RESEARCH ARTICLE

The Correlation between Anti-GAD65 and Coxsackievirus B-IgG (CVB-IgG) in Type 1 Diabetes-Coxsackievirus B (T1D-CVB) Patients

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ABSTRACT

Coxsackieviruses are one of the main causes of type 1 diabetes, due to the sequence similarity between the protein 2 C (P2-C) in the structure of the virus and the glutamic acid decarboxylase (GAD65) autoantigen in human beta-cells. The study aims to detect the anti-GAD65 and specific anti-CVB IgG in Type 1 Diabetes (T1D) patients to study the correlation between the levels of the GAD65 autoantigen and CVB- IgG autoantibody in T1D-CVB patients. A hospital-based cross-sectional study was carried out from November 2018 to July 2019 at Babylon Diabetic Center in Marjan Teaching city, Babylon teaching hospital for maternity and children and college of medicine at the University of Babylon. A total of 150 samples were obtained from diabetic patients and 50 samples from non-diabetic individuals as control. Diabetic mellitus (DM) patients diagnosed by clinical features, RBS test (above 200 mg/dL) and HbA1c test (above 6.5%). Anti-Gad and CVB-IgG detected by indirect enzyme-linked immunosorbent assays (ELISA) . The study showed the age group A2 (1-5 years), the females group (B1), and rural group (C1) more susceptible to T1D-CVB infection. The study exhibited a positive correlation between anti-gad and anti-CVB-IgG (r = 0.644**) in T1D-CVB patients.

Keywords: Coxsackievirus (CVB), Type 1 Diabetes (T1D), Glutamic acid decarboxylase 65 (GAD65), Immunoglobulin G (IgG), enzyme-linked immunosorbent assay (ELISA).

INTRODUCTION

Coxsackievirus are viruses that belongs to the Picornaviridae family and the *Enterovirus* genus, the important features included non-enveloped, linear, and positive-sense ssRNA *viruses. Enterovirus* also includes poliovirus and echovirus.[1]

Coxsackieviruses are divided into two groups A and B, depending on the pathogenesis in the mice. There are 23 serotypes (1–22, 24) of these viruses belonging to group A and 6 serotypes (1–6) belonging to group B.[2]

Type 1 diabetes is caused by insulin deficiency as a result of beta-cell damage in the pancreas; this occurs as a result of the autoimmune attack on the pancreas cells due to the great similarity between beta cells and some viruses, like Coxsackieviruses.[3]

Coxsackieviruses is one of the main causes of type 1 diabetes, due to the sequence similarity between the protein 2 C (P2-C) in the structure of the virus and the glutamic acid decarboxylase (GAD65) autoantigen in human beta-cells.[4]

This molecular mimicry leads to an immune response involving cellular and humoral immunity, which leads to the formation of autoantibodies such as IgM and IgG that attack autoantigens in beta cells such as GAD65.[5]

The study aimed to detect the anti-GAD65 and specific anti-CVB IgG in T1D patients, to study the correlation between the levels of the GAD65 autoantigen and CVB- IgG autoantibody in T1D-CVB patients.

MATERIALS AND METHODS

Samples Collection

A total of 150 serum samples were obtained from diabetic patients, and 50 serum samples from non-diabetic individuals as control. A hospital-based cross-sectional study was carried out from November 2018 to July 2019 at Babylon Diabetic Center in Marjan Teaching city, Babylon teaching hospital for maternity and children, and college of medicine at the University of Babylon. The samples collected from DM depended on the clinical features, such as, increased thirst, frequent urination, extreme hunger, and unexplained weight loss, random blood sugar (RBS) test (above 200 mg/dL), and hemoglobin A1c (HbA1c) test (above 6.5%). The age, gender,

residency, family history to DM, glycogen storage diseases, cortisone administration, were taken from the patient himself or the patient's companion during the questionnaire.

