

*Original Research Article*

# Immunological profile (GAD, ICA, insulin receptor antibodies) in type I DM patients, our experience at KHMC

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## Abstract

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The aim of this study was to determine the frequency of autoantibodies among Jordanian patients with diabetes mellitus type 1. A total of 128 subjects were included with age range between 1 year and 14 years, 45.3% (58) of which were female and 54.7% (70) were male. Samples were analysed for anti-Glutamic acid decarboxylase, anti-insulin, and anti-islet cell antibodies using mosaic pancreas (monkey)/cerebellum (monkey) instructions for the indirect immunofluorescence assay, Euroimmun, Germany. One hundred and twenty eight subjects with T1DM were enrolled in study; 58 female (45.3 %) and 70 male (54.7%). The overall frequency of any autoantibodies was 36.7% (47 of 128 subjects). Anti-GAD, anti- Islet cell and anti-insulin antibodies were detected in 30.46% (39 subjects), 25.78% (33 subjects), and 23.43% (30 subjects) respectively table-1. Anti-GAD and anti-islet cell antibodies were more frequent in male, and anti-insulin antibodies were more frequent in female's figure 1, 2, 3. Frequency of multiple antibodies more than one was observed in 38 patients (29.68%); whereas only one antibody (anti-insulin antibodies) was observed in 28 patients (21.87%). 18 of 19 subjects with positive anti-GAD antibodies had positive anti-islet cell antibodies. About one third of diabetes mellitus patients from Jordan had presence of autoantibodies such as anti-GAD, anti-islet cell, and anti-insulin in a single form or in a combination, which is suggest of having diabetes mellitus type 1. We recommend further large studies to be done in different areas of country.

**Keywords:** Diabetes mellitus, Autoimmunity, Autoantibodies

## INTRODUCTION

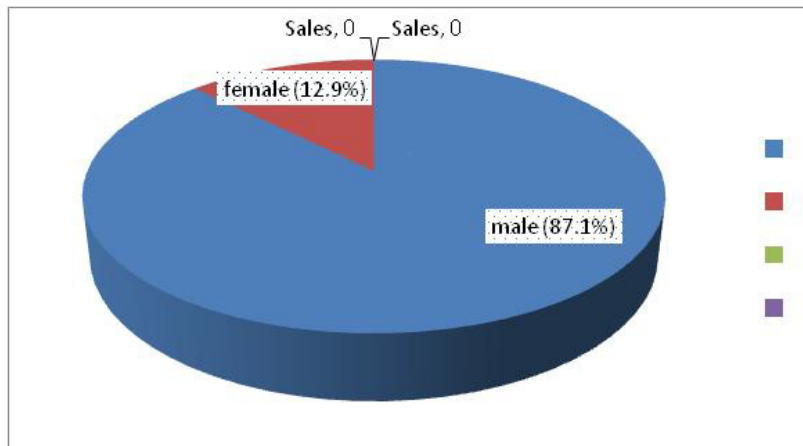
Type 1 Diabetes mellitus known as insulin-dependent or juvenile diabetes is a chronic organ-specific autoimmune disease. The majority of cases are diagnosed as type 1 diabetes within the first two decades of life (Abner and Ake, 2001). Diabetes mellitus type 1 is classified as type 1a (autoimmune) and type 1b (idiopathic). Islet cell antibodies are important differentiating factor

differentiating type 1a from type 1b diabetes (William et al., 2002).

The most frequent type is type 1 a (Young et al., 2013). Type 1a diabetes is caused by autoimmune process that leads to destruction of insulin producing  $\beta$  cells in the pancreatic islet and characterized by loss of insulin secretion (Narendran et al., 2005).

**Table 1.** frequency of anti-GAD, islet cell, and anti-insulin antibodies (total=128).

Auto-antibodies	Number	Percentage
Anti-GAD	39	30.46%
Anti-islet cell	33	25.78%
Anti-insulin	30	23.43%

**Figure 1.** Frequency of anti-GAD regarding sex (male and female)-total= 39.

As a result of autoimmunity several autoantibodies against autoantigens – ICA-2 (against islet cell), GAD 65 (against glutamic decarboxylase), and insulin are produced (Catherine et al., 2005). Detection of specific auto-antibodies in newly diagnosed diabetes is considered an immunological tool for diagnosis and for differentiating autoimmune type 1 diabetes and non-autoimmune diabetes. These autoantibodies include anti-GAD, anti-ISLET, and anti-insulin antibodies. The frequency of anti-GAD antibodies in DM type 1 is 80% and 62.9% for anti-ISLET antibodies (Pardini et al., 1999).

In this study we investigated the occurrence of the autoantibodies in type 1a DM in Jordanian population.

## METHOD

Our study was approved by ethical committee of the royal medical services, Amman-Jordan. Blood samples were collected and analysed in Princess Iman Centre for research and laboratory sciences, King Hussein Medical Center in a time period between 1/10/2016 and 30/3/2017 in a prospective manner. A total of 128 subjects were included with age range between 1 year and 14 years, 45.3% (58) of which were female and 54.7% (70) were male. 10 ml blood sample was collected, from each patient, into gel separator (with clot activator) tube. After sample clotting they were centrifuged at room temperature, and the sera were collected in cap-closed tubes. Haemolysed, icteric, and lipemic samples that may give erroneous results were set as criteria of exclusion.

