DEMONSTRATION OF PEPSIN ACTIVITY IN CIRCULATING IMMUNE COMPLEXES ISOLATED FROM DIABETIC PATIENTS

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ABSTRACT

Pepsin activity was demonstrated in circulating immune complexes, isolated from sera of 90 diabetic patients, which included 30 type 1, 60 type 2 diabetic patients and 40 control subjects, by the polyethylene glycol method. Pepsin activity was estimated by the proteolytic assay, using acidified haemoglobin as substrate. The mean polyethylene glycol indices in type 1 and type 2 diabetic patients were 21.9±3.6 and 24.9±5.3 respectively, when compared to controls which was 9.2±1.4. The mean pepsin activity is such complexes was found to be 772.5±222.2 units/ml/24hrs in type 1, 741.2±71.2 units/ml/24 hrs in type 2 and 593.3+59.0 units/ml/24 hrs in control subjects, respectively. The specificity of the pepsin assay was checked using pepstatin, a specific inhibitor of pepsin. Inhibition of pepsin activity was observed in a dose dependent manner by increasing the concentration of the inhibitor. Significance of this observation in clinical association with circulating immune complex is discussed.

KEY WORDS: Type 1 diabetes; Type 2 diabetes; Circulating immune complexes; Polyethylene glycol index, Pepsin assay.

INTRODUCTION

Circulating immune complexes are frequently observed in autoimmune diseases like rheumatoid arthritis, type 1 diabetes and systemic lupus erythromatosus (1-3). In an earlier report we demonstrated presence of circulating immune complexes (CIC's) in peptic ulcer cases (4). The presence of circulating immune complexes in peptic ulcer cases was supported by dissociating the complexes and characterizing the antibody present in CIC's. This was based on the findings of Ohnishi (1984)(5), who reported that pepsin has an immune complexase (breaks immune complexes) activity at neutral pH, without affecting the protein components other than the immune complexes. Ohinishi also demonstrated that pepsin is present in the mouse macrophages and has the same molecular weight and biochemical characteristics as the pepsin secreted from gastric mucosa. It is claimed that irrespective of its origin, whether from macrophages or gastric juice, this protease has the capacity to break CIC's at neutral pH. However, information on this aspect is scanty and restricted to experimental animals like the mouse. The present study was undertaken to investigate if pepsin could be detected in CIC's in clinical cases (not reported earlier) as it is claimed to break CIC's at neutral pH in the serum. In such an event, substantial proportion of CIC's should have pepsin bound to it, for the purpose of breaking the complexes. We performed the study in diabetic cases, as we are engaged in research on various aspects of this disorder. We found a considerable proportion of diabetic cases are positive for CIC's in the serum. Significance of the results obtained has been discussed, since formation and clearance of CIC's play a key role in immune complex mediated tissue damage, under diseased conditions.

MATERIALS AND METHODS

30 type 1 diabetic patients, 60 type 2 diabetic patients and 40 age and sex matched, healthy subjects, were included in the present study. The cases were selected from the Endocrinology division of Princess Durru Shehvar Hospital (Hyderabad), Gandhi General Hospital (Secunderabad), and Osmania General Hospital (Hyderabad). The clinical diagnosis of diabetes mellitus, as well as classification of cases into type 1 and type 2 diabetes, was done according to the criteria laid down by World Health Organization (6). Blood sugar levels were estimated by glucose oxidase method (Glucose oxidase kit, GOD-PAP/end point, Kaizen Diagonstics, Hyderabad). None of the cases were suffering from malnutrition. Type 1 cases were found to be positive for ketosis, upon withdrawal of insulin, while none of the type 2 cases showed ketosis. All the patients and controls were fasting at the time of blood collection. 5 ml of venous blood was collected from these cases for detecting CIC's as well as for estimation of pepsin activity in these complexes.

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Polyethylene glycol (PEG) index

Circulating immune complexes (CIC's) in the sera of patients and control subjects were quantified according to the method of Digeon et al (1977) (7). Briefly the procedure is as follows. To 2ml of 3.3% PEG, 0.2m1 of serum was added and incubated for 2 hours at 25°C and the precipitated CIC's were sedimented by centrifuagation at 2500 G for 30 minutes. The precipitates were then washed with 3% PEG, dissolved in 0.2m1 of distilled water, and the volume made up to 2ml with 0.1N NaOH. The protein content was estimated by Lowry's method (1951)(8), as a measure of PEG index.

