different age, gender and ethnic groups. Various studies from the west have shown that in adults, auto-antibodies can also appear in patients not classified as classical Type 1 diabetes.[2,3]

But there is limited data available on the immunological profile of youth onset diabetes mellitus in India.

It is with this background that the current study was conducted and our aim was to determine the auto-antibody profile in young diabetic adults and its utility in classifying these patients.

## MATERIALS AND METHODS

This cross sectional study was carried out in the department of Biochemistry, Amrita Institute of Medical Sciences, Kochi. The study was approved by the Institutional Ethics Committee in January 2020.

### Selection And Description Of Participants

The study was conducted on patients, aged 15-40 years, diagnosed with diabetes of less than 5 years duration who attended the hospital from January 2020 to December 2020. Patients with gestational diabetes and diabetes secondary to drugs were not included in the study. Based on the proportion of antibody ICA (61%) observed in an earlier publication[4] and with 20% allowable error and 95% confidence, the minimum sample size for the current study was estimated to be 61.

### Technical Information

All subjects fulfilled the American Diabetes Association (ADA) criteria for diabetes. The patients were grouped into Type 1 diabetes mellitus, Type 2 diabetes mellitus and Other types of diabetes based on their diagnosis and the samples were tested for Anti GAD 65, IA2A and ICA antibodies and fasting C peptide levels. Assay for Anti GAD antibody was done with "Anti- GAD ELISA (IgG)" kits manufactured by Euroimmun. IA2 antibody was assayed using the "Enzyme immunoassay for the determination of auto-antibodies to Protein Tyrosine Phosphatase IA2 in human serum" manufactured by Medizyme. ICA antibody was analyzed using "Human Islet cell antibody ELISA kit" manufactured by Bioassay Technology Laboratory. C peptide was assessed by ECLIA on Cobas 8000 immunoassay analyzer.

### Statistics

The statistical analysis was done using the IBM SPSS version 20.0 software (IBM SPSS, USA). For all the continuous variables the results are given as mean ± SD or Median (range) and for categorical variables as percentage. To test the statistical significance of the difference in the proportion of categorical variables between type 1, type 2 and other types of diabetes, Chi square test was used. To test the statistical significance of the difference in mean of continuous variable between more than two groups, Kruskal - Wallis test was used as the data was skewed. Bonferroni multiple comparison test was used for Pairwise comparison. A "p value" < 0.05 was considered statistically significant.

## RESULTS

The study sample consisted of 53 diabetic patients in the age group of 15-40 years. The mean age of the study group is 25.75 ± 6.96 years. There were 15 cases of Type 1 diabetes (28.3%), 17 cases of Type 2 diabetes (32.1%) and 21 cases of other types of diabetes (39.6%) which included 4 cases of Latent Autoimmune Diabetes of Adults (LADA), 15 cases of suspected Maturity Onset Diabetes of the Young (MODY) and 2 cases of pancreatic diabetes.

In patients diagnosed with Type 1 DM, 7 were females (46.7%) and 8 were males (53.3%).Type 2 DM comprised of 4 females (23.5%) and 13 males (76.5%). 3 males (14.3 %) and 18 females (85.7%) were present in other types of diabetes shown in Table 1.

| Type of diabetes | Gender | | Age (years) |
|---|---|---|---|
| | Female | Male | |
| Type 1 diabetes | 7 (46.7) | 8 (53.3) | 22.53 ± 6.696 |
| Type 2 diabetes | 4 (23.5) | 13 (76.5) | 27.35 ± 6.964 |
| Other types of diabetes | 3 (14.3) | 18 (85.7) | 27.33 ± 6.938 |
| Categorical data expressed as n (%) | | | |
| Continuous data expressed as Mean ± SD | | | |

**Table: 1 Characteristics of the study population**

Auto- antibody level in different types of diabetes is given in Table 2.

| Antibody | Type 1 DM | | Type 2 DM | | Others | | p value |
|---|---|---|---|---|---|---|---|
| | n | % | n | % | n | % | |
| GADA | 10 | 66.7 | 6 | 40 | 7 | 46.7 | 0.001 |
| IA2A | 0 | 0 | 0 | 0 | 1 | 5.9 | 0.001 |
| ICA | 4 | 19 | 1 | 4.8 | 1 | 4.8 | 0.001 |

