

# ANTI INSULIN ANTIBODIES AND MICROVASCULAR COMPLICATIONS OF DIABETES

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## SUMMARY:

The role of anti-insulin antibodies in the causation of microangiopathy in diabetes was studied in 280 insulin-treated patients. Prevalence of retinopathy and nephropathy among patients with low or high antibody titres were similar. Early onset of the complications (< 5 years of duration) also had no bearing on the antibody titres. Estimation of insulin bound immune complexes and free serum insulin also showed no correlation of these factors to the presence of the complications. A positive correlation was seen between the antibody titre and the insulin requirement. The results suggest that the insulin antibodies do not specifically contribute to the pathogenesis of microangiopathy through formation of specific antigen-antibody complex.

## INTRODUCTION:

The role of anti-insulin antibodies in the causation of microvascular complications of diabetes is a matter of controversy. While some reports show that insulin antibodies<sup>1</sup> and insulin anti-insulin complexes<sup>4</sup> play a role in causing microvascular complications<sup>2-5</sup>, others have found no such correlation.<sup>6</sup>

Andersen<sup>2</sup> has shown a positive correlation between the insulin antibody titres and the occurrence of microvascular complications. In a prospective study of patients treated with insulin, we noted that the rate of progression of retinopathy and proteinuria was slightly slower in those treated with monocomponent insulin (MC insulins) compared to those treated with conventional insulins (under publication). Patients treated with the MC insulins have lower

antibody titres, on account of the lower antigenicity of these insulins. This study was therefore taken up to see whether there is any correlation between the occurrence of insulin antibodies and insulin anti-insulin complexes and the presence of microvascular complications of diabetes.

## MATERIAL AND METHODS:

Two hundred and eighty insulin treated diabetic patients were studied. 140 of them were IDDM and the other 140 were NIDDM patients. There were 175 men and 105 women. The details are shown in Table 1. All patients in this study were treated with conventional bovine insulin. The daily dose of insulin varied from 20 to 120 units/day.

Table-1  
CLINICAL CHARACTERISTICS OF THE PATIENTS  
n = 280; M:F = 175:105

Duration of Years	Diabetes No. of Patients	Duration of Insulin Treatment Years	No. of Patients	Age at onset of Diabetes Years	No. of Patients
< 5	25	< 5	151	< 15	16
5-10	76	5-10	58	15-30	123
11-15	71	> 10	71	> 30	141
> 15	108				

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All the patients in the study had a complete biochemical work-up including liver and kidney function tests. A detailed retinal examination after full mydriasis was done by an ophthalmologist using both direct and indirect ophthalmoscopy. The Hammersmith Hospital grading system was used for gradation of retinopathy<sup>7</sup>. Those with a twenty four hour protein excretion of 500 mg or more in the absence of urinary infection or other causes of albuminuria were considered to have nephropathy<sup>8</sup>. Early onset of complications is taken to denote the development of the complications within the first five years of diabetes.

Insulin antibody index was calculated from the amount of unlabelled insulin required to displace 25 percent of bound I-125 insulin from the antibody, according to the procedure of Sebiakova et al<sup>9</sup>. Normal control sera showed only less than 10% binding of I-125 insulin.

Insulin anti-insulin complexes were assessed by calculation of the difference between the insulin binding of free antibody and total amount of insulin antibody. Binding capacity of free insulin antibodies was obtained by calculating the percentage of radioactive insulin (I<sup>125</sup>

binding capacity of free insulin antibodies was less than 25%.

Free insulin was estimated in the fasting state according to the procedure of Nakagawa et al<sup>11</sup> using 25% polyethyleneglycol (PEG) to extract free insulin.

Patients were classified into three groups on the basis of their insulin antibody titres (IAb).

Group I IAb < 1000 uU/ml  
Group II IAb 1000-5000 uU/ml  
Group III IAb > 5000 uU/ml

Statistical comparisons were done using 't' test, Chi square analysis or the Fischer's exact probability test. Correlation co-efficients were also calculated wherever relevant.

## RESULTS:

Table 2 shows the prevalence of retinopathy and nephropathy in the groups with different antibody titres. There was no difference in the prevalence of retinopathy or nephropathy among the three groups with different antibody titres ( $\chi^2 = 0.36$ ,  $P = 0.83$ , N.S. for retinopathy,  $\chi^2 = 0.1$ ,  $P = 0.95$ , N.S. for nephropathy).

