CIRCULATING IMMUNE COMPLEXES IN TUBERCULOSIS

A Arora, J P Wali, P Seth, J S Guleria, P Aggarwal

ABSTRACT

To examine whether any correlation exists between the levels of circulating immune complexes (CICs) and the activity of tuberculosis, CICs were measured in the sera of 75 patients with active tuberculosis and in 25 control subjects using polyethylene glycol method. The effect of drug treatment on the levels of CICs was also estimated in 25 patients. It was found that levels of CICs were elevated in most of the untreated patients (96%) of tuberculosis and the CICs levels fell to control values in 64% of patients at the end of treatment.

Keywords: Pulmonary tuberculosis, tubercular pleural effusion, tubercular lymphadenitis, circulating immune complexes.

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INTRODUCTION

Circulating immune complexes (CICs) have been demonstrated in sera of patients with a variety of diseases eg. malignancies[1,2], infectious diseases[3] and collagen vascular diseases[4]. In the pathophysiology of diseases like systemic lupus erythematosus, CICs play an important role[4]. Tuberculosis is a common disease in India. It can affect any organ of the body and can also occur in a disseminated form. The precise role, if any, of the CICs in the pathogenesis of tuberculosis is not clear. Moreover, the presently available methods of assessing disease activity, eg. chest X-ray, bacteriological evidence and Mantoux test have their own limitations. The present study was therefore undertaken to estimate the CIC levels in patients with tuberculosis and to find out whether any correlation exists between CIC levels, disease activity and drug treatment.

MATERIAL AND METHODS

Seventy-five newly diagnosed cases of tuberculosis were included in the study. The diagnosis was established by clinical, radiological, bacteriological or histological means. Out of 75 cases, 55 had pulmonary parenchymal tuberculosis, 13 had tubercular pleural effusion and 7 had tubercular lymphadenitis. All patients were followed up monthly with complete physical examination and erythrocyte sedimentation rate estimation after start of treatment and radiological evaluation was done every three months in patients with pulmonary tuberculosis and tubercular effusion. In twenty-five patients (15 with pulmonary disease and 10 with tubercular pleural effusion), CIC estimation was done every month for nine months.

The patients with parenchymal pulmonary tuberculosis were categorized into grade I, II, and III on the basis of radiological findings[5]. The levels of CICs were correlated with the radiological grading.

Five ml of blood was drawn by a sterile syringe from all the patients before and after the treatment, while in 25 patients, blood samples were obtained every month while on treatment. Serum was separated from these samples and stored at minus 20 degrees Centigrade until assayed. The level of CICs was estimated by polyethylene glycol (PEG) method using a simplified turbidometric assay based on the precipitation of complexed (but not free) Ig by low concentrations of PEG as described by Haskova et al[6] with some modifications[7]. Briefly, 2 ml of 4.166 percent PEG 6000 (Sigma Chemicals Co., USA) in 0.1M borate buffered saline, pH 8.4 (BBS) was added to 0.22 ml of sera diluted 1:3 in BBS to obtain a final concentration of 3.75% PEG and 1:30 of serum. A similar volume of diluted serum mixed with 2 ml of BBS served as a control. After incubation at 25° C for 60 minutes, the light absorbance of the test and the control samples was measured at 450 nm (E_{450}) by a spectrophotometer (UV-190, Schimdzu, Japan). The results were expressed as PEG index derived by the following formula:

$$\text{PEG index} = (E_{450} \text{ with PEG} - E_{450} \text{ with BBS}) \times 1000$$

We preferred the PEG method for estimation of CICs as it is simple, less time consuming and equally sensitive to the complement consumption assay[7]. Since the aim of our study was to correlate the levels of CICs before and serially after the chemotherapy and also to correlate it with the severity of the disease, we did not further characterise the PEG precipitates.

CIC levels were also estimated in 25 healthy individuals (controls). All these individuals were healthy volunteers with no history of fever or any other symptoms of systemic infection in the recent past. Majority of these cases were college students or office goers. None of them had any history of jaundice in the past. Haemogram and liver function including HBsAg were done in all of these control subjects and no abnormality was detected in any of these tests.

