Autoimmune Markers of Diabetic Patients in Zagazig University Hospital

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ABSTRACT

Type 1 diabetes (T1DM) results from cell mediated autoimmune destruction of the β cells of islets of Longerhans and the presence of islet autoantibodies confirms the autoimmune etiology of diabetes.Study of the autoantibodies in diabetic patients permits proper diagnosis and management of the disease or even avoid the dreadful complications which occur early or sometimes before the diagnosis of the diabetes. **Aim of the study:** Study the autoimmune markers of diabetic patients in Zagazig University Hospital and the relationship of these markers on the β cell function and glycemic control. **Subjects and methods:** Study screened 80 subjects which were classified into 3 groups: first group (group A) is patients group included 40 patients and divided into: adult group (group A_1) included 20 adults with type 2 DM not responding to oral hypoglycemic medications, children group (group A_2) included 20 patients with type 1DM. Second group (group B) included 20 siblings of diabetic father or mother or both (T1DM).Third group, control group (group C) included 20 healthy subjects, 10 healthy adult subjects (group C_1) and 10 healthy children subjects (group C_2). All subjects were fully evaluated clinically and subjected to routine laboratory investigations, determination of autoantibodies against Glutamic Acid Decarboxylase (GAD), Islet cell Antibodies (ICA),Insulin Auto Antibodies (IAA) and fasting serum C-peptide by ELISA technique,Glycosylated Hemoglobin A1c(Hb A1c) using ion-exchange chromatography and measurement of the expression of the cluster of differentiation CD_4/CD_{25} T-regulatory cells out of CD_4^+ by flowcytometry. **Results:** The most frequently encountered antibody in group A_1 was GAD65 in 20% of cases, followed by ICA 15% and IAA in 10% of cases. When GAD, ICA and IAA were taken together,they were detectable in 5% of cases. The most frequently encountered antibody in children group was GAD65 in 60% of cases, followed by ICA 40% and IAA in 30% of cases. The most frequently encountered antibody in risk group was ICA in 15% of cases, followed by GAD in 10 % and IAA in 10% of cases. There was significant increase in the level of serum C-peptide in patients with negative autoantibodies than those with positive autoantibodies ($P < 0.003$).There was significant increase in level of fasting C-peptide in patients with single autoantibody positivity than in patients with multiple autoantibodies positivity ($P < 0.001$). There was significant positive relation between individual autoantibodies and the level of HbA1c ($P < 0.001$). There were highly significant increase in the level of CD4/CD25 in control group than in the adult or children patients groups ($P < 0.001$).There was no statistical difference in the level of CD4/CD25 in risk group in comparison with control group ($P = 0.9$). There was significant decrease in percentage of CD4/CD25 in adult patients group and children patients group with positive autoantibodies than those with negative autoantibodies ($P = 0.019$ and < 0.001 respectively).**Conclusions & Recommendations:** All patients with type 1DM have one or more autoantibodies that are reactive to islet antigens. Some

patients with type 2DM have underlying autoimmune process and positive for at least one of the islet autoantibodies as in type 1DM. Screening of autoantibodies is indicated to identify type 1DM, predict the disease course in Latent Autoimmune Diabetes in Adult (LADA) and evaluate the risk in siblings of type 1 diabetic patients specially if associated with low level of fasting serum C-peptide of insulin.CD4/CD25 T regulatory cells activity suppress activation of the immune system and prevent pathological self reactivity which has a crucial role in type 1DM, but larger studies are recommended to prove if there is also a defect in the function of T. regulatory cells and the probability to correct these defects as a novel preventive and/or therapeutic lines against T1DM.
Keywords: Anti GAD antibodies, ICA, IAA, CD4/CD25, T-regulatory cells of DM.

INTRODUCTION

Diabetes mellitus is defined as the dysregulation of glucose metabolism characterized by chronic hyperglycemia resulting from defects in insulin secretion, decreased insulin sensitivity or a combination of both[(1)].

