A Study on the Presence of Islets Cell Autoantibodies in Non-Insulin Requiring Young Diabetic Patients

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Abstract: *Type 1 diabetes is hypothesized to pass through several stages where patients still do not need insulin but found to have serum autoantibody against islets cell. These patients were found to be in great risk of quickly developing insulin dependency. This study aims to find the presence of islets cell autoantibody in non-insulin requiring young diabetic patients of Bangladesh.*
To compare the Islets Cell Autoantibody (ICA) and Glutamic Acid Decarboxylase 65 (GAD 65) autoantibody level in the non-insulin requiring young diabetic patient and non-diabetic control group we have taken 120 non-insulin requiring diabetic patient and 60 age and sex matched non-diabetic control subjects. ELISA kit was taken from DRG inc.
There was moderately strong negative correlation between age of onset of diabetes mellitus and ICA level. But no correlation was found between age of onset and GAD 65 level. Therefore, we may conclude that ICA is present in young diabetic patient of our country and its value is negatively correlated with the age of the patient.
Key Words: *Type 1 diabetes, non-insulin, Islets Cell Autoantibody, Glutamic Acid Decarboxylase 65, correlation*

I. Introduction

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels. DM though previously considered less significant disease is now being seen as one of the most important threat to human health in 21st century. [1]

Especially in the last two decades there has been an explosive increase in the number of People diagnosed with diabetes worldwide. According to IDF, the global figure of people with diabetes has increased from 150 million in 2001 to 382 million in 2013. IDF has also predicted that number of diabetic patient will reach 592 million by 2035. According to IDF prediction there will be 55% increase of diabetic patient globally; whereas in the South East Asia region the increase will be 70.6% (IDF, 2013).

It was reported that Bangladeshis are more susceptible to develop obesity, diabetes, hypertension, and coronary artery diseases compared with other South Asian migrants (Indian, Pakistani) settled in United Kingdom.[2] This dangerous state is revealed with same importance when IDF estimated total diabetic patient in Bangladesh was more than 5 million with a prevalence rate of 5.52% in adult population. According to IDF statistics, total healthcare expenditures due to diabetes was more than 200 million USD in 2013 (IDF, 2013).

There are two main types of diabetes. Type I diabetes pathogenesis occurs primarily due to autoimmune-mediated destruction of pancreatic B-cell islets. This results absolute insulin deficiency and patients must take exogenous insulin for survival to prevent the development of ketoacidosis. The Expert Committee on the diagnosis and Classification of Diabetes Mellitus also mentioned idiopathic pathogenesis of type I diabetes mellitus (ADA, 2010).

Type II diabetes is characterized by insulin resistance and/or abnormal insulin secretion, either of which may be major determining factor. Type II diabetes patients do not dependent on exogenous insulin, but to control blood glucose level it may work as an important factor if this is not achieved with diet alone or with oral hypoglycemic drugs. Classifying diabetes is still elusive and nebulous; some study has suggested that a classification considering etiology is superior to clinical judgment. [3]

Young diabetic patient who is not insulin requiring at the time of diagnosis may need insulin and even become dependent on insulin for survival. [4] Islet-reactive T cells responding to multiple islet proteins have been found in Type 2 DM patients with or without islet cell antibodies, the historical hallmark of

islet autoimmunity. [5] Islet autoantibodies have historically been relied upon as indicators of the presence of islet autoimmunity in diabetes patients. The most common islet autoantibodies, which are islet cell autoantibodies (ICAs), Glutamic acid Decarboxylase (GAD) autoantibodies, insulinoma-associated antigen 2 (IA-2) autoantibodies, and insulin autoantibodies (IAA), are found in childhood Type I DM patients, and many of these patients demonstrate positivity for multiple islet autoantibodies. In fact, positivity for an increasing number of islet autoantibodies associated with a progressively greater risk of developing' insulin dependency. GAD autoantibodies and ICAs are much more common than insulin autoantibody (IAA), Insulinoma-associated antigen 2(IA-2), and zinc transporter 8 (ZnT8) autoantibodies.[6]

In the DAISY (The Diabetes Autoimmunity Study in the Young) cohort, 89% of children who progressed to diabetes expressed two or more autoantibodies. Age of diagnosis of diabetes is strongly correlated with age of appearance of first autoantibody and IAA levels. [7]

