ASSOCIATION OF AUTO ANTIBODIES AND GENETIC FACTORS IN TYPE 1 DIABETES MELLITUS

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Abstract: Data reported on celiac and thyroid autoimmunity in type I diabetes mellitus differ vastly. Therefore, we studied celiac and thyroid auto antibodies in relation to patients clinical characteristics (gender, age, duration of the disease, age onset and body mass index), beta cell antibody status (GADA & IA-2) and HLA DRBI and DQB1 types. The study included forty-five type 1 diabetic patients (19 males and 26 females) with age ranging from 6 to 25 years (mean age 12.956 ± 4.94) and fourteen healthy subjects as a control group. Serum samples were analyzed for anti-gliadin, anti-reticulin, anti-endomysium, anti-thyroid peroxidase, GADA and IA-2A. Polymerase chain reaction (PCR) techniques were used to amplify the second exon of DRB1 and DQB1 alleles, after which sequence specific oligonucleotide probe dot blot hybridization techniques were used to analyze the amplified products. Molecular typing of HLA class II alleles showed an increased frequency of DRB1 *04 and *0101 and DQB1 *02 in type 1 diabetic patients in comparison to control group (57.77% Vs 21.428%, P<0.05; 28.8% Vs 0%, P<0.05; 57.77% Vs 21.428%, P<0.05). Celiac and thyroid autoimmunity tended to be more prevalent in the subgroup of patients with GADA positivity compared with GADA negative patients. These subgroups of patients were of older age, higher age onset and had female preponderance. GADA were also positively associated with DRB1 *03 and BQB1 *02 and negatively associated with DRB1 *04 and DQB1 *03. Logistic regression analysis revealed that anti-gliadin status was determined by female gender (B=2.1404, P=<0.05) and GADA positivity (B=4.3515, P=<0.01). **Conclusion:** HLA genotypes, beta cell auto antibodies and patient's clinical characteristics could identify a different subset of type 1 diabetes that had increased prevalence of thyroid and celiac auto antibodies. Thus combining clinical, immunological and genetic factors could predict thyroid and celiac autoimmunity in type 1 diabetes.
[Abd El-Samea ER, El Chennawy F, El-Nahas MR: Association of Auto antibodies and Genetic Factors in IDDM. New York Science Journal 2011;4(5):1-10]. (ISSN: 1554-0200). http://www.sciencepub.net/newyork.

Keywords: Type 1 Diabetes Mellitus (IDDM), Insulin, auto antibodies, T cells, β cells

INTRODUCTION

Type I diabetes mellitus, arising through a complex interaction of immune, genetic and environmental factors, results from autoimmune destruction of insulin-producing β cells. Type I diabetes mellitus is characterized by the appearance of insulitis and the presence of β-cell autoantibodies. In up to one third of patients the autoimmune attack is not limited to β cells, but expands into an autoimmune polyglandular syndrome. Of type I diabetic subjects, 15 to 30% have autoimmune thyroid disease (Hashimoto's or Graves' disease), 5 to 10% are diagnose with autoimmune gastritis and/or pernicious anaemia (AIG/PA), 4 to 9% present with celiac disease (CD), 0.5% have Addison's disease (AD), and 2 to 10% shows Vitiligo. Type 1 diabetes is frequently associated with other autoimmune diseases such as celiac disease and autoimmune thyroiditis *(Barker JM, 2006).* However, the frequency of these associations varies widely in different populations. Thyroid peroxidase antibodies are present in 5-40% of type 1 diabetic patients *(Chang et al 1998, Prazny et al 1999, Sumnkiz et al 2006).* Celiac autoimmunity had been reported in 3 to 10.4% of type 1 diabetic patients *(Hansen et al 2001, Schuppan & Hahn 2001 and Tandon et al 2002).* Untreated celiac disease may be associated with long-term health risk e.g. intestinal lymphoma, so screening and early treatment with a gluten-free diet seem to be justified *(Schober et al 2002).* Moreover, the majority of patients develop celiac disease *(Barera et al 2002)* and thyroid disease *(Holl et al 1999)* several years after diabetes onset. Thus, extending screening programs for celiac and thyroid disease after the onset of type 1 diabetes is recommended, even in the absence of clinical symptoms *(Barera et al 2002 and Holl et al 1999).* Screening for associated autoimmune disease not only allows earlier diagnosis of these diseases but also allow better management of diabetes. *Fernandez-Soto et al (1997)(Brent GA 2008)* suggested that anti-TPO predicted poor metabolic control in pregnant type 1 diabetic patients. *Graham et al (2002)* concluded that genetic factors e.g. HLA-DQ need to be combined with islet antibody markers when evaluating the risk for type 1 diabetes development. *Field (2002)* concluded that the rewards of the genetic studies will enable clinicians to predict autoimmune disease in predisposed subjects in order to target them for preventative therapies. More than 95% of celiac patients share the DQ 2 or DQ 8

