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Original Research Article

Prevalence of Autoantibodies in Underweight Diabetic Adult Subject in Southern Rajasthan

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Conflict of interest: Nil

Abstract

Introduction: This study aims to estimate the autoantibody profile of underweight, physically active diabetic subjects, to know the exact etiology of their diabetes because this will help the medical staff for proper management with less morbidity and mortality.

Methodology: This study was conducted in the Biochemistry department, RNT medical college, Udaipur.

Inclusion criteria: Underweight diabetic subjects on oral antidiabetic drugs or insulin, Age 18 years to 60 years and Both sex.

Exclusion criteria: Obese or overweight diabetic individuals, Gestational diabetes mellitus, Secondary diabetes mellitus - pancreatitis, post pancreatectomy, steroid induce DM, lipodystrophy, congenital insulin resistance syndrome and Age less than 18 years and above 60 years.

Results: Out 110 of which 95 patients tested positive for only GAD antibodies and 5 patients tested positive for both GAD antibodies and tTGIgA. 10 patients were sero negative for either antibodies. 17 seropositive patients were demonstrating good control of blood glucose as evident by HbA1c levels (6.5%-7.5%) while 83 patients were having HbA1c levels above 7.6% experiencing poor control of blood glucose. The mean value of fasting blood glucose was higher in sero positive patients (176±57.18) mg% when compared with sero negative patients (163.81±36.66) mg%.

Conclusion: Antibodies can be present long before the clinical presentation of type 1 diabetes, and their detection can be useful in confirming a diagnosis of type 1 diabetes. AntiGAD Antibodies have the advantage because their titer remains relatively stable over a period of time.

Keywords: AntiGAD Antibodies, HbA1C, Type 1 Diabetes, tTGIgA

Introduction

The prevalence of diabetes in India is increasing day by day; not only of type 2 Diabetes Mellitus, but also of type 1 Diabetes. Progressively increasing sedentary life style & unhealthy eating habits can explain majority of type 2 diabetics. Thus, it is important to see contribution of their nutrition, dietary habits & environmental factors for pancreatic insufficiency as well as autoimmunity. Evaluation for antibodies against insulin or pancreatic beta cells and other gut antigens may be helpful in correctly classifying these individuals.

Gamma amino butyric acid (GABA) is a major inhibitory neurotransmitter of the brain, it is produced at high levels in pancreatic islets1.In the nervous system GABA is stored in, and secreted from, synaptic vesicles. The mechanism of GABA secretion from beta-cells remains to be elucidated.

GABA immunoreactivity was found to be concentrated in regions of beta-cells which were enriched in synaptic-like microvesicles. These findings suggest that in beta-cells synaptic-like microvesicles are storage organelles for GABA and support the hypothesis that storage of non-peptide signal molecules destined for secretion might be a general feature of synaptic-like microvesicles of endocrine cells[1].

While the total function of GABA in β cells is incompletely understood, its synthesizing enzyme GAD is possibly one of the most significant pancreatic islet β cell autoantigens. GAD is a primary target of autoantibodies and anti-GAD antibodies are associated with the development of diabetes[2].

Both Diabetes and Coeliac disease are polygenic disorders, in which more than 30 genetic loci have been described to be associated with the diseases, thereby contributing to the genetic susceptibility. This comorbidity can largely be attributed to overlapping genetic HLA risk loci; in both conditions, the HLA-DQ2 and DQ8 genes have been shown to be important determinants of disease susceptibility. We report that a single-nucleotide polymorphism (SNP) in the gene (PTPN22) encoding the lymphoid protein tyrosine phosphatase (LYP), a suppressor of T-cell activation, is associated with type 1 diabetes mellitus (T1D)[3].

Transglutaminase 2 (TG2) is the most widely distributed and most abundantly expressed member of the transglutaminase family of enzymes, a group of intracellular and extracellular proteins that catalyze the Ca2+ dependent posttranslational modification of proteins.

The pathogenesis of CD is largely attributed to trigger by wheat gluten. “Gluten” refers to the protein complex of wheat that may include more than 100 different molecules. When gluten is ingested, it is digested to form peptides. The proline- and glutamine-rich residues remain undigested[4].

These glutamine residues can be converted to negatively charged glutamic acids by tTG, which is a calcium-dependent enzyme that mediates deamidation of gliadins that eventually lead to the formation of epitope that binds efficiently to DQ2, which is then recognized by gut T cells[5].