Study Design

The patients divided into six groups depended on age/years, A1 (≤ 0-1), A2 (1-5), A3 (5-10), A4 (10–20), A5 (20–30), and A6 (≥ 30), two groups depended on gender, B1 (females), and B2 (males), and two groups depended on residency C1 (rural), and C2 (urban). The study excluded the patients with the following criteria: patient who doesn't want to participate in the study, hereditary diabetes, type 2 diabetic patients, glycogen storage diseases, and cortisone administration. The included criteria, type 1 diabetic patients, all ages ranging from premature babies to aging, gender of patients, and residency of patients.

Diagnosis of Type 1 Diabetic Patient's

Diagnosis of type 1 diabetic patient's, performed by detection of glutamic acid decarboxylase (GAD65) as a biomarker in patients serum. GAD65 antibodies detected by an indirect ELISA kit (Elabscience, USA).

Detection of CVB-IgG in Type 1 Diabetic Patient's (T1D)

CVB-IgG in T1D detected by an indirect ELISA kit (Elabscience, USA).

Statistical Analysis

Statistical Package for the Social Sciences (SPSS) software (version 26.0) was used to find the effect of different factors in the study parameters. The difference between study groups and control was analyzed by Independent (unpaired) samples t-test, and the correlation between the anti-GAD and CVB-IgG, were analyzed by Bivariate correlation. Values of $p < 0.05$ and $p < 0.01$ were considered statistically significant.

RESULTS AND DISCUSSION

Prevalence of Groups

The DM patients were diagnosed by clinical features, random blood sugar (RBS) test (above 200 mg/dL), and HbA1c (above 6.5%) test, then grouped according to the following criteria:

According to age, the age group A2 (1-5) years old has the highest percentage 26.6% (40/150), while the lowest percentage was among the age group A6 (≥30) years old 5.3% (8/150) as

Table 1: Distribution of DM patients according to age

Patients group	Age range/ years	No. of DM patients	Percentage
A1	≤ 0–1	26	17.3% (26/150)
A2	1–5	40	26.6% (40/150)
A3	5–10	28	18.6% (28/150)
A4	10–20	32	21.3% (32/150)
A5	20–30	16	10.6% (16/150)
A6	≥ 30	8	5.3% (8/150)

Table 2: Distribution of DM patients according to gender

Group	Gender	No. of patients	Percentage
B1	Females	84	56% (84/150)
B2	Males	66	44% (66/150)

Table 3: Distribution of DM patients according to residency

Group	Residency	No. of patients	Percentage
C1	Rural	89	59.3% (89/150)
C2	Urban	61	40.7% (61/150)
Group	Residency	No. of patients	Percentage
C1	Rural	89	59.3% (89/150)
C2	Urban	61	40.7% (61/150)

shown in Table 1. According to gender, the females' group has the highest percentage, which constituted 84/150 (56%) patients, while the males were constituted 66/150 (44%) patients, as shown in Table 2. According to residency, the rural group has the highest percentage constituted (89/150) (59.3%) patients, while the urban constituted (61/150) (40.7%) patients, as shown in Table 3.