Type 1 diabetes mellitus (T1DM) is a chronic disease associated with the selective destruction of pancreatic β-cells. The exact etiology of the disease is unclear however the insulin deficiency primary results from an auto immune destruction of pancreatic β-cells[(2)].

Type 1 diabetes results from the autoimmune destruction of insulin-producing β cells in the pancreas. Genetic and, environmental factors act together to precipitate the disease. Each individual appears to have a unique combination of these factors that allow for susceptibility to disease. Investigators have long searched for physiological mechanisms that could link diverse environmental events to inheritable genetic traits and the aberrant gene expression in immune cells. Much of this searches have focused on either uncovering elusive gene polymorphisms found in at-risk populations or on finding common elements in the lives of susceptible individuals that "trigger" their immune system toward a self-destructive pathway[(3)].

Environmental factors have been implicated in the pathogenesis of type 1 diabetes both as triggers and potentiators of β-cell destruction, although the contribution of any individual exogenous factor has not yet been definitely proven. Type 1 diabetes is considered to be a chronic immune-mediated disease with a subclinical prodrome of variable duration[(4)].

It is characterized by selective loss of insulin-producing β-cells in the pancreatic islets in genetically susceptible subjects. The most important genes contributing to disease susceptibility are located in the HLA class II locus on the short arm of chromosome 6. Nevertheless, only a relatively small proportion, i.e., <10%, of genetically susceptible individuals progress to clinical disease. This implies that additional factors are needed to trigger and drive β-cell destruction in genetically predisposed subjects[(4)].

Type 1 diabetes is an autoimmune disorder caused by autoreactive CD4+ and CD8+ T-cells that recognize pancreatic antigens such as insulin or GAD and subsequently

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destroy insulin-producing β-cells. Islet cell autoantibodies ICA are strongly associated with the development of type 1 diabetes. The appearance of autoantibodies to one or several of the autoantigens GAD65, ICA, or insulin autoantibodies IAA, signals an autoimmune pathogenesis of β-cell killing. A β-cell attack may be best reflected by the emergence of autoantibodies dependent on the genotype risk factors, isotype, and subtype of the autoantibodies as well as their epitope specificity[(5)].

It is speculated that progression to β-cell loss and clinical onset of type 1 diabetes is reflected in a developing pattern of epitope-specific autoantibodies. Although the appearance of autoantibodies does not follow a distinct pattern, the presence of multiple autoantibodies has the highest positive predictive value for type 1 diabetes[(6)].

Latent autoimmune diabetes in adults (LADA) is a genetically linked, autoimmune form of type 1 diabetes mellitus that is commonly seen after age 30 in patients who often have a normal body mass index without overt signs of metabolic syndrome. They have positive circulating antibodies reflecting the autoimmune nature of beta cell destruction, and they frequently are poorly controlled on oral anti-diabetic agents. Because they are older when first symptomatic, they are often diagnosed with type 2 diabetes. However, it is important to recognize patients with LADA because they often progress quickly to insulin dependence[(7)].

First-degree relatives of individuals with type 1 DM have an approximate 5% risk of developing the disease (independent of country)[(6)].

The objective of this study was evaluation of GAD65, ICA and IAA autoantibodies as autoimmune markers with determination of the percentage of $CD4^+/CD25^+$ out of CD4 cells in diabetic patients in Zagazig University Hospital and their relationship to residual beta-cell function determined by fasting C-peptide of insulin and glycemic control determined by Hb A1c.

