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GAD65 autoantibody positivity and its association with clinical and biochemical parameters among young onset diabetes mellitus

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Abstract

Background: Despite increasing the prevalence of young-onset (<30 years) diabetes mellitus (DM) in Bangladesh, its etiology remains uncertain. Determining the Glutamic acid 65-kilodalton isoform antibody (GAD65Ab) positivity may help to understand the pathogenesis.

Objectives: To assess the GAD65 antibody status and its association with clinical and biochemical features with young onset DM in Bangladesh.

Method: This single-center cross-sectional study was conducted among 50 [age (years): 19.34±7.07, m/f: 22/28] previously diagnosed patients with young-onset DM [type 1 DM (T1DM)/ type 2 DM (T2DM)/ gestational DM: 31/ 18 / 1, assigned depending on clinical features]. A pre-designed case record form was used to record baseline demographic variables, height, weight, glucose (fasting & 2-hour during oral glucose tolerance test), hemoglobin A1c (HbA1c), and GAD65Ab levels. Glucose was measured by hexokinase, HbA1C by HPLC, and GAD65Ab by chemiluminescence immunoassay.

Results: Fourteen (28%) participants had positive GAD65Ab autoantibody status. While 43% of T1DM and one participant with GDM had positive GAD65Ab, all the participants with T2DM had negative status. GAD65Ab levels negatively correlated with body mass index (BMI) (r=0.41, p=0.004) and HbA1C (r=-0.36, p=0.010) in the study participants. Binary logistic regression analysis showed that those with BMI <18.5 kg/m^2 (OR=18.1, 95% CI 1.7-192.8, p=0.016) and HbA1C <9% (OR=32.8, 95% CI 3.1-351.5, p=0.004) had independently higher odds for GAD65Ab positivity.

Conclusions: GAD65 autoantibody is not uncommon in Bangladeshi patients with young-onset DM with an inverse association with HbA1c and BMI. *[J Assoc Clin Endocrinol Diabetol Bangladesh, January 2024; 3 (1): 03-08]*

Keywords: Type 1 diabetes mellitus, Type 2 diabetes mellitus, GAD65 autoantibody, HbA1c, Young-onset diabetes mellitus

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Introduction

Diabetes is a global public health concern. It is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both.[1] Globally, diabetes prevalence in 2019 was estimated to be 9.3%, which may rise to 10.2% by 2030. The number of people with Diabetes in Bangladesh was 8.4 million in 2019 and the projected rise is 15 million by the year 2045.[2] In recent development the number of young people with diabetes is rising and this is of particular concern. Earlier onset of diabetes leads to longer lifetime exposure to hyperglycemia and so increases predilection to long-term complications. Almost 50%

of young adults with type 1 diabetes develop diabetes-related complications in their 20s, including retinopathy, neuropathy, and hypertension. Moreover, the course of diabetes in people under 30 years could be more rapid and disruptive than in patients who develop the disease later in life, leading to early morbidity and poor quality of life. DM in adolescents and young adults can adversely affect social life as to living with a chronic disease throughout patients' working life. Roughly, 23 million young adults under 30 years had diabetes worldwide in the year 2000 which by 2013 had increased to 63 million.[3,4] According to American Diabetes Association (ADA) Diabetes can be classified as type 1, type 2, gestational diabetes, and other specific type of diabetes. However, the traditional paradigms of type 2 diabetes occurring only in adults and type 1 diabetes only in children are no longer accurate, as both diseases can occur in both age groups.[5] Hence, subtypes such as latent autoimmune diabetes in adults (LADA) and maturity-onset diabetes in young (MODY) have been suggested.[6,7]

Autoimmunity is an important cause of young-onset diabetes. GAD65 is found on the vesicles of ß-cells of the pancreas. GAD65 antibody positivity acts as a strong risk factor for the development of type 1 DM. GAD65 antibody positivity is associated with a rapid destruction of ß-cells & insulin deficiency. Autoimmune mediated destruction of the β-cells of the pancreas with consequent insulin deficiency and insulin resistance are the leading causes of type 1 DM. In the case of young-onset diabetes, autoimmunity leading to β-cells destruction plays a significant role. Multiple genetic and environmental factors lead to the development of autoantigens against β-cells leading to an autoimmune attack mediated by different autoantibodies. The autoantigens are presented by HLA on antigen-presenting cells (APCs) to diabetogenic autoreactive T cells. Autoreactive CD4+ T cells then produce high-affinity autoantibodies against β-cells and also help diabetogenic CD8+ T cells to acquire cytolytic activity and attack β-cells through the release of cytokines. The released cytokines stimulate macrophages and other innate immune cells to further damage β-cells yielding a positive feedback loop to propagate further β-cell destruction and ultimately leading to the development of diabetes in under 30 populations.[8,9]

Our study aimed to assess the distribution of GAD65 antibody levels and find out its association with clinical and biochemical parameters in patients with young-onset diabetes mellitus (DM).

