RESULTS AND DISCUSSION

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4.1 Pulmonary Tuberculosis : The radiographic and bacteriological extent of the disease and response to chemotherapy in patients of North Eastern India

The present investigation deals with the radiographic extent and sputum status of pulmonary tuberculosis patients in this demographic region. The dynamics of chemotherapeutic response varied widely from rapid recovery (resolution of inflammatory infiltrative changes, disappearance of tubercle bacilli and cavities during the first six months of treatment) to slow recovery (slow regression of destructive lung changes).

It has been observed that high number of patients (n = 89) were having lesions of both the lungs and 41 of these were having severe extensive disease. The disease was considered 'limited' if it involved less than one third of the lung before and 'extensive' if the lesions were more profuse. 25.8% of the total patients were unilateral limited, whereas, there was no unilateral extensive patients.

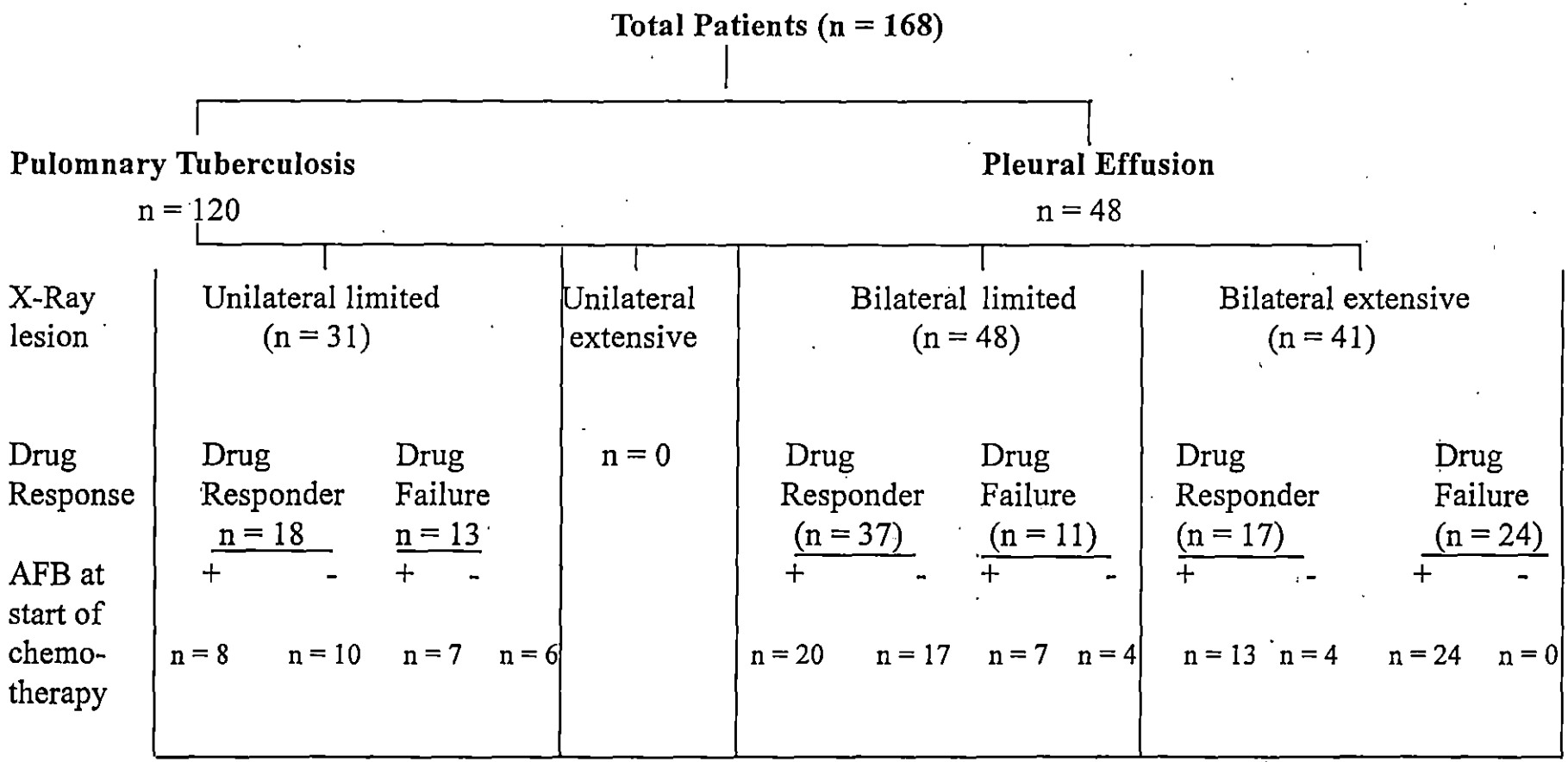
41.9% (13) of unilateral limited and 20.8% of bilateral limited were drug failure and 58.5% of bilateral extensive diseased groups were multidrug resistant.

34.16% of the patients were sputum negative when the chemotherapy was initiated (Table 6).

