



Original Article

Autoantibodies in Type 1 Diabetes Mellitus Patients and Their Siblings in Taiz, Yemen; A Hospital Based Study

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ABSTRACT

Aims and objectives: Type 1 Diabetes Mellitus (T1DM) is characterized by the presence of auto-antibodies, self-reactive T-cells. Measurement of islet autoantibodies can assist in the diagnosis of the disease, and the detection of auto-antibodies in non-diabetic individuals indicate that they are at high risk of subsequent development of the disease.

Materials and methods: This study is a cross sectional study which aims to determine the frequency of anti-glutamic acid decarboxylase autoantibodies (GADA) and anti-insulin autoantibodies (IAA) in patients with T1DM and their siblings in AL-Thawrah Hospital, Taiz city, Yemen. It was conducted from June to December 2005. Blood samples were collected from 44 T1DM patients, 52 siblings and 16 controls. Sera were obtained from collected blood samples and were analyzed for GADA and IAA using the immunoradiometric assay.

Results: GADA was present in 16 out of 44 (36.3 %) patients, 3 out of 52 (5.7 %) siblings and 0/16 (0.0 %) of the controls. While, IAA was positive in 28 out of 44 (63.6 %) patients, 6 out of 52 (11.5 %) siblings and 0/16 (0.0 %) controls.

Statistical analysis: Differences between groups were assessed using Chi-square test. Statistical tests were performed using SPSS version 20 and *P*-value less than 0.05 was considered significant. Significantly high positivity of GADA in patients versus siblings and controls was noted ($P < 0.001$).

Conclusion: High positivity of IAA in patients versus siblings and controls was noted as well ($P < 0.01$ and $P < 0.001$, respectively).



Introduction

Diabetes mellitus (DM) is an important non-communicable disease. Universally, it is expected that 382 million people are suffering from diabetes with a prevalence of 8.3 %. It was reported that higher prevalence rates were estimated in North America and the Caribbean (11 %), followed by Middle East and North Africa (9.2 %) and Western Pacific regions (8.6 %).^{1,2}

Measurements of islet autoantibodies can aid the diagnosis of autoimmune diabetes and the discovery of such antibodies in non-diabetic individuals indicates a considerable increased risk for subsequent development of Type 1 Diabetes mellitus (T1DM).³ T1DM is an organ-specific autoimmune disease that results in irreparable breakdown in insulin secretion.⁴ Hyperglycemia emerges when 80–90 % of the islet β -cells disappear.⁵ T1DM is principally insulinopenic diabetes that can be sub-classified as autoimmune T1DM (type 1a) along with several associated autoantibodies or idiopathic T1DM (type 1b).⁶ T1DM is strongly associated with both cellular and humoral immune responses to insulin producing beta cells.⁷

Individuals at risk can be identified on the basis of the positivity for islet autoantibodies. Therefore, diabetes risk is greater among people with more than one islet autoantibody or with higher titer of such antibodies.⁸ Human leukocyte antigens (HLA) genes are the main genetic factor that controls T1DM, and they appear capable of modulating the initiation and development of β -cell autoimmunity.⁹ The universal nature of antibodies in glutamic acid decarboxylase (GADA) and islet cell antibodies (ICA) was observed after the medical diagnosis of T1D, in addition to highly variable concentrations for GADA.¹⁰ Studies in Caucasian populations have revealed an 80 % - 90 % occurrence of autoantibodies in patients newly diagnosed

with T1DM.¹¹ Similarly, related studies from Asia have also established a 83 % - 90 % occurrence of GADA in Japanese patients affected with T1DM.¹²

The mechanisms that determine the age-dependent effect of islet autoantigen specific autoantibodies are not fully comprehended. T1D is twice as prevalent among men younger than 20 years.¹³ However, this disparity between sexes is not easily elucidated by the diagnostic sensitivity of the autoantibodies. The notion that the diagnostic sensitivity differs with age and occasionally with sex has significant outcomes when using autoantibodies to predict T1D. Previous studies have shown a decline in prevalence for all autoantibodies with older age for both sexes. The diabetes duration-related decline in the levels and occurrence of autoantibodies were also observed. This study revealed a reduction in prevalence for all autoantibodies in both sexes affected by diabetes exceeding 7 years. An inverse correlation was confirmed between GADA, ICA and diabetes duration in patients with Type 1 diabetes.¹⁴