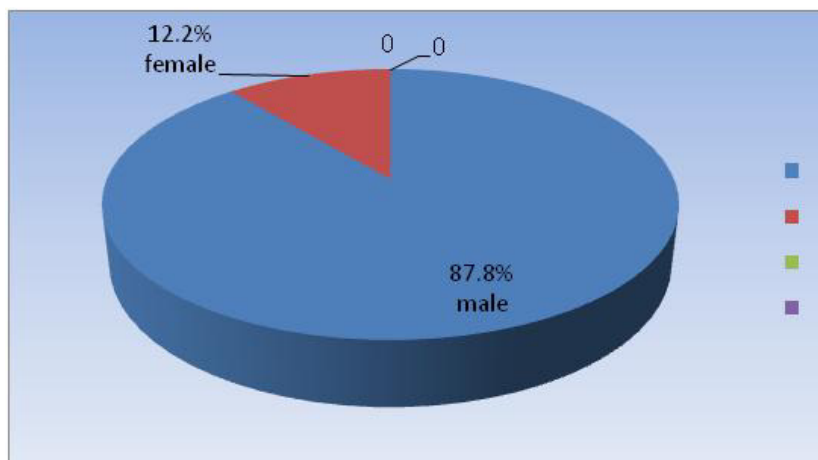
Samples were analysed for anti-GAD, anti-insulin, and anti-ISLET antibodies using mosaic pancreas (monkey)/cerebellum (monkey) instructions for the indirect immunofluorescence assay, Euroimmun, Germany. The principle of the test based on incubation of diluted patient sample with combination of primate pancreas (pancreas islet) and primate cerebellum (glutamic acid decarboxylase- GAD). If the reaction is positive, specific autoantibodies of classes IgM, IgG, and IgA attach to the antigens. In the second step, attached autoantibodies are stained with fluorescein-labelled anti human antibodies and made visible under fluorescein microscope. The titer of 1/10 and more considered as positive.

For anti-insulin antibodies detection we performed Enzyme linked immune reaction, ORGENTEC Diagnostica GmbH kit, Germany. Principle of the test based on incubation of patient sera with recombinant human insulin bound to microwells. Using Elisa reader samples reading were obtained. Samples with reading equal or more than 10u/ml considered as positive.

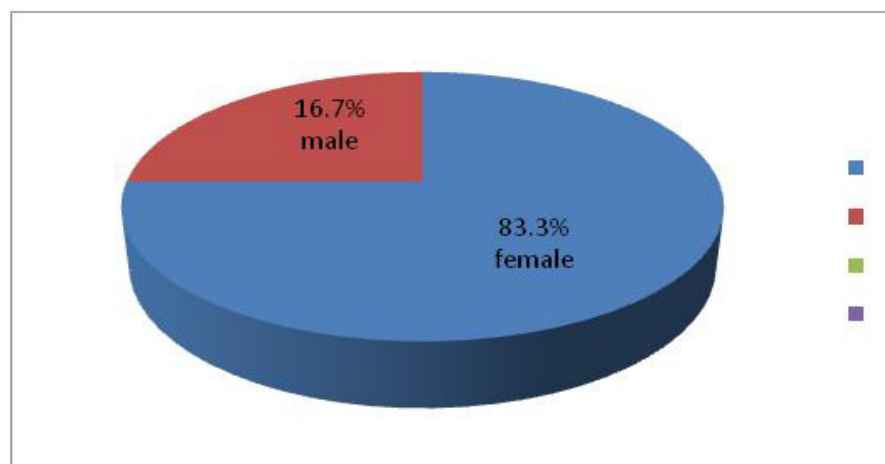
In both procedures positive and negative controls were included with each test. All sample results were analyzed using Microsoft excel sheet, windows 7 home premium.

## RESULTS

One hundred and twenty eight subjects with T1DM were enrolled in study; 58 female (45.3 %) and 70 male (54.7%). The overall frequency of any autoantibodies was



**Figure 2.** Frequency of anti-islet antibodies regarding sex. Total = 33



**Figure 3.** Frequency of anti insulin antibodies regarding sex. Total=30

36.7% (47 of 128 subjects). Anti-GAD, anti- Islet cell and anti-insulin antibodies were detected in 30.46% (39 subjects), 25.78% (33 subjects), and 23.43% (30 subjects) respectively Table 1. Anti-GAD and anti-islet cell antibodies were more frequent in male, and anti-insulin antibodies were more frequent in female's figure 1, 2, 3. Frequency of multiple antibodies more than one was observed in 38 patients (29.68%); whereas only one antibody (anti-insulin antibodies) was observed in 28 patients (21.87%). 18 of 19 subjects with positive anti-GAD antibodies had positive anti-islet cell antibodies.

## DISCUSSION

The frequency of diabetes mellitus is high among children worldwide and there is a need for early diagnosis, correct management and treatment. DM classified as type 1

diabetes (immune mediated) and type 2(non-immune mediated with insulin deficiency). Based on these findings, the autoantibodies are an important marker to differentiate between type 1A and 1B diabetes (Polly et al., 1997).

Although differentiation between T1DM and T2DM in young individuals is possible by such autoantibodies (GAD, Islet cell, and anti-insulin antibodies) (Dhanwal et al., 2014). Historically, anti- islet cell antibodies was the first autoantibodies associated with T1DM, whereas other antibodies such as anti-GAD and anti-insulin were defined later on (Taplin and Barker, 2008).

The occurrence of any autoantibodies in our study was 36.7%, which is keeping with 42.6% frequency in study conducted by Lutale et al. (2007). K. S. Aljabri et al in Saudi Arabia were found higher frequency of anti-GAD (53.8%), anti-islet (32.2%), and anti- insulin (76%) antibodies (Aljabri et al., 2013).

## CONCLUSION

About one third of diabetes mellitus patients from Jordan had presence of autoantibodies such as anti-GAD, anti-islet cell, and anti-insulin in a single form or in a combination, which is suggest of having diabetes mellitus type 1. We recommend further large studies to be done in different areas of country.

## Authors Declaration

We certify that the submitted work has not been published before and is not being considered for publication elsewhere. The work has been approved by all authors.

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