

## Original Article

# Traditional Islet Cell Autoantibodies In Diabetic Patients With and Without Long-Term Complications

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### Abstract:

The aim of the present work was to demonstrate the presence of the traditional islet cell related autoantibodies in the diabetic patients with and without long term complications and to identify relevant predisposing markers of pre-clinical diabetic complications. There was a significant difference ( $P < 0.001$ ) between the percentage of islet cell autoantibodies (ICA), glutamic acid decarboxylase autoantibodies (GAD-Ab), and insulin autoantibodies (IAA) positive subjects in the diabetic groups and their matched control and potential groups. Type-1 diabetic groups had a higher percentage ( $P < 0.05$ ) of subjects positive for ICA, GAD-Ab, and IAA than Type-2 diabetic groups. The concentration of ICA in the studied population strongly correlated with the duration of the disease ( $r=0.705$ ,  $p < 0.05$ ). There was no significant difference ( $P > 0.05$ ) between the percentage of islet cell antigen-2 autoantibodies (IA2-Ab) positive subjects in the different groups of diabetic population and their control. In conclusion the traditional islet cell antibodies have a role in the detection and development of diabetes especially Type-1 rather than the long-term complications. Other more specific autoantibodies and immune responses, which were not studied, may have a role in the etiology and pre-clinical appearance of these chronic complications. Key Words: Diabetes, Autoantibodies, Complications.

### Introduction:

Diabetic complications are the major causes of morbidity and mortality in patients suffering from diabetes mellitus. Microvascular complications are specific to diabetes and do not occur without longstanding hyperglycemia<sup>(1)</sup>. Microangiopathy including retinopathy, nephropathy and neuropathy are common. Metabolic, immunological, environmental and genetic factors are involved in their etiology. Both Type-1 and Type-2 diabetics are susceptible to angiopathy, but its prevalence as a function of the duration or severity of diabetes may differ markedly<sup>(2)</sup>. Some patients of both types of diabetes survive for many years without developing significant microvascular complications; on the other hand, other patients develop microvascular complications even if they are under good control of diabetes which reflects the complexity of pathogenesis of diabetic complications<sup>(3)</sup>. Prospective studies have demonstrated that diabetic patients with microalbuminuria, defined as urinary albumin excretion (UAER) rate between 20-200 g/min have a 20 fold higher risk of developing clinical nephropathy<sup>(4,5)</sup>. Many risk factors have been implicated in microalbuminuria development including duration of diabetes, sex, young age at diagnosis, cigarette smoking, blood pressure, poor glycaemic control, ethnic origin and hereditary factors<sup>(4,6)</sup>. Maintaining a controlled blood glucose level decreases or stops the progression of these complications<sup>(7,8)</sup>. There is a general agreement that Type-1 diabetes is an autoimmune disease; induced by an interaction between environmental agents and genetic susceptibility<sup>(9,10,11,12,13)</sup>. According to the previous studies, it is clear that diabetes mellitus and its complications are a growing health problem in many countries, with considerable morbidity and mortality<sup>(14,15)</sup>. The aim of the present work was to demonstrate the presence of the traditional islet cell related autoantibodies in

relation to duration of the disease and detection of the long-term complications of diabetes mellitus in Sudanese patients in order to identify relevant predisposing markers of pre-clinical diabetic complications.

### Subjects and Methods:

**Subjects** Three hundred and thirty seven Sudanese subjects were included in this study. They were divided into five groups, two were control (33 and 32 subjects for type 1 and type-2 patients respectively) and potential diabetic groups (32 and 31 for type 1 and type-2 patients respectively). They were matched for age with the diabetic patients. The other three diabetic groups were divided depending on duration and presence of long-term complications (microangiopathy). Two groups included diabetic patients with disease duration of 5 (39 type-1 and 39 type-2) and 5 years (30 of type-1 and 30 of type-2) but without diabetic complications. The third group included 32 type-1 and 38 type-2 patients with diabetic complications.

**Samples collection and storage:** Ten ml of blood was collected from each of the fasting subjects enrolled in the study from the outpatients of Khartoum and Khartoum North Teaching Hospitals. The sera prepared were stored at -20 °C. During samples collection all diabetic patients infected with parasitic diseases were excluded. Overnight fasting urine samples were tested for presence of macroalbuminuria on collection and then stored at -20 °C for estimation of microalbuminuria.

**Diagnostic Criteria** Patients were medically examined and were diagnosed and categorized to one of the above groups according to the WHO criteria<sup>(17)</sup>. Patients information was obtained by filling in the questionnaire.

**Statistical analysis** Statistical evaluation of different variables was performed using one way ANOVA,

Student's t-test and Pearson's correlation coefficient. **Estimation of renal function** Macroproteinuria was estimated by sulphosalicylic acid solution (10%). Microalbuminuria was measured by immunoturbidimetric assay according to <sup>(18)</sup>, RANDOX Lab. Ltd. UK).