The autoantibodies detected at the initiation of T1DM include: Islet cell autoantibodies (ICAs), initially described in the 1970s; IAA; 64-kDa autoantibodies (64-KAs); insulin receptor auto-antibodies; carboxypeptidase-H auto-antibodies; heat shock protein (HSP) autoantibodies (identified in the 1980s), and GADA recognized in the 1990s. Afterwards, an array of different islet autoantibodies was identified comprising 51-kDa aromatic-L-amino-acid decarboxylase auto-antibodies, chymotrypsinogen-related 30-kD pancreatic auto-antibodies, glima 38 auto-antibodies, tyrosine phosphatase-like protein autoantibodies (IA-2As) and others.³ The present study aims to determine the frequency of GADA and IAA in patients afflicted with T1DM along with their relations in Taiz, Yemen.

Material and Methods

Inclusion criteria

For all evaluated groups, participants with good health and age more than 6 years were enrolled in this study.

Exclusion criteria

Non-healthy patients suffering from chronic diseases such as renal failure, heart diseases or hepatic diseases were excluded or volunteers with age less than 6 years.

Study population

Population consisted of three groups of Yemeni subjects from Taiz City. The patients group consisted of 44 T1DM patients, siblings group consisted of 52 unaffected siblings, while the non-diabetic controls group consisted of 16 individuals. This study was approved by ethics committee at Hodeidah University, Hodeidah, Yemen.

Data and samples collection

Data collected from patients with T1DM, their siblings and controls includes age, gender and duration of the disease. Blood samples were collected from the study population during the period June to December 2005 at AL-Thawrah Hospital, Taiz, Yemen. After obtaining the informed consent, about 5 mL of venous blood were drawn from T1DM patients, their siblings and control into plain tubes. Serum samples were separated within half an hour by centrifugation at 1500 g at room temperature for 5 min, the sera were then aliquoted and stored at -20°C until analyzed.

Detection of IAA and GADA by immunoradiometric assay

Measuring of IAA and GADA was done using commercial kits ^{125}I IRM assay (Beckman coulter company, France). Manufacturer's instructions were followed in calculating and converting the results into

arbitrary units by extrapolation from a standard curve. Positive results were considered when the antibody concentration exceeds 1.0 U/mL.

Statistical analysis

Differences between groups were assessed by Chi-square test. Statistical tests were performed using SPSS version 20 (SPSS Inc., Chicago, IL, USA). *P*-value less than 0.05 was considered significant.

Results

A total of 112 participants [44 patient (mean age 15.4 ± 6.12 years), 52 siblings (mean age 13.97 ± 6.3 years), and 16 controls (mean age 12.86 ± 5.32 years)] were participated in this study. The average of participant's age ranged between 6.3-46 years. The minimum and maximum age of the patients and siblings ranged between 8.3-52 and 6.6-35 years, respectively. The minimum and maximum age of the control group ranged between 6.1-25 years.

Frequency of anti-glutamic acid decarboxylase autoantibodies and anti-insulin autoantibodies in different groups

As indicated in table 1, the frequency of GADA was 16/44 (36.3 %), and 3/52 (5.7 %) and 0/16 (0.0 %) in the patients, siblings and controls, respectively. Statistical significant difference was detected in the frequency of GADA between patients and siblings and between patients and controls ($P < 0.001$ and $P < 0.01$, respectively). The frequency of IAA was 28/44 (63.6 %), 6/52 (11.5 %) and 0/16 (0.0 %) in patients, siblings and controls, respectively. Also, significant differences were observed in the frequency of IAA between patients and siblings and patients and controls ($P < 0.001$) for both.