haplotype; patients negative for both types are unlikely to suffer from celiac disease, *Madrino Cobo et al 1999). De Block et al (2001)* found an association between anti-TPO and DQB1 *0301 status *(Barker JM et al 2005).* The aim of this work is to study celiac and thyroid autoantibodies in type 1 diabetic patients in relation to their clinical characteristics (gender, age, duration of diabetes, age at onset and body mass index) islet cell autoantibodies (GADA and IA-2) and HLA polymorphism.

SUBJECT AND METHODS

This study included forty-five type 1 diabetic patients (19 males and 26 females) with age ranging from 6 to 25 years (mean age 12.956 $\pm$ 4.94). type 1 diabetes mellitus started between 4 and 16 years of age. The mean age at the onset of type 1 diabetes was 9.178 $\pm$ 3.651 years. All patients presented at onset with hyperglycemia, polydepsia, polyuria and/or ketoacidosis. All patients began insulin therapy at time of diagnosis and remained insulin dependent thereafter. Fourteen healthy subjects were also included as a control group and their HLA DR and DQ alleles were determined. Control group was used to compare their alleles frequencies with that of diabetic patients. None of the control subjects was positive for the studied immunological markers.

METHODS

Thorough history taking and clinical assessment were done with special stress on manifestations pointing to functional thyroid disorders or gastrointestinal upsets suggestive of celiac disease. Serum samples of diabetic and control subjects were analyzed for glutamic acid decarboxylase-65 antibodies (GADA) using (MEDIPAN, GERMANY) RIA kit *(Petersen et al 1994).* Sera considered positive for GADA if they contained 0.9 U/ml or more of the antibody. Antibodies to protein tyrosine phosphatase-related islet associated-2 molecules (IA-2A) were detected by Immunoprecipitation of highly purified human recombitant IA-2 using (MEDIPAN, GERMANY) kit. Serum sample is regarded positive for IA-2 if the antibody is 0.75 U/ml or more. IgG anti-gliadin and anti-endomysial antibodies were tested by solid phase immunoassay using kit supplied by IMMCO-Diagnostic Company, USA. Indirect immunofluorescence testing for anti-reticulin antibodies were done by kit supplied IMMO-Diagnostic Company, USA. HLA class II alleles (HLA DRB1 and HLA DQB1) were determined using PCR amplified DNA and non-radioactive oligonucleotide probes. Genomic DNA was isolated from fresh peripheral blood drawn on EDTA anticoagulant using Instagene TM whole blood kit (Biorad, Italy). Amplification of nucleic acid by PCR was done. The DNA to be amplified by PCR is introduced in a reagent mixture containing an excess of dNTPs biotinylated primers and thermostable DNA polymerase. The primers by heating the two strands of the DNA helix are separated (Denaturation) in order to expose the target sequences to the primers, the primers are complementary to the regions flanking. The target sequence, therefore upon cooling to specific temperature, the primers will bind to their target region (annealing). At again another temperature and utilizing the dNTPs, the thermostable DNA polymerase will extend the annealed primers along the target Template (extension). Thus two exact biotinylated copies of the template sequence are produced after once cycle of denaturation, annealing and extension, after 35 cycles, a multi-amplified biotinylated target sequence is obtained. Alleles of the HLA DRB1 and HLA DQB1 genes were determined using PCR with 37 and sequence specific DNA probes *(Buyse et al 1993).* The HLA type was determined using INNO-LIPA to get the allele-specific amplicons.

