Diagnosis of autoimmune diabetes in adults using Immunological methods in Khartoum-Sudan

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Abstract

Adult's onset diabetes represent a heterogeneous mixture of type 1 and type 2 DM, often difficult to differentiate between. Such patients may actually have latent autoimmune diabetes in adults (LADA) which is an autoimmune form of DM, where beta-cell destruction is less aggressive, leading to a slower development of insulin dependency. Detection of glutamic acid decarboxylase auto antibodies (GADA) using Enzyme Linked Immunosorbant Assay (ELISA) method is a useful immunological method for diagnosis of (LADA). The aim of this study was to diagnose (LADA) patients using ELISA method to detect GADA and to identify patients who fit into the criteria of (LADA) from diabetic patients wrongly diagnosed as having type 2 DM. The study was cross sectional, conducted in El-Ribat University Hospital, in Khartoum, Sudan during the period April to May 2012. Fifty 40-60 years old patients diagnosed as type 2 DM were included; all had elevated fasting blood glucose (FBG) not responding to oral hypoglycemic agents. Results showed that 6% of these patients were GADA +ve thus proving that some patients with Type 2 DM not responding to oral hypoglycemic agents were in fact suffering from latent type1 DM; using immunological methods have a beneficial role in diagnosing such patients.

Keywords: Autoimmune, Diabetes, LADA, Diagnosis, Immunological

INTRODUCTION

Latent Autoimmune Diabetes of Adults (LADA), also known as Diabetes Type 1.5, is a term coined by Tuomi et al. (1993); Tuomi et al., (1993 ) to describe slow-onset type 1 autoimmune diabetes in adults. Adults with LADA are frequently initially misdiagnosed as having type 2 DM, based on age rather than etiology, however; it is distinguished from type 2 DM by the presence of islet auto antibodies that are common to type 1 DM. (Fasanmade et al., 2008), the autoimmune beta-cell destruction in LADA is less aggressive leading to a slower development of insulin dependency.

Patients with type 1 diabetes commonly have one or more of four islet auto antibodies: namely, islet cell antigens (ICAs), glutamic acid decarboxylase auto antibodies (GADAs), tyrosine phosphatase proteins (IA-2s), insulin auto antibody (IAA); whereas patients with
LADA typically have only one autoantibody, manifesting afar less incidence of IA-2 and ICA auto antibodies (Palmer et al., 2005).

Nowadays, the identification of autoimmune diabetes in adults represents a major interest because its prevalence is relatively high and seems to be underestimated. Also, accurate and timely diagnosis of LADA patients allows an early and accurate therapeutic intervention. (Hosszufalusi et al., 2004).

The major characteristics of LADA patients is represented by age of diagnosis greater than 40 years, positivity for auto-antibodies (ICA and/or GADA), clinical features similar with type 2 DM, glycemic control initially possible only with diet or diet and oral anti-diabetic agents. Insulin dependency can occur in a few months up to ten years or later (Zimmet et al., 2003).

Prevalence of lada

The prevalence of latent autoimmune diabetes of the adult (LADA) varies according to the population studied, criteria used and antibodies analyzed. In a series of 256 patients > 25 years, it was found that 26 (10.2%) were anti-GAD antibody (GADA) positive and 16 of them (6.3%) progressed without initial insulin requirement. Compared to GADA-negative diabetics, patients with LADA present a higher prevalence of other auto antibodies (anti-TPO, anti-21-hydroxylase and antibodies associated with celiac disease) and a higher frequency of genotypes and haplotypes indicating a risk for DM 1. Patients with high GADA titers may benefit from early insulinization and avoiding the use of oral glycaemic agents, delaying beta-cell failure. In contrast, patients with low GADA titers do not seem to have any disadvantage when managed as type 2 diabetic patients (GADA negative). (Calsolari et al., 2008).

Immunology of lada

The immunology of diabetes society released the following criteria to determine if patients have LADA, specifying that patients be: aged at least 30 years or older, positive for at least one of the auto antibodies found in type 1 diabetes and free from insulin treatment for the first six months after diagnosis. (Palmer et al., 2005).

Autoantibody positivity was associated most often with female gender (1.5 x higher frequency than men) and higher glycosylated hemoglobin (A1C) levels, as well as lower body mass index (BMI) and waist/hip ratios. (Unger et al., 2007).

Diagnostic tests for (lada) include

1) C-peptide (also known as insulin C-peptide, connecting peptide

This test measures residual beta cell function by determining the level of insulin secretion (C-peptide). β-cells produce proinsulin, which is then converted into C-peptide and insulin in equal ratios; hence C-peptide and insulin levels could be used as markers for β-cell function (Sara et al., 2012). Persons with LADA typically have low, although sometimes moderate levels of C-peptide as the disease progresses. Patients with insulin resistance or type 2 diabetes are more likely to, but will not always, have high levels of C-peptide due to an over production of insulin. Unlike insulin, C-peptide is subject to neither hepatic nor significant peripheral degradation, but is mainly removed by the kidneys. As a result, C-peptide has a longer half-life than insulin (Wahren et al., 2000).

