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Physiological Factors Affecting AIA in T2DM and Healthy Controls.

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Research Article

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ABSTRACT

Anti-insulin antibodies (AIA) have been found to be in circulation in normal individuals as well as diabetic patients. AIA are known to affect the physiological function of insulin. This study was under taken to determine the AIA status in normal subjects and in type-II diabetics and to correlate AIA titres with physiological factors like age and BMI. 40 normal individuals (mean age 48.83 ± 8.58) and 80 type II diabetics (mean age 49.86 ± 7.21) were selected from Department of Endocrinology, M.S.Ramaiah hospital, Bangalore. Blood samples were collected from the patients. Plasma glucose levels (FBS, PPBS), HbA1c levels using ELISA method and AIA levels using RIA method were determined. Height and weight of the subjects were recorded and BMI was calculated using standard method. The frequency of AIA positivity in type-II diabetics did not differ significantly from those of normal non-diabetic individuals (6/80 were AIA positive in type II diabetics, 3/40 were AIA positive in normal subjects, p= 0.618). Age, BMI showed negative correlation, as age (p= 0.010) and BMI (p= 0.275) increased AIA titres decreased. There is no significant difference in AIA titers in type-II diabetics and in normal individuals. AIA titer is more in younger and leaner individuals.

INTRODUCTION

Glycemic control is one of the primary functions of insulin. This is affected by a host of factors in normal subjects as well as in diabetics. One of the factors contributing to decreased insulin function is the presence of antibodies against insulin especially Anti insulin antibodies.

Diabetes mellitus is a clinical syndrome characterized by hyperglycemia due to absolute or relative insulin deficiency. Diabetes is clinically classified into 2 types

- Type I Diabetes mellitus (T1DM)
- Type II Diabetes mellitus (T2DM)

Traditional phenotypic and clinical criteria used to diagnose T1DM are 0-30 years as age of onset, with lean BMI, abrupt onset, incidental diagnosis, short history of symptoms, glucose levels being very high, ketosis most common complication. Results due to β cell destruction by immune inflammatory cells with serological evidence of antibodies against islet cells of pancreas and are Insulin dependent- treated by (subcutaneous) insulin only.

Whereas T2DM are more than 35 years of age, usually obese, often presents with symptoms for years, glucose levels being moderately raised, ketosis very rare and neuropathy, nephropathy, retinopathy being very common complications. Results due to Peripheral insulin resistance, Impaired insulin secretion, Increased glucose production and are insulin independent can be treated by diet management/OHA/insulin.

These days age cannot be considered as the main criterion because many cases of T1DM have been reported to occur even after 30 years. It can occur from 0 to 90 years. T2DM has been reported to occur in children and adolescents also. 8- 45% of children are known to present with T2DM. Obesity can also be no longer taken as an important criterion as many lean individuals have been reported to have T2DM.Likewise insulin dependency is

also not the criterion for T1DM as many T2DM have shown primary and secondary oral hypoglycemic drug failure (OHAF). Many T2DM require insulin therapy from the time of diagnosis (primary oral hypoglycemic drug failure) [1,5].

There is also a subgroup of T2DM patients of about 5-15% who have autoantibodies against Islet cells and Insulin that are similar to those of T1DM. The presence of these antibodies suggests that even T2DM may have an autoimmune process responsible for their insulin secretary deficit/insulin resistance. Even β inflammatory cells have been observed in T2DM patients apart from auto antibodies. It has also been found that 10-15% of T1DM are antibody negative. All these factors have made it difficult to classify diabetic patients into type-I and type-II diabetes mellitus [1,5].

It is believed that autoimmune β cell destruction process proceeds slowly in this form of type II diabetes mellitus [3].

Glutamic acid decorboxylase-65 antibody (GAD-65/ GADA), Anti insulin antibody (AIA), Islet cell antibody-512 (ICA- 512), Insulinoma associated antibody /Tyrosine phosphatase antibody (IA-2), Insulin receptor antibody (IRA) are all the diabetic associated antibodies which serve as markers of autoimmune process. These antibodies are found in a majority of (60-75%) of newly diagnosed T1DM, in a significant minority of T2DM individuals (15-20%), occasionally in individuals with gestational diabetes mellitus (<5%) and also in a small percentage of non-diabetic population (3-5%) [1,5,25,26,22]. Many research works have also been done and found Diabetes associated antibodies among 3-5% non-diabetic individuals [2,6,10,11,18].

Objectives

Our objective was to evaluate the presence of anti-insulin antibodies (AIA) in normal controls and in T2DM patients.(subjects). If present to evaluate the relationship between anti-insulin antibodies (AIA) and physiological factors like age and BMI.