Discussion

In this study there were altogether 168 patients of pluro- pulmonary tuberculosis among which 120 patients were found to be suffering from pulmonary parenchymal disease without pleural effusion. Among this group there were only 11 patients classified as suffering from unilateral limited disease whereas 89 patients had bilateral disease. Bilateral extensive involvement was noted in 41 patients. This shows that in these patients bilateral limited and extensive involvement were more common.

Table 6. Distribution of study subjects and clinical groups



When we consider the sputum AFB status of the same patients as many as 79 patients out of this 120 were found to be sputum positive at the initiation of chemotherapy.

This extension and bacteriological positivity may have several etiological basis:

1. Our patient population were all high landers and according to the concept of herd immunity this population has got rather recent exposure to mycobacteria. The evolutional list of tuberculosis as established by epidemiological methods shows a characteristic trend in a fixed group of population. In the initial stage the disease produce by mycobacteria is acute and devastating with high mortality. With passing of centuries the disease takes a more chronic form with less mortality and more morbidity. This second type of response is now seen in average Indian population while the high landers are still in the high group.

2. As this region is tuberculosis prone, most of the inhabitants are more or less exposed to *Mycobacterium tuberculosis*. Hence, they possibly developed a low zone tolerance to that antigen. This low zone tolerance may effect their clinical manifestation and only a high amount of antigen shows the sign of the disease.

3. Tuberculosis is a contagious disease spread by droplet infection. The droplets carrying bacilli after being expectorated from a patient may remain suspended in air for a long duration. The bacilli are very susceptible to sunlight, drying and desiccation. Closed indoor inhabitation, lack of ventilation and lack of adequate exposure to sunlight are the three criteria easily met in the dwelling of this patient population under study. The low socio-economic condition with associated under nutrition and thus undermining natural immunity is another contributory factor.

4. That the disease classification seems more as bilateral than unilateral denotes that we are getting the patients in advance stage of the disease as also substantiated by sputum bacteriological status. An interesting study revealed by Toman showed that extensive disease is not necessarily preceded by limited stage disease in most of the cases. Here in this group the first chest X-ray showed

bilateral disease in 89 out of 120 cases probably reflecting their susceptibility and lack of immunity.

5. The number of drug failures was 40%. In a study done on the same group (De Sarker, S., unpublished data) reported high percentage of multi drug resistant tuberculosis with high infectivity and geographical clustering. The data shows that the infectivity and again close contact indoor dwelling with lack of ventilation leading to the clustering.

4.2 Association of HLA

Study on the immunogenetic aspect of disease is most useful in identifying not only the mode of inheritance of a particular disease process but also in understanding the immunopathogenic mechanism underlying it. Incidentally, most diseases that show strong HLA association have unknown etiology and known mode of inheritance, for example various autoimmune and rheumatological diseases. In this respect, immunogenetic studies of mycobacterial infectious diseases are important since the causative organism is already known and the relevant peptides involved are being to be characterised. Association of HLA class-I and class-II antigens with the disease has been studied by serological method.

4.2.1 Association with HLA class I antigens

The phenotype frequencies of HLA class I antigens in patients with pulmonary tuberculosis were compared with that of healthy controls (Table 7). Significantly increased frequencies of HLA -A9 (33.3% vs-8.33, $p< 0.01$, RR = 5.63); HLA -B7 (33.3% vs 8.33, $p< 0.01$, RR = 5.63) and decreased frequency of HLA-A2 were observed (3.3% vs 25.0%, $p< 0.01$, RR = 0.10) in the patient group as compared to controls.

4.2.2 Association with class II antigens

Among the class-II antigens, only HLA -DR2 showed significantly deviated frequencies among patients as compared to controls. It occurred with a frequency of 36.6% in PTB patients as compared to 4.16% in healthy controls, $X^2 = 19.9$, RR = 13.02 (Table 8).

Table 7. Phenotypic frequency of HLA class I antigens in pulmonary tuberculosis and control.

HLA antigen	Phenotypic Frequency (%)		X^2	Relative risk
	PTB n = 120	Control n = 282		
A1	10.00	25.00	0.2	0.33
A2	3.30	25.00	5.48*	0.10
A3	13.30	4.10	1.90	3.79
A9	33.30	8.33	9.92*	5.63
A10	6.60	4.16	2.32	1.60
A11	16.60	8.33	0.90	2.20
A19	10.00	4.16	0.60	2.50
A28	3.30	8.33	0.80	0.30
A29	6.60	25.00	3.66	0.21
B5	20.00	20.83	0.30	0.94
B7	33.30	8.33	9.92*	5.63
B8	13.30	6.00	1.90	2.39
B12	3.30	4.16	0.40	0.77
B15	10.00	16.60	1.20	0.55
B16	16.60	6.00	2.50	3.11
B18	6.60	4.16	0.70	1.60
B22	3.30	4.16	0.90	0.77
B35	3.30	4.16	0.90	0.77
B37	3.30	8.33	0.60	0.30
B40	3.30	4.16	0.90	0.77

n = number of subject studies
X^2 = Chi square
* = $p < 0.01$

Table 8. Phenotypic frequency of HLA class II antigens in pulmonary tuberculosis and control.