Methods

This single-center cross-sectional study was done between 1st January 2021 to 30th June 2021 at the Dhaka Medical College Hospital, Dhaka, Bangladesh. Patients aged from 5 to 30 years of both sexes, diagnosed with diabetes according to the standard criteria of the American Diabetic Association 2021 were included in the study. Patients aged >30 years, severely ill, and unwilling to participate were excluded from the study. The sample size was 50. A pre-designed case record form was used to record baseline demographic variables. The GAD65 antibody, fasting plasma glucose (FPG), plasma glucose 2 hours after 75-gm glucose (2h-PG) during oral glucose tolerance test (OGTT), and hemoglobin A1c (HbA1c) were measured on eligible patients who met the inclusion and exclusion criteria. GAD65 antibody was measured by chemiluminescence immunoassay qualitative determination of glutamic acid decarboxylase antibody (GAD65) in human serum with a cut-off value of 17 IU/mL. Plasma glucose was measured by the hexokinase method and HbA1c was measured using high-performance liquid chromatography (HPLC) assays with venous blood.

Collected data were analyzed by the SPSS 23. Data were expressed in frequency (%) and mean±SD. Associations between two variables were done by Pearson's chi-square/Fisher's exact or unpaired t-test as appropriate. Correlation between two variables was done by Pearson's correlation test. Binary logistic regression analysis was done to predict positive GAD65Ab status. A two-sided p-value below 0.05 was considered statistically significant.

Informed written consent was obtained from all the participants or their parents. The study was done after the clearance of the ethical review committee of Dhaka Medical College Hospital.

Results

Among 50 participants, 14 (28.0%) had a positive GAD65Ab. The participants with positive GAD65Ab (Ab+ve) status were younger than the participants with negative GAD65Ab (Ab-ve) status. Those with an Ab+ve status had a higher frequency of polyuria, polydipsia, and weight loss but a lower frequency of acanthosis nigricans. Body mass index (BMI) levels

Table-I: Baseline characteristics of the study participants according to GAD65Ab status (n= 50)

Variables	GAD65Ab +ve (n= 14)	GAD65Ab-ve (n= 36)	p
Age, years	15.8±5.9	20.7±7.1	0.025
Age group			
≤20 years	11 (78.6)	16 (44.4)	0.030
>20 years	3 (21.4)	20 (55.6)	
Sex			
Male	9 (64.3)	13 (36.1)	0.072
Female	5 (35.7)	23 (63.9)	
Symptoms			
Polyuria	14 (100.0)	24 (66.7)	0.023
Polydipsia	14 (100.0)	27 (75.0)	0.047
Polyphagia	6 (42.9)	11 (30.6)	0.511
Weakness	14 (100.0)	31 (86.1)	0.304
Weight loss	14 (100.0)	26 (72.2)	0.045
Blurring of vision	2 (14.3)	8 (22.2)	0.704
Signs			
Muscle wasting	10 (71.4)	15 (41.7)	0.114
Acanthosis nigricans	1 (7.1)	16 (44.4)	0.018
BMI, kg/m^2	18.8±3.2	22.7±4.8	0.007
BMI category			
Underweight	9 (64.3)	6 (16.7)	0.002
Others	5 (35.7)	30 (83.3)	
Glycemic values			
Fasting glucose, mmol/L	18.6±3.4	16.3±4.7	0.072
2-h glucose, mmol/L	26.2±6.5	22.8±5.0	0.049
HbA1C, %	8.3±0.5	10.9±2.1	<0.001
Type of DM			
Gestational	1 (7.1)	0 (0.0)	
Type I	13 (92.9)	18 (50.0)	—
Type II	0 (0.0)	18 (50.0)	

Data were expressed in frequency (%) or mean±SD
Within parentheses are percentages over the column total
Pearson's chi-square/ Fisher's exact test or unpaired t-test was done as appropriate

were lower and the frequency of underweight was higher in the Ab+ve group. Though the OGTT glucose levels were higher, the HbA1C levels were significantly lower in the Ab+ve group than in the Ab-ve group. According to the type of DM, GAD65Ab was positive among 13 (41.9%) participants with T1DM, and one participant with GDM, but none in those with T2DM (Table-I). After removing one participant with GDM from the analysis, T1DM patients had significantly higher percentages of positive GAD65Ab status (p=0.002).