Statistical methods

Frequency, mean and standard deviation were used to describe data. Mann-Whitney test was used to test for significance of difference between each two groups in quantitative variables. Fischer exact test and relative risk (RR) were used to test for association between different variables. Logistics regression analysis was used to test for variables affecting anti-gliadin and anti-TPO. P value was considered significant if less than 0.05. These tests were run on an IBM compatible personal computer using statistical package for social scientist (SPSS) for windows (SPSS Inc., Chicago, IL, USA).

RESULTS

The frequency of DRB1 *0101 in diabetics was higher than that in the controls (28.8% Vs 0%, P<0.05), while the frequency of DRB1 *15011, *1001 and *1101 in diabetics were significantly lower than that in controls (4.44% Vs 50%, P<0.001; 0% Vs 35.7%, P<0.001; and 0% Vs 42.86%, P<0.001) (table 1). Although, individual DRB1 *04 subtypes in diabetic patients showed a non significant difference from controls, the total DR *04 alleles were significantly higher in diabetic patients in comparison to control subjects (57.77% versus 21.428%, P<0.05, RR=1.416). The frequency of DQB1 *0301, *0404, *0601, 0602, * 0603 alleles in diabetics were lower than that in the controls (0% Vs 28.6%, P<0.01; 0% Vs 28.6%, P<0.01: 0% Vs 21.4%, P<0.05; 2.2% Vs 21.4, P<0.05; and 0% Vs 35.7%, P<0.001 respectively) (Table 2). Although, individual DQB1 *02 subtypes in diabetic patients showed nonsignificant difference from control, the total DQ *02 alleles were significantly

higher in diabetic patients in comparison to control subjects (57.77% versus 21.428%, P<0.05, RR= 1.416). Comparison of GADA positive versus GADA negative patients (table 3) demonstrated that GADA positivity were associated with female gender, higher age at onset and older age. GADA were associated with anti-gliadin, anti-reticulin and anti-endomysial antibodies were significantly higher in GADA positive subgroup (24.138%, P<0.05). GADA were also positively associated with DRB1 *03 and DQB1 *02 and negatively associated with DRB1 *04 and DQB1 *03. Comparison of IA-2A positive versus IA-2A negative patients (table 4) revealed that IA-2A were positively associated with DRB1 *04 and DQB1 *03 and negatively associated with DRB1 *03 and DQB1 *02. Logistic regression analysis showed that anti-gliadin status was determined by female gender (B=2.1404, P=<0.05) and GADA positivity (B=4.3515, P=<0.01) but not by DR or DQ alleles or IA-2A status (table 5). On the other hand, anti-TPO status was not associated with any of the studied parameters.

Table 1: frequency of DRB1 alleles in type 1 diabetic patients versus control subjects:

