Journal of Diabetes and Its Complications 30 (2016) 1039–1042

Contents lists available at ScienceDirect

Journal of Diabetes and Its Complications

journal homepage: WWW.JDCJOURNAL.COM

# Islet cell-associated autoantibodies in Ethiopians with diabetes mellitus

Elias S. Siraj [a,*], Manjula K. Gupta [b], Helen Yifter [c], Abdurezak Ahmed [c], Tedla Kebede [c], Ahmed Reja [c], Jemal Abdulkadir [c]

[a] Lewis Katz School of Medicine at Temple University, Section of Endocrinology, 3322 North Broad St., Philadelphia, PA 19140, USA
[b] Cleveland Clinic, Department of Endocrinology, Diabetes & Metabolism, 9500 Euclid Avenue, Cleveland, OH 44195, USA
[c] Addis Ababa University, Endocrine Unit, PO Box 9086, Addis Ababa, Ethiopia

ARTICLE INFO

*Article history:*
Received 13 February 2016
Received in revised form 25 April 2016
Accepted 4 May 2016
Available online 7 May 2016

*Keywords:*
Islet cell antibodies
Ethiopia
Africa
Type 1 diabetes
GAD, ICA

ABSTRACT

*Background & aims:* Our understanding of the role of autoimmunity in the pathogenesis of diabetes in African populations is limited. This study aims to evaluate the prevalence of 4 different islet cell-associated antibodies in Ethiopian patients with diabetes and non-diabetic controls.
*Methods:* A total of 187 subjects from a diabetic clinic at an Ethiopian hospital were evaluated in a cross-sectional study. Fifty-five patients had type 1 diabetes mellitus (T1DM), 86 had type 2 diabetes mellitus (T2DM) and 46 were non-diabetic controls. Islet cell-associated antibodies were measured using 4 different assays for antibodies against islet cells (ICA), glutamic acid decarboxylase (GADA), insulin (IAA) and the protein tyrosine phosphatase-like IA-2 (IA-2A).
*Results:* Comparing the antibody positivity in subjects with T1DM versus T2DM, the results were as follows: 29% versus 3.5% for GADA; 21% versus 2.7% for ICA; 27% versus 16% for IAA. In the control group, the only positive result was for IAA at 2%. IA-2A was absent in all groups. The combi-assay for GADA and IA-2A detected all GADA-positive subjects. T2DM patients who were GADA positive had lower BMI, lower C-peptide levels and all of them were on insulin therapy.
*Conclusions:* Compared to Caucasians, Ethiopians with T1DM have less prevalence of islet cell-associated antibodies, but the rates are higher than in T2DM. GADA is present in Ethiopians, whereas IA-2A seems to be absent. GADA positivity in T2DM correlates with clinical features of T1DM, indicating the existence in Ethiopia of the subgroup, latent autoimmune diabetes in adults.



## 1. Introduction

The significance of autoimmunity in the pathogenesis of type 1 diabetes mellitus (T1DM) has long been established. The pathogenesis of type 2 diabetes mellitus (T2DM) on the other hand involves a combination of varying degrees of insulin resistance and relative insulin deficiency. But there is also increasing evidence now that autoimmunity to islet cell components is present in some individuals clinically diagnosed as T2DM. Most of these patients have been found to have several features of T1DM and the name latent autoimmune diabetes in adults (LADA) has been used to designate those (Fielding, Brophy, & Davies, 2007; Palmer, Hampe, Chiu, Goel, & Brooks-Worrell, 2005; Stenström, Gottsäter, & Bakhtadze, 2005). These findings raise the question whether there is a subset of T2DM that shows some features of autoimmunity or whether those are T1DM patients wrongly diagnosed as T2DM.