MATERIALS AND METHODS

40 healthy controls and 80 T2DM patients on Diet/Oral hypoglycemic agents and never on insulin aged between 35 – 60 years were included and Subjects with preexisting endocrinal, autoimmune diseases, Fever and other infections were excluded from our study. These subjects were recruited from the out patient diabetic clinic at the Division of Endocrinology M.S Ramaiah Memorial Hospital. A detailed physical examination was done. The study subjects were evaluated for blood pressure, body weight and height

Assays

Fasting and postprandial venous plasma glucose was determined by glucose-oxidase method using glucose autoanalyser. Glycosylated hemoglobin (HbA1c) was determined using ELISA method. AIA titres were determined using RIA (Radio immuno assay).

Statistical Methods

Student t test (two tailed, Independent)/, ManWhitney U test has been used to find the significance of AIA titres between the diabetics and controls. Analysis of variance has been used to find the significant change AIA titres with age and BMI.

Significant figures

+ Suggestive significance $0.05<P<0.10$
* Moderately significant $0.01<P \leq 0.05$
** Strongly significant $P \leq 0.01$
* **Statistical software:** The Statistical software namely SPSS 11.0, Stata 8.0 and Systat 11.0 were used for the analysis of the data and Microsoft word and Excel have been used to generate graphs, tables etc.

RESULTS

Statistical analysis of AIA in healthy controls and T2DM revealed no significance, depicting that the incidence of AIA is equally prevalent in both groups as shown in table 1.We found significant statistical correlation between AIA titers and age of type-II diabetics. AIA titers decreased significantly as the age of the subject increased, So there is a negative correlation between Age of T2DM with a p value of 0.010** as shown in table 2. We found a negative correlation between BMI and AIA even though not significant (p value 0.27) as shown in table 3. As BMI increases AIA decreases.

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Table 1: Comparison of AIA between Healthy controls and T2DM

	T2DM	Normal	P value
AIA!	6.26±0.76 (2.00-34.30)	6.53±1.23 (2.50-43.30)	0.618

! Non-Parametric test-Mann Whitney U test

Table 2: Association of Age with AIA

Age in years	AIA	
	Min-Max	Mean ± SE
35-40	3.10-34.30	11.43 ± 3.54
41-45	2.70-29.60	8.51 ± 2.40
46-50	2.80-16.65	5.28 ± 0.97
51-55	2.00-7.30	4.42 ± 0.28
56-60	2.70-5.10	3.89 ± 0.19
All cases	2.00-34.30	6.26 ± 0.76
Significance	F=3.576, P=0.010**	

As age increases the AIA decreases significantly

Table 3: Association of BMI (kg/m^2) with AIA

BMI (kg/m^2)	AIA	
	Min-Max	Mean ± SE
≤20	2.70-32.04	10.35±3.89
21-25	2.00-27.04	6.94±1.50
26-30	2.75-34.30	5.79±1.13
31-35	2.90-7.30	4.31±0.45
36-40	2.70-5.10	4.11±0.37
All cases	2.00-34.30	6.26±0.76
Significance	F=1.307, P=0.275	

DISCUSSION

There is a wide spectrum within diabetes syndrome. T1DM may have a slow progression with good residual insulin secretion and without autoantibodies, while phenotypic T2DM may have autoantibodies. A single patient may have traits of both TIDM and T2DM. The etiology is mainly unknown, but environmental factors play an important role in genetically predisposed individuals. The search for just one single cause for the manifestation of DM is confusing [10]. Causes of this mistake are varied and are not well understood. It is thought that some autoantibody production is due to a genetic predisposition combined with an environmental trigger (such as a viral illness or a prolonged exposure to certain toxic chemicals). While families may be prone to develop autoimmune conditions, individual family members may have different autoimmune disorders, or may never develop an autoimmune condition. The type of autoimmune disorder or disease that occurs and the amount of destruction done to the body depends on which systems or organs are targeted by them.

AIA is found in the majority of T1DM, few T2DM, in high-risk prediabetic population and also in about 8% of normal population. The presence of AIA in a diabetic patient may disturb glucose homeostasis in various different ways. First these antibodies may mediate hyperglycemia by binding insulin secreted during a meal and releasing it post-prandially. When the blood insulin dissociates from insulin antibody, it inappropriately raises the plasma free insulin resulting in post- prandial hypoglycemia. Binding of insulin, to insulin- antibody can also result in its decreased clearance from the blood predisposing to fasting hypoglycemia. AIA belongs to Ig G group of Immunoglobulins. With the exception of Anti insulin antibody (AIA) none of the above mentioned diabetic antibodies are β cell specific. AIA may influence the quality of the glycemic control in diabetic patients [5,7,23, 24].

