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PPD-Specific IgG and IgG Subclasses in the Sera of Pulmonary Tuberculosis Patients

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This study was performed to characterize the humoral immune responses with isotype profiles in Mycobacterium tuberculosis infection. PPD-Specific IgG and IgG subclasses were measured using ELISA in 212 patients with pulmonary tuberculosis.

The values of PPD-specific IgG were significantly higher in pulmonary tuberculosis patients than those in the control group, and were correlated to the severity of illness ($P<0.01$). The specificity and sensitivity of ELISA for IgG antibodies were 1.0 and 0.81, respectively as determined in 212 sera from tuberculosis patients and 44 from healthy controls. The positive predictive value was 1.0 (171/171), while negative predictive value was 0.52 (44/85). The values of PPD-specific IgG were significantly decreased after 2-4 months of treatment. Among the moderately and far advanced pulmonary tuberculosis patients, the values of PPD-specific IgG were significantly decreased in responders after 6 months of treatment. However, PPD-specific IgG in nonresponders was increased ($P<0.01$).

PPD-specific IgG subclass responses were evident to all four IgG subclasses. No changes of isotype response according to the severity of the disease were observed.

Key Words: *PPD-specific IgG, IgG subclasses, Tuberculosis*

INTRODUCTION

Tuberculosis was described on record as 'phthisis', a consumptive disease, even in the age of ancient Greece. The diagnosis of active tuberculosis in children and extrapulmonary tuberculosis are particularly difficult because symptoms are frequently absent or uncharacteristic and bacteriologic confirmation is seldom achieved. For these reasons, diagnosis is based largely on tuberculin skin test and clinical and X-ray findings, which have a low specificity and may produce a high proportion of false negative results. Recently, the increase of INH (isonicotinic acid hydrazide)-resistant populations of Mycobacterium tuberculosis and increased incidence of tuberculosis in patients with HIV infection made us take a growing interest in tuberculosis (Chaisson and Slutkin, 1989). Early diagnosis and accurate assessment of therapeutic response are essential for the management of tuberculosis.

Serologic diagnosis of tuberculosis has been tried since 1948, when Middlebrook et al (1948) detected anti-mycobacterial antibody in the serum of a patient with pulmonary tuberculosis using the hemagglutination method. Nassau et al (1976) reported the usefulness of ELISA in the diagnosis of tuberculosis in 1976. PPD-specific IgG and IgG subclasses were measured in the sera of tuberculosis patients using ELISA to clarify the humoral immune responses against Mycobacterium tuberculosis and for the better understanding of isotype profile of antibodies in tuberculosis.

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MATERIALS AND METHOD

Subjects and specimen

Two hundred and twelve patients with pulmonary tuberculosis and forty four healthy control subjects were enrolled in the study during a 2 year period from Jan. 1987 to Dec. 1988 (Table 1).

Diagnosis of tuberculosis was made by clinical and chest X-ray findings, tuberculin skin test, family history, smear and culture of sputem, and therapeutic responses. The patients were divided into 4 groups according to the American National Tuberculosis and Respiratory Disease Association classification. Group I, consisting of 65 patients with primary tuberculosis, Group II, 54 patients with minimal pulmonary tuberculosis, Group III, 58 patients with moderately advanced pulmonary tuberculosis, Group IV, 35 patients with far advanced pulmonary tuberculosis. Control subjects were healthy medical students with normal chest X-ray findings and negative tuberculin skin tests (with unclear BCG vaccination history) and all had no family history of tuberculosis. Table 2 shows the age and sex distribution of pulmonary tuberculosis patients. Most of primary tuberculosis patients were less than 5 years of age, patients over 16 years of age ammount to most of far advanced pulmonary tuberculosis patients. PPD-specific IgG was measured in the sera of all subjects and PPD-specific IgG subclasses were measured in the sera of 78 patients who showed high titer of PPD-specific IgG (O.D.: above 0.3). After taking blood samples, all sera were preserved with 2% sodium azide (10ul/ml serum) at −70°C until antibody assays were performed.

Table 1. subjects for detection of PPD-specific IgG in sera.