Frequency of anti-glutamic acid decarboxylase autoantibodies and anti-insulin autoantibodies according to gender

A total of 44 (26 male and 18 female) patients were tested for GADA; it was found that 9/26 (34.6 %) of male patients and 8/16 (46.1 %) of female patients were positive for GADA as shown in table-2. In addition, a total of 52 siblings with GADA (30 males and 22 females) were found to have 3/30 (10.0 %) of males were positive, while females showed negative results 1/22 (4.5 %). The controls neither males nor females showed positive results as shown in table 2.

On the other hand, 44 patients (26 males and 18 females) were tested for IAA; it was found that 19/26 (72.2 %) of males and 10/18 (55.6 %) of females were positive for IAA. At the same time, 44 siblings (30 males and 22 females) were tested for IAA, 2/30 (6.7 %) of males and 4/22 (18.2) of females were positive for IAA, while in control group there is no any positive results detected in males or females (0.0 %) (Table 2).

Frequency of anti-glutamic acid decarboxylase autoantibodies and anti-insulin autoantibodies according to age group

In age group 1, 2/5 patients (40.0 %) were positive for GADA, 11/30 (36.7 %) were positive in the age group 2, and 3/9 (33.3 %) were positive in the age group 3. Whereas, there is no any positive case in the control group 0/16 (0.0 %). In siblings, the positivity was seen in 2 individuals out of 23 (8.6 %) in group 2 and also 2 individuals were positive out of 11 in group 3. Controls showed negative results in all age groups (Table 3).

The frequency percentage of IAA in patients based on age was, 3/5 (60.0 %), 16/30 (53.3 %) and 5/9 (55.6 %) in the age groups 1, 2 and 3, respectively.

The frequency percentage of IAA in siblings based on the age was 2/10 (20.0 %), 2/23 (8.7 %) and 1/11 (9.1 %) in the age groups 1, 2 and 3, respectively (Table 3).

Association between duration of disease and frequency of anti-glutamic acid decarboxylase autoantibodies and anti-insulin autoantibodies

The frequency of GADA in the duration interval (0-5) years of disease onset compared with the intervals (6-10) and (>10) which was 12/27 (44.4 %) versus 5/11 (45.5 %) and 0/6 (0.0 %) for GADA. On the other hand, the frequency of IAA in the same intervals was 19/27 (70.4 %), 8/11 (72.7 %) and 3/6 (50 %), respectively. No significant differences were observed in the frequency of GADA and IAA in the different duration intervals of the disease ($P < 0.063$ and $P < 0.398$, respectively) (Table 4).

Discussion

Diabetes mellitus is a group of metabolic disorders which occur as a result of defects in insulin secretion, insulin action, or both.⁶ Recently, diabetes is classified into two types (T1DM and T2DM) depending on the etiopathogenesis according to the American Diabetes Association. T1DM is categorized by a destruction of β -cell, whereas in T2DM there is a resistance to insulin action and an inadequate compensative response in insulin secretion. Presence of diabetes antibodies such as GADA, ICA and IAAs give the sign that T1D is an autoimmune process.¹⁴

T1DM is primarily insulinopenic diabetes that is subclassified as autoimmune type 1 diabetes (type 1a) or idiopathic (type 1b).⁶ It has generally been considered that autoantibodies function as only markers of disease and has no role in the pathogenesis. This is confirmed by the fact that, blocking of β -cell function in experimental models

does not affect the development of disease and the removal of immunoglobulin from T1DM patients has only a minimal effect on disease severity.¹⁵ Measurements of islet autoantibodies can assist in the diagnosis of autoimmune diabetes and the detection of such antibodies in non-diabetic individuals indicates that a significantly increased risk for subsequent development of T1DM.³ Relatives at risk can be identified on the basis of positivity for islet autoantibodies. Diabetes risk is higher among relatives with more than one islet autoantibody or with higher titer of such antibodies.⁸ HLA genes are the major genetic determinant of T1DM and they seem to be capable of modulating the initiation and progression of β -cell autoimmunity.¹⁵