SUBJECTS AND METHODS

Study was carried out during 2011 and 2012 on 80 subjects selected from those attending diabetes Outpatient Clinic in Zagazig University Hospital and classified into 3 groups: **First group** is the patient group (group A) included 40 patients and is divided into: **Adult group (group Al)** included 20 adult patients diagnosed as type 2 diabetes but not responding to oral hypoglycemic medications,They were 8 males(40%) and 12 females (60%) their ages ranged from 20-60 years (Mean ± SD : 37 ± 12.3).**Children group (group A2**) included 20 patients diagnosed as type I diabetes according WHO criteria,They were 11 males (55%) and 9 females (45%),their ages ranging from 3-16 years (Mean ± SD: 10.6± 4) .**Second group (group B)** is a **risk group** included 20 siblings of diabetic father or mother or both (T1DM). They were 11 males (55%) and 9 females (45%) their ages ranging from 18-25 years (Mean ± SD: 21± 2.5).**Third group (group C)** is a **control group** included 20 subjects divided into: **(group Cl)** included 10 **healthy adult** subjects,they were 5 males(50%) and 5 females(50%) their ages ranging

from 25-60 years (Mean ± SD: 44± 13.5) and they have no family history of diabetes.**(groupC2)** included 10 **healthy children** subjects they were 5 females (50%) and 5 males (50%) ,their ages ranging from 5-16 years old (Mean ± SD: 10.8± 2.8) with no family history of diabetes.Patients were excluded from the study if they were suffering from hepatic or renal impairment, chronic diseases or taking medications known to affect immune system.

-After being informed on the purpose and procedures of the study, all subjects signed on informed consent form.

-All subjects were subjected to the following:

1- Complete history taking and full clinical examination with routine laboratory investigations.

2- Specific laboratory investigations:

-Determination of GAD autoantibodies by qualitative ELISA technique[8].

-Determination of ICA autoantibodies by qualitative ELISA technique[9].

-Determination of IAA autoantibodies by qualitative ELISA technique[10].

-Determination of C- peptide of insulin by quantitative ELISA technique[11].

-Glycosylated Hb using ion- exchange chromatography[12].

-Measurement of the expression of the $CD4^+$/ $CD25^+$ T-regulatory cells out of CD4+ by Flowcytometry[13].

Sample collection: from each selected patient and control 5 ml blood were aseptically withdrawn by sterile venipuncture and collected in 2 tubes:

-2.0 ml blood were added to tube containing EDTA (1.5mg EDTA/ml blood) for determination of HbAlc and flowcytometric analysis of CD4V CD25+ T regulatory cells.

-3 ml blood was left to clot and serum was separated by centrifugal ion and collected in aliquots for routine investigations as well as for detection of GAD65, ICA and IAA auto antibodies and C-peptide of insulin.

The data were tabulated and statistically analyzed using Epi-INFO (2005) and SPSS version-10 software package.

RESULTS

The demographic characteristic of all studied groups as regard age are shown in table (1) which revealed that the adult patients group (Group Al) has age range of 20-60 years with mean ± SD, 37±12.3which is comparable with control group (Group C l) which has age range of 25-60 years with mean ± SD, 44± 13.5.

The same was found in children group where children patients group (Group A2) has age range of 3-16 with mean ± SD, 10.6 ± 4, and control (group C2) had age range of 5-16 years with mean ± SD, 10.8±2.8.

As regard Gender, there were comparable distribution among both sex.

The prevalence of autoantibodies present in the study groups are shown in table (2) which revealed the following:

1. ***Adult patients group***: the most frequently encountered antibody in adult group was GAD65 in 20% of cases, followed by ICA, 15%. When taken together, both GAD65 and ICA, were detected in 10%. IAA was detectable only in 10% of cases. When both GAD65 and IAA were taken together, they were detected in 5% of cases also ICA and IAA were detected in 5% of cases. When GAD 65, ICA and IAA were taken together, they were detectable in 5% of cases.
2. ***Children patients group***: The most frequently encountered antibody in children group was GAD65 in 60% of cases, followed by ICA, 40%. When taken together, both GAD65 and ICA, were detected in 30 %. IAA was detectable only in 30% of cases. When both GAD65 and IAA were taken together, they were detected in 25 % of cases also ICA and IAA were detected in 15 % of cases. When GAD, ICA and IAA were taken together, they were detectable in 5% of cases.
3. ***Risk group***: The most frequently encountered antibody in risk group was ICA in 15% of cases, followed by GAD, in 10 %. When taken together, both GAD65 and ICA, were detected in 10 %. IAA was detectable only in 10% of cases. When both GAD65 and IAA were taken together, they were detected in 5 % of cases also ICA and IAA were detected in 5 % of cases. When GAD, ICA and IAA were taken together, they were detectable in 5% of cases.