Estimation of Pepsin Activity in the Dissociated CIC's

In order to estimate pepsin activity, if any, in the isolated CIC's, the circulating immune complexes were dissociated as follows. After precipitation and washing with 3% PEG, 0.2m1 of borate buffered saline was added and the solution was made to 2m1, with 0.45 Mm potassium thiocyanate. This was incubated at 20°C for about 18 hours and dialyzed against distilled water, to remove acid and used for estimation of pepsin activity in the dissociated immune complexes, according to the proteolytic method of Mirsky et al (1952) (9), using acidified hemoglobin as substrate.

RESULTS

Details of the results pertaining to immune complex levels and pepsin activity in immune complexes are given in Table 1 to 3. It is evident from Table 1 that circulating immune complexes, measured as PEG indices, are higher in type 1 as well as in type 2 diabetic patients, compared to controls. Mean PEG indices being 21.9±36 in type 1 and 24.9±5.3 in type 2 diabetic cases, which are significantly higher than that of the control subjects i.e. 9.2±1.4. The specificity of pepsin assay was checked by using pepstatin, a specific inhibitor of pepsin. In was observed that pepsin activity was inhibited by pepstatin in a dose dependent manner. This indicates presence of higher levels of CIC's in diabetic cases. Enhanced levels of CIC's have been reported in diabetic patients (10). Significantly higher levels of CIC's have also been reported in diabetic cases with complications (11) and this is in consonance with our study. A case was considered as positive for CIC if it had a PEG index more than mean ± 2SD of controls, which is 18.0. Frequencies of CIC positive and negative cases are shown in Table 2. It was observed that 11 (36.7%) out of 30 type 1 cases and 14 (23.3%) out of 60 type 2 diabetic cases, were positive for immune complexes, while only 5% of control subjects were positive for CIC's. Details of the results obtained regarding pepsin activity in immune complexes are given in the Table 3. Pepsinogen activity was found to be as follow: 772.5±222.2 units/ml/24hrs in type 1 diabetic patients, 741.2+71.2 units/ml/24 hours in type 2 diabetic patient and 593.3+59.0 units/ml/24hours in controls.

DISCUSSION

Our findings suggests that pepsin is bound to immune complexes and that it acts as an immune complexase. Presence of pepsin activity in immune complexes isolated both from clinical cases as well as from control subjects indicates that it is a normal function of pepsin to break such complexes. This appears to be accomplished by the binding of pepsin to Fc portion of immunoglobulins (particularly of IgG type) which are exposed by configurational changes brought about in lg molecule following interaction with an antigen. This is one of the few studies which highlights an important function of pepsinogen/ pepsin. Earlier Ohnishi et al (1984) have demonstrated immune complexase activity of pepsin in CIC's isolated from mouse. Further studies on this aspect will provide useful information on the possible use of pepsin for breaking immune complexes in vivo, in patients, as a therapeutic agent.

Table 1: Mean PEG Indices in Diabetic (DM) Cases and Controls

Category	n	Mean PEG Index+S.D
Type-1 DM	30	21.9 ± 3.6
Type-2 DM	60	24.9 ± 5.3
Total DM	90	23.4 ± 4.4
Controls	40	9.2 + 1 .4

Table 2: Frequencies of Circulating Immune Complexes Positive and Negative cases in Test and Control Subjects

	PEG positive		PEG negative	
Category	n	%	n	%
Type-IDM	11	36.7	19	63.3
Type-2DM	14	23.3	46	76.7
Total DM	25	27.8	65	72.2
Controls	2	5.0	38	95.0

Table 3: Pepsinogen levels in Dissociated Circulating Immune Complexes of Diabetes Mellitus (DM) and Control Subjects

Category	n	Mean ± S.D.
Type-1 DM	30	772.5 ± 222.2 *
Type-2 DM	60	741.2 ± 71.2 *
Controls	40	593.3 ± 59.0

*P<0.05 Controls vs Type 1 DM
*P<0.05 Type 2 DM vs Controls

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