Detection of Anti-Glutamic Acid Decarboxylase (GAD65) by ELISA

The current study revealed a high increase in the anti-gad level in DM patients in comparison to the healthy control group (168.6 ± 29.2 pg/mL compared to 11.3 ± 2.9 pg/mL). A high significant difference was found between the anti-GAD level of patients and control at p-value (0.05), as shown in Figure 1. The present results showed anti-GAD in 49% (74/150) of DM patients in all six groups. The age group A2 (1–5) years old has the highest percentage of 41.9% (31/74). While, the age group A6 (≥30) years old 2.7% (2/74) has the lowest percentage, as shown in Figure 2. The female group has the highest percentage, which constituted 42/74 (56.7%), while the males were constituted 32/74 (43.3%) of DM patients with positive anti-GAD antibodies, as shown in Figure 3. The rural group has the highest percentage
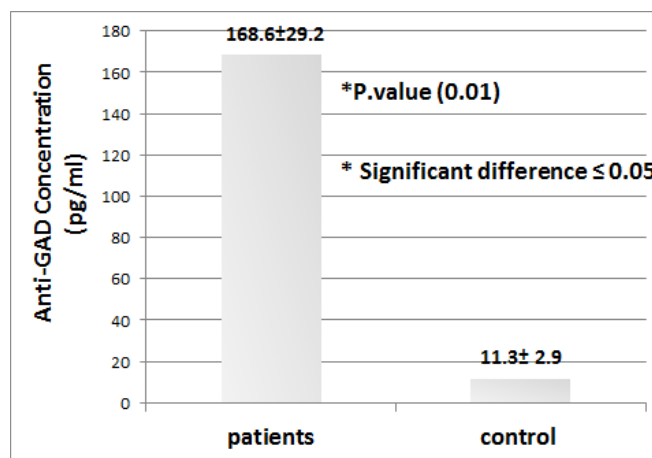

Figure 1: Anti-GAD level (study group) in DM patients in comparison to the healthy peoples (control group) with a significant difference at p-value (0.01)

constituted 54% (40/74), while the urban constituted 46% (34/74) of DM patients with positive anti-GAD antibodies, as shown in Figure 4.

Detection of Anti-Coxsackievirus B (anti-CVB) IgG by ELISA

The ELISA assay performed to detect the specific anti-CVB IgG T1D patients and to study the correlation between the high levels of the GAD and autoantibody (IgG) in T1D-CVB patients.

The study revealed a high increase in anti-CVB IgG in T1D patients compared to the healthy control group (323.18 ± 14.9 pg/mL compared to 12.9 ± 1.4 pg/mL). A high significant difference was found between the anti-CVB IgG of patients and control at p-value (0.02), as shown in Figure 5.

The present results had shown the specific anti-CVB IgG antibody in 81% (60/74) of T1D patients in all six age groups. Group A2 (1-5) years old have the highest percentage of 45% (27/60), while the age group A6 (≥ 30) years old has the lowest percentage 1.6% (1/60) in T1D patients with positive CVB-IgG, as shown in Figure 6. The female group has the highest percentage, which constituted 60% (36/60), while the males were constituted 40% (24/60) of T1D patients with positive CVB-IgG, as shown in Figure 7. The rural group had the highest percentage, which constituted 56.6% (34/60), while the urban constituted 44.4% (26/60) of T1D patients with positive CVB-IgG, as shown in Figure 8.

Figure 2: Distribution of DM patients with positive anti-GAD, according to age groups

Figure 3: Distribution of DM patients with positive anti-GAD, according to gender groups

