

# Circulating Immune Complexes in Diabetics

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*Circulating immune complexes (CIC) were detected by platelet aggregation test (PAT) in 40.0% of 45 diabetics and by polyethylene glycol precipitation — complement consumption test (PEG-CC test) in 30.6% of 36 diabetics as compared to 5% and 10% of 20 normal control subjects for each test. The prevalence of CIC in diabetics was significantly higher than in the normal controls ( $P < 0.05$ ). There were no correlations between the presence of CIC detected by PAT and the duration of the disease, insulin treatment, or diabetic complications. Thus multiple factors must contribute to the increase of CIC in diabetics. The role of these various factors needs to be studied.*

**Key Words:** Circulating immune complexes, platelet aggregation test, diabetics.

Circulating immune complexes (CIC) were reported to be increased in diabetics, especially in those who were type I insulin dependent near diagnosis (Irvine *et al.*, 1977) and in patients with severe microangiopathy (Irvine *et al.*, 1978a). In recent years, a vast amount of literature has accumulated reporting that soluble CIC are responsible for, or associated with, a variety of pathologic lesions (Koffler, 1967; Nydegger *et al.*, 1977; Wager *et al.*, 1978; Chalesworth *et al.*, 1982; Moonly *et al.*, 1983; Park *et al.*, 1984a, 1984b), but the role of CIC in diabetics is still controversial (Kilpatrick and Virella, 1982). In this study, we investigated the presence of CIC in the sera of diabetics and compared it to that found in normal controls. We also investigated association with insulin treatment and association with the occurrence of diabetic complications.

## MATERIALS AND METHODS

### Patients

Sera were obtained from forty-five diabetics who were admitted to Severance Hospital. The duration of diabetes in the patients was between 1 month and

25 years. Twelve had been treated with insulin for more than 1 year at the time of the study and thirty-three had been treated with diet or oral hypoglycemic agents. Insulin treated patients received ordinary commercial insulin (NPH, crystalline). The presence of microangiopathy and retinopathy was assessed by a careful routine physical and ophthalmological examination. Questionable abnormalities were further evaluated by ophthalmological consultation. Nephropathy was evaluated on the basis of BUN, serum creatinine levels and proteinuria (BUN > 50mg/100ml, serum creatinine levels > 2mg/100ml, and/or proteinuria > 150mg/24h were considered as an index of the kidney involvement). Twenty blood donors were included in the study as normal controls.

### Measurement of Soluble Immune Complexes (CIC)

**Platelet aggregation test (PAT):** Fresh human platelets were separated by differential centrifugation (Aster and Jandl, 1964a, 1964b) and adjusted to a final concentration of 200,000 platelets/mm (Penttinen *et al.*, 1971). The platelets were used on the day of preparation. The test sera were diluted in basal salt solution (BSS: pH 7.5) containing 300mg glucose per liter (Penttinen, 1977), starting with a dilution of 1/2 put into wells of disposable plastic U-plates (Flow Laboratories, Inc., U.S.A.). 25ul of test serum dilution were added to 25ul of BSS and 50ul of platelets suspension. Pipetting was done at room temperature. The results were read by naked eye in dark field illumination after

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**Table 1. The prevalence of circulating immune complexes in diabetics**

	Platelet aggregation test		PEG-CC test		PAT+PEG-CC	
	Number of subjects	PAT titer >1:10	Number of subjects	%CC> 25%CC	Number of subjects	Both positive
Control group	20	1( 5.0%)	20	2(10.0%)	20	1( 5.0%)
Diabetics	45	18(40.0%)	36	11(30.6%)	33	7(21.2%)

\* P&lt;0.05

overnight incubation at 5-9°C.

A smooth white bottom indicated a positive reaction, and the appearance of white platelet pellets indicated a negative reaction (Penttinen *et al.*, 1971; Myllyla *et al.*, 1971).

The Chi square test was used for the statistical analysis of the results.

**Polyethylene glycol precipitation-complement consumption test (PEG-CC test):** The details of the procedure are given elsewhere (Harkiss and Brown, 1979). Briefly, CIC are first isolated from serum by precipitation in 2.5% PEG and concentrated. They are then assayed functionally by measuring their ability to fix complement. Samples giving values of 25% or more of complement consumption are considered as positive in the PEG-CC test (Park *et al.*, 1984a).

## RESULTS

The CIC were assayed by PAT on forty-five diabetics and twenty blood donors, and by PEG-CC test on thirty-six diabetics and twenty blood donors (Table 1). Eighteen (40.0%) of the 45 diabetics and 1(5.0%) of the 20 blood donors showed a PAT positivity. Therefore CIC were significantly increased in diabetics ( $P>0.05$ ). Eleven (30.6%) of the 36 diabetics and 2(10.0%) of the 20 blood donors were also positive by the PEG-CC test. Table 2 shows that only 7 (50%) of the 14 PAT positive diabetics were also positive in PEG-CC test and 2 (10.5%) of the 19 PAT negative diabetics were positive in PEG-CC test. Agreement of the two tests was seen only in 24 (72.7%) of the 33 diabetics tested.

When the duration of diabetes was taken into account, there was no significant correlation between CIC and duration of disease (Table 3). However, when patients were divided according to the type of treatment, insulin treated diabetics showed a higher prevalence of CIC compared to the non-insulin treated diabetics; but this prevalence was not significant (Table 4). The prevalence of CIC in patients without

**Table 2. Detection of circulating immune complexes in diabetics by platelet aggregation test and PEG-CC test**

	Number of subjects	PAT	PEG-CC test
Control group (20)	1	+	+
	1	-	+
	18	-	-
Diabetics (33)	7	+	+
	7	+	-
	2	-	+
	17	-	-

**Table 3. Influence of duration of diabetes mellitus on circulating immune complexes**

Duration of diabetes mellitus	Number of patients	PAT titer more than 1:10
0-5 years	22	9 (40.9%)
6-10 years	12	2 (16.7%)
10 years or more	11	7 (63.6%)

**Table 4. Influence of insulin treatment on circulating immune complexes in diabetics**

	Number of patients	PAT titer more than 1:10
Control group	20	1 ( 5.0%)
Diabetics	45	18 (40.0%)
Insulin treated	12	7 (58.3%)
Non-insulin treated	33	11 (33.3%)

microangiopathy was 37.5% and with retinopathy and/or nephropathy was 38.9% (Fig. 1).



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