# Glutamic acid decarboxylase-IgG among T2DM patients with HCMV infection and HbA1c levels

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## Abstract

**Background:** Prophetic immune marker glutamic acid decarboxylase (GAD) autoantibody is a distinctive diagnosis between Type 1 Diabetes Mellitus and Type 2 Diabetes Mellitus. Individuals have diagnose as Latent Autoimmune Diabetes in adults (LADA) has such autoantibody, which those patients incorrectly diagnosed as T2DM.

**Objective:** To determines frequency of anti- GAD IgG antibody marker in LADA patients, were misdiagnosed as T2DM in relevance with a Cytomegalovirus infectin antibodies and Hemoglobine A1c levels.

**Patients and Methods:** Ninety five patients with T2DM chosen, arranged in two groups with and without cardio vascular diseases (CVD) and a garoup of 49 individuals as healthy control matched age and gender. Samples took from subjects that attended to Hawler Cardiac Center and Diabetic Centers at Erbil city in March 2014. Sera subjected to Enzyme‐Linked Immunosorbent assay (ELISA) and Glycohemoglobin assay for HbA1c.

**Results**: GAD islet cell autoantibody was seropositive in 3.05% diabetics and 2.04% controls. There were high titers of HbA1c with low C-peptide in diabetics. A weak negative correlation was found with significant difference between anti-GAD IgG and age (r = -0.282, P < 0.017 correspondingly ).

**Conclusion**: Appropriate diagnosis for LADA could help to preserve the residual Beta-cell function.

**Key words:** Anti-GAD-IgG, Anti-CMC-IgG, C-peptide, HbA1c.

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## Introduction

Human Cytomegalovirus (HCMV) is a β-herpes virus which in 1975 secluded [1]. Preponderance of inhabitants in the world is under risk infection of Human Cytomegalovirus (HCMV) [2]. The viruses are prospective environmental factors that have a secure basis in the medical literature that induce autoimmunity in T1DM [3].

The reasonable techniques that by HCMV-Stimulate distraction of β-cells

were establish to be either through apoptotic effects [4,5] cytotoxic effects [6]. or by induction of cytokines in the infected β-cells. Imitate auto-antigens by pathogenic microbes are intellect accountable for autoreactive T-cell making active; particularly human Cytomegalovirus has a fuction in these diseases pathogenesity [7]. Autoimmune diseases through molecular mimicry may be directed autoreactive T-cells could cross-reacted with neural cells GAD65 and primary prevention of these autoimmune diseases via attacking HCMV by vaccination or tolerance-inducing induction strategies" [7].Mainfold islet antibodies or GADA alone is prognosticating outlook of β-cell distraction at diagnosis of Diabetes [8]. Clinically, a number of diabetic patients have been reported to exhibit autoimmune antibodies independent of insulin treatment. Latent auto immune diabetes of adults clinically is a private set of diabetes, which is identical to T 2 DM once is occurring, but pathophysiologically is an advanced deficiency of impaired β-cell autoimmune-mediated [9]. Zimmet et.al. firstly inserted an eponym to describe adult patients that phenotypically T2DM, hwo were positive for auto antibodies as "latent autoimmune diabetes of adults (LADA)" [10]. Adult diabetic patients who were tested positive for GAD auto-antibody and clinically classified as type 2 diabetes mellitus [11]. Less severed to compare to type 1 diabetes mellitus which remains unclear. The IgG4

subclass is more frequent among all anti-GAD subclasses, in LADA patients [12].

The proposed criteria by International Diabetes Society (IDS) to establish definition of LADA include three conditions. Firstly, an age of above 35 years, secondly, at least one antibody test positive should be reported from four antibodies that commonly seen in T1DM patients; which are 1) islet cell autoantibodies (ICA), 2) anti-glutamic acid decarboxylase (anti-GAD), 3) Insulin-associated protein-2 antibodies (IA-2A), and 4) insulin autoantibodies (IAA), and thirdly, after diagnosis within the first 6 months should not be insulin dependent [13]. Immunologically patients with T1DM and latent autoimmune daibete of adults are sharing same characteristic of autoantibodies [14]. And Interleukin-1 Receptor antagonist (IL-1Ra), Interleukin-six (IL-6) and Tumor Necrosis-alpha (TNF-alpha) as a systemic cytokines [15].

Generally, it appears that, however some anthropologic properties have beneficial effects for the preparatory inspection of LADA patients in diabetic population, a corroboratory diagnostic markers for LADA can be done by determining C-peptide level and GAD-IgG autoantibodies. In addition, adequate treatment for LADA patients can be reserved by convenient diagnosis of LADA patients from patients with T2DM patients. In view of this fact, the residual β-cell function can be preserved and their postponed autoimmune-induced distraction to a great distance [16].

Frequently, B-cells that an orchestrated with autoantibodies suggested that leads to immune-mediated B-cell dysfunction, thus previously they ongoing insulin therapy through succession of the disease paralleled to T2DM patients with negative-autoantibody [17]. Accurately, urgent protection of β-cell destraction are require for LADA patients especially obese and insulin-resistant individuals, due to endogenously reduced insulin production and convert to insulin therapy [18].

The current study aimed at demonstrating the link between HCMV infection and HbA1c levels to the autoimmune anti-GAD-IgG among subjects that diagnosed as T2DM.