The Correlation between Anti-GAD and CVB-IgG in T1D-CVB Patients

The present study exhibited positive correlations between anti-gad and anti-CVB-IgG (r = 0.644**) in T1D-CVB patients, as showed in Figure 9. The results showed that DM patients with high levels (168.6 ± 29.2 pg/mL, as compared to
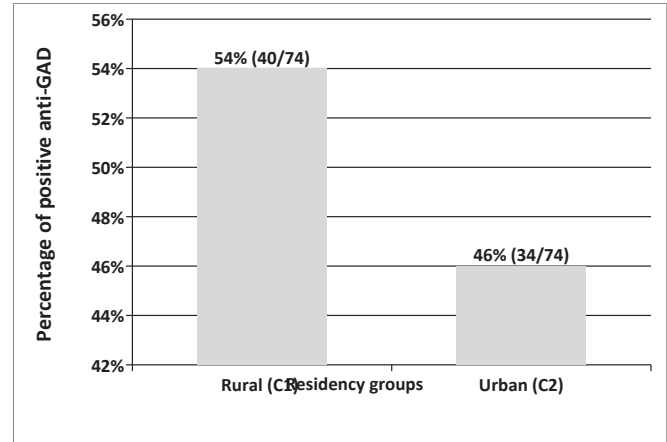
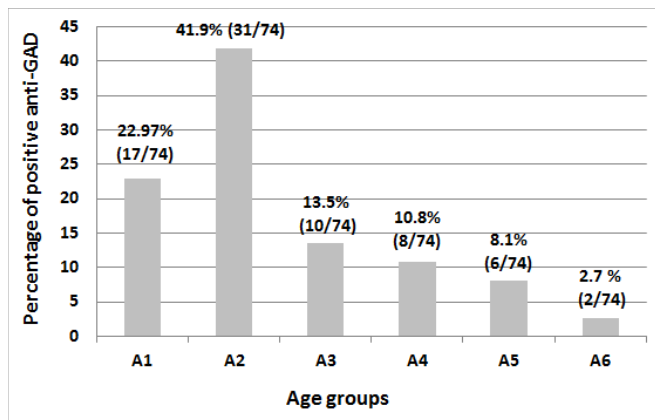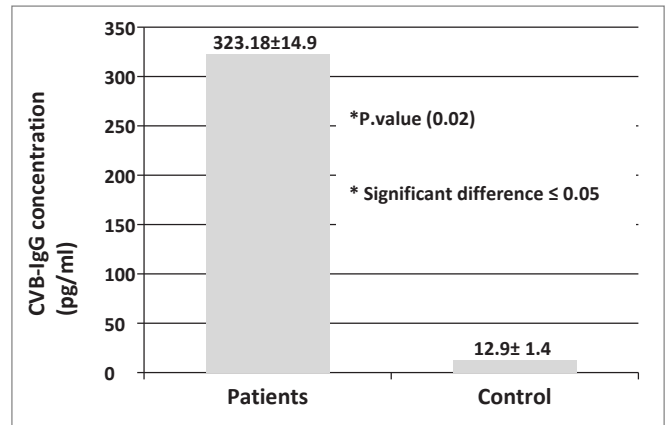**Figure 4:** Distribution of DM patients with positive anti-GAD, according to Residency groups.