HLA antigen	Phenotypic Frequency (%)		X^2	Relative risk
	PTB n = 120	Control n = 282		
DR1	13.30	8.33	1.80	1.73
DR2	36.60	4.16	19.90*	13.02
DR3	23.30	20.83	0.50	1.15
DR4	13.30	8.33	1.80	1.73
DR5	10.00	4.16	0.60	2.50
DR6	10.00	4.16	0.60	2.50
DR7	13.33	8.30	1.80	1.73
DR8	6.60	4.16	0.30	1.60
DR9	6.60	11.95	1.22	0.57
DQ1	13.30	33.33	3.58	0.31
DQ2	13.30	33.33	3.58	0.31
DQ3	53.30	37.50	1.70	1.90

n = number of subject studies
X^2 = Chi square
* = $p < 0.01$

4.2.3 HLA association with the radiographic extent of the disease

No significant correlation was found between HLA class-I antigens and the extent of chest X-ray lung lesions of the PTB patients. The DR2 distribution was correlated with different radiographic lung lesions (UL, UE, BL, BE). The data is summarized in Figure 7. Only 6 (19.3%) of UL and 14(30%) of BL were DR2 positive. On the contrary 21 of the 41 BE (51%) had DR2 positive.

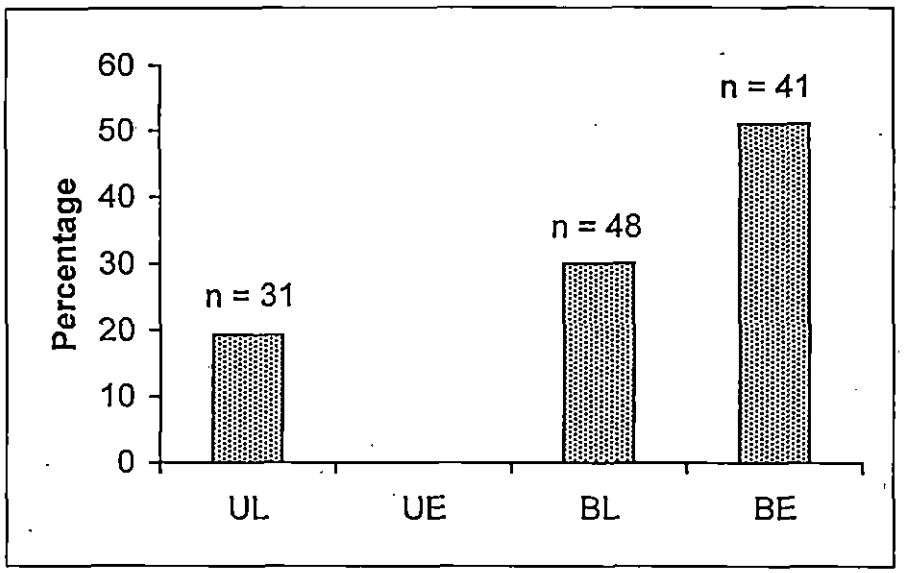


Fig. 7. Distribution of DR2 in the four clinical sub-groups, based on lung lesion.

4.2.4 HLA association with dynamics of chemotherapy

To determine whether chemotherapeutic response is related to host genetic make up, the distribution of HLA antigens in the drug responders and drug failure patients were analyzed.

No deviation was found in the distribution of HLA class I antigens between controls and drug responders and multidrug resistant patients.

HLA -DR2 was found to be most significantly higher in drug failure group of patients with a frequency of 62.5% as compared to 37.5% in drug responder group ($X^2 = 13.57$) giving a relative risk of 0.36. No significant difference was found in HLA class II specificities between the two groups [a. rapid recovery where cavities and all disappeared in the first six months of treatment, b. slow regression of destructive lung changes] of drug responders (Table 9 and Fig 8).

Table 9 Association of HLA -DR2 with drug responsive PTB and drug failure PTB.

	Total no.	No. of antigen bearing	Phenotype frequency (%)
Total patients	120	44	36.6
Drug responder	72	27	37.5
Drug failure	48	30	62.5