GADA – Glutamic Acid Decarboxylase antibody

IA2A – Insulinoma Antigen 2 antibody

ICA – Islet Cell Antibody

**Table 2: Auto- antibody profile in different types of diabetes**

A statistically significant association was seen between all antibodies and the different types of diabetes. Combination of GAD and IA2 antibodies were positive for 2 patients (13 %), GAD and ICA for 1 patient (6 %) and IA2 and ICA for 2 patients (13 %). All three antibodies were positive for 2 patients (13 %) with type 1 diabetes. In other types of diabetes, GAD antibodies alone were positive for 3 patients (14%) (LADA) and ICA alone for 1 patient (4 %) (MODY). Combination of GAD and ICA was seen for only 1 patient (4 %) (LADA).

Patient characteristics in different types of diabetes are given in Table 3.

| Parameters | T1DM (n=15) | T2DM (n=17) | OTHERS (n=21) |
|---|---|---|---|
| Age at diagnosis (years) | 21.07 ± 6.40 | 26.59 ± 7.38 | 25.62 ± 6.01 |
| BMI (kg/m2) | 20.44 ± 5.50 | 24.87 ± 5.04 | 21.48 ± 5.41 |
| DKA | 5(33.3) | 0(0.0) | 4(19) |
| Symptoms | 12(80) | 13(76.5) | 17(81) |
| Acanthosis nigricans | 2(13.3) | 13(76.5) | 2(9.5) |
| Autoimmunity | 3(20) | 2(11.8) | 4(19) |
| Family history | 8(53.3) | 10(58.8) | 18(85.7) |
| Plasma glucose at diagnosis (mg/dl) | 418.32 ±139.73 | 360.55± 159.80 | 314 ±108.86 |
| HbA1c at diagnosis (mg%) | 11.75 ± 3.60 | 10.84 ± 2.83 | 11.04 ± 3.51 |

Categorical data expressed as n(%), Continuous data expressed as Mean±SD

Symptoms – Polyuria, polydypsia, weight loss

Autoimmunity – (anti TPO antibodies, ulcerative colitis)

BMI – Body Mass Index, DKA- Diabetic ketoacidosis

**Table 3 : Characteristics of patients and subtypes of diabetes**

A statistically significant association between Diabetic ketoacidosis (DKA) with different types of diabetes was found (p = 0.041). Acanthosis nigricans also had a statistically significant association with types of diabetes.(p = 0.001).

## DISCUSSION

The etiology of diabetes in young adults varies in different ethnic groups. In European Caucasians, T1DM is the predominant etiology[5,6] while T2DM forms the major subtype in many Asian populations.[7,8] In previous studies from India, the proportion of different types of diabetes in young adults had varied widely.[9-11] In the current study, only 15 patients(28.3 %) were diagnosed with T1DM and 17 patients with T2DM (32.10 %). 21 patients (39.6 %)with other types of diabetes constituted the majority of the study population

Consistent with the previous studies on gender and diabetes, male subjects were in excess compared to females in the current study.[12] Compared to patients with T2DM and other types of diabetes , T1DM patients were younger.

Autoimmunity in youth onset has been reported earlier from India and elsewhere.[13-17] Sahoo et al reported antibody positivity for 30% in young diabetic adults from North India. We assessed anti-GAD antibodies, ICA and IA2 antibodies in the study population. 26.4 % of subjects had elevated anti-GAD antibodies which was the highest followed by ICA in 17 % patients, followed by IA2A in 13.2 %. 37.7 % of the study population had at least one antibody positive. As compared to previous studies, the percentage was found to be more in our study.

In the current study highest prevalence of anti GAD antibody was observed in T1DM (66.7 %). Mehra et al[18] and Sahoo et al had similar observations in those diagnosed with T1DM. Lan et al and Goswami et al[19,20] have reported increased positivity of anti-GAD antibodies. Certain studies have shown that low titre GAD antibody may be positive in T2DM also[21,22] however, none of the patients with type 2 diabetes in the current study were positive for anti GAD antibodies. In a study done by Lohmann et al[23] on LADA patients, 68 % were positive for anti GAD antibody. We found that 4 patients (19 %) with LADA included in other types of diabetes were positive for anti GAD antibody. 6 patients (40 %) of T1DM patients in our study had elevated IA2 antibodies. Prevalence for IA 2 in T1DM was found to be lower than for GAD antibody. Other studies from India have shown a similar observation.[13,14]