Among the clinical and biochemical variables, GAD65Ab levels were significantly but negatively correlated with only BMI (r=-0.41, p=0.004) and HbA1C (r=-0.36, p=0.010) in the study participants (Figure-1). Binary logistic regression analysis showed that those with BMI <18.5 kg/m^2 (OR=18.1, 95% CI 1.7-192.8, p=0.016) and HbA1C <9% (OR=32.8, 95% CI 3.1-351.5, p=0.004) had independently higher odds for being GAD65Ab positivity (Table-II).

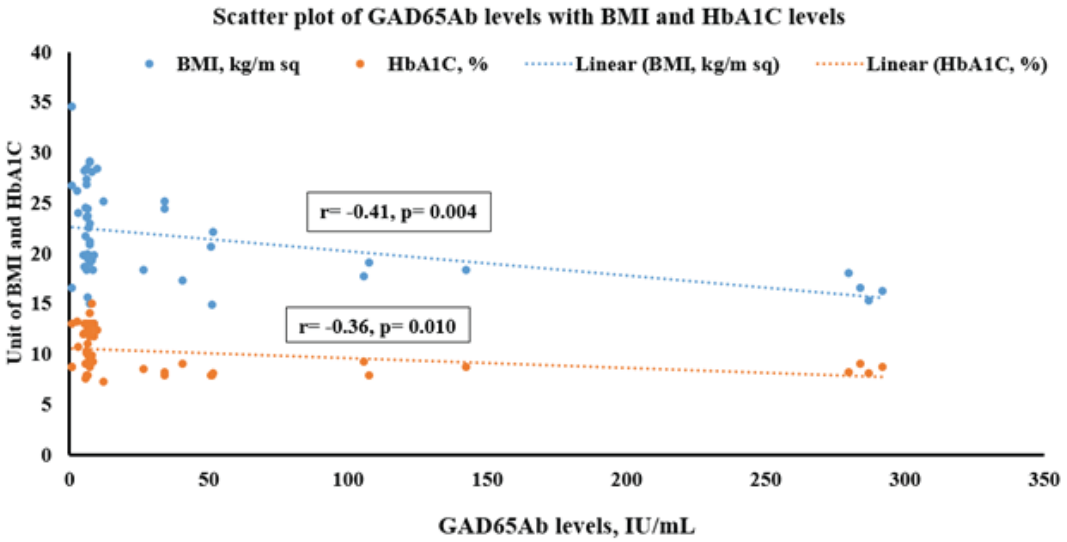

Figure 1: Correlations of GAD65Ab levels with clinical and biochemical variables (n= 50)
Pearson's correlation test was done

Table-II: Predictability of clinical and biochemical variables over GAD65Ab positivity as a dependent variable in the study participants (n= 50)

Variables	Univariate			Multivariate		
	Odds ratio	95% CI	p-value	Odds ratio	95% CI	p-value
Sex (Female)	0.31	0.09 – 1.14	0.078	0.84	0.14 – 5.10	0.853
Age (≤20 years)	4.58	1.09 – 19.27	0.038	2.51	0.28 – 22.43	0.411
BMI (<18.50 kg/m2)	7.46	1.90 – 29.34	0.004	18.09	1.70 – 192.79	0.016
HbA1c (<9%)	15.19	3.32 – 69.47	<0.001	32.79	3.06 – 351.48	0.004

Binary logistic regression analysis of GAD65Ab positivity as a dependent variable was done

Discussion

DM is one of the most prevalent and serious non-communicable diseases all around the world. It is one of the leading causes of death, disability, and economic losses, and is identified as a major threat to human health and development.[10] Worldwide 1.3 million deaths occurred in 2010 which was two times higher than the number of deaths in 1990.[11] Moreover, it can lead to a multitude of complications like heart diseases, stroke, neuropathy, renal failure, retinopathy, and blindness.[12,13] Therefore, it poses a major healthcare burden all over the world.[14] So, reliable markers are needed for the management of diabetes mellitus. In this regard, autoantibody against GAD65 may be a valuable predictor of risk and progression to overt autoimmune diabetes. After confirming type 1 Diabetes person should be counseled about insulin treatment for the rest of their life. It has been evidenced that type 1 diabetes is associated with increases in the risk of other autoimmune diseases. As type 1 diabetes is a component of autoimmune polyendocrine syndrome GAD65 positivity opens a big window for searching for other autoimmune diseases like autoimmune thyroid diseases, Addison's disease, celiac disease, and pernicious anemia. GAD65 positive Type 1 diabetes, autoimmune thyroid disease, and pernicious anemia are the most frequent GAD65 autoimmune associations. These diseases are associated with the presence of specific autoantibodies in blood which can be detected before the development of clinically overt disease. Since follow-up of our patients is

