

The Association between Anti-Glutamic Acid Decarboxylase IgG and Hemoglobin A_{1c} among Newly Diagnosed Type 1 Diabetes of Some Iraqi Children in Karbala City

Hiba Ali Al-Khadhi Hadi, Hayam Khalis Al-Masoudi, Mohammed Salih Mahdi¹

Department of Microbiology, College of Medicine, University of Babylon, Hillah, ¹Imam-Al-Hussein Medical Hospital, Employment in Karbala Teaching Hospital for Children, Karbala, Iraq

Abstract

Background: Type 1 diabetes mellitus (T1DM) is brought on by immune system dysfunction intolerance, invasion of beta cells by autoreactive immunological T cells, degradation of beta cell activity and survival, and extended exogenous insulin treatment. One of the circulation autoantibodies against antigens of pancreatic cell that produce insulin is glutamic acid decarboxylase (GAD IgG antibody) that indicate the stress and/or death of the islet cell in T1D. A blood glucose monitoring test called hemoglobin A_{1c} (HbA_{1c}) is used to determine the average blood sugar level over three months (February, March, and April of 2022). **Objective:** The purpose of this study is to assess the frequency of anti-GAD IgG and HbA_{1c} in T1DM children, as well as their correlation. **Materials and Methods:** Seventy individuals with new-onset T1DM (confirmed by clinical signs, including increased urination, intense hunger, intense thirst, and abrupt weight loss, along with a random blood sugar test that is higher than 200 mg/dL and a fasting blood sugar test that is higher than 126 mg/dL) are male and female, ranging in age from 1 to 12 years. Blood was drawn for the biochemical and immunological tests (including HbA_{1c}, fasting blood sugar, C-peptide, anti-islets antibodies, and anti-GAD antibodies). **Results:** Ninety-four percent of the T1DM patients had positive anti-GAD IgG with a mean concentration of 147 IU/mL, and 6% had negative anti-GAD IgG with a mean value of 9 IU/mL. At the same time, the findings of the current study's calculation of HbA_{1c} levels revealed that individuals with newly diagnosed T1DM had a total mean average of HbA_{1c} of 10.53. **Conclusion:** Specifically in T1D that had arisen in extremely young children, autoimmunity was discovered to take part in the glycemic index expressed by glycated hemoglobin average.

Keywords: Beta cells stress, diabetes type 1, GAD, HbA_{1c}

INTRODUCTION

Type 1 diabetes mellitus (T1DM) is brought on by immune system dysfunction intolerance, invasion of beta cells by auto-reactive immunological T cells, degradation of beta cell activity and survival, and extended exogenous insulin treatment.^[1] The loss of β -cells causes an insulin shortage, which results in life-threatening hyperglycemia.^[2] Similar to other autoimmune diseases, it is brought on by the interaction of genetic and environmental factors, both of which have been associated with a higher chance of developing T1DM.^[3] It has long been recognized that the immune system contributes to the development of illness, and proof of this includes the discovery of antibodies

against islets of Langerhans antigens in vulnerable people.^[4] One of these autoantibodies, antiglutamic acid decarboxylase (anti-GAD IgG), has been identified in 50%–80% of T1DM patients and is regarded as a crucial antigen for beta-cell autoimmunity to occur.^[5] GAD was first identified as a T1DM auto-antigen target in 1982 when 64,000 KD immune-precipitated from islets in

Address for correspondence: Dr. Hiba Ali Hadi Al-Khadhi,
Department of Microbiology, College of Medicine,
University of Babylon, Hillah, Iraq.
E-mail: hibaali8172@gmail.com

Submission: 23-Jan-2023 **Accepted:** 27-Feb-2023 **Published:** 02-Feb-2024

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: WKHLRPMedknow_reprints@wolterskluwer.com

How to cite this article: Hadi HAA-K, Al-Masoudi HK, Mahdi MS. The association between anti-glutamic acid decarboxylase IgG and hemoglobin A_{1c} among newly diagnosed type 1 diabetes of some Iraqi children in Karbala City. Med J Babylon 2023;20:706-8.