The comparison of prevalence of auto antibodies between patients groups with control group and between adult patients group with children patients group are shown in table (3) which revealed that: as regard the comparative study between adult patients group and control group there was no significant difference between two groups for prevalence of GAD65 autoantibody (P= 0.29), ICA autoantibody (P=0.46), or IAA autoantibody (P= 0.46).

The comparative study between children patients group and control group shows that there was significant difference between two groups for prevalence of GAD65 autoantibody (P = 0.018), and ICA autoantibody (P = 0.03). There was no significant difference between two groups for prevalence of IAA autoantibody (P = 0.11). The comparative study between risk group and control group shows that there was no significant difference between two groups for prevalence of GAD65 autoantibody (P = 0.46), ICA autoantibody (P = 0.46), and IAA autoantibody (P = 0.46).

The comparative study between adult patients group and children patients group, shows that there was significant difference between two groups for prevalence of GAD65 autoantibody (P = 0.09), and ICA autoantibody (P = 0.07), while there was no significant difference for prevalence of IAA autoantibody (P = 0.2).

The relations of fasting level of serum C-peptide of insulin to the presence of autoantibody and to the gender in patient groups are shown in table (4) which revealed

significant increase in the level of serum C-peptide in patients with negative autoantibodies than those with positive autoantibodies (P = 0.003).

With significant increase between level of fasting C-peptide in patients with single autoantibody positivity than in patients with multiple autoantibodies positivity (P = 0.001).

There was significant decrease in the level of serum C-peptide of insulin in females than males in patients groups (P = 0.006).

The correlation between presence of individual autoantibodies with level of fasting C- peptide of insulin and with HbA1c among patients groups, revealed in table (5) which showed; negative correlation between the presence of autoantibodies and C-peptide of insulin and positive correlation between the presence of auotantibodies and HbAlc level in patient groups.

Table (6) shows the comparative studies between control groups (Adult / Children) with patients groups (Adult / Children) and between children control group with risk group,according to the mean and SD of the percentage of $CD4^+/CD25^+$ out of CD4 cells.

In the adult patients group the mean and SD of the percentage of CD4/CD25 out of CD4 cells were 0.96 ± 0.55.In the adult control group the mean and SD of the percentage of CD4/CD25 out of CD4 cells were 2.85 $\pm$ 0.92.There was highly significant increase in the level of CD4/CD25 in control group than adult patients group (P< 0.001).

In the children patient group,the mean and SD of the percentage of CD4/CD25 from CD4 cells were 0.96$\pm$ 0.46, while in the children control group the mean and SD of the percentage of CD4/CD25 from CD4 cells were 2.96 ± 0.46. There was highly significant increase in the level of CD4/CD25 in control group than children patients group (P< 0.001).

In the risk group the mean and SD of the percentage of CD4/CD25 out of CD4 cells were 2.99$\pm$ 0.70.In the children control group the mean and SD of the percentage of CD4/CD25 out of CD4 cells were 2.96 $\pm$ 0.6.There was no significant statistical difference in the level of CD4/CD25 in risk group in comparison with children control group (P = 0.9).

The relation between the percentage of $CD4^+/CD25^+$ out of CD4 cell and presence or absence of autoantibodies in patients and risk groups shown in table (7) which revealed significant decrease in percentage of CD4+/CD25+ in adult patients group with positive autoantibodies than those with negative autoantibodies (P< 0.05) and highly significant decrease in percentage of CD4+/CD25+ in children patients group with positive autoantibodies than those with negative autoantibodies (P < 0.001).But,there was no significant difference in percentage of $CD4^+/ CD25^+$ in risk group with positive autoantibodies than those with negative autoanibodies (P = 0.57).

