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Original Research Article.

Association of Islets Cell Antibodies with Beta Cell Function in Type 2 Diabetic Patients of Bangladesh

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ABSTRACT

Background: A significant portion of type 2 diabetes patients may have the autoimmune condition hidden inside them for years and most of them may succumb to insulin dependence within a short span of time. There is no specific and established marker are available for differentiation of insulin dependent and non-insulin dependent diabetes.

Objective: The objective of the study was to assess the presence of ICA in T2D patients and positive for ICA at the time of diagnosis predicts deteriorating Beta-cell function which is measured by C-peptide.

Materials & Methods: It was a cross-sectional study. A total of 173 patients of newly detected T2D were enrolled in the study at, OPD, BIRDEM Dhaka from January 2015 to June 2016. Participants were divided into two groups. First, ICA positive antibody, and second, whom no ICA antibodies were detected. The patient's positive ICA antibody was selected to test the C-peptide level at the beginning and after a 6-month interval.

Results: In this study, 12.7% of T2D patients had been found ICA positive which was statistically significant (p<0.001). ICA positive T2D patient's mean value was 1.72±0.64 and ICA negative 0.55±0.16. The patient's positive for the ICA antibody was tested for the C-peptide level at the time of diagnosis and the mean C-peptide level was 8.20±4.78 ng/ml. After six months follow up the mean C-peptide was 3.93±3.38ng/ml. The changes of mean C-peptide at the time of diagnosis and after six months follow up was statistically significant (p<0.001) in ICA positive patient.

Conclusion: It can be concluded that deteriorating pancreatic β-cell function is reflected by a change in C-peptide level among patients with ICA positive T2D patients, who may need treatment with injectable insulin earlier.

Key Words: T2D, ICA, Peptide, Positive, Patients.

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INTRODUCTION

Diabetes Mellitus (DM) is a metabolic disorder with a continuous spectrum of symptoms and etiologies extending from a cell-mediated autoimmune disease of T1D to a non-autoimmune metabolic disorder namely, T2D.[1,2] The cell-mediated autoimmune pathology related with T1D has been anticipated for many years, however the immune cells involved in the β-cell demolition in humans and their β-cell targets have not yet been definitively identified. DM has proven to be one of the commonest non-communicable diseases that leads the largest contribution to morbidity and mortality worldwide. The lack of insulin or the inability of the cells to respond to insulin leads to hyperglycemia.

The onset of T2D is difficult to determine due to its progress slowly and the usual presentation without acute metabolic disturbance. T1D diabetes is caused by an autoimmune reaction where the body's immune system attacks the insulin-producing beta cell in the islets of the pancreas gland. In T2D, hyperglycemia is the result of inadequate production of insulin and the inability of the body to respond fully to insulin. T2D is most commonly seen in older adults, but it is increasingly seen in children, adolescents and younger adults due to rising levels of obesity, physical inactivity and poor diet.[3] In Bangladesh, the prevalence of diabetes among 20-79 years had increased