Access this article online

Quick Response Code:



Website:
<https://journals.lww.com/mjby>

DOI:
10.4103/MJBL.MJBL_72_23

newly diagnosed T1DM patients. Overall, 80% of T1DM patients have autoantibodies against GAD at the time of clinical presentation.^[6] Gamma-aminobutyric acid, a transmitter in the central nervous system, is produced thanks to the enzyme GAD. Two GAD subtypes, GAD65 and GAD67, are produced in human body tissues, with GAD65 being more common in beta cells of the pancreas.^[6] Glycation is the process by which sugar is chemically bonded to hemoglobin, creating the kind of hemoglobin known as hemoglobin A1c (HbA1c).^[7] The structure of the bloodstream's high glucose level, which is usually linked to diabetes mellitus, is demonstrated by the sugar-hemoglobin relationship. Since red blood cells only live for four months, the HbA1c test is only valid for a three-month median. The test is only valid for three months since various red blood cells have varied lifespans.^[8] Glycated hemoglobin is produced in normal amounts at normal glucose levels and predictably increases when plasma glucose levels rise on average.^[3,9] The purpose of this study was to assess the prevalence of anti-GAD (a measure for auto-immunity) and glycated hemoglobin (a measure for sugar management) in some of the newly onset T1DM children.

OBJECTIVES

This study seeks to assess the incidence of anti-GAD IgG and HbA1c and their relationship among T1DM children.

MATERIALS AND METHODS

From the start of February 2022 till the give up of December 2022, this observation changed into carried out. Youngsters with newly identified T1DM who visited the Imam Alhassan Middle for Endocrinology and Diabetics in Karbala, Iraq, had a total of 70 blood samples taken. Youngsters with newly identified T1DM of a range of a while, from 1 to 12 months antique to 15 years, comprised the target group. Whole blood was taken in a volume of 5 mL. The fresh blood was drawn immediately and divided into two parts; the first portion (5 mL) was drawn (2 mL into an anticoagulated ethylene diamine tetra acetic acid tube to measure the average of HbA1c) and three milliliters into gel tubes, let it coagulates, then centrifuged for 5 min at 4000 rpm, and the resulting serum was dispensed into two separate plain tubes: one tube was used to measure the level of glucose, the second-tube was used to measure the level of anti-GAD by enzyme-linked immune-sorbent assay (ELISA). The diagnosis was conducted by the consultant physician in the center, depending upon WHO guidelines. The patient's parents or a companion filled out a questionnaire that included questions on the patient's age, sex, T1DM, and other chronic illnesses in the family. Using an ELISA kit for quantification (EUROIMMUNE, Lubek, Germany), anti-GAD is identified in the patient's serum. The procedure was carried out following the kit's instructions. HbA1c measurement in T1DM patients^[10] by apparatus analysis of

the serum has been carried out using high-performance liquid chromatography.^[11] Technologies such as immunoassay and quantitative reflectometry were employed. The test uses an anti-antibody to HbA1c that identifies the first residues of amino acids of hemoglobin A's glycemic chain.

Statistical analysis

Statistical analyses—SPSS Version 24.0 (SPSS, IBM Company, Chicago, Illinois) had been done, and the outcomes were presented as (mean $\pm$ SD) to detect different factors and consequences of parameters. An independent *t* test was used to compare means and standard deviation (SD), and $P \leq 0.05$ was used to assess statistical significance.

Ethical approval

The Declaration of Helsinki's ethical guidelines, which guided the study's conduct, were followed. Before the sample was obtained, it was done with the patient's verbal and analytical consent. The study protocol, subject information, and consent form were reviewed and authorized by a local ethics committee following document number 6553 (including the number and date on September 12, 2022) to acquire this approval.