Table (1): Demographic characteristics of the studied groups:

Variable	***Group A***		***Group B (N=20)***	***Group C***	
	Al (N=20)	A2 (N=20)		C1 (N= 10)	C2 (N=10)
Age (years) Mean ± SD	37 ±12.3	10.6±4	21±2.5	44± 13.5	10.8±2.8
Range	20-60	3- 16	18-25	25-60	5- 16
Gender					
Male	8(40%)	1 1 (55%)	11(55%)	5 (50 %)	5 (50 %)
Female	12(60%)	9 (45%)	9 (45 %)	5 (50 %)	5 (50 %)

Table (2): Prevalence of autoantibodies present in the study groups

Groups	*Autoantibodies*	*Frequency*	*Percent*
Adult patient Group	GAD	4/20	20%
	ICA	3/20	15%
	IAA	2/20	10%
	GAD & ICA	2/20	10%
	GAD & IAA	1/20	5%
	ICA & IAA	1/20	5 %
	GAD&ICA&IAA	1/20	5%
Children patient Group	GAD	12/20	60%
	ICA	8/20	40%
	IAA	6/20	30%
	GAD & ICA	6/20	30 %
	GAD & IAA	5/20	25 %
	ICA & IAA	3/20	15 %
	GAD&ICA&IAA	1/20	5%
Risk Group	GAD	2/20	10%
	ICA	3/20	15%
	IAA	2/20	10 %
	GAD & ICA	2/20	10%
	GAD & IAA	1/20	5%
	ICA&IAA	1/20	5 %
	GAD&ICA&IAA	1/20	5%

Table (3): Comparison of prevalence of autoantibodies between the study groups.

Groups / ***Autoantibodies***	***Group A1 (Adult Patient)***	***Group C1 (Adult Control)***	***X^2***	***P***
GAD	4 (20%)	0 (0 %)	1.09	0.29
ICA	3 (15%)	0 (0%)	0.54	0.46
IAA	2 (10%)	0 (0%)	0.53	0.46
Groups / ***Autoantibodies***	***Group A2 (Children Patient)***	***Group C2 (Children Control)***	***X^2***	***P***
GAD	12 (60%)	0 (0 %)	5.51	0.018*
ICA	3 (40%)	0 (0%)	4.55	0.03*
IAA	2 (30%)	0 (0%)	2.44	0.11
Groups / ***Autoantibodies***	***Group B (Risk Group)***	***Group C (Control Group)***	***X^2***	***P***
GAD	2 (10%)	0 (0 %)	0.53	0.46
ICA	3 (15%)	0 (0%)	0.54	0.46
IAA	2 (10%)	0 (0%)	0.53	0.46
Groups / ***Autoantibodies***	***Group A1 (Adult Patient)***	***Group A2 (Children Patient)***	***X^2***	***P***
GAD	4 (10%)	12 (60 %)	2.85	0.09
ICA	3 (15%)	8 (40%)	3.13	0.07
IAA	2 (10%)	6 (30%)	1.63	0.2

* Statistically significant.

Table (4): Relations of fasting level of serum C- peptide of insulin to the autoantibodies in patient groups

Autoantibodies	***C- peptide***		***T***	***P***
	X ± SD	*Range*		
Negative (N= 16)	1.25 ± 0.42	0.85- 2.5	3.16	0.003 Sig
Positive (N= 24)	0.89 ± 0.26	0.55- 1.5		
Single	1.16 ± 0.3	0.85 – 1.8	7.5	0.001

Multiple	0.76 ± 0.15	0.5 – 1.05		Sig
Male (N = 19)	1.27 ± 0.49	0.55 – 2.5	2.86	0.006 Sig
Female (N = 21)	0.85 ± 0.19	0.65 – 1.5		

Table (5): Correlation between presence of individual autoantibodies with the level of fasting C- peptide and with Hb A_{1C} among patient groups.