substantially in the rural and urban area and increasing prevalence of T2D ranged between 4.5% to 35%.[4, 5] DM a chronic condition that occurs when there are raised levels of glucose in the blood due to the body cannot produce any or enough of the hormone insulin or use insulin effectively. Classification is very important for DM patients due to determining therapy.[6] Now a day's slowly progressive autoimmune diabetes is difficult to differentiate from classical T2D in adults. This form of diabetes, variably referred to as 'latent autoimmune diabetes in adults.[7]In earlier various studies it was found that autoimmune mechanism only involved in type1 diabetic patients but newer studies found that it also involves 10%-23% of T2D patients.[8,9] After extensive research, scientists have found several autoantibodies are related to diabetes. The presence of autoantibodies is associated with a greater risk of developing insulin dependency. A young diabetic patient who is not on insulin-requiring at the time of diagnosis may need insulin and even become dependent on insulin for survival.[9] The most common islet autoantibody which are islet cell autoantibody (ICA), glutamic acid decarboxylase (GAD) autoantibody, insulinoma-associated antigen 2(IA-2) autoantibody insulin autoantibody (IAA) are found in childhood T1D patients.[7] For clinical T2D patients, GAD autoantibodies and ICA are much more common than insulin autoantibody (IAA), insulinoma-associated antigen 2(IA-2) autoantibody and zinc transporter 8(znT8) autoantibodies.[9] ICAs and antigen-specific glutamic acid decarboxylase 65 antibody (GADA) are markers of islet autoimmunity present in most patients at the diagnosis of T1D.[10]In patients clinically believed to have type 2 diabetes, ICA and GADA predict future insulin dependency, a condition often referred to as latent autoimmune diabetes in adults (LADA).[11-15] Like GADA, protein tyrosine phosphatase-like protein antibodies (IA-2 antibodies [IA-2As]) are antigen-specific islet antibodies. IA-2As are detected in a high frequency at diagnosis in type 1 diabetic children[16], whereas the frequency is lower in adult-onset type 1 diabetic patient[17, 18] and in LADA patients.[19] Recently, we reported that, if combined with ICA or GADA, IA-2A in high levels predict ß-cell failure. Different from IA-2A, GADA positivity in low levels alone specifies a slowly enlightened ß-cell dysfunction of the first 5 years after diagnosis[20], whether low GADA levels are linked with complete ß-cell failure when the duration of diabetes increases is not known. After diagnosis, probably in parallel with ß-cell destruction[21,22], islet antibodies disappear in type 1 diabetic children when the duration of diabetes increases, ICAs more rapidly than IA-2As and GADAs.[23] It is unclear how long after the diagnosis of diabetes islet antibodies remains in adults and whether there are differences between the three antibodies in this respect. Allied with forthcoming low C-peptide levels, the development of GADA or ICA after the diagnosis of diabetes has been described in young adults.[24, 25] Whether development of islet antibodies after diagnosis occurs in elderly diabetic patients needs to be clarified. In the beta-cells of the pancreas, human insulin is first formed as preproinsulin in the rough endoplasmic reticulum, then cleaved immediately into proinsulin and transported to the Golgi apparatus where packaging into secretory granules takes place. Further, it cleaved into insulin and C-peptide in equimolecular amounts.[26] The great interest in C-peptide instead of insulin due to the variating hepatic handling of insulin. Presence of insulin binding antibodies may be disturbed to assess endogenous insulin secretion, so C-peptide is measured to look for the pattern of endogenous secretion of remaining β-cell function.[25-27] We undertook present study to reveal the islets cell antibodies in relation to β-cell function to predict insulin requirement at some time after diagnosis of T2D patients.

MATERIALS AND METHODS

In this cross-sectional study, a random sample of newly diagnosed T2D of 173 patients was enrolled who attended the outpatient department (OPD) of BIRDEM, General hospital, Dhaka from January 2015 to June 2016. Laboratory analysis was conducted in the Department of Immunology, BIRDEM. The patients between 30 to 55 years both male & female with a history of diabetes less than one-year duration was selected as the study population. Patients diagnosed as type 1diabetics, pregnant women, DM with chronic infection, received insulin were excluded in this study. BMI, blood pressure, waist and hip circumference were measured. Fasting blood for glucose, lipid profile, HbA1C, islets cell autoantibodies (ICAs), and C-peptide were performed at the time of diagnosis. Patients positive for the ICA antibody were selected to see the level of C-peptide at the time of diagnosis. After six months of an interval, we measured again the C-peptide level of ICA positive patients. Both islets cell autoantibodies (ICA) and C-peptide levels were measured by qualitative enzyme-linked immunosorbent assay (ELISA) method. The ELISA reagents used were from DRG international inc, USA. Data were analyzed by SPSS version 23.0. All values were conveyed in Mean ±SD. The correlation was tested with the Pearsons correlation coefficient analysis test. P-value <0.05 was considered for the significant test.

Table 1: ICA Status of the Respondents (N=173)

ICA Status	Frequency (n)	Percentage (%)	Gender			
			Male	%	Female	%
Positive	22	12.7	14	63.64	8	36.36
Negative	151	88.4	82	54.30	69	45.70
Base	173	100.0	96		77	

Table 2: Distribution of ICA status according to gender (N=173)

Gender	n	ICA Positive	ICA Negative	P Value
Male	96	14(14.6%)	82(85.4%)	0.411^{ns}
Female	77	8(10.4%)	69(89.6%)	
Base	173	22(12.7%)	151(87.3%)	