There are major geographic and ethnic differences in the occurrence of both T1DM and T2DM and their association with autoimmunity. Most studies dealing with the relationship between types of diabetes and autoimmunity were done in European or American populations (Atkinson & Maclaren, 1994). Despite some emerging literature in Africa dealing with autoimmunity and diabetes, our understanding still remains limited and incomplete (Elkadhi et al., 2002; Gill, Tekle, & Reja, 2011; Hawa et al., 2005; Lutale, Thordarson, & Holm, 2007; Magzoub, Abdel-Hameed, & Bottazzo, 1994; Oli, Bottazzo, & Doniach, 1981; Omar, Bottazzo, & Asmal, 1986; Panz, Kalk, Zouvanis, & Joffe, 2000; Peters, Lester, Kohnert, & Hildmann, 1986).

Traditionally, the islet cell antibody (ICA) assay has been used as the method of choice in assessing autoimmunity in diabetes (Knip & Akerblom, 1998). However, it is labor intensive, requires human pancreatic tissue and has poor precision and variable sensitivity in different laboratories. More recently, antibodies against glutamic acid decarboxylase 65 (GADA), antibodies against the tyrosine phosphatase-like protein IA-2 (IA-2A), and insulin autoantibodies

Disclosure statement: None of the authors had conflict of interest.

* Corresponding author at: Temple University School of Medicine, Section of Endocrinology, MOB, 3322 N. Broad St., Philadelphia, PA 19140. Tel.: +1 215 707 4746; fax: +1 215 707 5599.
*E-mail address:* elias.siraj@tuhs.temple.edu (E.S. Siraj).

http://dx.doi.org/10.1016/j.jdiacomp.2016.05.005
1056-8727/

(IAA) have been well studied as alternatives to ICA (Bonifacio & Bingley, 1997; Winter & Schatz, 2011). Furthermore, combi-assays for GADA and IA-2A have been shown to be as reliable but more convenient than single assays (Dittler, Seidel, Schenker, & Ziegler, 1998; Siraj, Rogers, Gupta, & Reddy, 2012; Wiest-Ladenburger et al., 1997).

The objective of this study was to investigate the role of autoimmunity in the pathogenesis of diabetes in Ethiopians and contribute to our understanding of the issue in African populations. Specifically, we used individual assays as well as the combi-assay for GADA and IA-2 and attempted to answer the following questions:

- What is the prevalence of the 4 autoantibodies [ICA, GADA, IA-2A and IAA] in the three groups of Ethiopians (T1DM, T2DM and controls)?
- How does the single-step combi-assay for GADA & IA-2A compare with individual assays for GADA and IA-2A?
- What is the concordance rate of GADA with ICA?
- In patients with T2DM, do those with positive antibodies demonstrate clinical features of T1DM?

## 2. Materials and methods

The study was performed by doing antibody assays on sera collected previously from Ethiopian subjects. Other studies from the same group of subjects were previously published (Seyoum, Siraj, Saenz, & Abdulkadir, 2008; Siraj, Seyoum, Saenz, & Abdulkadir, 2006; Siraj et al., 2002).

### 2.1. Subjects

A total of 56 subjects with T1DM and 97 subjects with T2DM (37 on insulin, 53 on oral agents, and 7 on diet alone) from the diabetes clinic of the Black Lion Hospital, Addis Ababa University, Ethiopia were included in the study. All consecutively seen subjects with either T1DM or T2DM, who volunteered to participate in the study and were available for laboratory testing, were included. Patients were classified as T1DM or T2DM, based on the WHO criteria (World Health Organization, 1985). Diagnosis of diabetes was made if FBG was ≥140 mg/dL or 2 hours post OGTT value was ≥200 mg/dL. Alternatively, random blood value of 200 mg/dL together with classic symptoms of diabetes such as polyuria, polydipsia etc. was used. The classification between T1DM and T2DM was primarily based on clinical characteristics, the most important of which was whether insulin requirement was seen at the time of diagnosis or not. T1DM patients did not respond to oral agents and required insulin. Most of them were not obese, had classic symptoms of diabetes and had severe hyperglycemia at the time of diagnosis.

In addition, 50 non-diabetic subjects were included as control subjects. These were healthy medical student volunteers ($n = 6$) or outpatients in the hospital being treated for minor medical problems ($n = 44$).