HLADRB1 alleles	Type 1 diabetic patients (n=45)		Control subjects (n=14)		Significance	Risk
	Frequency	%	Frequency	%		
*0101	13	28.8	0	0	<0.05	-
*01021	4	8.9	1	7.1	NS	1.054
*0103	7	15.6	0	0	NS	-
*0104	2	4.4	0	0	NS	-
*0301	2	4.4	1	7.1	NS	0.868
*03011	7	15.6	0	0	NS	-
*0305	2	4.4	0	0	NS	-
*1301	2	4.4	0	0	NS	-
*1303	0	0	1	7.1	NS	-
*13031	3	6.7	0	0	NS	-
*13032	2	4.4	0	0	NS	-
*15011	2	4.4	7	50	<0.001	0.258
*1504	2	4.4	0	0	NS	-
*15022	2	4.4	0	0	NS	-
*1505	2	4.4	0	0	NS	-
*0402	9	20	2	14.3	NS	1.019
*04022	2	4.4	0	0	NS	-
*0403	2	4.4	0	0	NS	-
*0404	2	4.4	1	7.1	NS	0.868
*0406	2	4.4	0	0	NS	-
*0414	3	6.7	0	0	NS	-
*0415	2	4.4	0	0	NS	-
*0422	2	4.4	0	0	NS	-
*0432	2	4.4	0	0	NS	-
*0701	7	15.6	4	28.6	NS	0.804
*0703	6	13.3	0	0	NS	-
*0704	2	4.4	0	0	NS	-
*1601	0	0	0	0	NS	-
*0901	0	0	1	7.1	NS	-
*1001	0	0	5	35.7	<0.01	
*1101	0	0	6	42.86	<0.01	

Table 2: Frequency of DQB1 in type 1 diabetic patients versus control subjects:

HLADRB1 alleles	Type 1 diabetic patients (n=45)		Control subjects (n=14)		Significance	Risk
	Frequency	%	Frequency	%		
*0102	0	0	0	0	NS	-
*0103	0	0	0	0	NS	-
*0104	0	0	2	14.3	NS	-
*0201	13	28.88	3	21.4	NS	1.092
*02013	3	6.6	0	0	NS	-
*0202	5	11.11	0	0	NS	-
*0203	5	11.11	0	0	NS	-
*0301	0	0	4	28.6	<0.01	-
*03011	2	4.4	0	0	NS	-
*0302	9	20	2	14.3	NS	1.091
*0303	0	0	2	14.3	NS	-
*03032	1	2.2	0	0	NS	-
*0304	7	15.6	2	14.3	NS	1.071
*0305	2	4.4	2	14.3	NS	0.825
*0307	6	13.3	0	0	NS	-
*0401	2	4.4	3	21.4	NS	0.625
*0402	6	13.3	2	14.3	NS	0.981
*0404	0	0	4	28.6	<0.01	-
*0501	0	0	1	7.1	NS	-
*05011	7	15.6	0	0	NS	-
*0502	4	8.9	3	21.4	NS	0.725
*05031	6	13.3	0	0	NS	-
*0531	0	0	0	0	NS	-
*0504	1	2.2	0	0	NS	-
*0601	0	0	3	21.4	<0.05	-
*06011	3	6.7	0	0	NS	-
*06012	3	6.7	0	0	NS	-
*06013	1	2.2	0	0	NS	-
*06015	1	2.2	0	0	NS	-
*0602	1	2.2	3	21.4	<0.05	0.313
*0603	0	0	5	35.7	<0.001	-
*0607	1	2.2	0	0	NS	-
*0613	1	2.2	0	0	NS	-
*0641	1	2.2	0	0	NS	-

Table 3: Comparison of GADA positive versus GADA negative diabetic patients:

	GADA positive patients	GADA negative patients	Significance
Number	29 (64.44%)	16 (33.56%)	
Sex (M/F)	7/22	9/7	<0.05
Age (years)	14.83 ± 4.69	9.56 ± 3.39	<0.001
Age at onset (years)	10.76 ± 3.33	6.31 ± 2.18	<0.001
Duration (years)	4.07 ± 2.31	3.25 ± 2.18	NS
Body mass index	20.39 ± 1.84	21.03 ± 1.78	NS
Fasting blood glucose (mg%)	192.6 ± 41.7	202.2 ± 48.4	NS
Anti-gliadin (+/-)	13/16	2/14	<0.05
Anti-reticulin (+/-)	8/21	1/15	NS
Anti-endomysial (+/-)	8/21	1/15	NS
Anti-celiac # (+/-)	7/22	0/16	<0.05
Anti TPO (+/-)	7/22	0/16	<0.05
DRB1 *03 (+/-)	11/18	0/16	<0.001
DRB1 *04(+/-)	10/19	16/0	<0.001
DQB1 *02(+/-)	22/7	4/12	<0.01
DQB1 *03(+/-)	13/16	14/2	<0.01