All the patients received a 3-drug anti-tubercular regimen

Department of Gastroenterology
All India Institute of Medical Sciences
New Delhi 110029
India

A Arora, MD, DM
Senior Resident

Department of Medicine
All India Institute of Medical Sciences

J P Wali, MD, MNAMS
Additional Professor

J S Guleria, MD, DM
Professor

P Aggarwal, MD, DNB
Assistant Professor

Department of Microbiology
All India Institute of Medical Sciences

P Seth, MD
Additional Professor

Correspondence to : Dr J P Wali

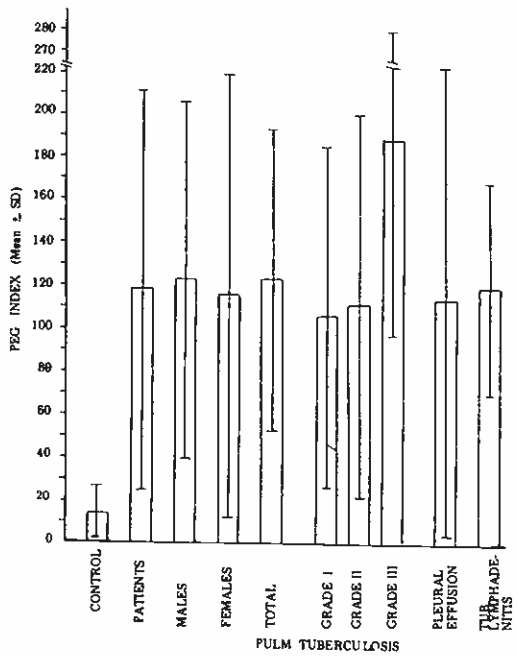
comprising isoniazid, rifampicin, and ethambutol for a period of nine months. In patients with tubercular lymphadenitis, the treatment was continued up to twelve months.

Statistical analysis was done using 't' test of significance and one and two way analysis of variance (a) to compare the differences in the CICs levels amongst the patients with pulmonary parenchymal tuberculosis, pleural effusion and tubercular lymphadenitis, (b) to compare the CIC levels in the three subgroups (grade I, II, III) of pulmonary parenchymal tuberculosis amongst themselves and (c) to assess the effect of treatment on the serial levels of CICs in the group of 25 patients followed up at monthly intervals. A 'p' value of <0.05 was considered as significant.

RESULTS

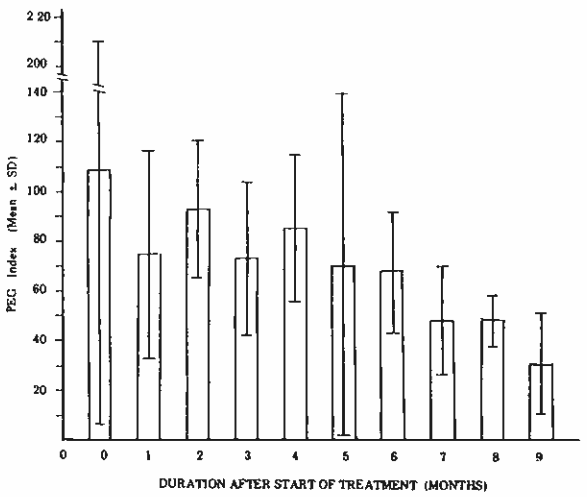
Out of 75 patients, 51 were male (mean age ± SD of 30.1 ± 10.7 years) and 24 female (27.2 ± 11.2 years). Control group had 22 males and 3 females with a mean age group of 32.6 ± 11.7 years which matched with the patient group. According to radiological grading of pulmonary parenchymal lesions, 20 patients had grade I, 25 grade II and 10 grade III lesions. In the control group, the mean PEG index (reflecting levels of CICs in the serum) was 14.2 ± 12.5 and only 3 individuals had a PEG index of more than 30. In accordance with the results in the control group, a mean PEG index greater than 30 was considered a positive index. The mean PEG index in 75 patients taken together was 117.8 ± 93.2 prior to the start of treatment. The levels in male and female patients were 122 ± 83.4 and 115.4 ± 103.2 respectively, the difference being not statistically significant. The PEG index in patients with pulmonary tuberculosis, tubercular pleural effusion and tubercular lymphadenitis were 122.1 ± 70, 111.6 ± 108.4 and 118 ± 49.3 respectively. In 55 patients with pulmonary tuberculosis, the PEG indices according to radiological grading were 105.2 ± 79.4, 109 ± 89.1 and 186 ± 91.4 in grade I, II and III lesions respectively, the difference being not significant statistically. These are depicted in Fig. 1.

Fig 1 - PEG index (mean± SD) in various groups of tuberculosis and controls.



In 25 patients, who were followed up for 9 months with CICs estimation every month, the PEG index before the start of treatment was 108.9 ± 101.9 and there was a gradual fall thereafter till a mean level of 31.3 ± 20.4 was documented at the end of treatment (Fig. 2). Two of these patients (8%) had negative PEG index, ie. a PEG index <30 before the start of treatment while at the end of nine months of treatment, 9 out of 25 patients (36%) still had elevated levels of CICs though the levels were not different statistically from that of the control group.