### 2.2. Collection of information

After obtaining informed consent from each subject, demographic information was collected by personal interview. Additional clinical information was collected from medical records. Blood pressure, height, weight, waist and hip circumferences were measured. Previously described techniques for measuring waist and hip circumferences were used. BMI and waist-to-hip ratio (WHR) were calculated.

Blood samples were collected from all the study subjects. After centrifugation of the blood samples, the sera were isolated and then frozen at −20 °C. Subsequently, the sera were transported to Germany and USA using dry ice-filled containers at temperatures around −70 °C. Within 24 hours, the specimens reached their destination and were kept frozen at −20 °C until the tests were performed.

### 2.3. Laboratory analysis

- **C-peptide assay:** The C-peptide assays were performed at the Endocrine Laboratory of the Center for Internal Medicine, University of Leipzig, Germany using radioimmunoassay kits from Diagnostic Systems Laboratories (Webster, TX).
- **Islet cell antibody (ICA):** ICA was determined at the Endocrine Laboratory of the Center for Internal Medicine, University of Leipzig, Germany using indirect immunofluorescence technique on human pancreas sections. For the purpose of this study, only those with unequivocal positive result were considered to be positive.
- **GADA and IA-2A:** Combined as well as separate GADA and IA-2A assays were performed on the sera at the Endocrine Laboratory of the Cleveland Clinic, USA. Both were done using radiobinding assays utilizing $^{35}$S-labelled GAD65 and IA-2. We have previously published the details of the assays used (Siraj et al., 2012).
- **Insulin auto antibody (IAA):** IAA was measured at the Endocrine Laboratory of the Cleveland Clinic, USA. It was done using a radiobinding assay utilizing 125-I labeled insulin (Amersham). We have previously published the details of the assays used (Siraj et al., 2012).

At the time of the study, zinc transporter 8 (ZnT8) antibody assays were not available and could not be tested.

### 2.4. Statistical analysis

From all subjects studied, complete data for analysis were available in 187 subjects (55 with T1DM, 86 with T2DM and 46 controls). Quantitative data were summarized by means and standard errors of mean (SEM). Categorical data were summarized using frequencies as well as proportions & percentages. Statistical analyses were performed using the non-parametric Chi-square test or Wilcoxon's rank sum test as appropriate.

## 3. Results

The key characteristics of the subjects in the study are described in Table 1. Subjects with T2DM were on average older than those with T1DM and control subjects. The mean duration of diabetes was longer in patients with T2DM than in patients with T1DM. Subjects with T2DM were relatively more obese (higher BMI) and had a greater degree of central obesity (higher WHR) than subjects with T1DM or controls. The mean basal C-peptide (BCP) level was lower in the T1DM group compared to that of T2DM and control subjects.

The prevalence of various antibodies in the 3 groups of subjects is described in Table 2. All antibodies except IA-2A were more prevalent

**Table 1**
Characteristics of subjects studied.

| | Type 1 DM $n = 55$ | Type 2 DM $n = 86$ | Controls $n = 46$ |
|---|---|---|---|
| Sex (male/female) | 34/21 [a] | 32/54 [b] | 29/17 [a] |
| Age, years | 29.9 ± 1.4 [a] | 52.1 ± 1.1 [b] | 28.5 ± 1.7 [a] |
| Duration of diabetes, years | 6.9 ± 0.8 [a] | 9.1 ± 0.7 [b] | - |
| Body mass index (BMI), kg/m2 | 20.2 ± 0.4 [a] | 24.6 ± 0.5 [b] | 19.9 ± 0.4 [a] |
| Waist-to-hip ratio (WHR) | 0.88 ± 0.01 [a] | 0.94 ± 0.01 [b] | 0.83 ± 0.01 [c] |
| Basal C-peptide level (BCP), nmol/l | 0.13 ± 0.04 [a] | 0.67 ± 0.04 [b] | 0.53 ± 0.05 [b] |

Data are shown as means ± standard error of mean (SEM). Different alphabetic superscripts identify significantly different values ($P < 0.05$) for each variable.