The correlation	***Autoantibodies***	***R***	***P***
Autoantibodies and serum fasting C-peptide	GAD	- 0.32	< 0.05 Sig
	ICA	- 0. 35	< 0.05 Sig
	IAA	- 0.36	< 0.05 Sig
Autoantibodies and level of HbAIC	GAD	0.55	< 0.001 HS
	ICA	0.4	< 0.001 HS
	IAA	0.38	< 0.001 HS

Table (6): Comparative studies between control groups and patients or risk groups according to the mean and SD of the percentage of $CD4^+/CD25^+$ out of $CD4^+$ cells:

Comparison between control &adult groups	**Variables**	**Adult group**	**Control group**	**t**	**P**
	Mean ± SD	0.96 ± 0.55	2.85 ±0.92	7.79	< 0.001 HS
	Range	0.2-2.7	1.09-3.90		
Comparison between control &children groups	**Variables**	**Children group**	**Control group**	**t**	**P**
	Mean ± SD	0.96 ±0.46	2.96 ± 0.62	11.45	< 0.001 HS
	Range	0.5-2.2	2.2-3.74		
Comparison between control & risk groups	**Variables**	**Risk group**	**Control group**	**t**	**P**
	Mean ± SD	2.99 ± 0.7	2. 96 ±0.6	0.01	0.9 NS
	Range	2.1 -4.0	2.2-3.74		

Table (7): Relation between the percentage of $CD4^+$ / $CD25^+$ out of $CD4^+$ cells and the presence of autoantibodies in patients and risk groups:

Groups	**Autoantibody**	**CD4 / CD25**		**T**	**P**
		X± SD	(Range)		
Adult group	Autoantibodies - ve (n=14)	1.399 ± 0.73	(0.5-2.2)	2.53	0.019* Sig
	Autoantibodies + ve (n=6)	0.625 ± 0.06	(0.56–0.7)		
Children	Autoantibodies -ve (n=2)	1.37 ± 0.27	(0.9 – 1.53)	4.5	< 0.001** H Sig
	Autoantibodies + ve (n = 18)	0.75 ± 0.26	(0.53 – 1.5)		
Risk group	Autoantibodies	1.36 ± 0.53	(0.56 – 2.1)	0.57	0.57

	-ve (n=16)			Non Sig
	Autoantibodies + ve (n = 4)	1.17 ± 0.74	(0.5 – 2.1)	

DISCUSSION

In this study, we tried to evaluate auto-immune markers of diabetes and their relationship to beta cell function.

As regard gender, the study showed that there was no significant difference between groups. This results was in agreement with that of ***Schiel and Muller*** [14], ***Zanone et al***. [15] and ***Chul et al*** [16].

As regard the prevalence of autoantibodies among patients group, the present study found that GAD65 autoantibody was present in 20% of cases in adult patients group followed by ICA 15% then IAA (10%). This results agree with that of ***Schiel and Muller*** [14] who found that GAD autoantibody present in 21% of their patients. This results also agree with ***Harvey et al.***[17], who found that GAD present in 24% of cases while ICA present in 11% of cases and IAA present in 13% of cases. On the other hand, **Takeda et al**. [18] reported that GAD antibodies was detected in 3.8% among their patients. This due to differences in number of studied subjects as well as difference in method of determination, as they detect GAD in cross-sectional study.

Also ***Chul et al.*** [16] disagree with our study as they reported that GAD present in 10% of diabetic patients.They concluded that,the presence of GAD antibody in type 2 diabetic patients can predict their course of B-cell function and identify in advance who are likely to require insulin treatment.***Richard el al.***[19] found that the percent of GAD65 in adult group was 51.0%, and this result are disagree with this study while ICA was 18.8% and IAA was found in 8.3% and this in agree with our study.