Table 3: Respondents age wise Distribution

Age in year	n	ICA Positive	ICA Negative	P Value
30-40	79	7(8.9%)	72(91.1%)	
41-50	92	13(14.1%)	79(85.9%)	
>50	2	2(100.0%)	0(0.0%)	
Mean ±SD		42.91±7.11	40.87±6.75	0.190ns

Table 4: Distribution of the Patients by BMI (N=173)

BMI (kg/m^2)	n	ICA Positive	ICA Negative	P Value
Normal weight	18	3(7.0%)	15(93.0%)	0.710^{ns}
Over weight	53	8(15.1%)	45(84.9%)	
Obesity	102	11(10.8%)	91(89.2%)	
Mean ±SD		24.56±3.96	24.90±4.07	

Table 5: Change of C-peptide level in ICA positive cases at the time of diagnosis and after six months.

	At time of Diagnosis (ng/ml)	After six months follow up (ng/ml)	P Value
Mean ±SD	8.20±4.78	3.93±3.38	<0.001*
Range	(0.94 - 15.5)	(0.35 -12.10)	

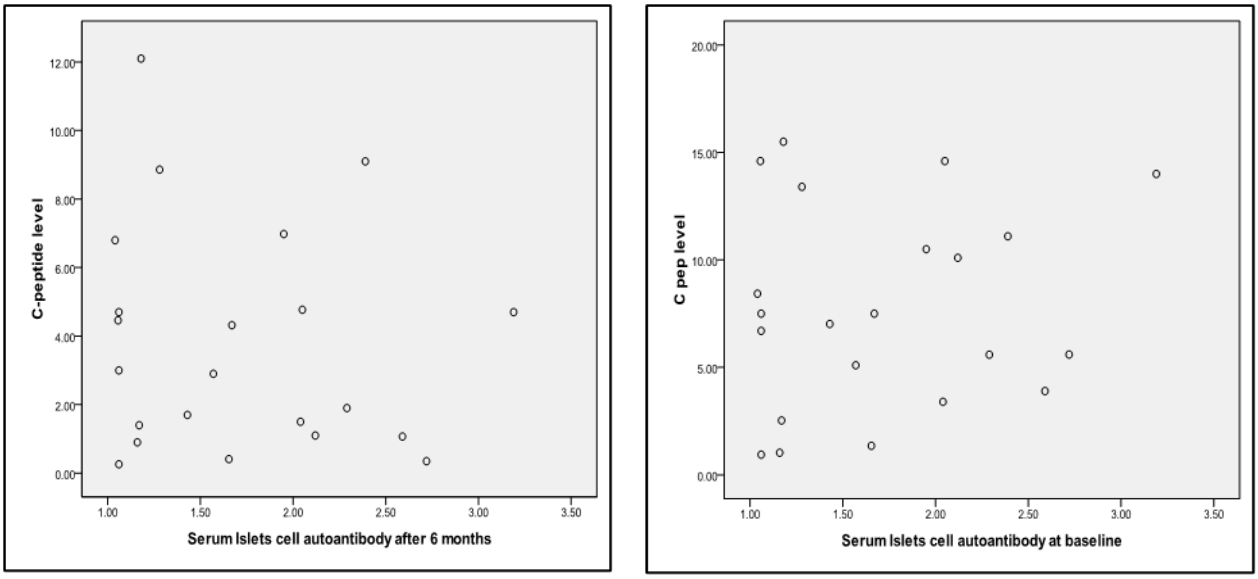

Figure 1 (a & b): Correlation between ICA and C-peptide at the time Diagnosis and after 6 months of follow-up

RESULTS

A total of 173 T2D patients were included in the study, of them 22 patients (12.7%) were ICA positive and rest 151patients (88.4%) were ICA negative.

In Table 1 shown that, among 173 T2D patients, 22 patients (12.7%) were ICA positive and rest 151 patients (88.4%) were negative. Gender distribution of ICA positive, 14 (63.64%) were male and 8 (36.36%) were female were ICA positive and regarding ICA negative 82 (54.30%) were male and 69 (45.70%) female.

In Table 2 shown that, among ICA positive cases, maximum patients were in 41-50 years age group followed by 30-40 years and above 50 years group respectively. Mean age was approximately close for 42.91±7 years in ICA positive cases and ICA negative cases 40.87±6.75 years.