Fig 2 - PEG index (mean± SD) at monthly intervals in 25 patients following start of treatment.



DISCUSSION

CICs have been shown to be elevated in sera of patients with granulomatous disorders eg. sarcoidosis[8] and Crohn's disease[9]. However, their correlation with disease activity and their role in the pathogenesis of various disorders remain to be established. Corticosteroids have been shown to inhibit the formation of CICs and they also ameliorate many of the extra-pulmonary symptoms including fever in patients with tuberculosis. In that case, is it possible that CICs may play an important role in the pathogenesis of systemic manifestations of tuberculosis and also in the occurrence of vasculitis in the central nervous system lesions? The present study attempts to clarify some aspects of CICs in relation to tuberculosis. Out of 75 patients with tuberculosis, 72 (96%) had elevated CIC levels in comparison to only 3 (12%) out of 25 controls ($p<0.001$). Moreover, there was no significant difference in the PEG index in male and female subjects both in the control and patient groups suggesting that circulating sex hormones in females do not influence the levels of CICs. In two other reports studying 40 and 96 patients with tuberculosis, elevated levels of CICs were found in 68% and 56% patients respectively[10,11].

Most of our patients (96%) with active tuberculosis had elevated CIC levels. This is in distinct contrast to two other studies mentioned above[10,11]. The reason for this could be that all our patients were freshly diagnosed and untreated cases of tuberculosis and 35 out of 55 cases with pulmonary tuberculosis had moderately severe parenchymal disease (grade II and III) and possibly had higher antigen and antibody load leading to the formation of CICs. Moreover, in the other 2 series[10,11], a significant number of patients were having either an inactive disease or were already on treatment before the CICs estimation

was done. Both of these factors could have been responsible for levels of CICs in these studies. Finally, in the subgroup with active untreated tuberculosis, Johnson et al[11] found elevated CICs levels in 78% of the patients. Bhattacharya et al[12] not only showed that CICs were significantly higher in tuberculosis patients (n=22) than in healthy control subjects (n=18) but also showed that these complexes were made up of immunoglobulins, albumin, complement components and mycobacterial antigens. They concluded that CICs levels could be a useful parameter in the diagnosis of active tuberculosis.

Circulating immune complexes may nonspecifically be raised in a number of infectious disorders including viral fevers but none of our patients had clinical evidence of any infection other than tuberculosis. So, we think that the elevated levels of CICs reflected the disease activity of tuberculosis.

PEG index is a sensitive method to detect the polymeric immune complex bound immunoglobulins in CICs. It is a simple, cheap and easily available test though it is not as sensitive as the Raji cell assay for the estimation of CICs. Even though it is mentioned that PEG precipitation method may precipitate many nonimmunological proteins[13], we presume that such a factor could not have been of any major significance in our study as none of our patients had any clinical evidence of conditions like rheumatoid arthritis or other immunological illnesses which would interfere with the PEG estimation of CICs.

In the present study, the mean PEG index in patients with grade III lesions was clearly higher than that in grade I or II indicating a possible correlation between the extent of the disease and the CIC levels. However, this difference was not statistically significant. This may possibly be due to (a) a small number of patients (n=10) in grade III category and (b) a wide standard deviation in the mean PEG index. Singh et al[14] studied 216 patients and showed increasing titres of CICs from reactive tuberculosis (micronodular, localized tuberculosis) at one end to nonreactive tuberculosis (acute miliary tuberculosis) at the other end of the immune spectrum of tuberculosis. This could be explained by the fact that both the number of tubercular bacilli and the levels of antibodies increase towards the non-reactive pole.

In our study, we did not find any significant different levels of CICs in patients with tuberculosis of various sites. This suggests that the CIC levels depend upon the activity of the disease rather than on the site of the disease. Similar results were found in another study[11].

The follow-up study in 25 patients revealed that 9 out 25 patients (36%) had elevated CICs at the end of nine months of treatment but they were approaching the control levels. In another report, 29% patients had elevated CICs after the treatment though there was a significant fall in their levels [15]. In a study by Carr et al[10], 15% of bacteriologically cured patients still had elevated CICs after treatment. This indicates that clinical and radiological clearance may precede the clearance of CICs from the circulation. It could also suggest the persistence of disease activity. It is tempting to speculate that sequential study of CIC levels in individual patients might give some guidance to the activity of the disease and provides useful information about the duration of the treatment necessary. However, to resolve this point, further studies and long term follow up with sequential measurement of CICs is necessary.

It is therefore concluded that the estimation of CICs does have a role in the diagnosis and management of patients with tuberculosis. Measurement of CICs could especially be useful where there is difficulty in culturing the tubercular bacilli.

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