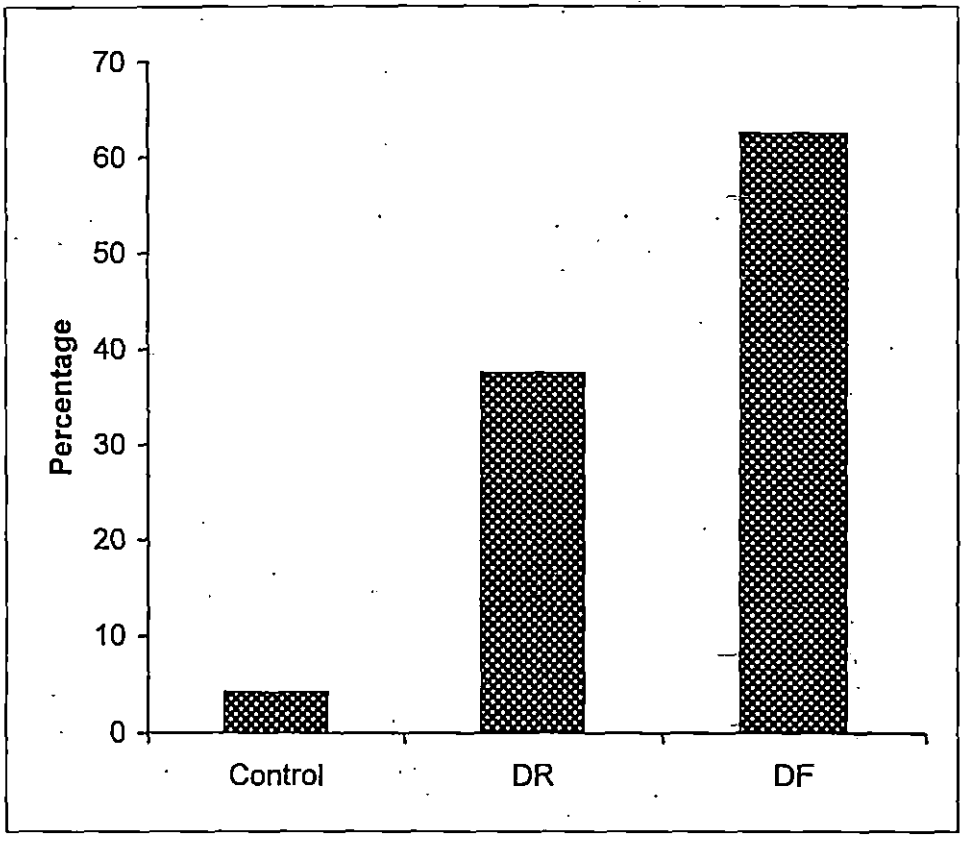


Fig. 8 Distribution of HLA DR2 in drug responder and drug failure group of PTB patients as compared to controls. DR and DF represents drug responder and drug failure respectively.

4.2.5 Discussion

The available literature shows a divergent result in HLA association with the disease (Table 4) which might be attributed to several factors including the ethnic variability of the HLA association and endemicity of the environment. The decreased frequency of HLA -A2 observed in the present study is in accordance with the finding reported in Kazakh patients (Khomenko *et al*, 1990). It is probable that HLA -A2 give some sort of resistance to the host against the disease. Molecular sub typing of this antigen may further lead to the identification of T cell receptor binding portion makes possible its involvement in the mediation of suppressor T cell activity. The higher incidence of A9 is a unique finding in this population. However, in Russian population an increase in B7 was noted (Khomenko *et al*, 1990) which is similar to the findings of the present investigation. Although various investigators have tried to correlate HLA phenotypes with clinical course and severity of the disease that is different stages of radiographic lung lesions, sputum smear positivity (Al- Arif, 1979; Brahmajothi *et al*, 1991) and breakdown to active tuberculosis of inactive pulmonary lesions (Hawkings *et al*, 1988), no report including the present study revealed consistent association of HLA class I antigens with the severity of disease in PTB. This class I molecules (HLA -A9, -B7) may present antigen to CD+ T cells and this might contribute to the risk of developing tuberculosis.

A positive association of HLA -DR2 with PTB patients in the population under study confirms by the findings of others (Singh *et al*, 1983a, 1983b; Mehra *et. al*, 1986; Bothamley *et. al*, 1989; Khomenko *et al*, 1990; Bramajothi *et al*, 1991; Pospelov *et al*, 1996) in other Asian population. However, in North American blacks (Hwang, *et al*, 1985), Mexican American (Cox *et al*, 1988) and Hong Kong Chinese (Hawkings *et al*, 1988) does not favour DR2 association with PTB.

When the patients were divided into drug failure and drug responder, a high frequency of DR2 was found among the drug failure forms of PTB patients. Generally, drug resistance occurs when a single drug is given and when the viable bacterial population in the lesions is large. Drug resistant mycobacteria are most

likely evolve through a chromosomal mutation mechanism to escape or prevent drug induced metabolic damage, probably by a mechanism of decreased permeability of the cell membrane against the drug, or decreased affinity of the drug binding sites, or due to the loss of a drug activating enzyme (Mitchison, *et al*, 1984). Immunomodulators particularly recombinant INF-γ have been shown in animal models to increase the efficacy of the early stages of chemotherapy of tuberculosis (Khor *et al*, 1986). The unresponsiveness or anergy - failure to react to purified protein derivative, PPD (Chan, 1991) has been described. DR2 may play a critical role in inducing anergy/an unresponsiveness to *M. tuberculosis* which leads to the development of infection in the drug resistant form of PTB. However, nature of the immunological imbalance in this drug failure cases needs intensive investigation.

In South Indian patients with PTB DR2 was found to be strongly associated with far advanced cases than those with minimal and moderate radiographic lung lesions (Bramojothi *et al*, 1991). In the present study extensive disease has been found to occur more significantly in whom DR2 was overwhelmingly present. Anergy induction role of DR2 may lead to a spurt in the bacillary population.