## Patients and Methods

This consideration 95 T2DM patients from Mala Afandi and Cardiac centers in Erbil were enrolled from January to July 2013 in addition to 49 individuals as a healthy controls to compare the tested parameters in the patients sera and controls. The age range was 25 – 60 years. Two groups were assigned as first group including 71 patients who only had T2DM, second group patients were 25 diabetics with history of cardiovascular disease. A questionnaire was filled by all participants who were in advance informed about the study and then an informed consent was obtained from each participant. The Ethics Committee of Hawler Medical University approved this study. About 7mL venous blood was taken of which 3mL was heparinized to measure HbA1c and the rest was centrifuged to separate serum. The sera were then stored at -20ºC for ELISA investigation of serum levels of anti-GAD IgG (EA 1022D UK A04, EUROIMMUN. UK), anti-CMV IgG (1201-2, Diagnostic Automation, Inc. USA). The experiments were done and measured as mentioned in the procedure provided in the kit.

Body Mass index can be calculated according to this mode:

BMI $=$(Weight (Kg))/(Height (m^2))

## Statistical Analysis

Statistical analysis and graphing were performed using SPSS (Statistical Package for Social Study) version 19.0 and Graph Pad Prism version 6.0 to attain descriptive and inferential statistics and illustrate the data. To determine the difference between numerical variables, student unpaired t-test and F-test (ANOVA) were performed. Also, Pearson correlation test was performed to designate the correlation between variables. When (P$\leq$ 0.05) is identified to be statistical significant.
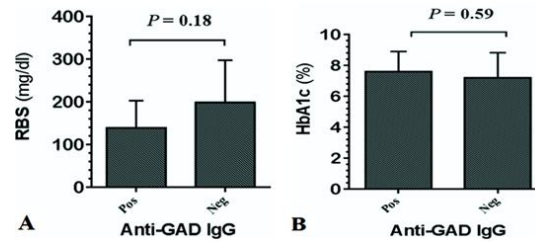
## Results

**Figure (1):** Random blood sugar levels and HbA1c percentage in relation of the total studied cases seropositive and seronegative for anti-GAD IgG.

**Table (1):** Demographic characteristic of diabetic and control subjects seropositive for anti-GAD IgG.

| Groups | Gender | anti-GAD IgG | C-peptide | Age year | BMI kg/m² | RBS mg/dl | HbA1C % | Duration of disease (year) | Insulin therapy |
|--------|--------|--------------|-----------|----------|-----------|-----------|---------|----------------------------|-----------------|
| Diabetic | Female | 10.8 | 4.7↑ | 45 | 27.3 | 210 | 8.7 | 12 | No |
| Diabetic | Female | 219.8 | 5.7↑ | 30 | 25.5 | 123 | 6.8 | 1 | No |
| Diabetic | Female | 10.4 | 0.3↓ | 60 | 24.4 | 201 | 8.7 | 10 | Yes |
| Diabetic | Male | 12.8 | 0.2↓ | 58 | 23.3 | 85 | 9.2 | 10 | No |
| Control | Male | 10 | 2.5↑ | 25 | 21.5 | 75 | 5.8 | Control | No |

**Table (2):** Frequency of anti- GAD IgG seropositivity in diabetic patients and healthy control.

| Serum Anti-GAD IgG | Diabetic | Diabetic & CVD | Healthy controls | Total |
|--------------------|----------|----------------|------------------|-------|
| Negative | 69 (97.2%) | 22 (91.7%) | 48 98% | 139 (97.2%) |
| Positive | 2 (2.8%) | 2 (8.3%) | 1 (2%) | 5 (2.8%) |
| Total | 71 | 24 | 49 | 144 |

**Table (3):** Frequency of anti-GAD IgG in anti-CMV seropositive and seronegative diabetics.

| Anti-CMV IgG | Anti-GAD IgG | | |
|---|---|---|---|
| | Seronegative | Seropositive | Total |
| Seronegative | 3 | 1 | 4 |
| Seropositive | 66 | 1 | 67 |
| Total | 69 | 2 | 71 |

## Discussion

The results of the present study showed there was no significant differences of the tested parameters namely RBS and HbA1c between anti-GAD seropositive and negative (p>0.05). Only two patients out of the total 5-seropositive for anti-GAD IgG revealed C-peptide ≥8.7 and BMI<25Kg/m². Anti-GAD IgG 2.8% seropositivity in patients with only T2DM, 8.3% in diabetics with CVD and 2% in control who were diabetes free.

The result of the present study disagree with an Iraqi study that report anti-GAD IgG frequency of 14.8% [19]. Other studies reported prevalence of 12.6% in Korea [20] and 9% in Finland [21].

In the present study some of the GAD autoantibody-positive patients had lower C-peptide level, references reorted diabetic patients with LADA that autoimmuned β-cell destruction may triggered in lessening insulin secretion ability [22].

Van Deutekom et al., award the appearance of manifold autoantibodies with or without higher anti-GAD titer, advansment disease proportion correlating with youth of age that, and elevated insulin reliance risk.

Chiefly, various autoantibody differences are found between T1DM and LADA [16]. Prevalently in LADA individuals, there are anti-GAD and ICA antibodies more than IAA, IA-2A, and zinc transporter 8 (ZnT8) antibodies in T1DM patients when compared [23].

Hwangbo *et al.* [24] referenced that patients are tending to insulin dependency when there are elevated anti-GAD titer manifest significante a reduce level of C-peptide. Hiemstra et al., accord that about 1, 5% of diabetic patients seropositive for anti-GAD IgG were seropositive for anti-CMV IgG antibodies.

## Conclusions

According to Data and current study can conclude anti-GAD-IgG has impact on diagnosis of Diabetes, especially Adult latent autoimmunity and to preserve islet cells from distraction and impress of cytomegalovirus infections on diabetes. Patients that diagnosed as Type 2 Diabetes Mellitus should deliver to GAD-IgG marker diagnosis to clarify whether they will suffer from T2DM or LADA.

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