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ANTI-ENDOTHELIAL CELL ANTIBODIES AND CIRCULATING IMMUNE COMPLEXES, A POSSIBLE PROGNOSTIC TOOL IN IDDM ANGIOPATHY

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ABSTRACT

52 patients suffering from IDDM (insulin-dependent diabetes mellitus) were studied for anti-endothelial cell antibodies (AEA) and circulating immune complexes (CIC).

20 had a high CIC level, and eight demonstrated AEA, of whom five had retinopathy and three did not show any obvious vascular complication. It has been shown that C3, C4 and CH50 levels were also decreased. AEA were evaluated by indirect immunoflourescent technique, using rat pancreas tissue as the antigen. To estimate the CIC level, PEG precipitating method was used.

It was suggested that by demonstrating AEA, we will be able to evaluate the prognosis of IDDM, especially the long standing phase, with no vascular complication.

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INTRODUCTION

Alterations in immunological function may play a role in both the pathogenesis and secondary complications of type I diabetes mellitus or IDDM (insulin-dependent diabetes mellitus).[14,18,19]

Circulating immune complexes (CIC) which have been shown in such patients to be mostly composed of islet cell antibodies (ICA), are considered as the cause of the development of vascular degenerative disease.[1-4,12,13,15,21]

In our study, looking for anti-islet cell antibodies in IDDM patients using the immunofluorescent technique, we found anti-endothelial antibodies (AEA) in some cases with a high CIC level and a low C3,C4 and CH50 activity.

As some of the AEA-positive subjects have not yet shown the retinopathy or any other vascular complications, it may be suggested that AEA determination will have prognostic vlaue in these patients.

MATERIALS AND METHODS

Sera of 52 subjects suffering from IDDM in various stages of the disease were sampled. 18 normal controls were tested as well.

Indirect Immunoflourescent Technique (IFT)

IFT was used with total anti-human conjugated antisera and monospecific anti-human antisera (anti-IgG or-IgM).

Antigen: Rat pancreas frozen sections were used as antigen.[7,9,20] Circulating immune complexes were evaluated by the PEG precipitating method,[10,15,21] and the class of the immunoglobulin involved was determined by radial immunodiffusion (RID).

C3 and C4 level was estimated by the RID and CH50 by the classic method.

Table I. Classification of patients.

	Control	Diabetic Patients (IDDM)			
		Recent-onset		Long standing-onset	
		Newly-diagnosed	Recent-onset	Few years	Several years
Number	18	10	20	19	3
Female/male ratio	0/18	5/5	12/8	6/13	1/2
Age (years)	(6-14)	3-5:3 7-14:7	6-8:11 11-15:8	7-17:18 26:1	14/18/23
Age at onset (years)	—	3-5:3	5-13 years	3-18	2-16
Duration of IDDM (Month or years)	—	1-2 month	5-18 months	2-10 years	10-16 years
Fasting blood Glucose	Normal	50-135 190-350	70-120 160-350	80-130 180-300	250-355
Urinary glucose	Negative	80-220 (-)-(+++)	80-220 (-)-(+++)	150-220 (+)-(+++)	180-220 (++)-(+++)
Known disease	—	—	12	8	2

RESULTS

52 patients suffering from IDDM were studied for several immunological alterations. Some of them are indicated in the present study.

Patient's status and classification are shown in Table I. They are divided into two categories; recent and longstanding onset. The former is subdivided to newly diagnosed and recent onset; and the latter also has two subdivisions, few years' standing and very long standing.

Table II indicates the 21 cases of high CIC levels.

Table II. Positive CIC frequency in two groups of IDDM patients.