RESULTS

Measurement of anti-GAD-IgG antibodies: The present results showed positive anti-GAD in 94% (66/70) with mean $\pm$ SD concentration (147 ± 92 IU/mL) and negative anti-GAD in 6% (4/70) with mean concentration (9 ± 0 IU/mL) in all three age categories, of all T1DM patients. The age group (6–10) has the largest percentage of GAD antibody positivity (48%; 34/70), with an average (mean + SD) ($129 + 92$ IU/mL), whereas, The age group (5) years old has the fewest positive GAD antibodies (12% 8/70) with (165 ± 84 IU/mL), while the mean and SD for the age group (11–15) years was (146 ± 103 IU/mL) with percentage of GAD antibody positivity (34% 24 /70) [Table 1].

The sex groups distribution, as seen in Table 2, is the patient groups (male and female). There were 48% (33/70) with anti-GAD mean (of 116 ± 90 IU/mL) in the male

Table 1: Positive and negative anti-GAD antibody levels in T1DM patients according to age group

Age groups	Anti-GAD positive number (%)	Anti-GAD negative number (%)	Anti-GAD positive mean $\pm$ SD (IU/mL)
<5 years	8 (12%)	0 (0%)	165 ± 84
6–10 years	34 (48%)	2 (3%)	129 ± 92
11–15 years	24 (34%)	2 (3%)	146 ± 103
Total	(66/70) 94%	(4/70) 6%	
P-value	($P \leq 0.04$)*S	($P \geq 0.14$)**NS	

*S: significant ($P \leq 0.05$); ** NS: nonsignificant ($P \geq 0.05$)

Table 2: Sex distribution of T1DM patients with positive and negative GAD test

Sex distribution	+ve GAD-antibody number (%)	-ve GAD-antibody number (%)	+ve GAD-antibody mean $\pm$ SD (IU/mL)
Female (37/70)	35 (50%)	2 (2%)	160 $\pm$ 96
Male (33/70)	31 (44%)	2 (3%)	116 $\pm$ 90
P-value	(P = 0.82) **NS (P = 0.90) **NS		
+ve: positive; -ve: negative; ** NS: nonsignificant (P $\geq$ 0.05)			

Table 3: The sex distribution of HbA1c level

Sex group	Glycated hemoglobin (mean $\pm$ SD)
Female	(10.76 $\pm$ 2.23)
Male	(10.28 $\pm$ 2.29)
Total	(10.53 $\pm$ 2.25)
T-test (P-value)	(P = 0.83) **NS

**NS: nonsignificant (P $\geq$ 0.05)

Table 4: HbA1c and anti-GAD IgG relationship

Study group	Glycated hemoglobin (mean $\pm$ SD)
Anti-GAD positive	(10.69 $\pm$ 2.21)
Anti-GAD negative	(8 $\pm$ 1.4)
T-test (P-value)	(P = 0.02) *S

*S: significant at (P $\leq$ 0.05)

group, while the female constituted 52% (37/70) with anti-GAD mean (of 160 $\pm$ 96 IU/mL).

The results of the level (HbA1c) in the current study reveal a total mean and SD of HbA1c (10.53 $\pm$ 2.25) in newly diagnosed T1DM. Distributed as 10.76 $\pm$ 2.23 in females and 10.28 $\pm$ 2.29 in males, with no statistical difference between males and females (P = 0.73), as described in Table 3.

The results of this study revealed that the anti-GAD positive group of children with T1DM had mean $\pm$ SD level of HbA1c (10.69 $\pm$ 2.21) and when compared to the level of HbA1c (8 $\pm$ 1.4) in the anti-GAD negative T1DM-group showed that the subjects with anti-GAD autoantibody positivity had substantially higher HbA1c levels than individuals with negative anti-GAD autoantibody with (P = 0.02) as shown in Table 4.

DISCUSSION

The statistical analysis of findings shows there is a substantial difference. There is a substantial difference between ages <5 years old and 11–15 years at (P < 0.04) and no significant differences between age groups <5 years old and 6–10 years old at (P $\geq$ 0.14) and also no significant differences (6–10) years old with 11–15 years (P $\geq$ 0.24).

Regarding sex, as shown in Table 2, there were similar male and girl distributions across patient groups (P = 0.82, P = 0.90).