A study from Czechoslovakia had reported a transient but highly prevalent presence of islet cell autoimmunity in MODY.[24] But we found 1 patient with suspected MODY (4.8 %) had elevated IA2 antibody in serum and no patients with Type 2 diabetes were positive for IA2 antibodies. The reactivity of ICA is proposed to consist of variable autoantigen-autoantibody reactions. Studies by Dhanwal et al[14], Mehra et al[18] and Lan et al[20] has shown an increased ICA positivity but in a study done in Carribean diabetic youths ICA were present in only 0.05 %. In our study, 7 patients with T1DM (46.7 %) and 1 patient with T2DM (5.9 %) and 1 patient with other types of diabetes (LADA) (4.8 %) had elevated ICA.

Only Dhanwal et al have analyzed all antibodies together for diagnosis of the type of diabetes in India. In their study, seven (14 %) patients showed presence of both anti- GAD and anti-ICA512/IA2 antibodies and five (9 %)

patients had both anti-GAD and insulin antibodies. Presence of all three pancreatic antibodies was observed in only three patients and anti GAD antibody was present in all antibody positive youth onset DM. In our study, a combination of GAD and IA2 antibodies were seen in 2 patients with T1DM, GAD and ICA in 1 patient with T1DM and 1 patient with LADA. Combination of IA2 and ICA was present in 2 patients with T1DM. All three antibodies were present only in two patients, both of them were T1DM.

Low values of mean C-peptide was observed in T1DM. Higher values were observed for patients with T2DM. The difference was statistically significant. Carina Torn et a[25], observed patients with autoimmune markers had a lower level of C-peptide, compared to non-autoimmune diabetic patients.

The clinical profile varied with the type of diabetes. 80 % of patients with type 1diabetes and 76.5 % patients with type 2 diabetes were symptomatic. 92.4 % GADA positive and 72.1 % GADA negative patients were symptomatic in a study by Katulanda et al[8]. Sahoo et al[13] observed that 95%, 55% and 94% of type 1, type 2 and FCPD patients had symptoms at diagnosis, respectively. In our study, the highest prevalence of ketosis and autoimmunity (anti TPO antibodies) were seen for T1DM patients followed by other types of diabetes. None of the patients with T2DM had DKA. Autoimmunity (ulcerative colitis and anti TPO antibody) was seen in 2 patients with T2DM. 81 % of patients with other types of diabetes had symptoms like polyuria, polydypsia and weight loss at diagnosis. The findings were similar to a study from North India where a higher prevalence of ketosis and autoimmunity were seen in T1DM patients and 7 % autoimmunity was seen in patients with T2DM.[13]

Prevalence of acanthosis nigricans was highest in patients with Type 2 diabetes (76.5 %) followed by type 1diabetes (13.3 %) and was least in other types of diabetes (9.5 %). In a study by Katulanda et al[8] 7.4% of GADA positive patients and 21.6 % of GADA negative patients had acanthosis nigricans.

Family history of diabetes was present in 53.3 %, 58.8 % and 85.7 % patients with type 1, type 2 and other types of diabetes (14 MODY, 2 LADA, 2 pancreatic dm), respectively. 26 % of T1DM, 60 % of T2DM and 38 % FCPD patients had a positive family history in a previous study.[13] Majority of patients with positive family history of diabetes grouped under other types of diabetes in the current study were suspected MODY patients (77 %).

The plasma glucose at diagnosis was higher in patients with T1DM across various studies.[7,13,14] Similar result was seen in the present study also. Hba1c at diagnosis was highest for T1DM and lowest for T2DM. Lower Hba1c was seen for type 2 diabetes in the study by Sahoo et al also.

**Limitation**

Lower sample size was obtained for the study as the patient load drastically decreased due to Covid -19 pandemic during the course of the study. Differences in the etiology of diabetes are likely between government and private hospitals, as well as in different regions of the country.

## CONCLUSION

By this cross- sectional study we have described the auto-antibody profile in young onset diabetes. We found that anti–GAD antibody was the most common antibody in young onset diabetes. Auto- immunity was more prevalent in Type 1 diabetes and multiple antibodies were positive in Type 1 diabetes. C peptide level was the highest in Type 2 diabetes and least in Type 1 diabetes. Auto-antibody profile along with history and clinical features aids to diagnose the type of DM in the young.

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