2) Diabetes mellitus autoantibody panel

Glutamic acid decarboxylase (GAD) auto antibodies, radioimmunoassay (RIA) (both commonly found in diabetes mellitus type 1) and insulin antibodies.

3) Islet Cell Antibodies (ICA) tests

Islet cells IgG Cytoplasmic Autoantibodies, IFA; Islet Cell Complement Fixing Autoantibodies, Indirect Fluorescent Antibody (IFA); Islet Cell Autoantibodies Evaluation; Islet Cell Complement Fixing Autoantibodies – these help in differentiating between (LADA) and type 2 diabetes. Persons with LADA often test positive for ICA, whereas type 2 diabetics seldom do (Rother et al., 2007).

4) Glutamic acid decarboxylase (GAD) Antibodies tests

Micro plate ELISA: Anti-GAD, Anti-IA2, Anti-GAD/IA2 Pool, in addition to being useful in making an early diagnosis of type 1 diabetes mellitus, GAD antibodies tests are used to differentiate between LADA and type 2 diabetes (American diabetes association; 2005), and may also be used for gestational diabetes risk prediction in immediate family members of type 1 diabetes patients, as well as a tool to monitor prognosis of the clinical progression of type 1 diabetes (Unnikrishnan et al., 2004).
5) Insulin auto antibodies (IAA) tests:

Radioimmunoassay, Anti-GAD, Anti-IA2, Anti-Insulin and Insulin Antibodies tests are also used in early diagnosis of type 1 diabetes mellitus, and for differentiating between LADA and type 2 diabetes, as well as for gestational diabetes risk prediction in immediate family members for type 1 diabetes, and to monitor the prognosis of the clinical progression of type 1 diabetes. Persons with LADA may test positive for insulin antibodies; persons with type 2 diabetes, however; rarely do so (Rother et al., 2007).

MATERIALS AND METHODS

This was a hospital based, cross sectional study that was carried out in Khartoum, Sudan at the Police Hospital (El-Ribat University Hospital), department of laboratories and blood bank.

The aim of this study was to diagnose (LADA) patients using ELISA method to detect GADA and to identify patients who fit into the criteria of (LADA) from diabetic patients wrongly diagnosed as having type 2 DM.

The study involved (50) females, diagnosed as type 2 diabetes mellitus, with an age of onset between (40 – 60) years. All were complying with dietary control for diabetes during the study duration as well as having oral hypoglycemic agents but still were not attaining normoglycaemia; on the contrary most of them had high fasting blood glucose level. Other groups included in the study were (15) adult patients with confirmed type 2 diabetes on oral hypoglycemic agents and controlled fasting blood glucose, those were negative control and a group of (15) children diagnosed with type 1 DM for detection of GADA auto antibodies.

Data was collected by a structured review questionnaire which included personal history of the patient, duration of the disease, history of diabetes, fasting blood glucose, calculation of body mass index in addition to clinical diagnosis and lab test results.

Blood samples were drawn under aseptic conditions in plane containers, plasma separated and kept in refrigerator at (2°C – 8°C) until use. Samples were tested for the presence of auto antibodies to glutamic acid decarboxylase (GAD) using ELISA method. Determination of the concentration of anti-GAD antibodies was performed using anti-GAD/IA2 ELISA kit: IgG (EUROIMMUN, Germany). After incubation, an automatic washer (Human GmbH, Germany) was used to wash the samples. GAD/IA2 (biotin-labeled GAD and IA2) added to the samples and further incubated and a second wash performed. An enzyme conjugate (peroxidase-labelled avidin) was added, sample was covered and incubated at room temperature. Then, the third wash was carried out after which a chromogen/substrate solution was added and samples incubated protected from direct sunlight, at the end a stop solution was added to stop the reaction. Photometric measurement of the color intensity was performed by Elisa reader (Human SH, Germany).

Results were evaluated semi-quantitatively by calculating the ratio of the extinction value of the control or patient sample over the extinction value of the calibrator 6. The test kit (EUROIMMUN) recommends interpreting results as follows:

≥The Cut-Off ratio was only one, therefore:

Ratio ≤ 1.0 Negative
Ratio ≥ 1.0 Positive

All this work has received an ethical approval from Sudan Academy of Science and El Ribat University Hospital as well as a signed consent from the enrolled patients.