These activated CD4+ T cells lead to the production of a number of cytokines that can in turn promote inflammation and villous damage in the small intestine. Activated gluten-specific CD4+ T cells can also stimulate B cell production of antigluten as well as anti-tTG antibodies[6].

Another feature of celiac disease is the high prevalence of various autoimmune disorders, especially type I diabetes, dermatitis herpetiformis, autoimmune thyroiditis, collagen diseases, autoimmune alopecia, and autoimmune hepatitis.

The purpose of this study is to evaluate the autoantibody profile of these underweight, physically active diabetic subjectsin Udaipur region, Rajasthan and to know the exact etiology of their diabetes.

Material & Methods:

This study was conducted in the Biochemistry department, RNT medical college, Udaipur (Rajasthan)after approval by ethics committee & obtaining due consent from subjects. It is a cross sectional study, include 110 underweight (BMI< 18.5 kg/m2) adult diabetic subjects (both existing & newly diagnosed).

Inclusion Criteria: Underweight diabetic subjects on oral antidiabetic drugs or insulin, Age 18 years to 60 years of both sexes.

Exclusion Criteria: Obese or overweight diabetic individuals, Gestational diabetes mellitus**,** Secondary diabetes mellitus - pancreatitis, post pancreatectomy, steroid induce DM, lipodystrophy, congenital insulin resistance syndrome, Age less than 18 years and above 60 years

Methods:110 consecutive study subjects were taken from OPD and wards of department of Medicine. Subjects were briefed about the study & its purpose and after obtaining due consent, 10 ml of blood sample was collected for biochemical analysis. A complete history was taken & Anthropometric measurements were done include height and weight. Parameters were recorded in Performa.

Investigations:

After overnight fasting following laboratory investigations was done in all subjects: Complete blood counts, Renal function tests- serum urea, serum creatinine, Liver function tests, Fasting blood glucose & post prandial blood glucose, Anti-GAD (glutamic acid decarboxylase) antibodies, anti TTG (Tissue transglutaminase) antibodies and USG abdomen to rule out pancreatic calcification.

Biochemical analysis were done in Siemens Autoanalyser using Flex® reagent cartridges. Anti-GAD antibody & Anti TTG antibody will be measured by ELISA.

Observations:

Here, In our study out of 110 patients 100 were GAD positive and out of 110 patients 5 were TTG positive. And, among seropositive patients of which 63 patients in 18-39 years of age and 42 patients in 40-60 years. Among GAD positive patients 64 were male and 36 were female. And, among TTG positive patients 4 were male and 1 was female.

Table 1: shows the biochemical parameters among seropositive and negative patients and among them we found that random glucose, globulins and A/G ratio, were significantly different among seropositive and negative patients as p-value <0.05.

		MEAN±SD		P-value
		Positive patients	Negative patients	
Age Of Diagnosis (Years)		26.36±13.27	33.4±15.53	>0.05
Glucose	Random	490.68±160.59	378±169.15	<0.05
	Fasting	176±57.18	163.81±36.66	>0.05
	Postprandial	352.12±133.68	334.72±104.73	>0.05
Liver Function Test	Total Protein (gm%)	7.78±0.62	7.63±0.56	>0.05
	Albumin (gm%)	3.94±0.53	4.2±0.58	>0.05
	Globulins (gm%)	3.85±0.62	3.43±0.68	<0.05
	A/G Ratio	1.02±0.26	1.23±0.38	<0.05
Renal Function Test	Urea (mg%)	30.41±12.73	32±15.22	>0.05
	Creatinine (mg%)	0.80±0.23	0.80±0.20	>0.05

	Uric acid (mg%)	3.87±1.04	3.58±0.67	>0.05
Lipid Profile	Ldl Cholesterol	104.53±33.20	97.90±18.89	>0.05
	Hdl Cholesterol	55.70±22.35	54.36±18.14	>0.05
	Triglycerides	142.63±127.63	133.63±86.63	>0.05
	Total Cholesterol	183.87±45.01	180.45±33.85	>0.05
micro elements	Calcium	9.10±0.64	8.87±0.72	>0.05
	Phosphorus	3.71±0.65	3.33±0.54	>0.05
CBC Profile	Haemoglobin (gm/dl)	10.45±2.05	11.10±1.77	>0.05
	White Blood Cells (*103 /microlitre)	7.21±1.8	6.79±1.7	>0.05
	Red Blood Cells (*106 /microlitre)	5.17±0.56	5.24±0.60	>0.05
	Platelets (*105 /microlitre)	2.28±0.76	2.38±0.73	>0.05

Table: 2 and 3 shows Distribution of patients based on tTGIgA and GAD antibodies concentration. And we found that based on tTGIgA antibodies concentration in which 5 patients were seropositive (>12 u/ml), 105 patients were seronegative (<8 u/ml) and no patient was in indeterminate range. And, based on GAD antibodies concentration in which 10 patients were seronegative (<1.00 u/ml), 100 patients were positive (>1.05) and no patient was in indeterminate range.