Anti-insulin antibodies have been found in T2DM who have or have never received exogenous insulin.It is found more frequently in T2DM patients who require Insulin therapy in association with GAD-65. Presence of both AIA and GAD-65 antibodies has proved to be almost gives 100% positive prediction for Insulin dependency [1].

AIA has been proposed to be particularly useful in predicting the onset of disease. Anti insulin antibodies (AIA) are one of several markers for autoimmune diabetes, but alone deserve special attention because unlike the other diabetic associated antibodies their ligand is unique to the β cell. AIA is the first marker to appear during the asymptomatic period, which precedes diabetes and they are present in the vast majority of young children destined to develop diabetes [7].

In the present study we have compared AIA in normal control and T2DM population. 3 out of 40 (7.5%) in normal controls and 6 out of 80(7.5%) among T2DM were positive for AIA, the incidence of AIA is almost equal in the two groups. In fact, the highest titer of AIA was found among one of the healthy control. May be due to high beta cell reserve, this healthy individual has not developed diabetes.

Our results are similar to the study done by Maruyama T et al who observed that the frequency of GAD-65 and AIA positivity in T2DM did not differ significantly from those of healthy controls [18]. Alok Kanuga et al observed in their study the frequency of GAD-65 was not significantly different among T2DM and healthy controls [3].

Three out of hundred normal individuals express one or more of these diabetic associated antibodies and 3 out of 1000 develop diabetes. This suggests that in the majority of antibody positive individuals, diabetes never develops or may develop late in life. The frequency of these diabetic associated antibodies in control population is usually higher than the incidence of the disease- 0.1 to 0.5%. Several types of diabetic associated antibodies have been reported in non diabetic patients and other autoimmune diseases [1,22,23].

The prevalence of GAD-65 was 1.6% and ICA was 0.1% in healthy controls in the study conducted by Niskanen L K et al [10], 3% were positive for GAD-65 and 1% for IA- 2 among healthy controls in the study conducted among Eastern Indians by Sanjeevi C B et al. There are individuals with extremely high levels of GAD-65 antibodies and no evidence of progression to diabetes [4].

At present the measurement of diabetic related antibodies in non diabetics is a research tool because no treatment has been approved to prevent the occurrence or progression of antibody positive T2DM.

In our study we excluded T2DM on insulin treatment and all were on diet/or OHA (oral hypoglycemic agents), as many studies have reported the occurrence of AIA in T2DM on Insulin treatment [1,5,22]. We also excluded subjects with auto-immune diseases and fever, as AIA is known to be associated with Graves' disease, stiff man syndrome, pernicious anemia, and other autoimmune disorders and in febrile conditions like chickenpox, mumps etc [1].

In our study we found the frequency of AIA in normal control population and type-II diabetics was same. So the possession of antibody per se cannot be taken as completely predictive of disease (diabetes) development. It is possible that antibody positive individuals who do not develop diabetes may have some degree of ß-cell destruction, which does not achieve the severity required for the appearance of abnormalities in glucose metabolism (diabetes mellitus) [2]. Pre-diabetics may pass through a stage of impaired glucose tolerance or even non-insulin requiring diabetes mellitus before frankly becoming insulin dependent [4].

In our study we observed that AIA titer is linearly associated with age of the patient. It is a well-known fact that adaptation plays a role in the level of the antigen-antibody reactions. This could be hypothesized to be the cause for the presence of higher titres of AIA being present in younger individual compared to those who were older.

Age is not a criterion in classification of diabetes although T1DM most commonly develops before the age of 30, an autoimmune beta cell destructive process can develop at any age. The autoantibodies detected at diagnosis of juvenile onset diabetics are not found in 15% of otherwise typical patients, and in the other 85% the antibodies disappear over the next year. Likewise type-2 diabetes mellitus more typically develops with increasing age but it also occurs in children, particularly in obese adolescents. Antibodies in T2DM are associated with age of the patient at onset of diabetes mellitus [5].

A UKPDS study found the proportion of patients with ICA and GAD-65 decreased with increasing age at diagnosis. They also found that antibody positive T2DM<35 years had low BMI and low HbA1C compared to older antibody positive T2DM>35 years. They concluded that among young adults with T2DM, the phenotype of those with antibody positive was similar to that of classic T1DM. In older antibody positive T2DM, the phenotype was closer to that of antibody negative diabetics [13]. Many studies have shown that the frequency of diabetic associated antibodies is high among younger compared to older type II diabetes mellitus patients [12,18].