Anti-GAD antibodies in the blood have been suggested as T1DM risk indicators.^[12] The findings of the current study concur with those of an Iraqi investigation, which showed that 88.6% of T1DM patients had GAD-antibody positive.^[13] According to a Tunisian study's findings that 84.6% of kids with newly onset diabetes showed anti-GAD antibodies (6 months after diagnosis).^[14] It is in line with an international investigation that used the anti-GAD IgG ELISA test and discovered that individuals with new-onset T1DM had an (89%) seropositivity rate.^[15] Other Iraqi research, however, showed a lower level of anti-GAD seropositivity.^[16,17] The results might be explained by the various cutoff levels used by diagnostic kits to establish the test's sensitivity.^[11] and/or changing the rate of autoimmune disease development from person to person among various groups.^[18]

Several investigations on the age distribution of anti-GAD IgG found that the prevalence of anti-GAD was 78.3% in patients with a disease duration of less than 5 years and started to fall in those have more than 12 years of illness duration due to the steady decrease of islet cell autoantibodies over age progression.^[19] In line with recent research on the distribution of sex, which indicated nonobvious variations in means of GAD-autoantibody IgG levels between male and female T1D patients, despite a modest tendency toward higher values in females.^[17,20]

The determination of HbA1c concentration is regarded as a significant biochemical test that was frequently used as a monitoring technique and screening tool for T1DM. The most recent findings showed that all of the patients who participated in this study had elevations above the typical standard levels. Values below 6.5% are recommended by the American Diabetes Association as an indication for the diagnosis of diabetes mellitus.^[21] It has been shown that the difficulty T1DM patients have maintaining glycemic control is because T1DM develops due to β -cell loss rather than insulin- resistance, in the case of classic T2DM.^[19] HbA1c levels were evaluated in T1DM patients in parallel research. One such study found that newly diagnosed diabetic children had HbA1c values of above 7.8%.^[22] Additionally, research shows HbA1c values higher than (9.4 %) in newly diagnosed T1DM patients before starting insulin treatment^[22] and research demonstrating that glycated hemoglobin >10%.^[23] Both the present study's findings and those of the preceding studies may point to poor glycated hemoglobin management in those patients with DM, which might be attributed to those individuals' undetected diabetes up until they manifested severe clinical symptoms or a condition known as diabetic ketoacidosis. The new results might be viewed as important proof that autoimmunity has a role in the glycemic indices reflected by T1DM patients' HbA1c levels. This study was comparable to the one that found that patients with autoantibody positivity had substantially higher HbA1c levels than those with

autoantibody negativity.^[23] However, these results ran counter to those of two other studies.^[24-26]

CONCLUSIONS

The anti-GAD IgG data showed that these autoantibodies are more prevalent in T1DM patients who were first afflicted. Furthermore, compared to lower levels of anti-GAD IgG at the outset of T1DM patients, the data demonstrated an enhanced concentration level of anti-GAD IgG related to high HbA1c levels.

Acknowledgments

We would like to express our gratitude to all patients for their cooperation and patience during this effort; without their assistance, this work would not have been possible.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