4.3 Antibody levels in pulmonary tuberculosis

Although PTB has been characterised by cellular immune reactions (Kaufmann, 1993), the importance of humoral arm in the pathogenesis has been recognised by the presence of *Mycobacterium tuberculosis* specific antibodies both in patients (Grange *et al*, 1980) as well as in healthy contacts (Pitchappan *et al*, 1991). An immune spectrum has been demonstrated in tuberculosis where in the cell mediated and humoral immunity are mutually exclusive, at opposing ends of the spectrum (Daniel *et al*, 1981).

The present investigation have been undertaken to determine antibody levels in patients with PTB in relation to various clinical manifestations. The status of HLA antigens in patients was also correlated with the level of these antibodies in order to understand the HLA- linked genetic involvement of the host for generation of these antibodies.

4.3.1 IgG and IgM antibodies in PTB patients

The level of IgG and IgM were compared with that of healthy controls and the data is presented in Figure 9. The base levels of IgG and IgM in healthy

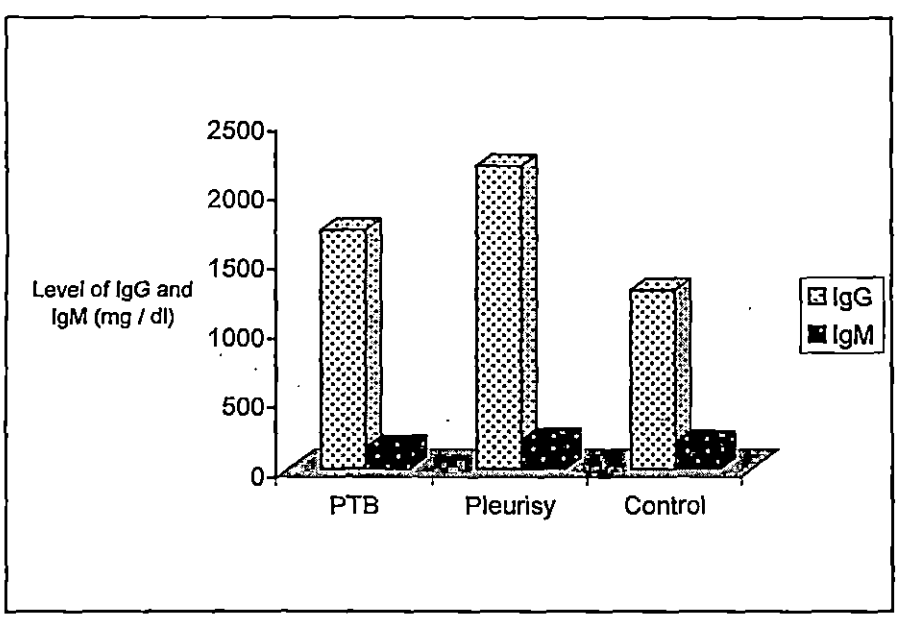


Fig. 9 The level of IgG and IgM antibody in PTB, Pleural effusion and healthy controls.

controls were 1288.6 mg/dl and 190.52 mg/dl respectively. PTB patients showed increased level of IgG (1723 mg/dl, t = 0.1%). Interestingly the level of IgM was lower among patients (154.5 mg/dl, t = 0.1%).

4.3.2 IgG and IgM antibodies in Pleural effusive patients

When the data was compared with pleural effusive patients it was found that the levels of IgG (2188 mg/dl, t = 0.1%) and IgM (210 mg/dl, t = 0.1%) were increased in relation to healthy endemic control (Fig.9).

4.3.3 IgG and IgM antibodies and sputum examination

To evaluate a possible association of IgG and IgM antibodies with disease activity, the PTB patients were further grouped on the basis of sputum culture examination. The levels of IgG were significantly increased in both AFB positive (2651 mg/dl, t = 0.1%) as well as negative patients (2653 mg/dl, t = 0.1%) compared to healthy controls (Fig. 10). The levels of IgM did not differ significantly in the two groups of patients from that of healthy controls.