Figure 5: CVB-IgG level (study group) in T1D patients in comparison to the healthy peoples (control group), with a significant difference at p-value 0.02
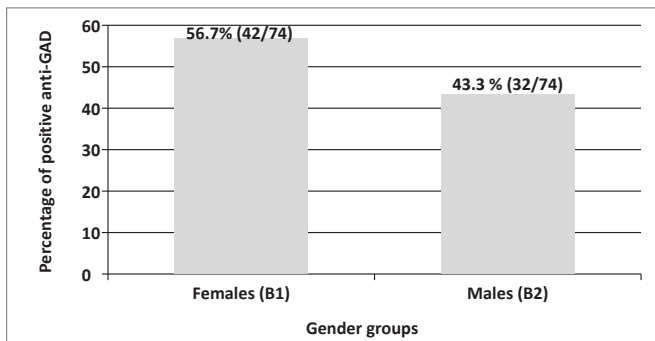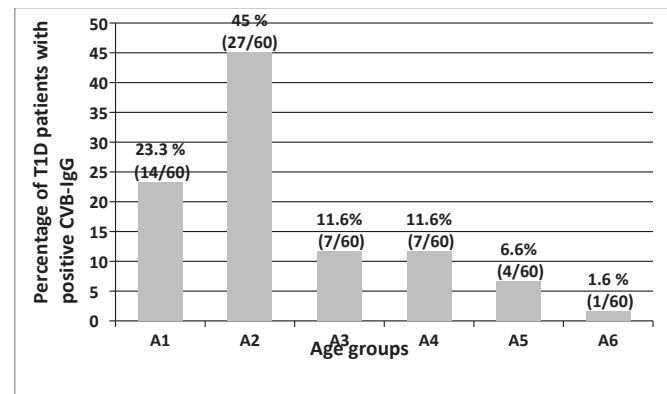
Figure 6: Percentage of T1D patients with positive CVB-IgG, according to age groups
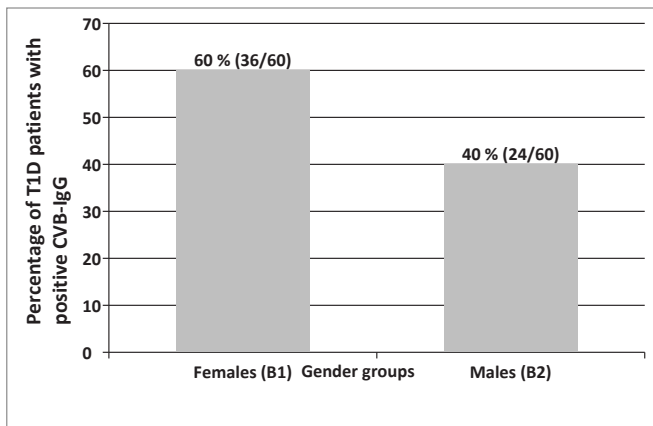
Figure 7: Percentage of T1D patients with positive CVB-IgG, according to gender groups.
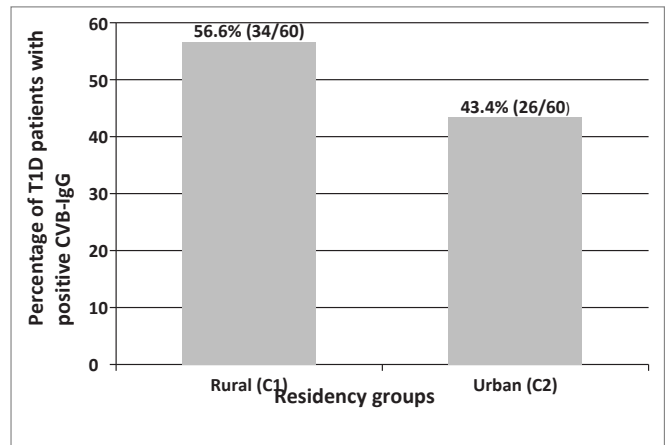

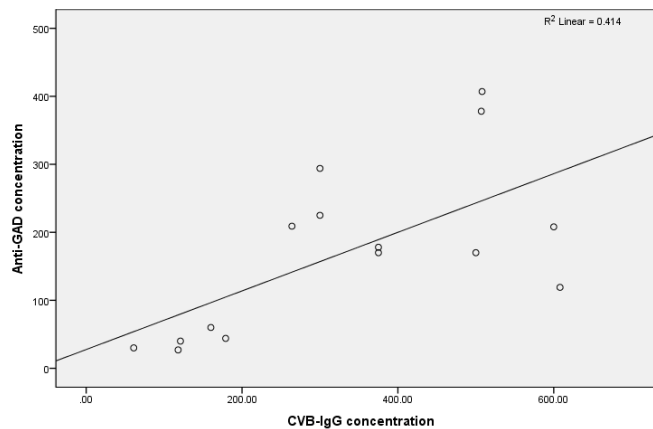**Figure 8:** Percentage of T1D patients with positive CVB-IgG, according to Residency groups.

Figure 9: The correlation between anti-GAD and CVB-IgG (** correlation is significant at the 0.01 level).

11.3 ± 2.9 pg/mL) of the anti-GAD65 with percentage 49% (74/150), they also have high levels of CVB-IgG autoantibody (323.18 ± 14.9 pg/mL in compared to 12.9 ± 1.4 pg/mL), with percentage 81% (60/74) in T1D-CVB patients.

DISCUSSION

The current study was based on several previous studies, such as,[6] who showed that GAD65 considered a biomarker to T1D patients. The detection of anti-GAD65 antibodies in serum of DM patients recognize the T1D from other DM types,[7,8] who explained that autoantibodies against GAD65 are found in 80% of type 1 diabetic patients, while only 11 to 18% of type 1 diabetic patients are positive for autoantibodies against GAD67, and another study by June et al., 2002 showed that the GAD is considered to be one of the strongest candidate autoantigens involved in triggering β-cell-specific autoimmunity.