- Mirmira RG, *et al.* Biomarkers of β -cell stress and death in type 1 diabetes. *Curr Diabetes Rep* 2016;16:1-9.
- Atkinson MA, Eisenbarth GS, Michels AW. Type 1 diabetes. *Lancet* 2014;383:69-82.
- Díaz-Flores M, Baiza-Gutman L. The Diabetes Textbook. New York, USA: Springer; 2019.
- Gianani R, Eisenbarth GS. The stages of type 1A diabetes: 2005. *Immunol Rev* 2005;204:232-49.
- Patterson CC, *et al.* Incidence trends for childhood type 1 diabetes in Europe during 1989–2003 and predicted new cases 2005–20: A multicentre prospective registration study. *Lancet* 2009;373:2027-33.
- Awchi DW, Rasool SN. Evaluation of anti-GAD65 and HbA1c prevalence among newly diagnosed type 1 diabetes of some Iraqi children. *Iraqi J Ind Res* 2022;9:125–130-125–130.
- Rutledge N. Diabetes education empowerment program (DEEP): Evidence-based practice measures for self-care management. Ph.D Thesis, Jacksonville State University. 2021.
- Miedema K. Standardization of HbA1c and optimal range of monitoring. *Scand J Clin Lab Invest* 2005;65(sup240): 61-72.
- Chehregosha H, *et al.* A view beyond HbA1c: Role of continuous glucose monitoring. *Diabetes Ther* 2019;10:853-63.
- Mahdi HA, Al-Humairi AK. Assessment of knowledge about hypertension among hypertensive patients in Babylon Province. *Med J Babylon* 2022;19:31.
- Horváth C. High-performance liquid chromatography: Advances and perspectives. Amsterdam, Netherlands: Elsevier. 2013.
- Zanone M, *et al.* Glutamic acid decarboxylase and ICA512/IA-2 autoantibodies as disease markers and relationship to residual β -cell function and glycemic control in young type 1 diabetic patients. *Metabolism* 2003;52:25-9.
- Sheet MM, Khudhair HAA. Beta-cell death and/or stress biomarkers in diabetes mellitus type. *Al-Kufa Univ Jr Biol* 2019;11:108-119.
- Elkadhi A, *et al.* Prevalence of anti-GAD autoantibodies in Tunisian children with type 1 diabetes. *La Tunisie Médicale* 2002;80:281-5.
- Palomer X, *et al.* Evaluation of two nonisotopic immunoassays for determination of glutamic acid decarboxylase and tyrosine phosphatase autoantibodies in serum. *Clin Chem* 2004;50:1378-82.
- Kareem AS, Almola GA, Alsalihi OJ. The correlation between anti-GAD65 and coxsackievirus B-IgG (CVB-IgG) in type 1 diabetes-coxsackievirus B (T1D-CVB) patients. *Int J Pharm Qual Assur* 2020;11:208-13.
- Mohammed AJ. Estimation of insulin and glutamic acid decarboxylase autoantibodies in patients with type 1 diabetes mellitus. *Diyala J Med* 2019;16:83-90.
- Thabit MF, Abduljabbar HA, Abid SG. Prevalence of immunological marker (anti-GAD) in patients with type 1 diabetes: Hospital based study. *J Faculty Med Baghdad* 2012;54:331-4.
- Mahdi NK, *et al.* The role of autoantibody and antioxidant enzymes in patients with type 1 diabetes. *Med J Islamic World Acad Sci* 2015;109:1-16.
- De Block CE, *et al.* The presence of thyrogastric antibodies in first degree relatives of type 1 diabetic patients is associated with age and proband antibody status. *J Clin Endocrinol Metab* 2001;86:4358-63.
- Association AD. 2. Classification and diagnosis of diabetes: Standards of medical care in diabetes—2019. *Diabetes Care* 2019;42(Supplement_1):S13-S28.
- Mitchell DM, *et al.* Elevated HbA1c is associated with altered cortical and trabecular microarchitecture in female with type 1 diabetes. *J Clin Endocrinol Metab* 2020;105:e1648-e1656.
- Urbanová J, *et al.* Positivity for islet cell autoantibodies in patients with monogenic diabetes is associated with later diabetes onset and higher HbA1c level. *Diab Med* 2014;31:466-71.
- Mezher IAS, Al-Khalidy NTiT, Nsiat AS. Study of the prevalence of anti-glutamic acid decarboxylase antibody in Iraqi children and adolescent with type 1 diabetes mellitus. *Al Mustansiriyah J Pharm Sci* 2011;10:114-22.
- Lee Y-S, *et al.* Prevalence of ICA and GAD antibodies at initial presentation of type 1 diabetes mellitus in Singapore children. *J Pediatr Endocrinol Metab* 2001;14:767-72.
- Zainal IG. Study the profile of some antioxidant markers in diabetic mellitus and non-diabetic patients with cardiovascular disease. *Med J Babylon* 2022;19:653-8.