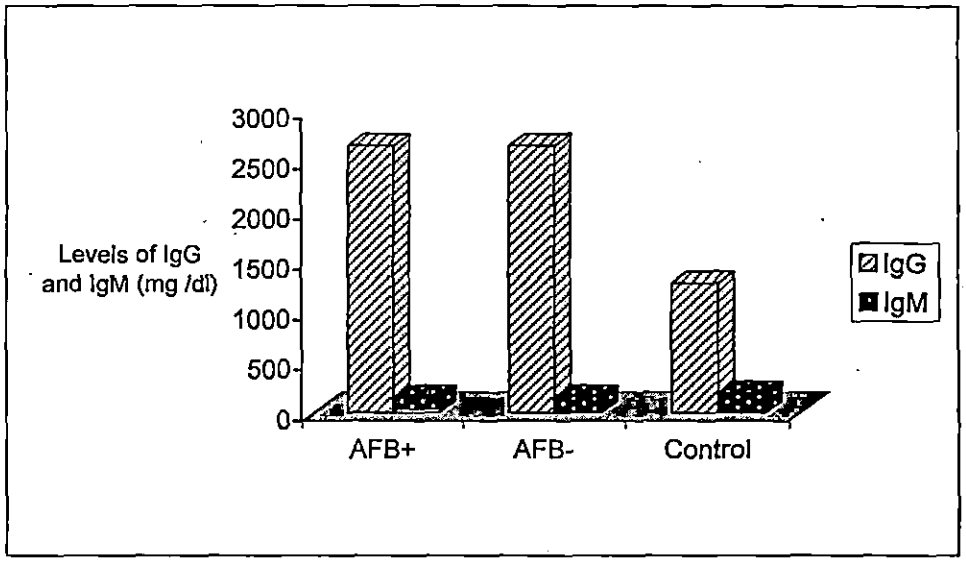


Fig 10 The level of IgG and IgM antibody in patients with pulmonary tuberculosis and controls in relation to sputum examinations.

4.3.4 IgG and IgM antibodies and clinical manifestation

The levels of antibody correlated with the extent of pulmonary lesions based on chest roentogram examination of the patients (Table10). The level of IgG was increased with increasing extent of the disease as judged clinically and radiologically. The increase was significant in all three groups (UL, BL and BE) as compared to healthy controls. Patients with bilateral extensive disease had higher levels than those with other groups but the IgM level did not differ significantly among the three groups.

Table 10 Antibody levels in PTB patients with various chest X-ray lesions

Chest X-ray lesions	IgG (mg/dl)	IgM (mg/dl)
Healthy controls	1288.6	190.52
Unilateral limited	1614	161.73
Bilateral limited	1803.1	126.58
Bilateral extensive	2653	144.5

4.3.5 IgG and IgM antibodies and antituberculosis chemotherapy

Concentration of IgG was significantly increased in both the drug responder (1658.8 mg/dl, t = 0.1%) as well as drug failure (1928.8 mg/dl, t = 0.1%) when compared to healthy controls. The increased was more pronounced in the drug failure cases. The significant difference was not observed in the levels of IgM between drug failure and drug responders (Fig.11).

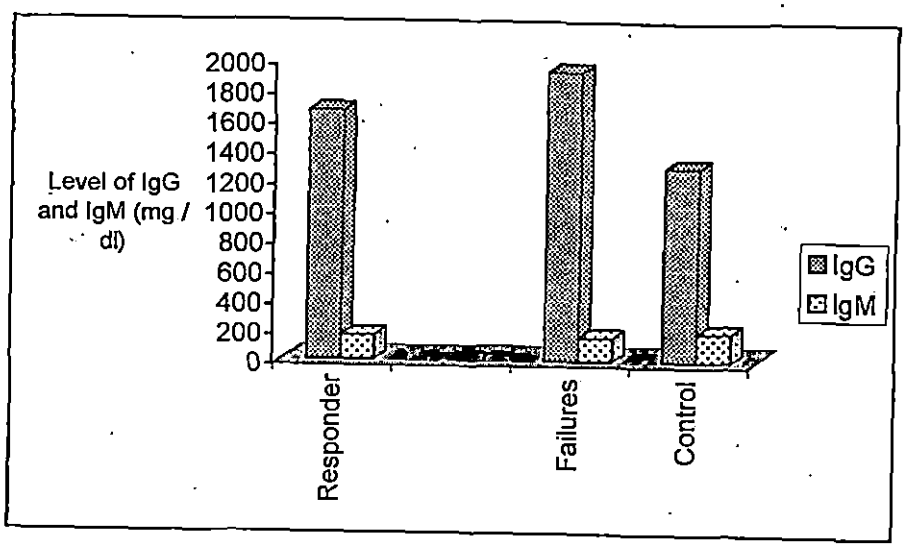


Fig. 11 IgG and IgM antibodies in PTB patients and controls in correlation with antituberculosis chemotherapeutic response.

The impact of chemotherapy on the levels of serum antibody was studied in 10 patients who had all three types of X-ray lesions found in our study. The serum samples were drawn before the start of chemotherapy as well as at 3, 5 and 12 months following chemotherapy (Figs.12 and 13). Among the 6 patients who showed clinical improvement following institution of antituberculosis chemotherapy, the serum IgG levels were significantly decreased from 2703 mg/ dl to 1497 mg/dl ($t = 0.1\%$) after 3 months of therapy. The dynamics of decrease was variable in individual patients. In 2 of them levels of IgG return to normal within three months of treatment both of which were in limited status of X-ray lung lesion. The remaining 4 patients, the levels of IgG dropped to 1831.12 mg/ dl. In remaining 4 patients another serum sample was obtained after 5 months of the start of chemotherapy. Although these patients did not take any specific anti-tuberculosis therapy in the next two months, the levels dropped further and returned to normal level in 3 cases. The remaining patient had a bilateral extensive lung lesion. On the other hand, the 4 drug resistant patients did not reveal any significant alteration in their antibody level after three months of chemotherapy as compared to their levels at the start of treatment. In 1 of the 4 patients studied in this group, the IgG levels were measured again after 12 months of the start of chemotherapy. Again no significant deviation was observed.

