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GAD-65 AUTOANTIBODIES AS A NOVEL MARKER IN THE DIAGNOSIS OF LATENT AUTOIMMUNE DIABETES IN ADULTS (LADA)

Biochemistry

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ABSTRACT

Latent autoimmune diabetes in adults (LADA) accounts for 2%-12% of all cases of diabetes. Patients are typically diagnosed in young adults and are often misdiagnosed as type II DM. The aim is to study the association of GAD-65 autoantibodies as a marker in the diagnosis of LADA and to compare the clinical parameters, & metabolic factors in diabetic Patients. We studied 300 diabetic patients. A multiple regression was run to predict role of GAD 65 antibodies in LADA diagnosis from Age, HbA1c, BMI, Cholesterol & C-peptide. These variables statistically significantly predicted GAD 65 antibodies, $P < 0.5$. The results of our study in a diabetic population revealed that the prevalence of LADA (17.3%). while some anthropologic characteristics can be useful for the preliminary screening of LADA patients in a diabetic population, GAD 65 autoantibodies determination can be considered as confirmatory diagnostic markers for LADA.

KEYWORDS

LADA, Diabetes mellitus, GAD 65 autoantibodies

INTRODUCTION

The global prevalence of diabetes among adults over 18 years of age has risen from 4.7 % in 1980 to 8.5 % in 2014.[1] Latent autoimmune diabetes in adults (LADA) accounts for 2%-12% of all cases of diabetes. Patients are typically diagnosed in young adults and are often misdiagnosed as type II Diabetes Mellitus (DM). Glycemic control is initially achieved with sulfonylureas but patients eventually become insulin dependent more rapidly than with type II DM patients. Although they have a type II DM phenotype, patients have circulating beta (β) cell autoantibodies, a hallmark of type I DM. With regards to its autoimmune basis and rapid requirement for insulin, it has been suggested that LADA is a slowly progressive form of type I DM. However, recent work has revealed genetic and immunological differences between LADA and type I DM. The concept of LADA was first introduced in 1993.[2,3,4,5,6]

So, the present study was conducted in a Tertiary care Hospital in Navi Mumbai in an attempt to establish LADA as a distinct, heterogenous clinical entity, its prevalence in presenting population and to conclude that testing for anti-GAD antibodies in adult-onset diabetic patients may be a routine procedure in order to detect latent insulin-dependency at the earliest possible stage. This study deals with the contribution of the immunological and metabolic components involved in the patho-physiology of LADA and recent approaches in its screening and diagnosis

AIMS AND OBJECTIVES:

To study the association of GAD-65 autoantibodies as a marker in the diagnosis of LADA. To assess the Beta cell function (C-peptide levels) in patients with LADA, & Type 2 DM. To study & compare the clinical parameters, & metabolic factors in diabetic Patients. Our study attempts to separate LADA from NIDDM more clearly & accurately to aid investigators & clinicians in diagnosing & treating diabetes more efficiently.

MATERIALS AND METHODS: Period of Study: August 2014 to December 2016. Place of Study: Biochemistry Department, Medicine & Diabetology Department MGM Hospital in Navi Mumbai. Study Design: Observational & Prospective study. The Study was carried out on 300 patients presenting to Diabetology OPD having any type of diabetes as per the Study Protocol approved by the Ethics Committee. Inclusion criteria: Patients above the age of 18 yrs. Patients attending OPD confirmed as diabetic as per WHO criteria1 will be included in the study. Exclusion Criteria: 1) Patients having gestational diabetes/ IGT/alcoholic pancreatitis /any other autoimmune disorders /steroid or drug induced Pancreatitis. All patients were given a Patient information sheet, explained about the study and an informed consent was obtained. Under aseptic precautions 8 ml blood was collected from patients with 10-12 hrs fasting for various biochemical parameters. The diagnosis of LADA is currently based on three criteria: Adult age at onset of diabetes;(2) The presence of circulating islet autoantibodies (GAD-65 autoantibodies); and (3) Lack of a requirement for insulin for at least 6 months after diagnosis. Islet autoantibodies (GAD-65 autoantibodies) are markers of beta cell autoimmunity that distinguish LADA from type 2 diabetes. A period of insulin independence after diagnosis is meant to distinguish LADA from classic type 1 diabetes. Clinical & Biochemical parameters evaluated for the study: Age, Gender, Age at onset of diabetes; BMI; Onset & Duration of diabetes; Family history of diabetes, if any; Diet and lifestyle; Estimation of Fasting & Post prandial blood glucose levels, Estimation of HbA1c levels, Estimation of Cholesterol levels, Estimation of C-Peptide Levels by Sandwich ELISA, Estimation of GAD-65 Auto antibodies (Islet cell antibodies) by ELISA.