Group	Patients	No. of cases
Healthy Control*		44
Pulmonary tuberculosis		212
I	Primary tuberculosis	65
II	Minimal tuberculosis	54
III	Moderately advanced tuberculosis	58
IV	Far advanced tuberculosis	35
Total		256

Table 2. Age and Sex distribution of the pulmonary tuberculosis patients.

Age (yr) \ Sex	Number of Patients		
	Male	Female	Total
– 1	3	2	5
2 – 5	23	20	48
6 – 10	13	16	29
11 – 15	20	23	43
16 –	54	38	92
Total	113	99	212

Antibody Assay

The measurement of PPD-specific IgG and IgG subclasses was performed using ELISA as postulated by Voller et al (1976).

PPD-specific IgG

Micro-ELISA plates (Immulon II, Dynatech Lab., USA) were coated with 100ul of PPD antigen (1mg/ml, Denmark, 1:800 dilution with carbonate-bicarbonate buffer) for 18 hours at 4°C. The plates were then washed with PBST three times and stored at 4°C for no longer than 2 months. 100ul of serum (1:100 dilution with PBST containing 0.5% BSA) was added to duplicate wells. The plates were incubated for 2 hours at room temperature, and then washed three times with PBST.

100ul of goat anti-human IgG conjugated with alkaline phosphatase (1:1000 dilution with PBST, Sigma, USA) was added to the wells, the plates were incubated for 1 hour in the dark at room temperature, and again washed with PBST three times.

One hundred microliters of *p*-nitrophenyl phosphate disodium (1mg/ml, pH 9.8, Sigma, USA) in 10% diethanolamine buffer was added as substrate. The ELISA plates were incubated for 1 hour in the dark at room temperature, and optical density was measured using an ELISA reader (ELIDA-5, Physica, USA) at 405 nm.

PPD-specific IgG subclasses

Micro-ELISA plates (Immulon II, Dynatech Lab., USA) were incubated overnight at 4°C with 100ul of PPD at a concentration of 1.25ug/ml. The plates were than washed with PBST three times. 100ul of serum (1:100 dilution with PBST containing 0.5% BSA) was added in duplicate wells and the plates were incubated for 2 hours at room temperature, and then washed three times with PBST. 100ul of monoclonal mouse anti-human IgG subclasses (Unipath, England, IgG1, IgG3; 200ng/100ul/well in PBST, IgG2, IgG4: 100ng/100ul/well in PBST) were added to the wells and incubated for 2 hours at room temperature. After three

more washings with PBST, 100ul of rabbit anti-mouse antibody conjugated with alkaline phosphatase (1:1000 dilution with PBST, Sigma, USA) was added and the plates were incubated in the dark for 1 hour at room temperature. Finally the enzyme substrate application and measurement of optical density were performed with the same method as in the PPD-specific IgG assay.

Each serum was tested at least twice on different days to minimize temperature variations and to verify the reproducibility of results.

Small plate to plate variations were corrected according to the values of standard positive and negative pooled sera.

Statistical Method

The values of the PPD-specific IgG and IgG subclasses were presented with mean optical densty ± S.E. The unpaired student t-test was used to determine the significance of differences in IgG and IgG subclass levels in various groups, and the relationship between IgG and IgG subclasses was analyzed by simple correlation and regression tests.

