ISSN (O): 2349-5332
CODEN: IRJPHY

MEASUREMENT OF AUTO ANTIBODIES IN LADA PATIENTS AND THEIR SIBLINGS AND HLA IN SELECT PATIENTS AND SIBLINGS

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Submitted on: 14.05.2024; **Revised on:** 21.05.2024; **Accepted on:** 27.05.2024

INTRODUCTION

Diabetes mellitus is now a raging life style non infective disorder which has gained momentum. It is characterized by high blood glucose levels (hyper-glycemia) and the inability to produce and/or target organ resistance More than 240 million persons worldwide have diabetes mellitus, and health officials estimate that this figure will exceed 300 million within the next 10 years.

There are four types are determined by the underlying mechanism, each demonstrating different levels of glycaemia and each type having a different mechanism of cause. As a well known fact the first one occurs in children and are generally insulin dependent and second one is adult onset which is controlled by drugs. There is a group which is LADA who are started on drugs but may need to be shifted to insulin if they have auto antibodies. As it has a genetic origin siblings of the diabetic patients can acquire the disease. The autoantibody estimation in both diabetic and their siblings were done to assess the importance these markers

KEYWORDS: insulin antibodies, insulin auto-antibodies, newborn infant, pregnancy, radio binding assay, type 1 diabetes mellitus

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Indian Research Journal of Pharmacy and Science; 39(2024)3011-3015;
Journal Home Page: https://www.irjps.in

INTRODUCTION

AIM

a) Estimation of Autoantibodies – GAD, IA2, C peptide, adiponectin in LADA patients.
b) Determine the mode of treatment.
c) HLA pattern in Siblings of such patients –to determine their susceptibility to the disease if lifestyle needs to be changed early.

Inclusion Criteria

i) Diabetes patients on insulin, (above the age of 20)
ii) Diabetes on anti diabetic drugs or diet (age 20 – 40)
iii) Controls i.e., patients attending hospital for other disease, proved to be non Diabetic.
iv) Siblings of overt diabetes whose blood glucose levels are normal

Exclusion criteria

1. Diabetic patient less than age of 20
2. Patients who are more than the age of 40

Materials

Samples were collected from different defined groups of patients attending Diabetic Outpatient and unknown groups in GH

i) 5-7ml of blood collected in plain tube for separation of serum to be tested for the various Auto-antibodies like, GAD65 isomer, IA2, C Peptide, insulin assay, adiponectin by ELISA
ii) 5-10ml of blood collected in heparin vials for HLA Low resolution A,B,DR typing
iii) Transported to our lab and stored at -20˚c for further evaluation

Methods

5ml of serum separated and tested by ELISA for all the given antibodies.
The RSR ELISA Version 2 kit is used for both GAD and IA 2 Autoantibody test. CALBIOTECH ELISA kit is used for the C-Peptide test and Elabscience ELISA Kit for Adiponectin.
HLA done by PCR SSP (combo (A,B,DR),low resolution kit) in AB systems 7500

Table 1: Patient Data with antibodies

Markers	Overt (all age group)		Siblings (not diagnosed with diabetes)		LADA (pts on oral drugs but un-controlled between 20-30)	
	1202	%	136	%	134	%
GAD positive	252	21	24	18	87	65
IA2 positive	42	3.5	4	3	69	52
C peptide Positive	264	10	7	5.5	16	12
GAD +IA2 Positive	12	1	nil	--	04	3
Insulin assay	216	18	6	8	77	58
Adiponectin	84	7	nil	nil	22	17