**Measurement of Insulin autoantibodies level** Insulin autoantibodies (IAA) level was measured by Radioimmunoassay in Clinical Biochemistry Department, Royal London Hospital according to Dixon, 1975 <sup>(19)</sup>.

**Determination of the islet cell autoantibodies (ICA) level** Islet cell autoantibodies level was measured by the indirect immunofluorescence method in the laboratories of Professor G. F. Bottazzo-University of London <sup>(20,1)</sup>.

**Measurement of glutamic acid decarboxylase autoantibodies (GAD-Ab) and islet cell antigen-2 autoantibodies (IA-2-Ab) levels.**

The concentrations of GAD-Ab and IA-2-Ab were determined by Radioligand Immunoprecipitation assay <sup>(22,32,4)</sup>.

## Results:

**Autoantibodies profile in Type-1 diabetic patients** There was a significant difference ( $P < 0.001$ ) in the percentage of ICA positive subjects in the Type-1 diabetic groups 1, 2 and 3 of 50%, 40% and 11.1%, GAD-Ab of 46.2%, 13.3% and 11.1% and IAA of 57.7%, 66.7% and 66.7% respectively, and their matched control and potential groups ICA positive subjects of 6.3% and 12.5%, GAD-Ab of 0% and 0% and IAA of 6.3% and 8.1% respectively. The percentage of ICA and GAD-Ab Type-1 diabetic positive subjects started to decrease with increasing duration of the disease. It was higher in the non-complicated group than the complicated group. The percentage of IAA Type-1 positive subjects in the diabetic groups was higher than in the control group. This percentage was comparable in different Type-1 Sudanese diabetic groups. There was no significant difference ( $P > 0.05$ ) in the percentage of IA-2-Ab positive individuals in the different groups of Type-1 diabetic population and their control group (Table-1).

**Autoantibodies profile in Type-2 diabetic patients** The percentage of ICA, GAD-Ab and IAA positive subjects in Type-2 diabetic groups 1, 2 and 3 (16.7%, 15.4% and 6.3), (0%, 18.5% and 15.6%) and (13.3%, 7.7% and 0%) respectively, were significantly higher ( $P < 0.05$ ) than the positive subjects in their control and potential groups (5.9% and 8.5%), (5.9% and 0%) and (0% and 3.1%) respectively (Table 2). The percentage of ICA and GAD-Ab positive subjects decreased with the duration of the disease. The complicated group had a lower positive percentage than the non-complicated Type-2 diabetic groups. There was no significant difference ( $P < 0.05$ ) in the percentage of IA-2-Ab positive subjects in the control and Type-2 diabetic population groups. There was a significant ( $P < 0.05$ ) difference in the percentage of positive subjects of ICA, GAD-Ab and IAA in Type-1 diabetic

groups and Type-2 diabetic groups. Type-1 diabetic groups had a higher percentage of positive subjects of ICA, GAD-Ab and IAA than Type-2 diabetic groups.

## Relationship between duration of diabetes and autoantibodies:

There was no significant correlation ( $P > 0.05$ ) between the duration of diabetes and the percentage of IA-2-Ab and IAA positive patients as shown in Table 3. A significant difference  $P < 0.05$  was noticed between duration of diabetes of less than 5 years and greater or equal 5 years and the percentage of ICA and GAD-Ab positive subjects of 34.9%, 17.2% and 37.2%, 6.2% respectively. Duration of the disease was strongly correlated ( $P < 0.05$ ,  $r = -0.705$ ) with the concentration of ICA in the studied population. An inverse relationship was noticed between the duration of diabetes and percentage of positive subjects with ICA in the population of Sudanese diabetic patient. No correlation was noticed between concentration of GAD-Ab and IAA and the duration of the disease ( $P > 0.05$ ,  $r = 0.1$ ).

## Long-term complications profile in the studied patients

The percentage of diabetic nephropathy, retinopathy and neuropathy in the complicated Type-1 diabetic group and Type-2 diabetic group were (31%, 23% and 46%) and (13%, 41% and 46%) respectively. 50% of the complicated Type-1 diabetic patients had 2 complications while 50% had only one complication. On the other hand, 21.9% of Type-2 complicated diabetic group had two complications and 78.1% had only one complication.

## Discussion:

The result of the different autoantibodies showed a significant difference between the diabetic groups in both types of the disease and their matched control. It is clear that these autoantibodies have an important role in the pathogenesis of the disease itself, but not its long-term complications, especially in Type-1 diabetes which is considered as autoimmune disease (13). The percentage of ICA positive subjects was high in the first group where the duration of the disease was  $< 5$  years. On the other hand this percentage was lower in group 2 where the duration was more than or equal to 5 years. In the group with complications the percentage of the positive subjects was lower than other two groups, showing that these autoantibodies have no role in the pathogenesis of the diabetic long-term complications.