In Table 3 shows that, according to age group of the patients in ICA positive highest was 7 (8.9%) of 30-40 years and in ICA negative highest was 79 (85.9%) of 41-50 years. The mean value was 42.91±7.11 & 40.87±6.75, p value was0.190$^{ns.}$

In Table 4 shows that, regarding BMI of ICA positive & ICA negative among total 173 T2D patients, the men score of normal weight, overweight & obesity was 24.56±3.96 and in ICA negative it was 24.90±4.07. P value is 0.710$^{ns.}$

In Table 5 shown that, at the time of diagnosis, mean C-peptide level was 8.20±4.78 in ICA positive patients. After six months follow up, mean C-peptide level was 3.93±3.38. There was statistically significant difference (p= <0.001) after six months follow up, which is significant that reduced C-peptide level than the level at the time of diagnosis.

The scatter diagram (a) of both ICA and C-peptide levels at diagnosis showed higher levels in ICA positive patients respectively. Pearsons correlation coefficient analysis showed small positive correlation (r=0.14; p>0.05) between ICA and C-peptide level. The scatter diagram (b) of correlation between ICA and C-peptide level after six month showed C-peptide levels were lower in ICA positive subjects after 6 months. Pearsons correlation coefficient analysis showed negative correlation (r=-0.13; p>0.05) between ICA and C-peptide.

The initial β-cell lesion in T1D is believed to result from islet autoimmune destruction. In contrast, the initial β-cell lesion in T2D is believed to result in a non-autoimmune lesion. The initiating factor in T1D is unknown, whereas the islet autoimmune development in T2D is believed to result from chronic systemic inflammation associated with obesity. In both pathways, inflammatory and effector immune cells reactive to islet proteins result and islet autoimmune destruction occurs. Chronic inflammation, insulin resistance along with β-cell defects/stress/impairment and amyloid are believed to be associated with T2D pathogenesis. It is unknown which of these factors, if any, also are associated with T1D pathogenesis. The resulting islet autoimmune diseases bridge the two forms of diabetes into one encompassing autoimmune phenomenon. "Double Diabetes" results from both T1D and T2D.[30]

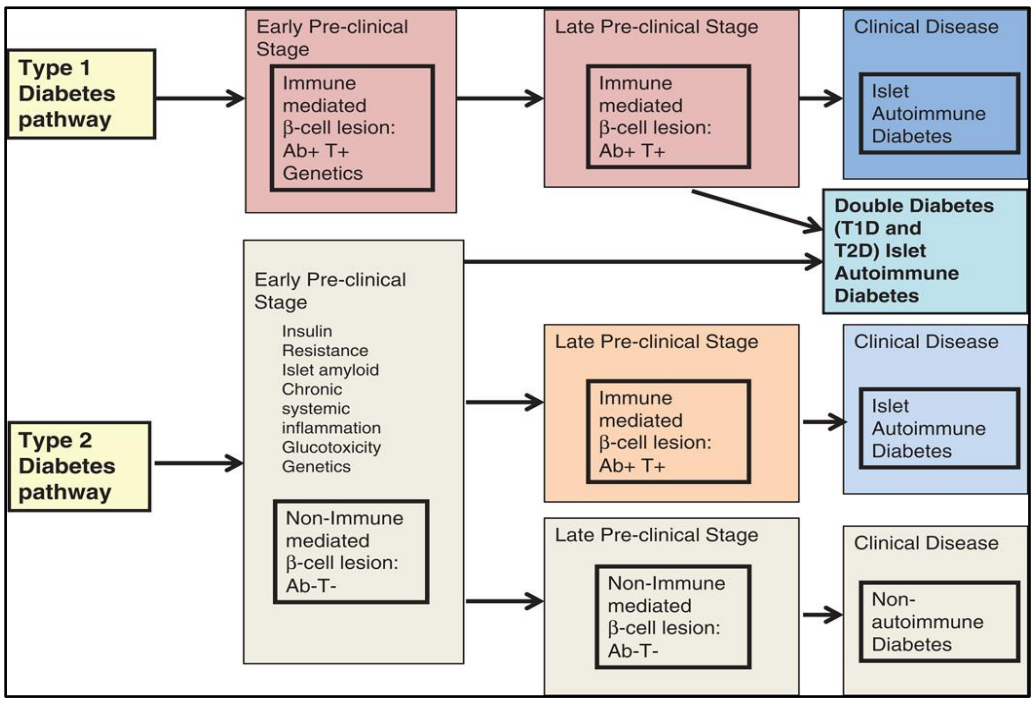

Figure 2: Pathways of type 1 (T1D) and type 2 diabetes (T2D) development[30]