J. R. Sa et al found that antibody positive T2DM with OHAF were younger at age than antibody negative OHAF [6]. Arikan E et al have found that GAD-65 positive T2DM had significantly earlier diabetes onset ($p< 0.001$) [13]. Alok Kanuga et al in their study divided T2DM into different groups and found maximal IA-2 positivity in 20- 30 age group (73%) and in 50–60 age groups (60%) [3]. Juneja R et al found that more than 90% type-II diabetes mellitus overlapped with BMI and age irrespective of their antibody status [14].

Antibodies decrease with age, so antibodies developing soon after diagnosis are transient.

The identification of diabetes type is sometimes difficult in non-obese (lean) adults aged 25-50 years. A subset of these patients may develop oral hypoglycemic agent failure (OHAF) rapidly. 1/3rd of these patients have a late onset and slowly evolving autoimmunity against β cells and others may have a dysfunction of β cells and/or a decrease in insulin sensitivity induced by chronic hyperglycemia [9]. Type-II diabetics in developed countries are predominantly obese; those in India are often non-obese [15].

Many studies have reported that diabetic associated antibody positive T2DM have low BMI (lean) compared to antibody negative T2DM [13,14,16,17,19]. Lohman T et al concluded that T2DM with more than one antibody are lean compared to a single antibody [17]. Alok Kanuga et al reported in their study that GAD-65 positive T2DM were of normal weight compared to GAD-65 negative T2DM who were obese [3].

We found a negative correlation between BMI and AIA titres even though not significant. AIA titers were high in T2DM, with less BMI compared to T2DM with more BMI. Beyan H et al concluded that antibody positive T2DM are lean so they are similar to T1DM compared to antibody negative T2DM [10]. Type II diabetics in developed countries are predominantly obese; those in India are often non-obese/ lean [17].

CONCLUSION

In our study we have observed that the incidence of anti insulin antibodies (AIA) is equal in type-II diabetic and healthy control population (7.5%). So AIA alone cannot be used as a specific antibody test to prove autoimmunity in type-II diabetes mellitus, even though it is β cell specific. It has been observed that AIA titers were high in younger and leaner individuals.

Many studies have been conducted to determine the occurrence of diabetes-associated antibodies in healthy individuals as well as in diabetic patients Most of the studies have been done to determine GAD-65 as it is the most common and also most sensitive among the diabetic related antibodies. So GAD-65 assay would give a significant result. Also assay of combination of antibodies (GAD + ICA-512 + AIA) would give better results than a single antibody titer.

REFERENCES

1. Frances M Ashcroft and Stephen JH Ashcroft. Insulin: Molecular Biology to Pathology. University of Laboratory of Physiology and Clinical Biochemistry, Oxford UK.
2. Horton V, Stratton I, Bottazzo GF, Shattock M et al "Genetic heterogeneity of autoimmune diabetes age of presentation in adults is influenced by HLA DRB1 and DQB1 genotypes (UKPDS Group). U K Prospective Diabetes Study. Radcliffe Infirmary, Diabetes Research Laboratories, UK. Diabetologia. 1999 May;42 (5):608-16
3. Alok kanungo, CB Sanjeevi. IA-2 Autoantibodies Are Predominant in Latent Autoimmune Diabetes in Adults Patients from Eastern India. Ann NY Acad Sci. 2003;1005:390–394.
4. R David G Leslie, Michela Delli Castelli et al. Age dependent influence on the Origins of Autoimmune Diabetes" Institute of Cell and Molecular Science, Queen Mary College, University of London, U.K. Diabetes. 2004;53;3033-40.
5. P Read Larsen, Henry M Kronenberg, Shlomi Melmed, Kenneth S Polonsky. Williams Textbook of Endocrinology. Tenth Edition. Harvard Medical University, Boston,Massachusets.
6. JR Sa, RC Silva, F Nasri et al. Non-obese adult onset diabetes with oral hypoglycemic agent failure: islet cell autoantibodies or reversible beta cell refractoriness?. Braz J Med Biol Res. 2003;36(10):1301-1309.
7. Potter KN, Wilkin TJ et al. The molecular specificity of insulin autoantibodies. University of Southampton, UK. Diabetes Metab Res Rev. 2000;16(5):338-53
8. Ueno H, Nishiyama A, Akita M, Watanabe T et al "The measurement of insulin antibodies and insulin autoantibodies by enzyme-linked Immunosorbent assay using recombinant human insulin antigen and its clinical application. Nippon Naibunpi Gakkai Zasshi. 1994;70(6):585-96.
9. Panczel P Hosszufalusi N, Bornemisza B Horvath L et al. Detection of antibodies against pancreatic islet cells in clinical practice." III. Belgyogyaszati, Klinika, Semmelweis Orvostudomanyi Egyetem, Budapest. Orv Hetil.1999;140(48):2695-701.
10. Sanjeevi CB, Balaji M, Balaji V, Seshaiah V. Autoantibodies to GAD65 and IA-2 antibodies are increased but not tissue transglutamatminase (TTG-Ab) in type 2 diabetes mellitus (T2DM) patients from South India." Department of molecular medicine, Karolinska institute, Karolinska Hospital, Sweden. Ann NY Acad Sci. 2003;1005:387-9
11. Juneja R, Hirsch IB, Naik RG, Brooks-Worrell BM et al "Islet cell antibodies and glutamic acid decarboxylase antibodies, but not the clinical phenotype, help to identify type 1(1/2) diabetes in patients presenting with type 2 diabetes. Department of medicine, University of Washington, Seattle, WA 98108, USA. Metabolism 2001 Sep (9):1008-13.