Zanone and others <sup>(25)</sup> showed that GAD antibody was not a marker of symptomatic autonomic neuropathy. The same result was recorded about ICA and GAD in relation to diabetic neuropathy and other complications <sup>(26)</sup>. No titers of the GAD-Ab were associated with the degree of neuropathy <sup>(27)</sup>.

About IAA, which was high in the different diabetic groups, this might be partly due to the exogenous insulin used by Type-1 diabetic patients and some of Type-2 diabetic patients occasionally. It is normal for the titer of the other autoantibodies to decrease with

duration of diabetes because the target, in the islet cells is destroyed day by day. Autoimmunity to a variety of the islet cell constituents has been

described, including carboxypeptidase H, anti-proinsulin autoantibodies and anti-insulin-receptor autoantibodies<sup>(2,8)</sup>. It was suggested that IA-2 is a

Table 1. Result of autoantibodies profile in Type-1 diabetic patients.

	ICA % positive	GAD-Ab % positive	IAA % positive	IA-2-Ab %positive
<b>Control</b>	6.3*	0**	6.3**	0***
<b>Potential</b>	12.5*	0**	8.1**	3.1***
<b>Group 1</b>	50*	46.2**	57.7**	11.5***
<b>Group 2</b>	40*	13.3**	66.7**	0***
<b>Group 3</b>	11.1*	11.1**	66.7**	0***

ICA: Islet cell autoantibodies  
 GAD-Ab: Glutamic acid decarboxylase autoantibodies  
 IAA: Insulin autoantibodies  
 IA-2-Ab: Islet cell antigen-2-autoantibodies  
 \* P <0.0001 (One way ANOVA)  
 \*\* P<0.001 (One way ANOVA)  
 \*\*\* P >0.05 (One way ANOVA)

The above abbreviations will be used in the next tables.

Table .2 Autoantibodies profile in Type-2 diabetic patients.

	ICA %positive	GAD-Ab % positive	IAA % positive	IA-2-Ab % positive
<b>Control</b>	5.9*	5.9*	0**	0***
<b>Potential</b>	8.5*	0*	3.1**	3.1***
<b>Group 1</b>	16.7*	13.3*	0**	0***
<b>Group 2</b>	15.4*	7.7*	18.5**	7.7***
<b>Group 3</b>	6.3*	0*	15.6**	0***

Table.3 Relationship between diabetes duration and prevalence of autoantibodies

Duration	ICA % positive	GAD-Ab % positive	IAA % positive	IA-2-Ab % positive
<b>&lt; 5 years</b>	34.9*	37.2*	34.9**	7.0**
<b>≥ 5 years</b>	17.2*	6.3*	40.6**	1.6**

\* P <0.05  
 \*\* P >0.05

major islet cell autoantigen in Type-1 diabetes<sup>(2,9)</sup>. Tuomi and his colleagues suggested that annually 1.2% of Type-2 diabetic patients become insulin deficient and certain Type-2 diabetes patients developed an absolute insulin deficiency within a few years, the so-called latent Type-1 diabetes. These patients showed many features of classical Type-1 diabetes as low C-peptide level, low body weight, presence of ICA and other organ specific auto-

antibodies<sup>(3,0)</sup>. So it was not surprising to find some of Type-2 diabetic Sudanese population had autoantibodies against the islet cell, glutamic acid decarboxylase, insulin and other islet cell related antigens.

In the present study the highest complication in both types of diabetes was neuropathy then nephropathy in Type-1 diabetic patients and retinopathy in Type-2 diabetic patients. These complications were more

common in the older patients and in those with longer disease duration. It was clear that Type-1 diabetic patients were more susceptible to diabetic long-term complications. Most investigators now agree that diabetic microvascular complications result from the interaction of multiple metabolic, genetic, and other factors, of which chronic hyperglycaemia is the most significant<sup>(2)</sup>. From the results of the present and previous studies mentioned above; it was clear that the traditional autoantibodies had no role in the pathogenesis of diabetic long-term complications. Other previous studies suggested the role of more specific autoantibodies against lens protein crystalline, sympathetic ganglia, vagus nerve, and phospholipids; as well as activated cellular immune response in the pathogenesis of microangiopathy<sup>(3, 1, 23, 33, 4)</sup>.

In a more recent study an increase in IgA level were noticed in the sera of diabetics with vascular complications suggesting a possible role of IgA in the pathogenesis of the vascular complications of diabetes mellitus<sup>(3, 5)</sup>.

The precise cause of diabetic long-term complications remains unclear, although it is believed to be due to the interaction between the biochemical, immunological, genetic and environmental factors. We suggest that a longitudinal study should be performed on the diabetic patients to further investigate the effect of those biochemical and immunological markers on the prevalence and development of diabetic complications. This might give a better indication as to the pre-clinical value of these markers.

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