ISSN 0975-6299 Vol.1/Issue-3/Jul-Sep.2010

International Journal of Pharma and Bio Sciences

ROLE OF C- PEPTIDE IN IDENTIFICATION OF PATIENTS SUSPECTED OF HAVING LATENT AUTOIMMUNE DIABETES IN ADULTS (LADA) IN NORTH INDIAN TYPE 2 DIABETES MELLITUS POPULATION.

SHAKTI AGGARWAL*[1], ATUL GOEL[2] AND ANJU JAIN[1]

[1]Department of Biochemistry, Lady Hardinge Medical College and associated hospitals, New Delhi - 110001,India.
[2]Department of Medicine, Lady Hardinge Medical College and associated hospitals , New Delhi -110001, India.

Corresponding Author: shaktiaggarwal@gmail.com

ABSTRACT

Aims: The objective of this study was to utilize C - peptide levels to identify the patients suspected of having latent autoimmune diabetes in adults from clinically diagnosed type 2 diabetics. *Methods*:100 type 2 DM patients and 100 healthy controls were selected. All patients were assessed for fasting C-peptide and insulin levels. Patients having C-peptide level<0.7 ng/ml were classified as suspected LADA subgroup. All patients were followed up six months later for fasting C- peptide and insulin levels along with insulin resistance. *Results*: 34% of the patients in the study group were classified as suspected LADA group while the rest of the patients in the study group (n = 66) were named as classic type 2 DM. There was a significant difference in the levels of C – peptide and insulin as well as insulin resistance between the suspected LADA group and classic type 2 DM patients, which was maintained on, follow up. *Conclusions*: C-peptide can be used as an important screening tool for autoimmunity.

KEY WORDS

Latent autoimmune diabetes in adults, type 2 Diabetes Mellitus, Glutamic Acid Decarboxylase antibody, C – peptide

INTRODUCTION

Latent autoimmune diabetes in adults (LADA), a term given by Zimmet et al comprise of clinically diagnosed type 2 Diabetes Mellitus (type 2 DM) patients who are positive for circulating autoantibodies to glutamic acid decarboxylase (GADA), islet cell cytoplasm (ICA), insulin (IAA), tyrosine phosphatase like molecule (IA-2A) [1]. It is characterized by slower beta cell destruction as compared to type 1 DM. The manifestation of LADA is later than type 1 DM and is often misdiagnosed as type 2 DM. Initially, glycemic control is achieved with oral hypoglycemics but eventually there is marked autoimmune destruction of the pancreatic beta cells and insulin dependency is seen within 3-5 years after diagnosis. Many studies on LADA show a prevalence of 10- 30% and the commonest autoantibody in these patients has been reported to be GADA and or ICA [2- 5]. Hence, many workers have advocated GADA and or ICA testing

to identify LADA patients [3, 4, 8, 9]. It has been documented that a high titre and or presence of multiple autoantibodies correlates with severe and faster β cell dysfunction [6-8]. However, the presence of autoantibodies, irrespective of the titre and number indicates a need for future insulin therapy. Early identification of these patients would enable earlier institution of insulin therapy to prevent rapid β cell dysfunction, a hallmark of autoimmune diabetes. Therefore it is important to identify these individuals in diagnosed type 2 DM patients.

However various studies in LADA have shown a negative association of autoantibody positivity with residual β cell function assessed by C- peptide levels [3,10, 11]. In the study by Monge et al, this negative association was independent of BMI and duration of disease [3]. Xia et al also observed that the heterogeneity in the severity of LADA (rate of β cell deterioration) is associated with the titre of autoantibodies irrespective of the duration of the disease [12]. As fasting C- peptide level is an important and reliable indicator of beta cell function, fasting C-peptide levels can indirectly assess the severity of the autoimmune destruction of the beta cells.

In this study, C-peptide levels were used to screen suspected LADA patients in diagnosed type 2 DM patients.

MATERIAL AND METHODS

The study was conducted in the department of Clinical Biochemistry and Department of Medicine, Lady Hardinge Medical College and Smt sucheta Kriplani hospital, New Delhi, India. The study was approved by the institutional ethical Committee.