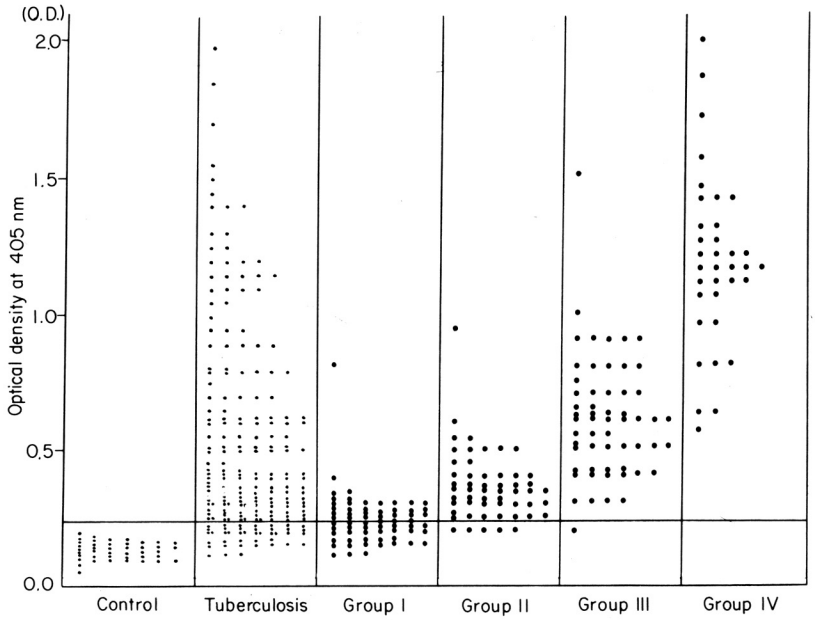

Fig. 1. Distribution of PPD-specific IgG in sera of pulmonary tuberculosis patients.
Group I : Primary
II : Minimal
III : Moderately advanced
IV : Far advanced

Table 3. Values of PPD-specific IgG in sera of pulmonary tuberculosis patients.

Group	No. of cases	PPD-specific IgG a)
Control	44	0.126 ± 0.030
Pulmonary Tuberculosis	221	0.535 ± 0.179*
Primary tuberculosis	65	0.228 ± 0.093*
Minimal tuberculosis	54	0.356 ± 0.136*
Moderately advanced tuberculosis	58	0.599 ± 0.239*
Far advanced tuberculosis	35	1.181 ± 0.354*

a) The values are mean O.D. ± S.E.
*$P < 0.01$

RESULTS

PPD-specific IgG

The values of PPD-specific IgG in different groups of patients are shown in Table 3., Fig.1.

PPD-specific IgG was significantly higher in pulmonary tuberculosis patients (0.535±0.179) than in controls (0.126±0.030). The values of PPD-specific IgG in primary tuberculosis, minimal tuberculosis, moderately advanced tuberculosis and far advanced tuberculosis were 0.228±0.093, 0.356±0.136, 0.599±0.239 and 1.181±0.354 respectively. A cutoff point of two times of the mean O.D. value of the healthy control populations was selected (0.126×2=0.252). According to this criterion the specificity of the ELISA for IgG antibodies was 1.0 as determined in the 44 sera from healthy controls. The sensitivity of the ELISA was 0.81 when applied to all 212 tuberculosis patients. But the sensitivity was gradually increased according to the extent of disease. The sensitivities of the ELISA in primary, minimal, moderately advanced and far advanced tuberculosis were 0.45, 0.91, 1.00, 1.00 respectively.

The positive predictive value was 1.0 (171/171), while negative predictive value was 0.52 (44/85).

Among 212 tuberculosis patients, 40 patients were followed up for more than 12 months. Changes of PPD-specific IgG in tuberculosis patients after treatment are shown in Table 4.

Values of PPD-specific IgG were significantly decreased after 2-4months of treatment in all tuberculosis patients except the far advanced pulmonary tuberculosis group. Moderately and far advanced tuberculosis patients were divided into two groups, responders and non-responders, according to the evidence of clinical and radiological improvement after 12 months of treatment.

PPD-specific IgG in responders was significantly decreased after 6 months of treatment from 0.887±0.068 to 0.572±0.041. However, PPD-specific IgG in nonresponders was increased from 1.225±0.114 to 1.694±0.119 (Table 5., Fig.2.).

PPD-specific IgG subclasses

The values of PPD specific IgG subclasses in the sera of pulmonary tuberculosis patients are shown in Table 6, Fig.3.

The values of PPD-specific IgG1, IgG2, IgG3, IgG4 were 0.288±0.034, 0.217±0.021, 0.124±0.007 and 0.104±0.004 respectively. Among 78 subjects, an optical density above 0.1 (2-2.5 times O.D. of healthy controls) of PPD-specific IgG1 was observed in 48 cases (62%), IgG2 in 65 cases (83%), IgG3 in 52 cases (67%) and IgG4 in 41 cases (53%). All four IgG subclasses were above 0.1 in 21 cases (27%).