Table-2  
INSULIN ANTIBODY TITRES AND THEIR RELATION TO  
MICROVASCULAR COMPLICATIONS OF DIABETES

Group	Number of patients	Normal*	Retinopathy**	Nephropathy***
I	133	80 (60%)	35 (26%)	23 (17%)
II	57	29 (51%)	14 (24%)	9 (16%)
III	90	50 (55%)	26 (29%)	16 (18%)

Differences in prevalence rate between the three groups

\*  $\chi^2 = 1.48$   $P = 0.48$  N.S.

\*\*  $\chi^2 = 0.36$   $P = 0.83$  N.S.

\*\*\*  $\chi^2 = 0.1$   $P = 0.95$  N.S.

insulin) bound to the patient's plasma in the absence of unlabelled insulin<sup>9</sup>. Total amount of insulin antibodies were estimated by acid treatment of the serum and removing the free insulin by charcoal precipitation by the method of Dimario et al<sup>10</sup>. Insulin anti-insulin complexes were estimated in 35 patients, whose

Prevalence of the complications increased with the duration of diabetes. However, no correlation was observed between the antibody titres and the presence of the complications. There was also no correlation between the duration of diabetes and the antibody titres (Fischer's probability test Non-

significant): Early onset of microvascular complications also had no bearing on the antibody titres.

Acid treatment of the serum samples to dissociate the immune complexes and to expose the binding sites did not alter the binding capacity. The binding capacities were  $21 \pm 16\%$  and  $22.5 \pm 21.5\%$  respectively with and without acid treatment. This indicated that the complexes containing insulin were present in negligible amount.

complications was similar in those with low, medium and high titres of insulin antibody. Coleman et al<sup>4</sup> have reported that insulin anti-insulin complexes are deposited in the basement membrane of the vasculature of insulin treated patients. This theory is also supported by some experimental studies<sup>12,13</sup>. The absence of insulin anti insulin complexes in a group of patients, and the absence of correlation of insulin antibody titres with the microvascular complications, suggest that the role

Table-3  
FREE INSULIN CONCENTRATIONS AND THE ANTIBODY TITRES  
IN RELATION TO VASCULAR COMPLICATIONS

Group	Free IRI uU/ml	Antibody Index mU/ml Mean $\pm$ SD	Antibody Index range mU/ml
I	$31 \pm 17$	$0.1 \pm 0.2$	Negligible to 0.5
II	$32 \pm 22$	$2.1 \pm 1.0$	1.1 to 3.7
III	$17 \pm 18^*$	$46.4 \pm 52.8$	6.3 to 177

\*P < 0.05 compared to I & II

Table 3 shows the fasting free insulin concentrations in the three groups of patients having different antibody titres. The free insulin concentration was significantly lower in those having high antibody titres ( $P < 0.05$ ). A negative correlation was observed between the free insulin concentration and the anti-insulin anti-body index ( $r = -0.33$ ,  $P < 0.05$ ). A positive correlation was observed between insulin anti-body index and insulin requirement ( $r = 0.2$ ,  $P < 0.05$ ). However, there was no correlation between insulin dose and free insulin concentration ( $r = 0.03$ ).

The fasting free insulin concentrations in those with and without complications were  $26 \pm 18$  uU/ml (range negligible to 56 uU/ml) and  $27 \pm 20$  uU/ml (negligible to 60 uU/ml) respectively.

## DISCUSSION:

We have not been able to observe any correlation between presence of the anti-insulin antibodies and the presence of microvascular complications of diabetes in a group of insulin treated patients. The prevalence of

of these antibodies in formation of immune complexes is probably negligible. There was also no correlation between fasting free insulin and microvascular complications. This suggests that circulating free insulin levels have no role in the causation of microvascular complications.

Irvine et al<sup>6</sup> have suggested that the immune complexes may contribute to the development of microvascular complications but they have not observed any correlation between insulin antibodies and the antigen antibody complexes in insulin treated patients, thereby suggesting that the immune complexes need not be comprised of insulin and its antibodies. They have demonstrated that the immune complexes are elevated in insulin treated as well as in non-insulin treated diabetic patients with microangiopathy. This again suggests that insulin antibodies do not specifically contribute to the pathogenesis of microangiopathy through formation of specific antigen-antibody complex formation.

In this study, the early onset of the complications defined as occurrence of complications

within 5 years of diagnosis of diabetes is also found to be independent of the insulin antibody titres. This observation is in contrast to that of Andersen<sup>1</sup> who has observed that the insulin antibody titres are higher with early onset of the complications.

In conclusion, anti-insulin antibodies may not have a direct or a significant role in the causation of microvascular complications of diabetes.

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