Table 2: Distribution of patients based on tTGIgA antibodies concentration

Number of patients	Less than 8 (u/ml)	8-12 (u/ml)	Above 12 (u/ml)	TOTAL
	105	0	5	110

Table 3: Distribution of patients based on GAD antibodies concentration

Number of patients	Less than 1.00 (u/ml)	1.00-1.05 (u/ml)	Above 1.05 (u/ml)	TOTAL
	10	0	100	110

56 patients were presenting with osmotic symptoms, 28 patients with ketosis, 11 patients with weakness and 5 patients complained of weight loss (Fig:1).

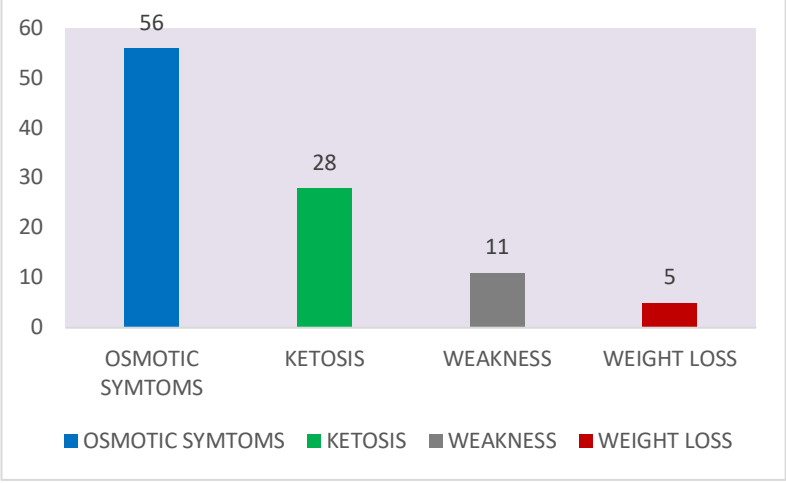

Figure 1: Distribution of patients based on clinical presentation

Discussion

Our current study estimated the autoantibody profile of underweight diabetic subjects as this will assist in proper management with decreased morbidity.The patients with lean diabetes in comparison to classical obese type 2 diabetes are characterized by younger age at onset, earlier and more prevalent use of insulin and higher prevalence in males. It was initially shown that patients with diabetes who are leaner have higher mortality rate in comparison to the obese (the obesity paradox).

Our study group consists of 110 patients out of which 77 (70%) patients were residents of tribal areas. This part of Rajasthan is a tribal belt whose inhabitants are often malnourished. Considering treatment, the common experience is that these patients do not fit in type 2 DM but they are atypical for type 1 DM also. In order to correctly classify these patients, our current study aimed at detecting antibodies against GAD (Glutamic acid decarboxylase) and tissue Transglutaminase (tTGIgA).

Mohan V et al studied around 10000 diabetics from India in which around 3.5% patients were lean with a BMI < 18.5 kg/m2 and highlighted the fact that HbA1c, fasting and postprandial blood glucose levels were higher among those in the lean group7.

Our study endorsed the findings of Mohan V et al[7] as we found that lean diabetics (BMI< 18.5 kg/m2) exhibited higher fasting (176 mg %) and postprandial (352.12 mg %) blood glucose levels. It emerges from this study that only 17% of patients were showing good control of blood glucose as evident by Hba1c levels whereas 83% patients were in poor control category (hba1c >7.6%).These findings supported the study done by Mohan V et al[7].Out of 110 patients studied, 100 (91%) patients exhibited positivity to GAD antibodies while only 10 (9%) patients were seronegative. Our study corroborated the findings of Singh AK et al[8].Singh AK et al in 2000 stated that immunological studies on patients with malnutrition-modulated diabetes mellitus in India have shown that many are positive for GAD Antibodies[8].