OBSERVATION & RESULTS: After recording the details of patients, the data was sorted into different groups according to the type of diabetes (Type 2 diabetes and LADA). The mean values were calculated for each parameter and compared in these groups. Descriptive statistical analysis has been carried out in the present study. All results were expressed as mean ± SEM or as percentage. Results on continuous measurements are presented on Mean, SD, and results on categorical measurements are presented in number (%). Significance is assessed at 5% level of significance. Data were considered significant at $P < 0.05$ and highly significant at $P < 0.001$. Student t test (two tailed, independent) has been used to find the significance of study parameters. Unpaired t test and p value test has been used to find the significance of study parameters on categorical scale between the groups. Spiros fourlanos in diabetes care 2006 has published a retrospective study and formulated a LADA clinical risk score.10 1) Age of onset < 50 years. 2) Acute symptoms of hyperglycemia. 3) BMI < 25 Kg/sqmt.4) Personal history of autoimmune disease. 5) Family history of autoimmune disease.

The presence of at least two of these distinguishing clinical features that is LADA risk score ≥ 2 had 90% sensitivity for identifying LADA. In the present study all the 52 patients with GAD-65 antibody Positive had LADA risk score ≥ 2. Also with reference to WHO report on diagnosis and Classification of diabetes Mellitus, presence of GAD-65 antibodies is marker for immune destruction leading to pancreatic B cell destruction which indicates Autoimmune Diabetes mellitus3. Our results showed that out of 300 diabetic patients, 52 (17.3%) were positive for GAD- 65 antibodies.

Table 1: Comparative evaluation of clinical and biochemical parameters in different type of diabetes including LADA using 'unpaired t' test.

Clinical and biochemical Parameters	LADA	TYPE 2 DM	P value (Sig 2 tailed)
Mean age in yrs	34.13	38.18	0.0001
Mean BMI	23.7	25.2	0.0001
Mean Age of onset	31.18	33.6	0.0001
Average duration of diabetes	2.95	4.57	0.0001
Mean negative family History of diabetes %	0.92	0.61	0.0001
Mean History of Insulin or Mixed treatment	0.07	0.62	0.0001
Mean HBA1c	10	8	0.0001
Mean Cholesterol g/dl	267	194	0.0001
Mean C peptide ng/ml	0.3463	2.08	0.0001
Mean GAD -65 antibodies ng/ml	40.34	9.705	0.0001

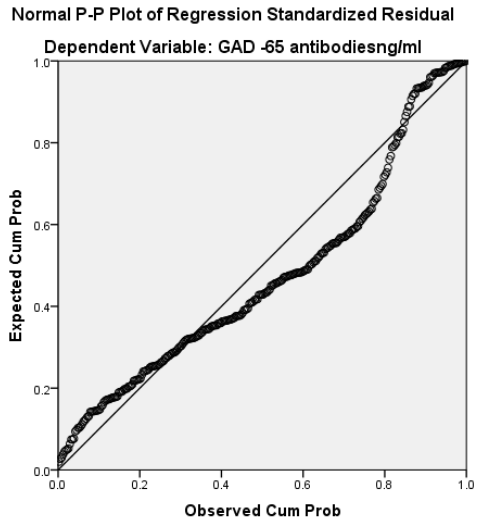
Table 3: ANOVA a

Model	Sum of Squares	df	Mean Square	F	Sig.
Regression	30309.450	5	6061.890	123.498	.000b
Residual	14430.939	294	49.085		
Total	44740.390	299			

a. Dependent Variable: GAD -65 antibodiesng/ml

b. Predictors: (Constant), C -peptide ng/ml, Age, BMI, Cholesterol, HbA1C

Fig.1



Summarizing the regression analysis of our data:
A multiple regression was run to predict role of GAD 65 antibodies in LADA diagnosis from Age, HbA1c, BMI, Cholesterol & C-peptide. These variables statistically significantly predicted GAD 65 antibodies, F (5, 95) = 123.49, p < 0.05, R2= 0.677. All the five variables added significantly to the prediction, p < 0.5. The results of our study in a diabetic population revealed that the prevalence of type 2 DM was higher (72.7%), followed by LADA (17.3%). According to reports, type 2 DM constitutes a major part of diabetes mellitus, that is, 80–90% of the total diabetic population1,2. Previous studies shows that LADA accounts for 2-12 % of the total diabetes population8,11. This implies that our findings are in accordance with the reported data[4,8,9,11,13].