missed sometimes, we should cover this in our initial visit. GAD65 positivity in patients with diabetes needs screening of other siblings to find out type 1 diabetes and also needs to evaluate for the presence of other autoimmune diseases.

Among participants, 28.0% had a positive GAD65Ab. Consistent with the finding of this study, Rodacki et al found 45% of their study population GAD65 positive;[15] However, Al-Abadey et al found a relatively higher prevalence of 67% of GAD65 autoantibody positivity among their study DM cases in contrast to the finding of this study. This discordance might be due to the large sample size of their study.

The participants with positive GAD65Ab status were younger than the participants with negative GAD65Ab status. Studies conducted by Praveen et al, Abady et al and Feltbower et al also found type 1 diabetes as the commonest type of diabetes in early life.[16-18] Those with a positive GAD65Ab status had a higher frequency of polyuria, polydipsia, and weight loss but a lower frequency of acanthosis nigricans. Similar findings were observed by Mayegra et al.[19]

BMI levels were lower and the frequency of underweight was higher in the antibody-positive group, a study conducted by Maya Nitecki et al found people with high BMI had a greater risk for incidence of type 1 DM with positive antibody.[20] This discordance may be due to the fact that a heterogeneous population was included in our study. To understand the correlation between BMI and antibody positive group, further study may require with larger population size.

GAD65Ab levels were significantly but negatively correlated with only BMI and HbA1C in the study participants. However, according to Anita L Grubb et al, HbA1C was significantly increased and BMI was decreased in the antibody-positive group.[21] This discordance may be due to the duration of diabetes (median 3 years of DM duration).

The main limitations of this study were a smaller sample size, no control group and it was conducted in a single tertiary care hospital which may not represent the entire community. In future, the study should be conducted with a larger sample size and data should be collected from multiple centers with proper randomization. Other ancillary tests like serum insulin, C- peptide, islet cell antibody, and antibody to insulin should be included in the study to explore the polygenic nature of young-onset diabetic patients.

Conclusion

In this study, we found around one-third of the young-onset diabetes population showed GAD65 positivity. This antibody also showed a negative correlation with HbA1C and BMI in the study population. The number of research about GAD65 autoantibody for young onset diabetic people is limited in our country. This research will be a helpful tool for a better understanding of the pathophysiology of young-onset diabetes and its correlation with antibody.

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Disclosure

The authors declare that no conflict of interest could be perceived as prejudicing the impartiality of the research reported.

Financial Disclosure

The authors received no financial support for the study.

Data Availability

Any inquiries regarding supporting data availability of this study should be directed to the corresponding author and are available from the corresponding author upon reasonable request.

Ethical Approval and Consent to Participate

This study was approved by the Ethical Review Committee (ERC) of Dhaka Medical College, Reg: No. ERC-DMC/ECC/2020/97, Approved on 24-06-2020. All procedures performed in studies involving human participants were in accordance with the ethical standards of the IRB and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed written consent was obtained from each of the participants included in the study.

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References

1. American Diabetes Association. 2. Classification and diagnosis of diabetes: Standards of medical care in diabetes—2021. Diabetes Care 2021;44(1): S15–S33. DOI: 10.2337/dc21-S002.

2. Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, et al. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. Diabetes Res Clin Pract 2019;157(107843): 01–10. DOI: 10.1016/j.diabres.2019. 107843.
3. Lascar N, Brown J, Pattison H, Barnett AH, Bailey CJ, Bellary S. Type 2 diabetes in adolescents and young adults. Lancet Diabetes Endocrinol 2018;6(1):69–80. DOI: 10.1016/S2213-8587(17)30186-9.
4. Monaghan M, Helgeson V, Wiebe D. Type 1 Diabetes in young adulthood. Curr Diabetes Rev 2015;11(4):239–50. DOI: 10.2174/1573399811666150421114957.
5. American Diabetes Association. 2. Classification and diagnosis of Diabetes: standards of medical care in diabetes-2019. Diabetes Care 2019;42(1): S13–S28. DOI: 10.2337/dc19-S002.
6. Brophy S, Davies H, Mannan S, Brunt H, Williams R. Interventions for latent autoimmune diabetes (LADA) in adults. Cochrane Database Syst Rev 2011; 2011(9):CD006165. DOI: 10.1002/14651858.CD006165.pub3.
7. Ho MS, Weller NJ, Ives FJ, Carne CL, Murray K, vanden Driesen RI, et al. Prevalence of structural central nervous system abnormalities in early-onset type 1 diabetes mellitus. J Pediatr 2008;153(3):385–90. DOI: 10.1016/j.jpeds.2008.03.005.
8. Giwa AM, Ahmed R, Omidian Z, Majety N, Karakus KE, Omer SM, et al. Current understandings of the pathogenesis of type 1 diabetes: Genetics to environment. World J Diabetes 2020;11(1):13–25. DOI: 10.4239/wjd.v11.i1.13.
9. Ilonen J, Lempainen J, Veijola R. The heterogeneous pathogenesis of type 1 diabetes mellitus. Nat Rev Endocrinol 2019;15(11):635–50. DOI: 10.1038/s41574-019-0254-y.
10. Murray CJL, Vos T, Lozano R, Naghavi M, Flaxman AD, Michaud C, et al. Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990–2010: A systematic analysis for the global burden of disease study 2010. Lancet 2012;380(9859):2197–223. DOI: 10.1016/S0140-6736(12)61689-4.
11. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: A systematic analysis for the Global Burden of Disease Study 2010. Lancet 2012;380(9859):2095-128. DOI: 10.1016/S0140-6736(12)61728-0.
12. Oldroyd J. Diabetes and ethnic minorities. Postgrad Med J 2005; 81(958):486–90. DOI: 10.1136/pgmj.2004.029124.
13. World Health Organization. World health statistics 2013 a wealth of information on global public health 2013. 01-06.
14. Forouhi NG, Wareham NJ. Epidemiology of diabetes. Medicine (Abingdon) 2014;42(12):698–702. DOI: 10.1016/j.mpmed.2014.09.007.
15. Rodacki M, Zajdenverg L, Albernaz MS, Bencke-Gonçalves MR, Milech A, Oliveira JEP. Relationship between the prevalence of anti-glutamic acid decarboxylase autoantibodies and duration of type 1 diabetes mellitus in Brazilian patients. Brazilian J Med Biol Res 2004;37(11): 1645–50. DOI: 10.1590/s0100-879x2004001100008.
16. Praveen PA, Madhu S V, Viswanathan M, Das S, Kakati S, Shah N, et al. Demographic and clinical profile of youth onset diabetes patients in India- Results from the baseline data of a clinic based registry of people with diabetes in India with young age at onset- Pediatr Diabetes 2020;7(19):01-07. DOI: 10.1111/pedi.12973.
17. Al-Abady HL, Mahdi NK, Al-Naama LM, Mahdi JK. The prevalence of autoantibodies among relatives for type 1 and 2 diabetic patients. J Pak Med Assoc 2016;66(9):1064-67.
18. Feltbower RG, Mckinney PA, Campbell FM, Stephenson CR, Bodansky HJ. Type 2 and other forms of diabetes in 0–30 year olds: a hospital based study in Leeds, UK. Arch Dis Child 2003;88(8):676-9. DOI: 10.1136/adc.88.8.676.
19. Mayega RW, Rutebemberwa E. Clinical presentation of newly diagnosed diabetes patients in a rural district hospital in Eastern Uganda. Afr Health Sci 2018;18(3):707–19. DOI: 10.4314/ahs.v18i3.29.
20. Nitecki M, Gerstein HC, Balmakov Y, Tsur E, Babushkin V, Michaeli T, et al. High BMI and the risk for incident type 1 diabetes mellitus: A systematic review and meta-analysis of aggregated cohort studies. Cardiovasc Diabetol 2023;22(1):300. DOI: 10.1186/s12933-023-02007-y.
21. Grubb AL, McDonald TJ, Rutters F, Donnelly LA, Hattersley AT, Oram RA, et al. A type 1 diabetes genetic risk score can identify patients with GAD65 autoantibody-positive type 2 diabetes who rapidly progress to insulin therapy. Diabetes Care 2019;42(2):208-21. DOI: 10.2337/dc18-0431.