In today's clinical practice it has become increasingly difficult to distinguish type T1MD from T2DM in pediatric diabetic patient as many children with T1MD are overweight at diagnosis. Numerous recent publications note a significant proportion of physician-diagnosed T2DM youth with evidence of pancreatic autoimmunity, exemplifying the challenges in distinguishing between TlDM and T2DM. The clinical implications of the phenomenon of antibody positivity in phenotypic T2DM youth, also referred to as "latent autoimmune diabetes in youth" (LADY), and "hybrid diabetes," are unclear at present.[8]

In Bangladeshi population, previously done study has found that 60 percent of type 1 diabetes patient and thirty five: percent of type 2 diabetes patients were positive for Islets cell autoantibody (Chowdhury, 2011).

We undertook the present study to see the presence of Islets cell autoantibodies in non-insulin requiring young diabetic patients in Bangladeshi population and compare the findings with the non diabetic controls.

II. Objective

To see the ICI(Islets Cell Autoantibody) and GAD 65 (Glutamic Acid Decarboxylase) value of non-insulin requiring young diabetic patient.

III. Methodology

Type of study:
Cross-sectional analytical study

Place of study:
1. Outdoor patients' Department (OPO) of BIRDEM Hospital, Dhaka, Bangladesh.
2. Department of Immunology, BIRDEM.

Cases:
Non Insulin requiring diabetic mellitus patients of under 35 year of age.
Known diabetes patients fulfilling the diagnostic criteria (Fasting plasma glucose and 2h-Plasma glucose levels) set by WHO/ADA.
Criteria for the diagnosis of diabetes mellitus:
1. Fasting plasma glucose (FPG) ≥ 126 mg/dl (7.0 mmol/l). Fasting is defined by no
calorie intake for more than 8 hours.
2. Two-hour plasma glucose ≥200 mg/dl (11.1 mmol/l) during an OGTT. The test should be performed as described by WHO using glucose load containing the equivalent of 75gm anhydrous glucose dissolved in winter.

The diabetic patients under study who were selected were all registered in the outdoor patient department (OPO) of BIRDEM hospital. They had all full blown diabetes. Each of the patients under the study had a guide-book his/her own.

TABLE 1: Sample Distribution

Subjects	Number
Non-Insulin requiring young diabetic patients	120
Controls	60

Exclusion criteria:
General
1. Pregnancy
2. Any form of severe diseases,
3. Age: ≥35years;
4. Insulin required in previous 1 year.
5. History of ketosis in previous 1 year.

Controls:
Controls were taken from BIRDEM Outpatient department attending persons who carne as suspected diabetic patient based on previous random blood sugar test result! but found non-diabetic in OGTI test.

Characteristics:
1. Non-diabetic
2. Age less than 35 years.
3. No history of other autoimmune disease.
4. No history of other chronic disease.
Though statistically acceptable sample size was 384 but due to time and resource constraint we had to limit our sample size to 120. As all the samples are collected from the BIRDEM Hospital and in this age group more patients are Insulin requiring than not. So we have taken 60 controls in a ration of 2: 1.

Design
A total of 120 diabetic patients were enrolled for the ease-control study. 60 non-diabetic subjects were chosen as controls. The cases were registered patients in the Outdoor Patients Department (OPO) of BIRDEM Hospital. Controls were suspected diabetic patient who found non-diabetic in OGTT test. Systematic random sampling was done to collect the cases. Every alternate outdoor patient fulfilling the criteria was taken as case.

IV. Results

Age distribution of study subjects:
The study was done on 120 diabetic patient with the mean age of 27.3 years and 60 non-diabetic controls with the mean age of 26.9 years. As shown in Figure 1 the patient and controls were age matched in different age groups.
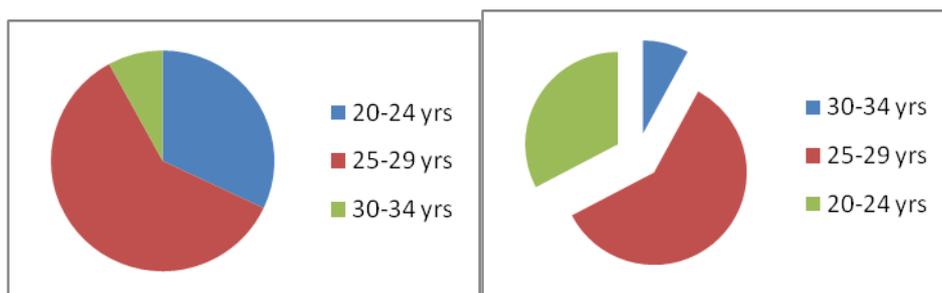

Figure 1-Age distribution of Patients and Controls

Gender distribution of the study subjects:
The study was done on 120 diabetic patients with male and female participants of 69/51 respectively and 60 non-diabetic controls with the male and female participants 36/24 respectively.