Discussion: Previous Studies showed that the average age in different diabetic populations varied significantly.[2,4,6,9] In our study, The average age of LADA (34.13 years) patients was found to be significantly lower than type 2 DM (38.18yrs) (P < 0.001). The average age at onset is a very critical factor in determining the type of diabetes as well as the treatment regimen to be decided for the patient. According to reported data, one of the important identifying characteristics of LADA is adult age at onset (>25 years)[4,6,7,8]. Our study showed that the average age at onset of diabetes in patients with LADA (31.18 years) was significantly higher than patients with type 2 diabetes patients (33.6years) (P < 0.0001). In an effort to correlate BMI values with the type of diabetes, it was observed that a majority of patients with type 2 diabetes were overweight (52.9%). This implies that the findings are in agreement with the existing literature which states that obesity is one of the prime factors leading to development of type 2 diabetes and insulin resistance3,4,9. Furthermore, the BMI values of LADA patients (23.7) were significantly lower than patients with type 2 diabetes (25.2) (P < 0.001) confirming the nonobese nature of these patients. None of the patients were obese which rules out the involvement of obesity and insulin resistance in development of LADA. Most of the patients (94%) displayed BMI values corresponding to healthy individuals suggesting that the gradual β-cell destruction in LADA is insufficient to cause significant weight loss. Reports suggest that BMI values are lower in LADA patients compared to patients with type 2 diabetes. Thus our findings were in accordance with the reported data4,6,8,9,10. The HbA1c levels determination in diabetes gives an idea about the glycemic control during the last three months. Generally values above normal values (7%) indicate a poor glycemic control. HbA1c levels determined in patients with different types of diabetes showed that LADA patients (10%) had significantly higher levels compared to type 2 diabetes (8%) (P < 0.01). This was in agreement with the published data13,14. This shows poorer glycemic control in LADA patients compared to type 2 diabetes. Determination of serum cholesterol levels revealed that LADA patients (mean=267 mg/dL) had significantly higher cholesterol levels than patients with type 2 diabetes (mean=197mg/dL), which was found to be in accordance with existing data4. This indicates that LADA patients are at a higher risk of developing cardiovascular complications than patients with type 2 diabetes.

Mean C-peptide levels in Type2 DM group is 2.08 and in LADA is 0.346. Independent sample test (unpaired t test) applied. T value is 24.61, the 2 tailed P value <0.01, this difference is considered to be highly statistically significant. C-peptide is secreted at equimolar concentrations with insulin and is not degraded as rapidly as insulin.15 The results of the C-peptide determination showed significantly low levels of C-peptide in LADA patients (0.34ng/mL) compared to patients with type 2 diabetes (2.08 ng/mL) (P < 0.01). This is in accordance with existing data4. Many other studies4,6,8,9 suggested that LADA (also known as GAD 65 Ab positive type 2 diabetes) has lower C-peptide levels than patients with GAD 65 Ab negative type 2 diabetes. Low C-peptide values in LADA suggest insulin deficiency due to autoimmune β-cell destruction. Patients with type 2 diabetes displayed higher C-peptide values (2.08 ng/mL) which show hyperinsulinemia due to compensatory increase in insulin secretion due to insulin resistance in type 2 diabetes. Mean GAD -65 antibodies in Type2 DM group is 9.705 and in LADA is 40.34. Independent sample test (unpaired t test) applied. T value 51.90, the 2 tailed P value <0.01, this difference is considered to be highly statistically significant. Results of GAD 65 autoantibodies determination revealed marked presence of GAD 65 autoantibodies in LADA patients as compared to Type 2 DM. This strongly depicts the autoimmune nature of the disease, LADA, which is significant in accordance with the current existing data.4,6,8,9 The absence of GAD 65 autoantibodies in type 2 DM implicates nonimmune nature of the disease, proving a significant association of GAD 65 autoantibodies with LADA.4,6,10

In conclusion, it appears that, while some anthropologic characteristics can be useful for the preliminary screening of LADA patients in a diabetic population, C-peptide levels and GAD autoantibodies determination can be considered as confirmatory diagnostic markers for LADA. Appropriate diagnosis of LADA would prevent misdiagnosis as type 2 diabetes and would help in optimum treatment of LADA patients so that residual β-cell function is preserved and the further autoimmune destruction of β-cells is delayed. Prevalence of LADA was found to be 17.3% of the total diabetes patients enrolled in our present study. This study is done in one tertiary care Hospital so it gives only an approximate prevalence of LADA in diabetes. It is necessary to do a large multicentre study all over India to find out the correct prevalence of LADA. Furthermore, genotyping of LADA patients will prove to be substantial in understanding the role of genes involved in the disease.

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