Table 4 : Comparison of IA-2 negative diabetic patients

	IA-2 positive patients	IA-2 negative patients	Significance
Number	19 (42.22%)	26 (57.78 %)	
Sex (M/F)	9/10	7/19	NS
Age (years)	9.84 ± 3.2	15.23 ± 4.8	<0.001
Age at onset (years)	6.37 ± 1.8	11.23 ± 3.28	<0.001
Duration (years)	3.47 ± 2.06	4.0 ± 2.43	NS
Body mass index	21.25 ± 1.9	20.15 ± 1.65	NS
Fasting blood glucose (mg%)	198.5 ± 56	194.2 ± 33.6	NS
Anti-gliadin (+/-)	7/12	8/18	NS
Anti-reticulin (+/-)	5/14	4/22	NS
Anti-endomysial (+/-)	4/15	5/21	NS
Anti-celiac # (+/-)	4/15	3/23	NS
Anti TPO (+/-)	2/17	5/21	NS
DRB1 *03 (+/-)	1/18	10/16	<0.05
DRB1 *04(+/-)	18/1	8/18	<0.001
DQB1 *02(+/-)	3/16	23/3	<0.001
DQB1 *03(+/-)	17/2	10/16	<0.001

Table 5: Logistic regression analysis for variables affecting anti-gliadin and anti-TPO

	Independent Variables	*B* (partial regression coefficient)	Standard error of *B*	Odds ratio	P
Anti-gliadin	Constant	-2.2791	2.2963		
	Age	-0.3328	0.2178	0.7169	NS
	Sex	-2.1404	1.108	0.1176	<0.05
	Duration	0.6651	0.3911	1.9447	NS
	GADA	4.3515	1.6404	77.5968	<0.01
	IA-2A	1.5687	1.5259	4.8005	NS
	DR *03	-0.1782	1.1587	0.8368	NS
	DR *04	1.3277	1.1147	3.7724	NS
	DQ *02	0.7699	1.1863	2.1596	NS
	DQ *03	-1.1452	1.107	0.3182	NS
Anti-TPO	Constant	-23.8628	104.8917		
	Age	0.400	0.3733	1.4914	NS
	Sex	-0.9145	1.1856	0.4007	NS
	Duration	-0.6622	0.5335	0.5157	NS
	GADA	18.6914	104.735	13109.35	NS
	IA-2A	12.9125	68.8698	405363.87	NS
	DR *03	0.6418	1.3207	1.8998	NS
	DR *04	-1.0502	1.4333	0.3499	NS
	DQ *02	0.8070	1.7117	2.2411	NS
	DQ *03	-9.517	68.8101	0.001	NS