In children group we found that percent of GAD65 antibody is 60% more higher than ICA, IAA (40%, 30%) respectively.In agreement with our results are that of ***Amany et al*** [20] as they found that GAD 65 present in 62.7%, ICA 17.6%.On the other hand,***Zanone et al*** [15] found that the prevalence of ICA is more higher (70%), while GAD was present in 56% and IAA was positive in 63%, also ***Urakami et al.***[21], reported that the GAD was found in (70%), of cases. This due to difference in number of studied subjects as well as difference in methods of determination.Also the results of this study are in disagreement of ***Yih- Hsin et al.*** [22] who found that percent of IAA, GAD antibodies in children group were 23.6% and 47% respectively.

The results of ***Schiel and Muller***[14], were more or less comparable with present study where GAD antibody was positive in 55% of type 1 diabetic patients.

Falorni and Brozzetti[23], concluded that GAD antibody assay should be offered to every diabetic patients and in cases of positivity screening for other autoimmune diseases should be carried out.

The present study showed that,in adult group presence of GAD and ICA together were present in 10% of cases,while GAD and IAA together were present in 5% of cases,lastly ICA and IAA together were present in 5% of cases.The presence of three antibodies were detected in 5% of cases.These results were in agreement with ***Harvey et al***. [17] who found that the presence of GAD and ICA antibodies together was 11% of cases and the three antibodies were present in 3% of cases. In contrary our results disagree with ***Richard et al***.[19] found that the percent of GAD and ICA antibodies together was 43.1% of cases, while ICA and IAA was present in 26.6 % and lastly,GAD and IAA was present, in 11 %, while the three antibodies where present in 11 % of cases.

In children group,presence of GAD and ICA together was present in 30% of cases,while GAD and IAA was present in 25% of cases,lastly ICA and IAA were positive in 15%.The presence of all three antibodies

were detected in 5% of cases. These results agree with that of ***Zanone et al.*** [15] who found that GAD and ICA was present in 38% of cases, while they found that percent of three antibodies found in 37% of cases and this disagree with the present study.Also the results of the present study disagree with ***Amany et al.***[20] as they found that the percent of ICA and GAD was 13.7%.

This study was carried out on 20 at risk group individuals which are siblings of diabetic father or mother or both (T1DM) to study humoral autoimmune markers, GAD, ICA and IAA in comparison to healthy control subjects. We found that ICA antibody was present in higher frequency in risk group (15%) followed by IAA , GAD present in 10% of individuals, also GAD and ICA present in 10%, GAD and IAA in 5%, ICA and IAA in 5% but three antibodies were found in 5%. Our results differ from that of ***Ying et al***[24], ***Willian et al*** [25] ***and Jennifer et al*** [26] as they studied large groups and use different methods of determination.

Human proinsulin C-peptide is a cleavage product of insulin in the beta cells of the islets of langerhans. It is released in amount equal to insulin into portal circulation. Its main function is to enable the folding of the proinsulin molecules by facilitating the formation of disulphide bonds of the α and β chains[27].

There was significant relation between the level of loss of C-peptide and gender where females showed more loss of C-peptide (P=0.006).This in agreement with that of ***Zanone et al***.[15]

A comparison between auto antibody-positive and auto antibody-negative patients and level of C-peptide of insulin demonstrated that there was significant increase in the level of serum C-peptide in patients with negative autoantibodies than those with positive autoantibodies (P = 0.003). This result was in agreement with that of ***Takeda et al.***[18] ***Rowley et al.*** [28] ***and Richard et al.***[19]. The result was contradictory with that of ***Zanone et al***.[15],they found that, there was no significant relation between level of C-peptide and antibodies positivity.

There was significant increase of level of fasting C-peptide in patients with single autoantibody positivity than in patients with multiple autoantibodies positivity(P<0.001) and this in agreement with ***Richard et al*** [19].