As shown in table 1, the frequency of GADA in Yemeni patients was lower than that reported in Prague, Germany, Belgium, and Sweden, which were (46 %, 52 %, 68 %, and 72 %, respectively).¹⁶⁻¹⁹ However, the frequency of GADA in this study was higher compared to 34.1 % and 33.8 % in Syria and Turin, Italy, respectively.^{20,21} Studies in other countries such as Jordan, Brazil, Gaza strip and Denmark also exhibited higher frequency of GADA which were 49 %, 67.5 %, 76 % and 72 %, respectively compared with the results of this study.^{20, 22-24}

In this study, the frequency of IAA among T1DM was 63.6 % which is higher than that reported in Germany and Sweden which were 43 % and 56 %, respectively^{17, 25} and it was lower than 69 % in Britain, but it was consistent with that reported in Australian population.^{26, 27}

The difference in the frequency of GADA and IAA between that obtained in the current study and that reported in other studies from different countries may be due to different factors such as ethnicity, socioeconomic status, environmental conditions, variation in sample size (number

of patients in this study is small compared with other studies), type of the technique used and involvement of patients with long standing disease. Titres and frequencies of the different autoantibodies showed to be declined with time.²⁸

This study showed that 5.7 % of siblings were positive for GADA. This figure is lower than that reported in Syrian and Finland and Jordanian which were 20 % and 8.0 % and 23 % respectively.^{20, 29} A frequencies of approximately 5 % was reported in Australia, Canada and USA.^{18, 30}

This study also shows a frequency rate of 11.5 % for IAA in siblings which is higher than that reported in Finland 3.7 %.³¹ However, it is being much lesser than that reported USA (34.5 %).³²

Prior study reported 53.8 %, 32.2 % and 76 % frequency rates for GADA, ICA and IAA, respectively among 171 T1DM patients (71 males (41.5 %) and 100 females (58.5 %)). It was found that GADA and IAA were non-significantly more frequent in females and GADA and IAA were significantly more frequent in patients less than 20 years of age. Higher levels of ICA and IAA were observed in GADA positive (42.4 % vs. 20.3 %, $P= 0.003$) and (79.3 % vs. 72.2, $P = 0.3$), respectively compared to GADA negative.¹⁴

The frequency rate of GADA was higher in females (44.4 %) than in males (34.6 %). However, this difference was not statistically significant ($P<0.470$). This indicates that there is no relationship between gender of patients and positivity to GADA. This finding is in line with that observed in previous study in Syria (30.4 % of male versus 38.4 % of female patients, $P<0.4$) and in Jordan (51 % males versus 47.9 % females $P<0.7$).²⁰ Hanifi-Moghaddam et al, also reported a non-significant gender and positivity to GADA.³³ A previous study in Brazil, showed that the frequency of GADA was 53.8 % in women

and 32.3 % in men, but this difference was not significant ($P < 0.07$).³⁴

As shown in table 3, GADA occurred in 40.1 % of patients in the age group 1, in 36.7 % and 33.3 % in the age groups 2 and 3 respectively. While, IAA frequency was 60.6 %, 53.3 % and 55.6 % of patients in the same groups, respectively. These findings indicate that there is no significant relationship between the age of onset and the frequency of either autoantibodies (GADA and IAA) ($P < 0.926$ and 0.987), respectively. These findings are in agreement with previous studies which found that no significant association of GADA and IAA with age ($P < 0.82$ and $P < 0.77$, respectively).^{25, 34}

The most important risk factor for the development of T1DM is the expression of multiple anti-islet autoantibodies especially in the presence of loss of First Phase Insulin Response (FPIR) on intravenous glucose tolerance tests.³⁰ All prospective studies on relatives of DM1 patients have shown that the combination of two or more autoantibodies gives a higher positive predictive value (PPV) than any single autoantibody.³⁵ It has been reported that, the increase in PPV depends on the number and titer of autoantibodies.³⁵ First-degree relatives without any islet autoantibodies had a 5-years risk for T1DM of only 0.2 %³⁴ and the risk increased to 15 %, 44 % and 100 % when the relative was positive for one, two or three antibodies, respectively.^{35,37}