Out of the 6 drug responder patients, 5 showed normal IgM level after three months of treatment. In the remaining 1, though initially there was a slight increase but no further change in IgM level was observed in subsequent months. In another 4 patients categorised later as 'drug failure cases', the level of IgM were unchanged even after one year of treatment from the levels at the start of chemotherapy.

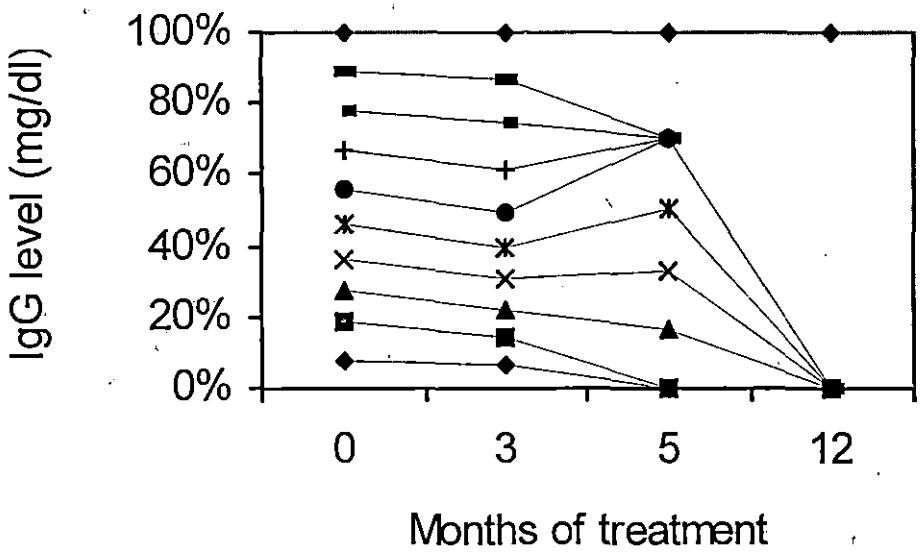


Fig. 12. Levels of IgG antibodies in PTB patients before and after anti-tuberculosis treatment

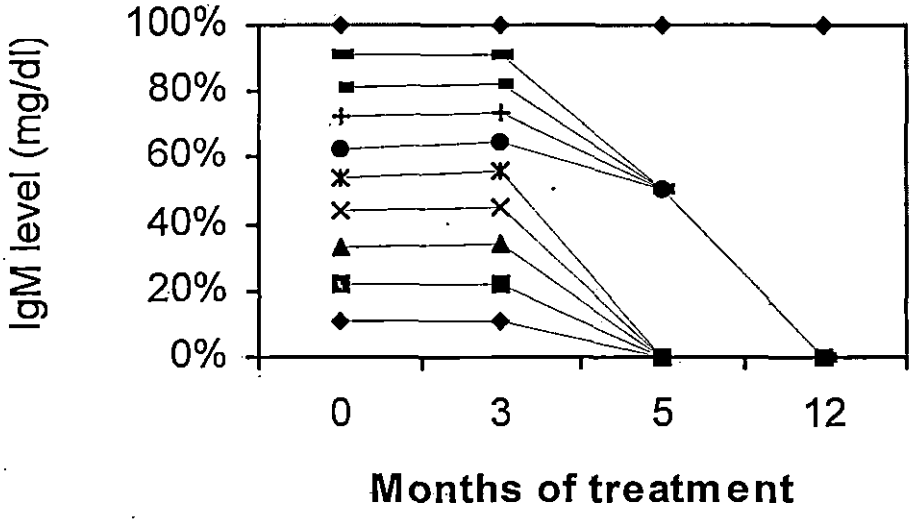


Fig. 13. *Levels of IgM antibodies in PTB patients before and after anti-tuberculosis* treatment

4.3.6 HLA and its role in the levels of IgG and IgM antibodies

Among the HLA class-I and class-II antigens studied, only HLA-DR2 was found to be positively correlated with the levels of IgG antibodies. The data were shown in Table 11. In HLA -DR2 positive patients, a statistically significant increase of IgG levels were observed (2946.6 mg/dl) as compared to DR2 negative patients (1678 mg/dl, t = 0.1%).

Table 11. Association of HLA.-DR2 and IgG and IgM antibodies in patients with pulmonary tuberculosis.