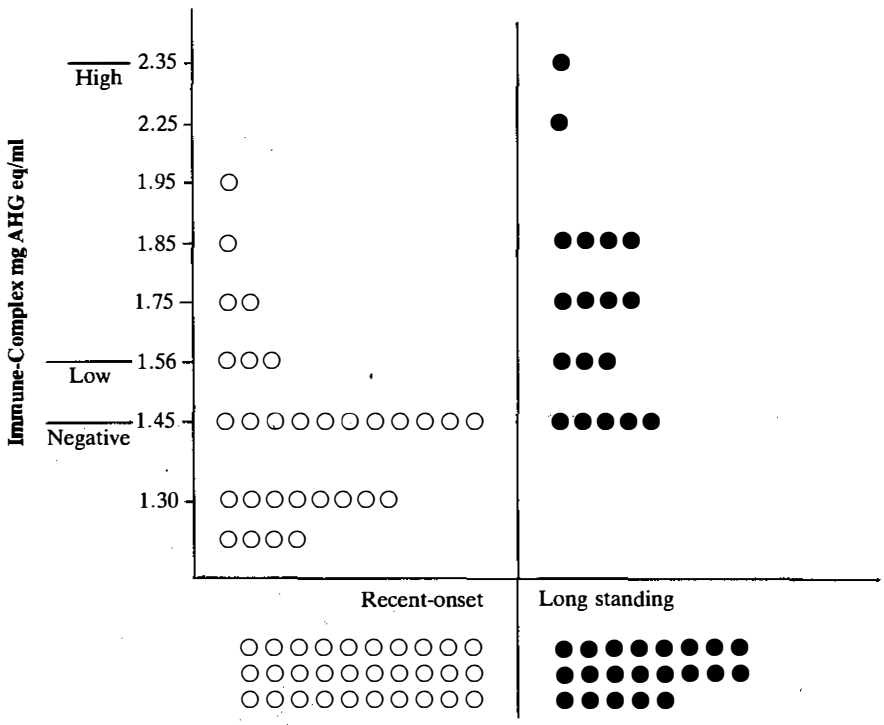
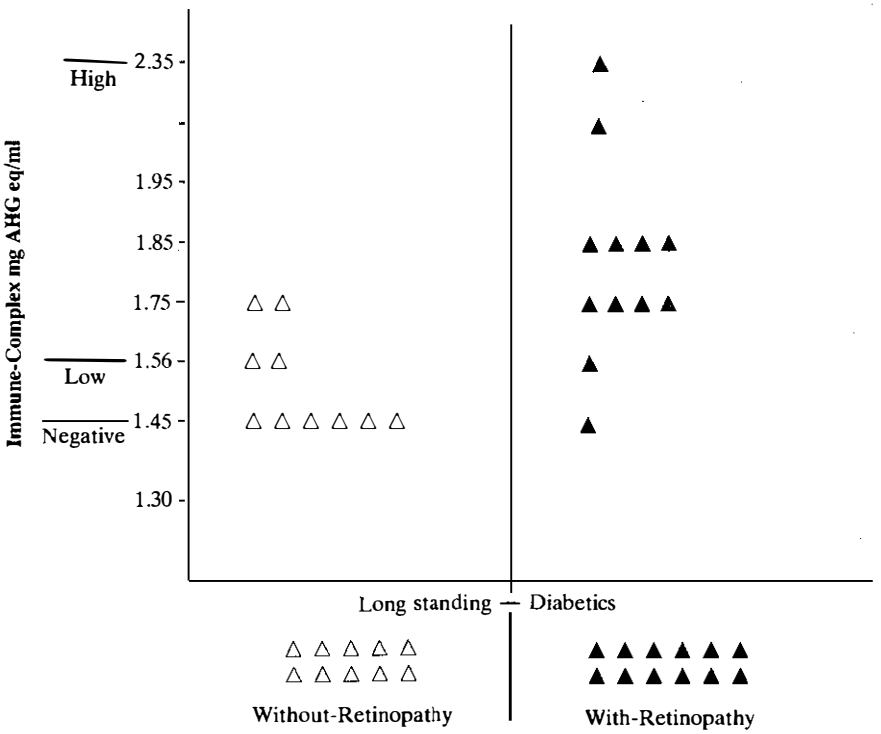


Table III. Positive CIC frequency in two groups of long standing IDDM patients.



Highest titers of CIC (more than 1.45 mg/dl), were seen in longstanding cases with retinopathy (Table III).

CIC levels in IDDM patients comparing with normal individuals showed significant differences. According to statistical apporaches, using "Z" distribution and "L" distribution, P value is: $P<0.0001$. Statistical analysis is demonstrated in the following table:

	CIC
N	$\bar{X} \pm SD$
Normal 18	0.48 ± 0.22
Patients 52	1.32 ± 0.43
	$Z=14$ $P<0.00001$ $t=7$ $P<0.0001$

Eight of 52 subjects had AEA (Fig.1). Five AEA-positive patients had vascular complications and three were without any obvious angiopathy (Table IV).

Using binominal distance known as "Bernoli Test" $p(x \leq 8) = 0.59$ was derived. Therefore the probability for a patient with high CIC levels to demonstrate AEA is 59% and the probability for the AEA-positive cases to show vascular complications is 64% $[p(x \leq 5) = 0.64]$.

Fig. 1 Immunoflourescence photograph of AEA.

Table IV. Comparison of various immunological factors in IDDM patients

	recently-diagnosed	recent-onset	Long standing	
			Few years	Several years
I.C.A*.	↑↑↑↑↑↑↑	↑↑↑↑	↑	—
C.I.C*	↑↑↑	↑	↑	↑↑↑
C3,C4	↑↑	—	—	↑↑↑
CH_{50}	↑↑↑	—	—	↑↑↑
AEA	—	↑↑↑	↑↑↑↑↑	—