It emerges from present study that 67 (67%) of antibodies positive patients were diagnosed below the age of 30 years whereas age of diagnosis for diabetes mellitus for 33% of patients was above 30 years. Ahuja MMet al[9]categorized a broad subset of patients mostly of Asian and African ethnicity under the following criteria: (1) blood glucose > 200; (2) onset < 30 years of age; (3) BMI < 18 kg/m2; (4) absence of ketosis on insulin withdrawal; (5) poor socio-economic status or history of childhood malnutrition9.

In our study, mean level of random blood glucose at the time of diagnosis for antibodies positive patients was found to be 490.68 mg% which was statistically significant ($p<0.05$) when compared to sero negative patients. These findings corroborated with study of Ahuja MMet al[9]. Fekadu S et al described phenotypic similarities demonstrating a male preponderance with the most extensive data being described from Ethiopia[10].This is in accordance with our study where 64 (64%) of patients were male who demonstrated GAD antibodies.

It emerges from this study that 91 (91%)patients either require insulin at initial stage or later as disease progresses with or without oral antidiabetic drugs for glycemic control and survival. These results are in close agreement with study by WHO[11].Zimmet PZ et al[12] studied 65 patients who presented with 'adult-onset' diabetes after the age of 30 years. Of these patients, 19 required insulin therapy. Moreover, the insulin-treated subjects had a higher mean concentration of antibodies to glutamic acid decarboxylase (GAD).

It emerges from our study that out of 38 patients who presented with diabetes after

the age of 30 years, 33 (87%) patients were tested positive for anti-GAD and 30 patients required insulin with or without oral hypoglycaemic drugs for blood glucose control. Moreover, the insulin-treated subjects (1.59±0.40) U/ml had a higher mean concentration of antibodies to glutamic acid decarboxylase (GAD).These results are in close agreement with findings of Zimmet PZ et al[12].Bhadada SK et alreported prevalence of tTGA (11.1%) from populations that have wheat or barley as their staple crop in North India [13].In comparison with study of Bhadada SK et al 70 (70%) patients in our study group were of tribal areas and maize was found to be staple food grain for them. Interestingly, patients who reported positive for tTGA were also seropositive for GAD antibody. PulikkalA et al[14] studied 258 participants and reported that 12 (4.65%) were found to be positive for IgA tTG antibodies. Distribution of IgA positivity was equal in both sexes.

Our study group demonstrated 5 (4.5%) patients having tTGA antibodies with male preponderance (80%) and 4 (80%) of tTGA-positive patients were in age group of 18-39 years. Our results are in close agreement with findings of Pulikkal A et al[14].Similar type of study and observations are also done by Sharma B et al[15] and Singh S et al[16].

Sharma B et al[15] reported the prevalence of anti-tTG IgA antibodies positive against autoimmune diseases was 20.7%.Singh S et al[16] studied various autoantibodies in thirty four T1DM patients and found 14.7% were positive for Anti-TTG. Though 5 (4.5%) patients were having tTGA antibodies but they were asymptomatic for coeliac disease at the time of study.Jacob A *et al* in 2009 conducted a study on 100 diabetics for coeliac disease and reported higher prevalence of CD in patients with T1DM than the general population in southern Kerala. This data lend support to recommend regular screening for CD in all patients with T1DM[17].

Agrawal RP*et al* studied 101 patients and reported 17.8% seroprevalence of IgA tTG. Titre more than 100 U/ml and in the range of 10-50 U/ml were observed in 55.56% and 38.89% patients respectively[18].

Our study group demonstrated 60% patients were having titre levels more than 100 U/ml and 40% patients in the range of 10-50 U/ml. Our findings are comparable with study of Agrawal RP et al[18].The present study confirms that diabetes is frequently associated with other organ specific autoantibodies.

Conclusion

Antibodiescan be present long before the clinical presentation of type1 diabetes, and their detection can be useful in confirming adiagnosis of type 1 diabetes. Anti-GAD Antibodies have the advantage because their titer remains relatively stable over a period of time. Clinical features of Coeliac Disease in Type 1 Diabetes patients may be subtle, atypical, or may be completely lacking. Periodic serological screening for Coeliac Disease in Diabetes is mandatory because it may be the only way to detect asymptomatic patients.

From our results, it is concluded that GAD antibodies and tTGIgA are prevalent in diabetics with former positively correlated in lean diabetics with insulin dependence. Screening for antibodies in diabetics should be encouraged for prompt diagnosis and better management.

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