The values of PPD-specific IgG were significantly correlated with PPD-specific IgG3 levels, but were not significantly correlated with other IgG subclasses (Fig. 4,5,6,7).

Individual isotype variation was minute in IgG subclass responses, and no changes of isotype response according to the extent of the disease were observed (Fig.8).

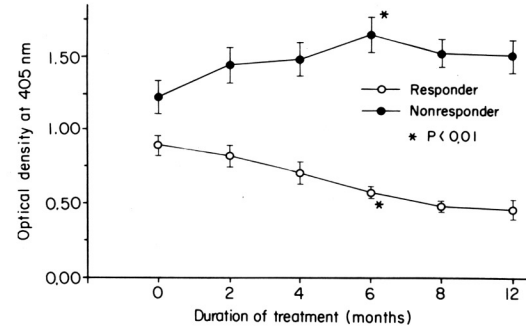

Fig. 2. Changes of PPD-specific IgG in sera accroding to the response of treatment.

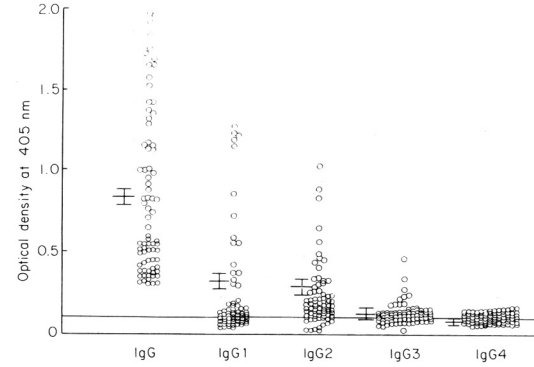

Fig. 3. Distribution of PPD-specific IgG and IgG subclass in sera of pulmonary tuberculosis patients. Each bar represents mean ± S.E.

DISCUSSION

Serologic diagnosis of tuberculosis has developed since 1948 when anti-mycobacterial antibody was detected for the first time using the hemagglutination technique (Middlebrook and Dubos, 1948). It was especially helpful for the diagnosis of extrapulmonary

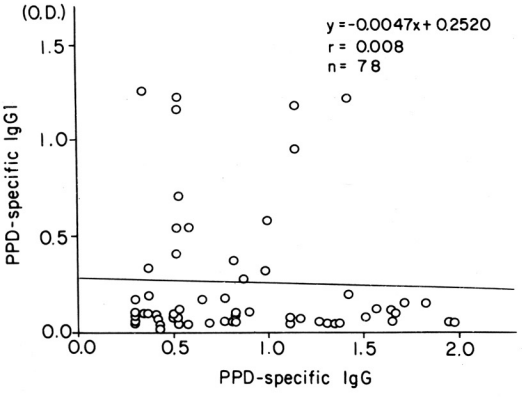

Fig. 4. Relationship of PPD-specific IgG and IgG1 in sera of pulmonary tuberculosis patients.

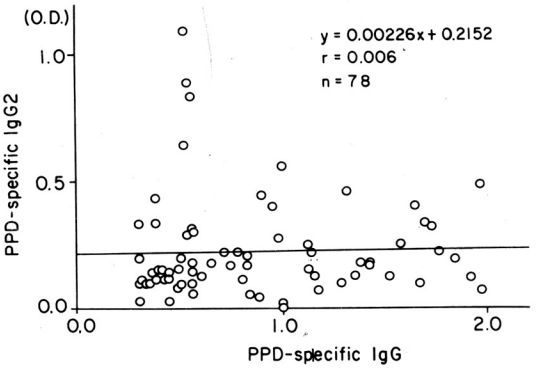

Fig. 5. Relationship of PPD-specific IgG and IgG2 in sera of pulmonary tuberculosis patients.