RESULTS

All study patients were clinically diagnosed as having Type-2 diabetes. The mean age of the sample patients was 50.6 ±5.6 years and the duration of the disease ranged from 0.25 to 20 years with a mean of 7.4 ±5 years. Body Mass Index (BMI) ranged from 21.3 to 42.9 Kg/m² with a mean of 26 ±3.3 Kg/m². FBG test results had a range of 276 mg/dl with a minimum of 88 and a maximum of 364 mg/dl. The mean FBG was 200 ± 68.6 mg/dl. A family history of diabetes was reported by 50% of the enrolled patients.

All patients were tested for anti-GADA auto antibodies, three (0.06 or 6%) out of the total number of patients were anti-GADA + ve. An exact (Clopper-Pearson) 95% CI for this proportion is 0.013 - 0.165, (1.3% to 16.5%). Table 1 and 2.

The mean age of the anti-GADA -ve patients were 50.2 ± 5.7 years and the median age was 50 years. Patients with anti-GAD auto antibodies were aged 52, 59 and 60 years. A visual comparison of the minimum, maximum, median and interquartile range,. Although the median age is higher in the GADA + ve group, due to the small number of cases it cannot be ascertained, to what extent this could be attributed to chance. The same also applies to the duration of diabetes and the body Mass Index. Fasting blood Glucose, however, has a slightly lower median in the GADA + ve group (185 compared to 193).

Since a sample size of 50 produces a two-sided 95% confidence interval with a width equal to 0.152 when the sample proportion is 0.06 (i.e. a 95% CI of 0.013 - 0.165), a much larger study is needed to narrow down the 95% confidence interval to an acceptable level and to reveal if the apparent differences between the two groups are genuine.

DISCUSSION

According to the previously described LADA characteristics,
3 out of the 50 patients (6%), were positive for pancreatic autoantibody (GAD 65/IA2) and have become non responsive to oral hypoglycemic agents after a few months or years after being diagnosed with diabetes, they had high fasting blood glucose levels, therefore they can be classified as LADA.

The percentage is similar with some of the published data, where the percentage of LADA cases varied as follows: Niskanen et al, Schernthaner et al, (5-10%), (Niskanen et al., 1998; Schernthaner et al., 2001). Thai et al (5-30%) (Thai et al., 1997; Ziegler et al.; 1989). (6-50%), depending on ethnic group, and higher in young people (Landin-Olsson et al., 2002).

Schiell et al. have found that 55% from all type 1 DM patients and 21% of insulin-treated type 2 DM patients have GADA, suggesting that the prevalence of LADA is underestimated. (Schiell et al., 2000). Mean age at diagnosis of LADA patients (44 ± 4 years) did not differ significantly when compared with that of type 2 DM patients (44.8 ± 4.5 years) but was significantly greater than in type 1DM (38.7 ± 6.2 years). Mean BMI in LADA patients was 27.5 ± 4.8 kg/m², significantly lower than in type 2 DM (30.9 ± 6 kg/m²), and significantly higher than in type 1 DM patients (24.6± 3.8 kg/m²). Mean fasting plasma glucose at diagnosis was similar in LADA and in type 1 DM patients (313.9 ± 132.7 mg/dl and 324.1 ± 116.5 mg/dl, respectively), but significantly higher than in type 2 DM patients (233.7 ± 90.8 mg/dl).

Depending on age and BMI, the current study agreed with studies by Turner R. and others (1997) reported that GAD 65 auto antibody were positive in 10% of cohort of >5000 patients. GAD 65 was positive in 34% of patients aged 25 – 34 years (younger), while in these study patients aged 40 – 60 years and the prevalence of GAD (+) auto antibody titer found to be higher in adults. This is because (GADA +ve) patients were aged >50 years (Turner et al., 2000).

Also, the current study agreed with an Italian study that evaluated 881 type 2 DM with mean disease duration of 8 years. GAD (+) patients was 6.6%. Most often GAD (+) auto antibody patients were associated with female gender and low mean BMI (Genovese et al., 2006).

### CONCLUSION

The study reported the prevalence of GAD antibody among Sudanese patients clinically classified as type 2 diabetes at diagnosis and were on oral hypoglycemic agents. Patients with a high titer of GAD auto antibody and long duration of diabetes had an increased risk of future insulin therapy requirement. Immunological method (ELISA) was useful in diagnosing and differentiating between LADA patients and Type 2 DM.

### RECOMMENDATIONS

1. The current study recommends testing for auto-antibodies to Glutamic Acid Decarboxylase in all patients with type 2 DM not responding to oral hypoglycemic treatment.
2. Making use of ELISA as a reliable immunological method in differentiation between LADA patients and type 2 DM is of great benefit to such patients.

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REFERENCES

Sara C, Kim AC (2012). Washington University how valuable is measurement of C-Peptide and Insulin Levels in Type 2 Diabetes? Volume 52 – Issue 7 July Diabetes Q&A.