DISCUSSION

A total of 173 clinically diagnosed T2D patients were included in this study. The age group with a maximum number of patients was 40-50 years. The mean age of the study group was 42.91±7.11 years in ICA positive and 40.87±6.75 years in ICA negatives patients. T2D patients with a duration of diabetes less than one year were enrolled in this study. Based on BMI, 19 of the 22 ICA positive patients were having a BMI >25 kg/m^2 or more, regard as obese as per American Diabetic Association, Clinical Practice Recommendation 2002.[6] From this study, we infer that 86% of the study population with T2D patients were obese. This view again build-up that obesity is one of the important factors in the development of T2D. In this study 22 (12.7%) out of a total of 173 T2D patients had been found to be ICA positive which was statistically significant. But the previous study by diagnosed T2D patients, 15 % (6 cases) have been found to be GAD positive, and 12.5% ICA (5cases) positive 27.5% found of 40 clinically patients were positive with ICA.[29] This positivity suggesting that they might fit into a category. In the present study, we also found the islets cell autoantibodies in young diabetic patients. Moreover, the sample size of the present study was larger but selected age can be a limiting factor for ICA positivity. The number of patients was less due to the absence of ICA autoantibodies that were excluded from this study. Researchers have suggested that autoantibodies are present in 10%-23% of T2D patients which correlated with our finding. T2D patients are more heterogeneous than type1 with respect to aetiopathogenesis. It was earlier presumed that the autoimmune process does not play any role in this pathogenesis, but later studies suggest otherwise. This positivity suggested that they had an immunological contribution in their pathogenesis. In a study, which analyzed, the patients considered T2D with ICA, beta-cell function progressively decreased after diagnosis and after 3 years was similar to T1D patients, whereas beta-cell function in T2D patients without ICA was unchanged.[7] In our study, we assessed beta-cell function by measuring C-peptide level in ICA positive T2D patients at the time of diagnosis and after six months follow up. However, at diagnosis, the mean fasting C-peptide level was 8.20 ±4.7ng/ml and was higher than the normal level. Studies suggested that raised C-peptide is a marker of insulin resistance.[28,29] Insulin resistance occurs when the body does not respond as well to the insulin that the pancreas is creating and glucose is less capable to enter the cells. At high plasma insulin, the hepatic insulin clearance decreases markedly, probably due to saturation of insulin receptors in the liver. The degree of glucose potentiation is overestimated if plasma insulin measured instead of plasma C-peptide.[26] It has been

hypothesized that the clinical signs of diabetes may appear at an earlier phase of islets beta-cell destruction in the presence of insulin resistance.[28,29] Thus the higher C-peptide at the time of diagnosis may be explained by the insulin resistance in ICA positive type 2 diabetic patients. After six months follow up the mean C-peptide was 3.93±3.38ng/ml. C-peptide levels in ICA positive patients were decreased compared to the time of diagnosis. The statistical test revealed a small positive correlation ($r=0.14$; $p>0.05$) between ICA and C-peptide level at diagnosis and after six months showed a small negative correlation ($r=-0.13$; $p>0.05$) analysis by Pearson's correlation coefficient. Fasting C-peptide level <0.6 ng/ml was considered as an indicator of poor insulin reserve. This study suggests that a significant portion of Bangladeshi T2D patients may have the autoimmune condition hidden inside and slowly progress to insulin dependency.

CONCLUSION

The phenomenon of autoimmunity is an integral part of the pathogenesis of diabetes. It is no more confined to T1D patients but also found in T2D patients.

We found that deteriorating pancreatic β-cell function reflected by change in C-peptide level among patients with ICA positive T2D patients. They may need insulin much earlier for effective control of diabetes.

This is further supported by the very fact that, in distinction to patients with multiple antibodies, a ß-cell function was often moderately unchanged with a certain years after diagnosis in most of the patients categorized as having slow-onset diabetes/LADA. Certainly, the few patient's multiple antibody-positive at diagnosis was low but well-maintained ß-cell function. In this context, it would be relevant to ask the overall belief that ~15% of type 1 diabetic patients are islet antibody negative.

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