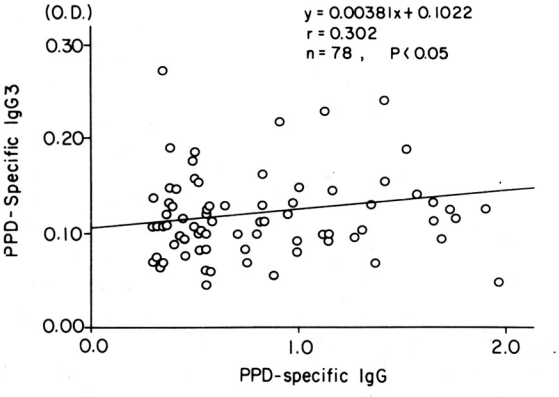

Fig. 6. Relationship of PPD-specific IgG and IgG3 in sera of pulmonary tuberculosis patients.

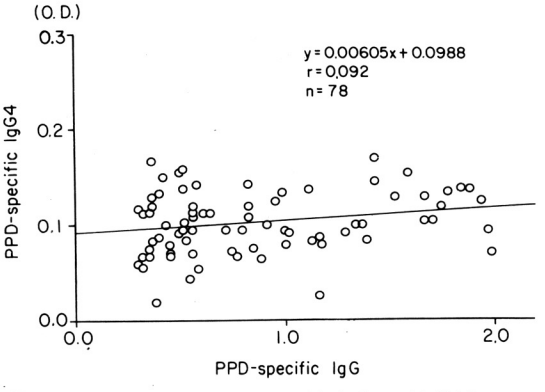

Fig. 7. Relationship of PPD-specific IgG and IgG4 in sera of pulmonary tuberculosis patients.

Table 4. Changes of PPD-specific IgG in the sera of tuberculosis patients after treatment.

Group	No of cases	Duration of treatment (months) 0	2	4	6	8	12
I	12	0.274±0.022	0.216±0.021	0.191±0.019*	0.185±0.016	0.164±0.011	0.170±0.015
II	7	0.340±0.023	0.278±0.021	0.233±0.019*	0.229±0.023	0.169±0.034	0.186±0.044
III	11	0.769±0.046	0.646±0.040*	0.595±0.038	0.549±0.045	0.460±0.044	0.457±0.062
IV	10	1.225±0.095	1.342±0.102	1.290±0.118	1.253±0.180	1.107±0.206	0.767±0.177*

Group: See Figure 1.
The values are mean O.D. ± S.E.
*$p<0.01$

tuberculosis and childhood tuberculosis which had little help from general diagnostic methods such as chest X-ray, sputum smear and culture.

It is well known that the sensitivity and specificity of serologic methods are largely affected by the antigenic components and methods of measurement. The diagnostic efficacy of serologic methods depends on the selection of profer antigen and technique. PPD has been used as a standard antigen for skin test and serologic diagnosis of tuberculosis even though it contains

Table 5. Changes of PPD-specific IgG in the sera according to the response of treatment.

Group	No of cases	Duration of Treatment (months) c)					
		0	2	4	6	8	12
Responder a)	15	0.887±0.068	0.815±0.077	0.698±0.073	0.572±0.041*	0.477±0.039	0.458±0.063
Nonresponder b)	6	1.225±0.114	1.440±1.221	1.481±0.111	1.649±0.119*	1.525±0.093	1.505±0.109

a) Patients with clinical and radiological improvement after 12 months of treatment
b) Patients without clinical and radiological improvement after 12 months of treatment
c) The values are mean O.D. ± S.E.
* $P < 0.01$

Table 6. Values of PPD-specific IgG and IgG subclasses in sera of pulmonary tuberculosis patients.

Total No of cases: 78

Group	No of cases	IgG	IgG1	IgG2	IgG3	IgG4
I	9	0.433±0.053	0.157±0.050	0.141±0.017	0.105±0.007	0.105±0.007
II	20	0.499±0.032	0.206±0.057	0.236±0.054	0.110±0.008	0.107±0.010
III	27	0.774±0.058	0.208±0.053	0.222±0.041	0.118±0.008	0.099±0.005
IV	22	1.387±0.096	0.301±0.084	0.225±0.026	0.150±0.021	0.106±0.004
Total	78	0.811±0.053	0.288±0.034	0.217±0.021	0.124±0.07	0.104±0.004

The values are mean O.D. ± S.E.
Groups: See Figure 1.

few polysaccharides and shows cross reaction with other acid-fast bacilli. Daniel et al (1975) and Anderson et al (1978) reported that antigen 5 exists only in human and bovine tubercle bacilli and recommended using antigen 5 for the serologic diagnosis of tuberculosis after examining the specificity of various antigenic components of Mycobacterium tuberculosis. Tandon et al (1980) and Grange et al (1980) got satisfactory results with high specificity using PPD antigen like antigen 5 or antigen 6.