The present results had shown anti-GAD65 in 49% (74/150) of DM patients to diagnose T1D patients. The current result

was lower than that obtained through the same assay by C.S. Hampe, et al.,[9] who referred that the prevalence of 74% (46/62) samples tested positive for GAD65Ab, and the highest prevalence of GAD65 antibody was showed in age group (1-5) years old. Also, lower from C. Rahmati, et al.,[10] who referred that 84% (64/76) from diabetic patients were positive for GAD65Ab.

The present study has found that anti-CVB-IgG prevalence rate at the TID patients was 81% (60/74) within CVB infection suspected individuals, by using specific Anti-CVB IgG, with the highest mean in children A2 (1–5), females, and rural, than other patient groups, as mentioned previously.

Present result was lower than that obtained through the same assay by R. Al- Suhail, et al.,[11] who referred that the prevalence of anti-CVB IgG within T1D patients was 88% (44/50), and the highest prevalence of CVB antibody was showed in age group 6 to 17 years and high prevalence of CVB infection, reported by males, rather than females.

On the other hand, the present result more excellent than the result of M. Abdel-Latif, et al.,[12] which revealed the prevalence of anti-CVB-IgG within T1D patients was 64% (244/382), with age group 2–16 and males more than females. Also, greater than[13] that revealed the anti-CV IgG achieved in their study was 64%.

According to the present result, we proposed the positive correlations between (Anti-Gad and CVB-IgG) may refer to the ability of CVB to induce autoantibody production, which may participate in β-cell destruction.

The present result agreed with K. Sadeharju et al., D. Hober et al., G. Frisk et al., and C. Bason et al.,[14–17] which showed there was observably increased of autoantibody (anti-Gad) in seropositive T1D-CVB infected patients.

However, the present study did not agree with L. Heino et al.,[18] which revealed that was no difference between autoantibody in diabetic patients who infected with CVB and control. Also, this did not agree with T. Kawashima,[19] who showed the viral loads revealed there was no positive CVB patient with a high level of anti-GAD antibodies.

The high level of anti-gad that showed within T1D-CVB infected patients compared to health, may be referred to as the specific correlation between them. CVB may perform polyclonal B-cell activation that is leading to the production of autoantibody upon infection. Partial homology has been suggested, which in turn may induce a harmful immune response against pancreatic cells. The continuous exhibition of related auto antigen-autoantibody could maintain prolonged immune response leads to serious tissue damage.

It was proposed there was a cross-reactivity, as a result of molecular mimicry, between CVB antigens and β-cell endogenous proteins. Due to partially resembles of the P2-C protein sequence of CVB with human GAD, that could trigger autoreactive and antiviral T-cells response against CVB infection which might be potent stimulator that may enhance vigorous autoimmune response. When CVB infects pancreatic β-cells, it will induce an inflammatory immune response, which leads to the destruction of β-cell and release of self-antigens, which in turn subject to phagocytosis and presentation by antigen-presenting cells (APCs), which in turn maintain an autoimmune process.[15,20]

Moreover, the complement activation by autoantibody could occur through the classical pathway, which can lead to direct cell lysis and damage, as well as, recruitment of leukocytes to enhance inflammatory response[21] further.

CONCLUSION

- CVB as a main non-genetic causative factor in causing the T1D.
- The age group A2 (1–5) years old more susceptible to T1D-CVB infection than other groups.
- The female group more susceptible to T1D-CVB infection than the male group.
- The rural group is more susceptible to T1D-CVB infection than the urban group.
- There is a high increase in the anti-GAD level in DM patients in comparison to the healthy control group.
- There is a high increase in anti-CVB IgG in T1D patients, compared to the healthy control group.
- There is a positive correlation between anti-GAD and anti-CVB-IgG in T1D-CVB patients.

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