The difference of ICA values in between the subject and control:
As both the ICA 512 and GAD65 are semiquantitive test. These gave both a quantitive value and also a categorical positive and negative answer. Statistically significant difference was found in between the quantitive ICA values in the study subjects and the control population; p= 0.004 (<0.05) when equal variances of the both the samples was assumed.

Table 2: ICA Test statistics

	Mean ICA level	Standard deviation	P value	Mean Difference	95%CI
Patient	0.714	0.343	0.004	0.143	(0.046-0.239)
Control	0.571	0.227			

ICA: Islets Cell Autoantibody; CI: Confidence Interval

Categorical result of ICA:

Categorical result of the ICA in different age group shows that most of the ICA positive patients are from 20-24 age group. Also it shows that in 30-34 age group no ICA positive was in both patients and controls.

Table 3: Categorical value of ICA according to age category in patients and controls

Age Group	Type Participant	Categorical value of ICA		Total
		Positive	Negative	
20-24	Control	9	1	10
	Patient	18	21	39
	Total	27	22	49
25-29	Control	42	3	45
	Patient	69	4	73
	Total	111	7	1I8
30-34	Control	4	0	4
	Patient	9	0	9
	Total	13	0	13
Total	Control	55	4	59
	Patient	96	25	121
	Total	151	29	180

Association of age of onset of diabetes and ICA value:

The moderately strong negative association was found between age of onset of Diabetes mellitus and value of ICA level(r=-0.45). The significance of this correlation was p<0.001. Following figure shows that diagnosis age is negatively associated with the total value of ICA in patients.

Categorical analysis of ICA:

Analysis of categorical values have shown that there is statistically significant difference between non Insulin requiring young diabetes and non diabetic control in respect of Positive ICA result (p=0.015).

Difference in ICA autoantibody in different age group:

Different ICA autoantibody positivity was found in different age group. Only statistically difference is present in 20-24 age group (p= 0.013). In two other groups no statistically significant difference between patient and control was found. Most significant finding was that not a single case was found positive over 30 years of age in both patient and control group.

The difference of GAD 65 values in between the subject and control:

Statistically significant difference was not found in GAD 65values of non insulin requiring young diabetic patients and non diabetic controls (p = 0.32) when equal variances of both the samples, was assumed.

Association of age of onset of diabetes and GAD 65 value:

No correlation was found in between age of onset of diabetes mellitus and total GAD 65 values($r=-.176$). The significance of this correlation was $p<0.01$. Following figure shows that no association is present in diagnosis age of diabetes and total GAD 65 values.

V. Discussion

In this study we have found that ICA level has statistically significant difference between patient and the non diabetic controls. But previously done like verge et al (1989), concluded that ICA level has, the least significant result among all five autoantibodies they have used. This discordance between the works may be due the difference in sample collection. [9] As we have taken our subject from the non insulin requiring diabetic patients but they have taken type I diabetic patient as their subjects. This gives an insight that non insulin requiring and type I diabetic patient may have different autoantibody marker. Irvine et al (1977) found that duration of diabetes has strong correlation with Islets cell autoantibody level. But in this research no statistically significant correlation between duration of diabetes and autoantibody markers was found. Irvine et al (1977) also found that autoantibody has strong correlation with coexisting autoimmune disease. But we excluded any patient with other autoimmune disease. In this respect our study gives better logical standing as it is free of this bias. As one autoimmune disease increases chances other autoimmune autoantibody formation. So, the study which has taken patients with autoimmune disease includes a confounding variable.

VI. Conclusion

This study result showed us the difference of autoantibody profile among non-insulin requiring young diabetic patients and non-diabetic controls. This study also showed the different level of autoantibody in different age of onset. ICA positivity indicates the presence of antibody against common epitope of all Islets cell antigen. But statistically significant level of GAD65 was not found. This indicates other autoantibodies may be the cause of this positivity. Future studies should include other Islet cell autoantibody like IAA, IA-2, Zn 8.

Acknowledgements

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