Table V. Complement levels in two groups of IDDM patients

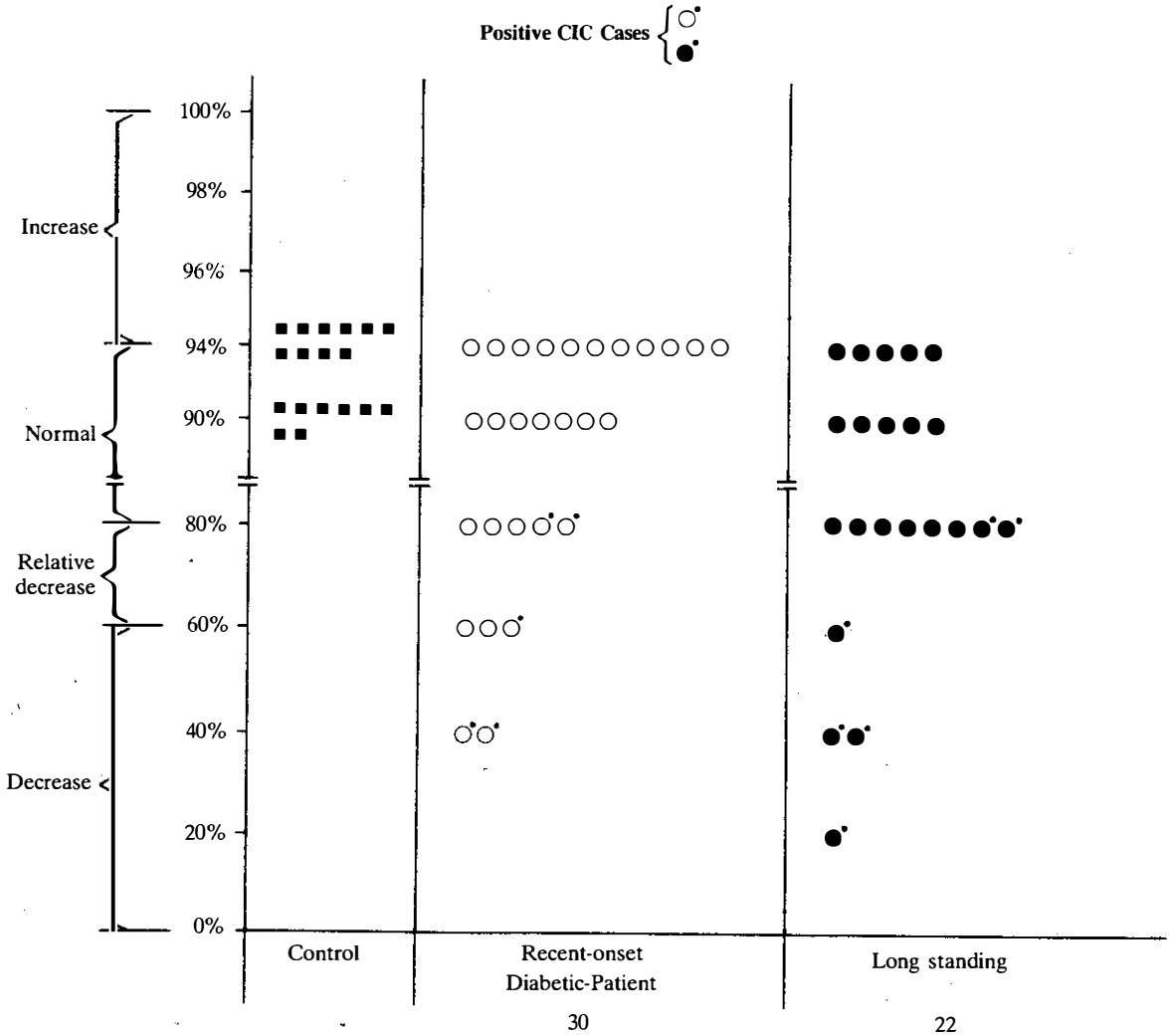


Table VI. Comparison betweendifferentstatus ofIDDM patients and their C3,C4, and CH50 levels.

Patients Status	Total No.	C3	C4			CH50
		129 mg/dl	49.2 mg/dl	8-16.8 mg/dl	80%	Normal
Recent onset	30	2*	5	4*	11	5
Long standing onset	22	2*	11	2*	12	6
Repeated infections	10	–	7	–	5	2
Retinopathy	12	2*	–	2*	10	10
Control	18	–	–	–	–	–

* Immune Complex Positive Cases

C3, C4 and CH50 activity were decreased in patients with high CIC levels (Table V). Table VI shows the various levels of C3, C4 in patients with complications.

DISCUSSION

Several features of diabetes mellitus may contribute to an increase in CIC levels. As postulated, denatured insulin binding to native IgG and complement fixation makes this immune complex active to damage the tissues. Delepess has hown an increase in CIC in IDDM subjects with anti-islet cell antibodies (ICA) and other auto-antibodies.[6]

Thus the increased prevalence of auto-antibodies may contribute to the load of immune complexes in IDDM.[5,7,8,9,18]

In this survey we looked for ICA, CIC, complement components, and many other immunological factors which will be published in the future. Using immunofluorescent technique for ICA, we found anti-endothelial cell antibodies (AEA) in eight of 52 patients. Five patients had retinopathy and three others had no complication.

High CIC level was shown in 21 subjects. All eight cases with AEA were in this category. By statistical analysis, we have shown that there is a significant difference between normal controls and IDDM patients ($p < 0.0001$). The probability of high CIC titers being accompanied by AEA is 59%, and the probability for AEA-positive cases to demonstrate vascular complications is 64%. Both C3, C4 level and CH50 activity were diminished in the above group.

We may suggest that some CIC in IDDM patients might be due to AEA, especially in those cases which are complicated with angiopathy, and measurement of AEA will be helpful to evaluate the prognosis of IDDM.

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