But it is generally known that antigen 5 has a relatively high specificity, and sonicated antigen and antigen 6 show a somewhat lower specificity than PPD antigen (Daniel and Debanne, 1987).

Although many methods have been developed, radioimmunoassay and ELISA are known as the most sensitive methods with high reproducibility. The clinical symptoms of tuberculosis depend largely on the immune reaction of the host rather than the virulence of the tubercle bcilli.

Beginning with Nassau et al (1975), who tried serologic diagnosis for tuberculosis using ELISA for the first time in 1975, Tandon et al (1980), Grange et al (1980), Zeiss et al (1984) and Daniel et al (1981) reported that ELISA could Be used effectively for the diagnosis of tuberculosis.

Bhatanger et al (1977) found out the reversed correlation between cellular and humoral immunity in tuberculosis infection. In addition, Lenzini et al (1977) reported high antibody titers in serious mycobacterial infection with lower cell mediated immune response.

In this study the values of PPD-specific IgG were increased compare to those of controls and correlated to the severity of illness. We obtained a sensitivity of 0.81 and specificity 1.0 in 212 tuberculosis patients and 44 healthy controls. The accuracies of positive and negative prediction values of ELISA for IgG antibodies were 1.0 and 0.52 respectively. The diagnostic efficacy of ELISA for PPD specific IgG in the sera of pulmonary tuberculosis was good in all groups of tuberculosis except primary tuberculosis, Because of the previous BCG vaccination in adults and children has no significant effects on ELISA results (Kardjito et al., 1980; Barreral et al., 1989), ELISA for PPD specific IgG, unlike the PPD skin test, can also be helpful for the diagnosis of tuberculosis in a vaccinated populations. The values of PPD-specific IgG were significantly decreased after 2-4 months of treatment in all groups of pulmonary tuberculosis patients except far advanced pulmonary tuberculosis patients. The values of PPD-specific IgG were significantly decreased in responders after 6 months of treatment, while PPD-specific IgG in nonreponders were increased. It can be suggested that serial measurement of PPD specific