DISCUSSION

Type I diabetes mellitus is a chronic inflammatory disease which leads to selective destruction of β cells in pancreatic islets. Such cells are affected by a process involving cellular and humoral autoimmunity mechanisms against their antigens. A failure of regulatory T cells on this process is involved *(Chatila TA 2009).* The present study confirmed the association between certain HLA class II alleles and type I diabetes. Analysis of the frequencies of alleles in our patients compared to control group revealed that DRB1 *04 and *0101 and DQB1 *02 were positively associated with type I diabetes, while DRB1 *15011, *1001 and *1101 and DQB1 *0301, *0404, *0601, 0602 and *0603 alleles were significantly increased in controls. Thus DRB1 *04 and *0101 and DQB1 *02 confer susceptibility while DRB1 *15011, *1001 and *1101 and DQB1 *0301, *0404, *0601, 0602 and *0603 confer protection to type I diabetes in our subjects. Previous studied reported variable association of DR and DQ type 1 diabetes in different populations. DR4 and/or DR3 have been shown to be strongly associated with type 1 diabetes *(Wolf et al 1983)(Sumnil Z et al, 2003).* Among DR4 subtypes, DRB1 *0401 has been reported to be strongly associated with the disease *(Sheehy 1992). Mbanya et al (2001)* found a higher frequency of the alleles DRB1 *03, DRB1 *1301 in Cameroonian type 1 diabetes, without significant association with DRB1 *04. *Abed Kamoun et al (2002)* showed that DRB1 *0405 predisposed to type 1 diabetes in the Tunisian population, whereas DRB1 *0403 has a protective effect. *Ronningen et al (2001)* suggested that type 1 diabetes is most strongly associated with HLA-DQ genes. DQB1 *02 particularly *0201 alleles had been repeatedly reported to be associated with type 1 diabetes in different populations *(Sang et al 2001, Perez-Bravo et al 2001and Zhang et al 2001). Kawabata et al (2002)* revealed heterogenecity of the contribution of HLA-DR and –DQ haplotypes to susceptibility to type 1 diabetes in Japanese population. *Ronningen et al (2001)* suggested that variation in the incidence of type 1 diabetes in Europe is explained by variations between populations in the distribution of particular DQ genotype which confer a high risk of type 1 diabetes in the general population. *Heward et al (2002)* determined that comparison of genetic association with type 1 diabetes in races provides information as to the exact determinant of disease susceptibility. The structural and functional properties of HLA-DQ and –DR molecules that confer susceptibility to several common autoimmune diseases have been defined. The relevant polymorphisms directly affect interaction with peptides *(Wucherpfennig 2001)(Aly TA et al, 2008).* The assessment of classical immunological markers such as