The study group consisted of 100 clinically diagnosed type 2 DM patients based on the criteria– diagnosis at > 25 years of age, initial six months of insulin independence. Patients with history of ketoacidosis at the time of initial diagnosis /intake of diabetogenic drugs /gestational diabetes and or other secondary causes of diabetes were excluded from the study.

The control group consisted of 100 healthy subjects matched for age and sex with the study group.

All patients included in this study were enrolled after informed consent. A detailed history and clinical examination was carried out in all the subjects. The body mass index (BMI) of each subject was also calculated using the formula BMI= Weight (Kg) / Height (m^2).

Sample collection and processing

After an overnight fast of 12 hours, venous blood samples were collected from all the patients and serum was separated by centrifugation. Serum was then stored in 2 aliquots at -20ºC until batch analysed for C- peptide and Insulin.

Vials containing sodium fluoride (NaF) and potassium oxalate powder were used for obtaining plasma. Fasting plasma glucose was measured using a kit based on glucose oxidase method (Randox, Antrim, Crumlin, UK) on Synchron CX-9 autoanalyser (Beckmann Coulter, Fullerton,CA,USA).

For estimation of Glycosylated hemoglobin (%HbA_{1c}), sample was collected in EDTA vials and analysis was done by an ion exchange chromatographic – spectrophotometric method. (FAR Diagnostics, Pescantina ,Verona, Italy).

Determination of C-peptide, Insulin

Fasting C-peptide was quantified using a direct sandwich ELISA (Mercodia, Uppsala, Sweden). Fasting insulin levels were also measured by sandwich ELISA using commercial kits and calibrators from DRG International, Marburg, Germany. The assay had a sensitivity of 1.76µU/ml and had negligible cross reactivity with human proinsulin (0%).

Determination of Insulin Resistance

Insulin resistance was calculated using Homeostasis Model Assessment (HOMA-IR) by using the formula [13]

HOMA-IR = (Fasting glucose x Fasting insulin)/405

(Glucose is given in mg/dL and Insulin is given in µU/mL).

Follow-up

All patients in the study group were followed up six months later (from the time of enrollment). Fasting plasma glucose, C- peptide and insulin levels were determined again and HOMA-IR was calculated again.

Statistical Analysis

Statistical analysis was carried out using SPSS for windows 10.0 software (SPSS Inc., Chicago, IL, USA). Data are expressed in Mean ± Standard Error of Mean. Controls, suspected LADA cases and classic type 2 diabetes mellitus were compared using one-way Anova and Kruskal - Wallis test where it was indicated. The suspected LADA cases were compared with the type 2 diabetes mellitus patients using the independent't' test or Mann – Whitney U test. p values <0.05 and < 0.01 were considered significant and highly significant respectively.

RESULTS

The evaluation of the C-peptide levels suggested that the type 2 DM patients in this study comprised of two distinct subgroups.34% of the patients in the study group had C peptide < 0.7 ng/ml and they were classified as suspected LADA group while the rest of the patients in the study group (n = 66) were named as classic type 2 DM. There was a highly significant difference in the level of C peptide between the two subgroups (Table 1). Likewise, a significant difference in fasting insulin levels and insulin resistance (HOMA- IR) was also found between the suspected LADA subgroup and type 2 DM (Table 1).

Table 1.
Comparative analysis of beta cell function and insulin resistance

	Suspected LADA[1] (n=34) Mean ± S.E.	Classic type 2 DM [2] (n=66) Mean ± S.E.	Controls[3] (n=100) Mean ± S.E.	P value [1 versus 2 versus 3]	P value [1 versus 2]
Fasting insulin (µU/ml)	10.42 ± 0.73	16.09 ± 1.13	13.85 ± 0.55	0.003	0.001
Fasting C - peptide (ng/ml)	0.39 ± 0.03	1.54 ± 0.09	0.87 ± 0.03	0.000	0.000
HOMA-IR	4.19 ± 0.53	7.3 ± 0.74	3.06 ± 0.13	0.000	0.006

n comparing the demographic profile between the two groups, suspected LADA patients had a mean age of 38.71 years ± 7.51 at the time of diagnosis as compared to 42.98 years ± 8.32 for the classic type 2 DM patients. A lower mean BMI was observed in suspected LADA patients than classic type 2 DM and the difference was significant. The mean fasting blood glucose and $\%HbA_{1c}$ were lower in suspected LADA group than in type 2 DM but the difference was not significant (Table 2).