12. Unnikrishnan AG, Singh SK, Sanjeevi CB. "Prevalence of GAD-65 antibodies in lean subjects with type n diabetes" Ann N Y Acad Sci.2004 Dec; 1037:118-21.
13. Lohmann T, Kellner K, Verlohren Hj, Krug 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). Department of Internal medicine III Leipzig, Germany. Diabetologia. 2001;44(8):1005-10
14. Hathout EH Thomas W, EL-Shahawy M, Nahab F et al. Diabetic autoimmune markers in children and adolescents with type 2 diabetes. Pediatr.2001;107(6):E102
15. Chong Hwa Kim, Ji Hyan Park, Tae Sun Park and Hong Sun Back. Autoimmune Hypoglycemia in a Type II Diabetic Patient with Anti-Insulin and Insulin Receptor Antibodies" Division of Endocrinology and Metabolism, Chonbuk, National University Medical School, Chonju, Korea, 561-712.
16. Dennis L.Kasper, Eugene Braunwad, Anthony S. Fauci. Harrisions Principles Of Internal Medicine. Vol-II, 16th Edition. Harward Medical School:Boston..
17. Akira Kasuga, Taro Maruyama, Shinya Nakamoto, Yukako Ozawa et al. High-Titer Autoantibodies against Glutamic acid decorboxylase Plus Autoantibodies against Insulin and IA-2 Predicts Insulin Requirement in Adult Diabetic Patient. Department of Internal medicine Tokyo, Japan: 12th April 2002.
18. Maruyama T, Kasuga A, Ozawa Y, Nagata A et al. Glutamic acid decorboxylase65(GAD65)antibodies and insulin auto-antibodies in Japanese patients with non-insulin-dependent Diabetes mellitus. Endocr J.1997 Feb:44(1):43-51
19. Henrik Borg, Anders Gottsater, Per Fernlund, and Goran Sundkvist. A 12 Year Prospective study of the Relationship Between Islet Antibodies and β-Cell Function At and After the Diagnosis in Patients With Adult-Onset Diabetes. Diabetes 2002;51:1754-1762.
20. Pedro Weslley Souza Rosario, Janice Sepulveda Reis, Ricardo Amim et al. Comparison of Clinical and Laboratory Characteristics Between Adult-Onset Type 1 Diabetes and Latent Autoimmune Diabetes in Adults. Diabetes Care. 2005;28:1803-1804.
21. Kobayashi T, Tanaka S, Harii N, Aida K, Shimura H et al. Immunopathology and genetic features in slowly progressive insulin-dependent diabetes mellitus and latent autoimmune diabetes in adults. Third department of internal medicine, School of Medicine, University of Yamanashi, Tamaho, Yamanashi 409-3898, Japan.
22. Michiaki Fukui, Koji Nakano, Etsuko Maruya et al. Diagnostic Significance of Antibodies to Glutamic Acid Decarboxylase in Japanese Diabetic Patients with Secondary Oral Hypoglycemic Agents Failure. Department of Internal medicine Tokyo, Japan: 18th April 2002.
23. Efimov AS, Malyzhev VA, Sakalo EA. Anti-insulin antibodies and circulating immune complexes in patients with diabetes mellitus. The Endocrine Society Ter Arkh. 2004;59(11):15-8.
24. Gambelunghe G, Forini F, Laureti S, Murdolo G et al. Increased risk for endocrine autoimmunity in Italian type 2 diabetic patients with GAD65 autoantibodies. Department of Internal medicine and Endocrine & Metabolic sciences, University of Perugia, Italy. 1: Clin Endocrinol (Oxf). 2000;52(5):565-73.