islet cell antibodies or anti-insulin antibodies has been recently completed by the screening of new promising markers such as GAD or IA-2 antibodies. The presence of these markers confirms the autoimmune component of the disease and thus supports the diagnosis of type 1 diabetes *(Luyckx et al 2000)(Wenzlau JM et al, 2007).* In this study, 29 patients (64.44%) were GADA positive and 19 patients (42.22%) were IA-2A positive. GADA and IA-2A positivity differ greatly in different population. *Pranzy et al (1999)* reported positivity of GADA in 58% and IA-2A in 25% of 55 randomly selected type 1 diabetic patients. *Chan et al (1996)* reported GADA positivity in 23% of 39 Chinese patients. *Savola et al (1998)* found that IA-2 antibodies were detected in 85.9% of IDDM. *Mendoza-Morfin et al (2000)* reported that the frequency of anti-GAD in Mexican and Asian type 1 diabetic patients was lower than that of the European patients. *Thai et al (1997)* showed that only 39.6% of Chinese type 1 diabetic patient has GAD antibodies and they suggested that autoimmunity may not be a major factor in the pathogenesis of type 1 diabetes in Chinese patients. In this study, we found that positivity for GADA but not IA-2A were associated with older age, higher age of onset and female gender. The relationship of either GADA or IA-2A to patient's clinical characteristics is a matter of controversy. *Graham et al (2002)* found that males were more likely than females to be negative for GADA and IA-2. Higher prevalence and levels of GADA but not IA-2 was observed in diabetic patients with postpubertal disease onset. GADA was more prevalent in adults and IA-2 more frequent in children *(Hermitte et al 1998). Serrano-Rios et al (1996), Thai et al (1997) and Chen et al (2001)* found no significant difference in gender, diabetes onset and duration between GADA positive and GADA negative patients. On the other hand, *Chan et al (1996), Hansen et al (2001)and Kukko M et al (2005)* found that anti-GAD positivity were associated with younger age and earlier age of clinical onset. In this study, GADAs were significantly associated with HLA DRB1 *03 and DQB1 *02, whereas, IA-2 antibodies were significantly associated with HLA DRB1 *04 and DQB1 *03. *Sabbah et al (1999)* showed a strong association of IA-2 antibodies and DQB1 *0302, while GAD specific humoral autoimmunity is linked to the DQB1 *02 alleles. *Graham et al (2002)* found that GADAs were associated with HLA-DQ2 in young but not in older patients, whereas IA-2 were negatively associated with DQ2 but positively associated with DQ8. *Schlosser et al (2002)* found that subjects with GADA or IA-2 revealed an increased frequency of the diabetes-associated HLA DQB1 alleles *0302 and/or *02 as well as decreased frequency in the protective allele *0602. *Chen et al (2001)* found no significant difference in frequencies of HLA DR3 or DR4 between GADA positive and GADA negative patients. IA-2 antibodies were found to be more prevalent in patients with HLA DR4 *(Savola et al 1998).* HLA molecules may influence antibody status and can determine the tissue to which an autoimmune process develops. Positivity for anti-gliadin and anti-TPO were detected in GADA positive subgroup with significantly higher frequency of DR *03 and DQ *02. Thus, the association between type 1 diabetes and anti-gliadin anti-TPO could be explained by a common immunogenetic basis, possibly related to HLA genotype. In this study, positivity for anti-gliadin, anti-reticulin and anti-endomysium antibodies were found in 33.33%, 20% and 20% of patients respectively. Patients with concominant positivity for anti-gliadin, anti-reticulin and anti-endomysial antibodies represent 15.55% of our patients. *Roldan et al (1998), Perritin et al (2004) and Ludvigsson JF at al (2006)* suggested that the combined determination of anti-gliadin and anti-endomysial antibodies is the test of choice in screening for celiac disease in diabetic patients. Anti TPO antibodies are found in 15.55% of our patients. None of our patients with thyroid or celiac autoantibodies had clinical manifestations of thyroid dysfunction or gastrointestinal upsets. This is consistent with previous studies that documented that autoimmune thyroid disease are usually subclinical *(Roldan et al 1999)* and celiac disease is usually silent or had atypical manifestation *(Schuppan and Hahn 2001) (Rewers M 2005).* Celiac and thyroid autoimmunity even in the absence of clinical manifestations need frequent observations as these patients are liable to develop celiac or thyroid disease after several years *(Barera et al 2002 and Holl et al 1999).* The frequency of anti-gliadin, anti-reticulin, anti-endomysium and anti TPO antibodies were significantly higher in GADA positive versus GADA negative patients. No such association was found with IA-2A. this finding is in agreement with previous data *(De Block et al 2001).* The association of GADA with thyroid and celiac autoimmunity might be explained by the fact that GADA-65 is not exclusively present in the pancreas but can also be found in other tissues *(Kawasaki et al 1994). Sabbah et al (1999)* suggested that GADA might represent a propensity to general autoimmunity. Logistics regression analysis revealed that anti-gliadin positivity was associated with female gender and GADA status. Previous studies suggested that coexistence of type 1 diabetes and other autoimmune disease may be related to gender *(De Block et al 2001),* age *(Holl et al 1999 and De Block et al 2001),* HLA genotype *(Madrino Cobo et al 1999, De Block et al 2001 and Kaukinen et al 2002).* The relationship of general autoimmunity to beta cell immunity in type 1 diabetes is a matter of controversy. *Chen et al (2001)* suggested that anti TPO was not

correlated with anti GAD in Chinese type 1 diabetic patients. These conflicting results suggest a genetic heterogenicity in the role of autoimmunity of type 1 diabetes mellitus and other autoimmune disease among races *(Caillat-Zucman S 2009)* . From this study, it is concluded that beta cell autoantibody status, patient's clinical characteristics and HLA genotypes could be identify a different subset of type 1 diabetes These patients should be thoroughly evaluated for associated autoimmune disease and continuous screening program for autoantibodies is essential as the prevalence of these autoimmune processes increase with aging. Thus, combining clinical, immunological and genetic factors could improve your capability to predict celiac and thyroid autoimmunity in type 1 diabetic patients.

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28/02/2011