A 10-years diabetic risk of 52 ± 17 was reported with positivity to GADA and IAA.⁸ Although, there were differences in the frequency of GADA and IAA in the various duration intervals of the disease, but these differences were not statistically significant ($P < 0.063$ and $P < 0.398$, respectively). These findings are in concordance with that reported by Rodacki et al. (2004) who found that the frequency of

GADA in the age groups: 1-5, 6-10, 11 -15 and >15 years was 45.8 %, 42.1 %, 52 %, and 40 %, respectively.³⁴ There were no significant differences between groups ($P < 0.874$).

Conclusions

GADA and IAA were found to be higher in patients rather than the siblings. High significant positivity of GADA in patients versus siblings and controls ($P < 0.001$) was observed. As well as, high positivity of IAA in patients versus siblings and controls was noted ($P < 0.01$ and $P < 0.001$, respectively).

Compliance with Ethical Standards

This study was approved by ethics committee in Hodeidah University, Yemen and informed consent was obtained from volunteers before sample collections.

Conflict of interest

The authors have declared no any conflict of interests in this study.

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Table 1. Distribution of anti-glutamic acid decarboxylase autoantibodies and anti-insulin autoantibodies in the different groups

Group	GADA ⁺		IAA ⁺	
	No.	(%)	No.	(%)
Patients	16/44	36.3	28/44	63.6
Siblings	3/52	5.7	6/52	11.5
Controls	0/16	0.0	0/16	0.0

GADA; Anti-glutamic acid decarboxylase autoantibodies, IAA; Anti-insulin autoantibodies.

Table 2. Distribution of anti-glutamic acid decarboxylase autoantibodies and anti-insulin autoantibodies among patients, siblings, and control groups according to gender

Positivity		Patients		Siblings		Controls	
		Male	Female	Male	Female	Male	Female
GADA	Number	9/26	8/18	3/30	1/22	0/9	0/7
	%	34.6 %	44.4 %	10.0 %	4.5 %	0.0 %	0.0 %
IAA	Number	19/26	10/18	2/30	4/22	0/9	0/7
	%	73.1 %	55.6 %	6.7 %	18.2 %	0.0 %	0.0 %

GADA; Anti-glutamic acid decarboxylase autoantibodies, IAA; Anti-insulin autoantibodies

Table 3. Distribution of anti-glutamic acid decarboxylase autoantibodies and anti-insulin autoantibodies in patients, siblings, and control groups according to age group

		GADA ⁺		IAA ⁺	
		No.	(%)	No.	(%)
Patients	G1	2/5	40.0	3/5	60.0
	G2	11/30	36.7	16/30	53.3
	G3	3/9	33.3	5/9	55.6
Siblings	G1	0/10	0.0	2/10	20.0
	G2	2/23	8.6	2/23	8.7
	G3	2/11	18.2	1/11	9.1
Controls	G1	0/4	0.0	0/4	0.0
	G2	0/8	0.0	0/8	0.0
	G3	0/4	0.0	0/4	0.0

G1: age group 1(0-9 years), G2: age group 2 (10-19 years), G3: age group 3 (>19 years),
GADA; Anti-glutamic acid decarboxylase autoantibodies, IAA; Anti-insulin autoantibodies

Table 4. Frequency of anti-glutamic acid decarboxylase autoantibodies and anti-insulin autoantibodies in patients depending on duration of disease

Duration of the disease (years)	GADA ⁺		IAA ⁺	
	No.	(%)	No.	(%)
0-5	12/27	44.4	19/27	70.4
6-10	5/11	45.5	8/11	72.7
>10	0/6	0.0	3/6	50

GADA; Anti-glutamic acid decarboxylase autoantibodies,
IAA; Anti-insulin autoantibodies