Table 2: HLA Type

Sl. No.	ANTI-GAD TEST RESULT	ANTI-IA2 TEST RESULT	HLA TYPING RESULT
1	Negative	Negative	A2B4*6,DRB1*0401,DQB*0301
2	Negative	Negative	B*15,A2,DRB1
3	Positive	Negative	A1*0501, DQBI*, DQA1*0301, DQ1*0302,
4	Positive	Negative	A*010201,DQ*0506,DRB1*0312
5	Positive	Negative	B*0406,DRB1*0402,A1*2011
6	Positive	Positive	A1*2010.DB*0107,DRB1*0312
7	(sibling)Positive	Positive	DRB1*0401,A*0203,B*0405
8	(sibling)Positive	Positive	B1*0602,A1*0102,DQA*0301

HLA TYPING – 2 samples with both antibodies negative ,3 with either positive, 1 with both positive and 2 siblings with both positive

RESULTS:

Out of 1202 overt diabetic samples, 252 (21%) were tested GAD positive and IA2 positive in 42 (3.5%).

Two antibodies (GAD and IA2) were positive in 12 (1%) and it was found that their blood sugar levels were not controlled by oral anti-diabetic drugs for over a period of one year (combination drug).

C-peptide was positive in - 264 (10%) usually C Peptide values are inversely proportional to GAD values. But this was not consistent with our study.

216 (18%) Insulin assay were positive. This shows that in spite of insulin being present this has not reached the target cells.

84 (7%) were tested Adiponectin positive. This did not have any correlation with LADA patients. This parameter plays more significance in pregnant and antenatal cases. This centre did a pilot study on this aspect.

Out of 136 Siblings sample (these siblings are not overt diabetes i.e. their sugar levels are well within limits and are not on any drugs), out of which 24 (18%) GAD positive, 4 (3%) IA2 positive, 7 (5.5%) C-Peptide positive and 6 (8%) Insulin assay positive.

Out of 134 LADA samples (age group between 20 to 40 who were uncontrolled by oral diabetic drugs), 87(65%) were GAD positive, 69 (52%) were IA positive, 16 (12%) were C- Peptide positive.

In this group 4 (3%) were both GAD and IA2 positive.

77 (58%) Insulin assay were positive and 22 (17%) Adiponectin were tested positive.

HLA typing were done in 8 samples of which 2 samples with both antibodies negative ,3 with either positive, 1 with both positive and 2 siblings with both positive.

Comparing the two sample who are negative for both it is found that A2 and DRB1 were present in both the significance could be that they have protected the individual from going for insulin dependency through subtypes could not be deciphered for the second patient.

Samples positive for either one antibody showed no similarity for samples who had both positive there was no similarity seen.

But in siblings (first one was sibling of sample 1) the HLA of the first matched with one of that of DRB1*0401 which showed that the sibling may get type 2 diabetes later as he has one of the HLA in common. This could be proved if the parent having diabetes also had this gene .

The second sibling of the sample (sibling of sample 4) had none of the alleles in common and is unlikely to get the disease .

DISCUSSION

At diagnosis, both ICA and GAD antibodies were shown to be predictors of insulin dependency, but GAD antibodies appeared to have higher sensitivity as predictors than ICA. (2,4,5&6) 21 % of the patients sample.

It shows that GAD auto antibodies are present in a large amount in circulating blood serum of an individual with LADA. This infers that GAD has a prominent role in determining the occurrence of LADA individuals.

The individuals showing positive test results for Anti-IA2 20.4% were comparatively less, than the number of Anti-GAD positive patients.

The presence of equal amount of both GAD and IA2 further strengthen the fact that presence of GAD auto antibodies are if not most prominent – important factors for depicting the presence of LADA . Thus, GAD and IA2 can be used as effective markers for diagnosing LADA.

C peptide positive patients are considered to be insulin deficient.
Siblings with lower values of both antibodies were informed about the status and given life style changes to postpone the occurrence of the disease.
Adiponectin did not have significant correlation .

Most of the patients have DRB1*03, DQB1*0201 or the DRB1*04, DQB1*0302 haplo type.

The sample size has to be increased to come to any concrete conclusion.

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CONFLICT OF INTEREST REPORTED: NIL; SOURCE OF FUNDING: NONE REPORTED