Table 2

Comparative analysis of anthropometric and biochemical parameters

	Suspected LADA[1] (n=34) Mean ±S.D.	Classic type [2] DM2 (n=66) Mean ±S.D.	Controls[3] (n=100) Mean ±S.D.	P value [1 versus 2 versus 3]	P value [1 versus2]
BMI (kg/m2)	22.34 ± 0.56	26.73 ± 0.53	25.74 ± 0.54	0.000	0.000
Fasting blood sugar (mg/dl)	165.35±16.77	176.45 ± 9.35	89.45 ± 0.97	0.000	0.53
HbA1c (%)	7.92 ± 0.19	8.23 ± 0.17	4.83 ± 0.07	0.074	0.26

On follow up of the patients in the study group, 5 were lost to follow up from the suspected LADA group and 22 from the classic type 2 DM. The remaining patients in both the subgroups (n = 29 for suspected LADA group and n = 44 for classic type 2 DM) were again subjected to fasting insulin and C peptide levels and HOMA- IR was also calculated. There was no significant difference between the initial and the follow up values for the respective subgroups. However there was a significant difference between the mean of the follow up values of these parameters between the suspected LADA and classic type 2 DM group (Table 3).

Table 3.
Follow up

	Suspected LADA[1]		Classic type [2] DM2		P value [I versus 2]	
	Initial (n=34)	Follow up (n=29)	Initial (n=66)	Follow up (n=44)	Initial	Follow up
Fasting insulin (µU/ml)	10.42±0.73	10.18±0.89	16.09±1.13	15.17±0.84	0.001	0.000
Fasting C - peptide (ng/ml)	0.39±0.03	0.33±0.04	1.54±0.09	1.43±0.01	0.000	0.000
HOMA-IR	4.19±0.53	4.09±0.69	7.3±0.74	7.45±0.45	0.006	0.000

Values are mean ± S.E of mean

DISCUSSION

Based on C-peptide levels (< 0.7 ng/ml), 34 of the 100 patients in the study group were classified as

suspected LADA while the rest (66) were classified as classic type 2 DM.

When fasting C peptide and insulin levels along with HOMA – IR were compared between the two subgroups, it was a significantly lower in the suspected LADA subgroup than in classis type 2 DM patients (Table 1). A lower mean age of diagnosis was found in the suspected LADA subgroup in comparison to the classic type 2 DM and this is in agreement with other findings [8, 17].We also observed a significantly lower BMI in the suspected LADA patients. Tuomi et al, the UKPDS data, Isomaa et al and Hosszufalusi et al also reported similar findings [2, 4, 14, 15]. The suspected LADA patients showed a lower mean fasting blood glucose and HbA_{1c} in comparison to the classic type 2 DM group. Tuomi et al and Yang et al documented a higher mean fasting blood sugar in LADA patients [2, 16]. The observation in the present study could be due to higher obesity and associated insulin resistance in the classic type 2 DM patients.

The Immunology of Diabetes Society has given three criteria to define LADA – diagnosis at > 30 years, presence of at least one circulating autoantibody (GADA/ICA/IAA/IA-2A), and initial insulin independence for the first six months [17]. Though we were unable to diagnose LADA in our study group on the basis of antibody testing but we were certainly able to identify two groups of population based on their c-peptide levels. The high agreement of fasting C-peptide, fasting insulin profile and HOMA – IR of suspected LADA patients with those of definite LADA patients in other studies, indicates that these patients cannot be merely dismissed as type 2 DM [14,16]. Also, on follow up of the patients in the study group (suspected LADA versus classic type 2 DM), a highly significant difference was observed for all these three parameters and it can be commented that the study group definitely consisted of two distinct subgroups – suspected LADA and classic type 2 DM.

This approach does have its limitations as a screening tool in the general population as it cannot differentiate LADA from classic type 2 diabetes mellitus in the initial stages of the disease .C- peptide levels do not differ significantly between the two types of diabetes in the earlier phase of the disease process, however, a more rapid decrease in C peptide level is seen later in LADA as compared to type 2 diabetes mellitus [15].

CONCLUSION

Although a bigger sample size and testing for autoantibodies such as GADA, ICA, IA-2A is needed to give a definite conclusion, we emphasize the potential role of fasting C-peptide level to identify LADA patients in type 2 diabetes mellitus patients especially in populations with low seropositivity and in developing countries where cost-benefit ratio is a huge point of concern.