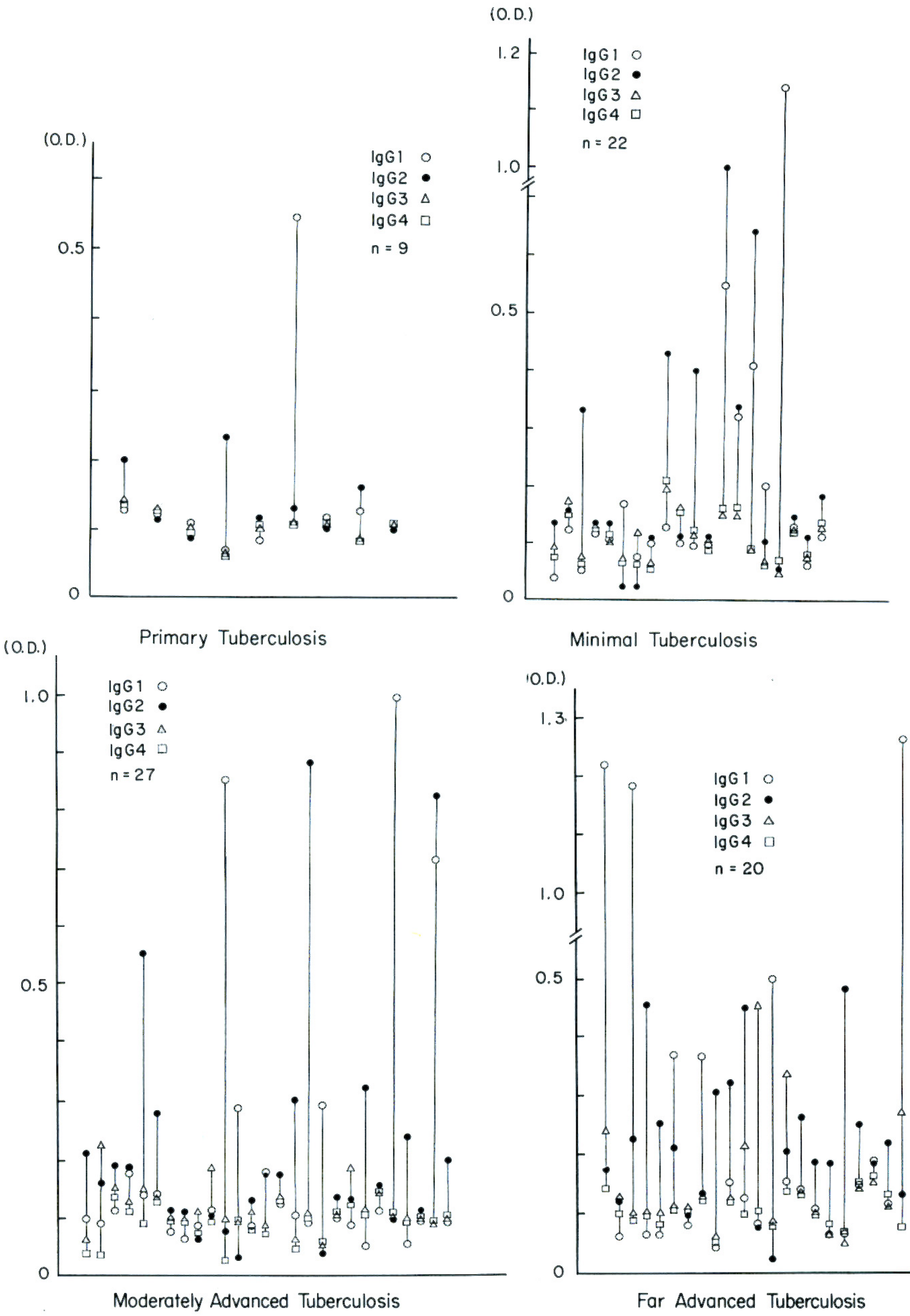

Fig. 8. Distibution of individual PPD-specific IgG subclasses in pulmonary tuberculosis patients.

IgG is helpful to evaluate the effectiveness of treatment and prognosis of patients. These results were similar to those reports of Favez et al (1968) and khomenko et al (1984), while Kaplan et al (1980) reported increased antibody titers during the early stage of treatment.

Four subclasses of IgG (IgG1, IgG2, IgG3, IgG4) are isotypes which differ in heavy chain antigenicity and biological properties. In general IgG subclass response in the body shows differences according to the antigenic components. IgG2 response against bacterial polysaccharide (Siber and Schur, 1980), IgG1 and IgG3 for protein antigen (Skvaril and Schiet, 1984), increase of IgG4 in allergic and parasitic disease

(Heiner, 1984) and the role of IgG3 in GBS infection (Kim et al 1989) were demonstrated.

IgG subclass responses on various diseases or after vaccinations like tetanus (Kim et al, 1989), hepatitis (Morell and Roth-wicky, 1983) and rubella were also reported. Gibson et al (1987) reported that PPD-specific IgG were increased in 51% of patients, while one or more IgG subclasses were increased in 90% of patients after measuring specific IgG and IgG subclasses in 107 sputum confirmed tuberculosis patients.

Our study revealed that all four IgG subclasses were enrolled on the immune responses against PPD antigen in tuberculosis (62% on IgG1, 83% on IgG2, 67% on IgG3, 53% on IgG4). All four IgG subclasses were increased in 27% of patients.

The values of PPD-specific IgG were significantly correlated with only PPD-specific IgG3 levels. Individual variation of IgG subclass responses was minute, and no changes of isotype response according to the severity of illness were observed.

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