There was negative correlation between individual autoantibody and the level of C-peptide of insulin and this in agreement with ***Zanone et al*** [15].

In our study there was positive correlation between the presence of individual autoantibody and HbAlc, and this is in agreement with ***Zanone et al*** [15] and ***Chul et al***.[16].

Dejaco et al.[29] reported that in patients with autoimmune diseases, reduced level of circulating CD4+ /CD25+ T cells were described, specifically in individuals with juvenile idiopathic arthritis, psoriatic arthritis, hepatitis virus C associated mixed cryoglobulinaemia,auto immune liver disease and systemic lupus erythermatosus. The lower level of circulating CD4+/CD25+ T cells also correlate with a higher disease activity or poorer prognosis.

It has been proposed that the reduced level may be caused by the impaired proliferation of peripheral CD4+/CD25+ T cells. Thereby the balance between pro-inflammatory and regulatory T cells, this balance would be disturbed, leading to the break down of self- tolerance.

Brusko et al.[30] demonstrated functional detects of CD4+CD25+ T-Cells in Type 1 Diabetes, while ***Longhi et al.***[31] demonstrated that regulatory CD4+ CD25+ T cells are defective numerically and functionally in autoimmune diseases. These results go hand in hand with the results of the present study in which we found

in adult patients group (LADA) that the percent of CD4+/CD25+ out of CD4 cells was lower than control group. The difference between control and study group according to the mean and SD of the percentage of CD4+/CD25+ from CD4 cells was statistically highly significant (P<0.001).This in agreement with ***Yang el al***.[32] who showed that level of $CD4^+/CD25^+$ T cells in the peripheral blood of LADA patients was highly significantly lower than normal control ($P < 0.001$).

Also in the present study we found in children patients group that the percent of $CD4+/CD25^+$ out of CD4 cells was lower than control group. The difference between control and study group according to the mean and SD of the percentage of $CD4^+/CD25^+$ from CD4 cells was statically highly significant (P<0.001).This results in agreement with ***Dang and Shi***[33], they reported that the level of $CD4^+/CD25^+$ cells in the peripheral blood of IDDM was significantly lower than normal control (P<0.05). These data suggest that CD4+/CD25+ T cells might play an essential role in the pathogenesis of IDDM patients.

The present study also found no significant differences in the percentage of $CD4^+/CD25^+$ out of CD4 cells between risk group and control group.

There was highly significant negative relation between percent of $CD4^+/CD25^+$ out of CD4 cells and the presence of autoantibodies in the children patients group ($P< 0.001$).There was significant negative relation between percent of CD4+/CD25+ out of CD4 cells and the presence of autoantibodies in the adult group (P<0.05).But,there was no significant relation between percent of CD4+/CD25+ out of CD4 cells and the presence and absence of autoantibodies in the risk group .

So,autoantibodies against islet antigens are found in most patients with type 1 diabetes and are now established markers for the clinical diagnosis and the preclinical phase of this disease [34,35,36].

We can say that, at the time of diagnosis almost all patients with type 1 diabetes have one or more autoantibodies that are reactive to islet antigens.

Before coming to an end it is worthy to mention that some patients with phenotypic type 2DM have underlying autoimmune process and positive for at least one of the islet autoantibodies as in type 1DM.Screening of autoanibodies GAD, ICA and IAA is recommended to identify type 1DM, predict the disease course in LADA and evaluate the risk of typ 1DM in siblings of diabetic parents type 1 specially if associated with low level of fasting serum C-peptide of insulin.

CD4CD25 T.regulatorey cells actively suppress activation of the immune system and prevent pathological self reactivity which has a crucial role in type 1DM, but more studies are recommended to prove that there is also a defect in the function of T. regulatory cells and not only a defect in their percentage and the probability to correct these defects as a novel preventive and/or therapeutic line in T1DM.

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