REFERENCES

1. Zimmet PZ, Tuomi T, Mackay IR et al., Latent autoimmune diabetes in adults (LADA): The role of antibodies to glutamic acid decarboxylase in diagnosis and prediction of insulin dependency, Diabetes Med 11:299-303, 1994.
2. Tuomi T, Groop LC, Zimmet PZ, Rowley MJ, Knowles W, Mackay IR.,Antibodies to glutamic acid decarboxylase reveal latent autoimmune diabetes mellitus in adults with a non insulin dependent onset of disease, Diabetes 42: 359-362,1993.
3. Monge L, Bruno G, Pinach S et al., A clinically oriented approach increases the efficiency of screening for latent autoimmune diabetes in adults (LADA) in a large clinic based cohort of patients with diabetes onset over 50 years ,Diabetes Medicine 21: 456-459,2004.
4. Turner R, Stratton I, Horton V et al .UKPDS 25, Autoantibodies to islet cell cytoplasm and glutamic acid decarboxylase for prediction of

insulin requirement in type 2 diabetes. Lancet 350: 1288-1293,1997.
5. Palmer JP, Hirsh IB, What's in a name: Latent autoimmune diabetes of adults,type 1.5,adult-onset and type I diabetes. Diabetes Care 26:536-538,2003.
6. Lohmann T, Kellner K, Verlohren J et al,Titre and combination of ICA and autoantibodies to glutamic acid decarboxylase discriminate two clinically distinct types of latent autoimmune diabetes in adults (LADA),Diabetologica 44: 1005-1010,2001.
7. Verge CF, Gianani R, Kawasaki E et al.,Number of autoantibodies (against insulin, GAD or ICA 512/IA2) rather than particular autoantibody specificities determines risk of type I diabetes ,J Autoimmun 9:379-383,1996.
8. Genovese S, Bazzigaluppi E, Goncalves D et al., Clinical phenotype and β – cell autoimmunity in Italian patients with adult onset diabetes, Eur J of Endocrinol 154:441-447,2006.
9. Fourlanos S, Perr C, Stein MS, Stankovich J, Harrison LC, Colman PG, A clinical screening tool identifies autoimmune diabetes in adults, Diabetes Care 29: 970- 975, 2006.
10. Arikan E, Sabuncu T, Ozer EM, Hatemi H,The clinical characteristics of latent autoimmune diabetes adults and its relation with chronic complications in metabolically poor controlled Turkish patients with type 2 diabetes mellitus, J Diabetes Complications 19: 254-258,2005.
11. Panczel P, Hosszufalusi N, Bornemisza B et al, Latent autoimmune diabetes in adults (LADA): part of the clinical spectrum of type 1 diabetes mellitus of autoimmune origin, Orv Hetil 142: 2571-2578,2001.
12. Xia L, Lin Y, Zhiguang Z, Gan H, Xiang Y, Glutamic acid decarboxylase 65 autoantibody levels discriminate two subtypes of latent autoimmune diabetes in adults, Chinese Medical Journal 116: 1728-1732,2003.
13. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and B-cell function from fasting plasma glucose and insulin concentrations in man, Diabetologia 1:28: 412–419, 1985.
14. Isomaa B,Almgren P,Henricsson M et al, Chronic complications in patients with slowly progressing autoimmune type I diabetes (LADA), Diabetes Care :22 1347- 1353,1999.
15. Hosszufalusi N, Vatay A, Rajczy K et al, Similar genetic features and different islet cell autoantibody pattern of latent autoimmune diabetes in adults (LADA) compared with adult onset type I diabetes with rapid progression, Diabetes Care 26: 452-457,2003.
16. Yang L, Zhou Z,, Huang G, Ouyang L, Li X, Yan X , Six year follow up of pancreatic β cell function in adults with latent autoimmune diabetes, World J of Gastroenterol 11: 2900-2905,2005.
17. Palmer JP, Hampe CS, Chiu H, Goel A & Brooks – Worrell B, Is latent autoimmune diabetes in adults distinct from type 1 diabetes or just type 1 diabetes at an older age?, *Diabetes*:**54** 62-67,2005.