	HLA	Healthy Subjects	PTB patients
IgG (mg/dl)	DR2+	1282	2946.5
	DR2-	1288	1678
IgM (mg/dl)	DR2+	195.5	154.15
	DR2-	194.0	163.8

4.3.7 Discussion

In the present study it has been observed that both the IgG and IgM immunoglobulin levels in case of pleural effusion were higher than the PTB patients. It is well known that in case of the chronic illness both primary and secondary immune responses may be triggered and therefore the levels of both IgM and IgG may be increased. Interestingly we did not get any statistically significant difference of IgG and IgM level between the PTB patients of AFB^+ and AFB^-, indicating that exposure to pathogen rather than the bacterial load may induce the humoral immunity. Therefore, from that point of view possibly

the question does not arise whether the pleural effusive patients were AFB^+ or AFB^-. But indeed it was essential to know what was the ratio of T and B cells in peripheral blood circulation among the pleural effusive and PTB patients. We have observed that in our case the percentage of T cells were migrated to the pleura and therefore the ratio between T and B cells were diminished in the peripheral blood circulation. This has been discussed in detail in Section 4.4 (Cellular Immunity). Therefore it seems that due to encapsulation of the bacteria in the pleura which is having less blood supply may act like a store house of antigens and giving the continuous supply of bacterial antigens slowly by diffusion to the immune system of the patients.

It has also been documented from the present study that the level of IgG was kept on increasing with the increment of the degree of infection which supports the existence of an immune spectrum in PTB, wherein the cell mediated and humoral immunity are mutually exclusive, at opposing ends of the spectrum (Daniel *et al*, 1981). Further support to the hypothesis of immune spectrum comes from the observation of the present study on the increased levels of IgG antibodies in the drug responsive vs drug failure group of patients. The latter are mostly severe cases with bilateral extensive lung lesion and high mycobacterial antibody titer. Antibody levels were declined as DTH responses increase during the course of treatment but it was found that the levels remained unaltered even after three months of antituberculosis chemotherapy, indirectly indicating that the production of the immunoglobulins in tuberculosis is independent of treatment.

Though in case of *M. tuberculosis* infection antibodies have no known protective value and may even antagonise protective immunity (Averbach,1980), the data suggests that these antibodies are related to infection. Similar increased activity of IgG documented by others (Khomenko, *et al*, 1990; Ellner,1996) suggesting that a spurt in the activity of this antibody is a consequence of disease. Non-deviating values of IgM in different groups of patients suggesting an absence of IgM antibody mediated B cell immune deviation in pulmonary tuberculosis.

Following initial studies demonstrating existence of immune response (Ir)

and /or immune suppression (Is) genes in the major histocompatibility complex (Mc Devitt and Chinitz, 1969; Mc Devitt and Benacerraf, 1969), several HLA alleles have been found to be positively associated with antibody production in malaria (Osoba *et al,* 1979), rhesus immunization (Durandy *et al,* 1986), insulin antibody production (Reeves *et al,* 1984) and influenza H3 hemagglutinin response (Durandy *et al,* 1986). It was found that the patients those were having DR2 positive showed higher level of IgG among the PTB patients. The same was also noted by Bothamley *et al* in 1989. As such it is really very difficult to comment clearly what is the relationship between the DR2 antigen and the higher level of IgG but it suggests a possible role of HLA-DR2 or a linked gene in the production of antibodies. However, a detailed study using an *in vitro* system is necessary to make the relationship clear. It is also necessary to characterise the various sub-population of immunoglobulins before examining their likely role as immunoregulatory factors in pulmonary tuberculosis.

4.4 Cellular Immunity

Pulmonary tuberculosis is a disease regulated entirely by the cell-mediated, delayed type immune response of the host against *Mycobacterium tuberculosis*. Inspite of the attention for several years the individual role and mode of action in disease resistance or response is still unknown. T cells with their different subpopulations participate in both cellular and humoral immune response to the disease with a helper or suppressor role. So it is important to study the different cell types, compare them with various markers of disease like roentgenographic lung lesions, AFB status, antituberculous chemotherapy and finding the factors which influence their response to *M.tuberculosis*.

For a quantitative assessment for the extent of sensitization with *M.tuberculosis* an *in vitro* cell culture was performed where peripheral blood mononuclear cells (PBMC) from PTB infected patients and PBMC and pleural fluid from pleural effusive patients were cultured with PHA.

T cells

4.4.1 T cells in PTB, Pleural Effusive patients and Controls

The percentage of T cells were 53% in pleural fluid (PFL) from the tuberculous pleurisy, 51% in peripheral blood from the same patients and 64% in controls. Percentage of the T cells in the PBMC of pulmonary tuberculosis patients were 59.8%, which was higher than the tuberculous pleurisy patients but still lower than the healthy controls (Table 12).

Table 12 Percentage of lymphocytes and blasts in case of pulmonary tuberculosis, pleural effusive patients and controls.

Immunologic Parameters	PTB (%)	Pleural Effusive PBMC (%)	Pleural Effusive PFL(%)	Control (%)
Total Lymphocyte	2.0	2.1	1.3	3.2
T-cell	59.8	51.0	53.0	64.0
B-cell	34.0	39.0	44.0	16.0
T-blast	29.0	29.4	23.0	3.0
B-blast	19.0	19.3	25.0	

4.4.2T cells and X-ray lung lesion

Taking the radiographic extent of the disease as a criteria it has been observed that lower percentage of T cells in bilaterally extensive group (the differentiation has already been defined in previous chapter) of patients (56%). Most nearer to normal were unilateral limited (60%) and bilateral limited groups (59.5%) (Fig.14).

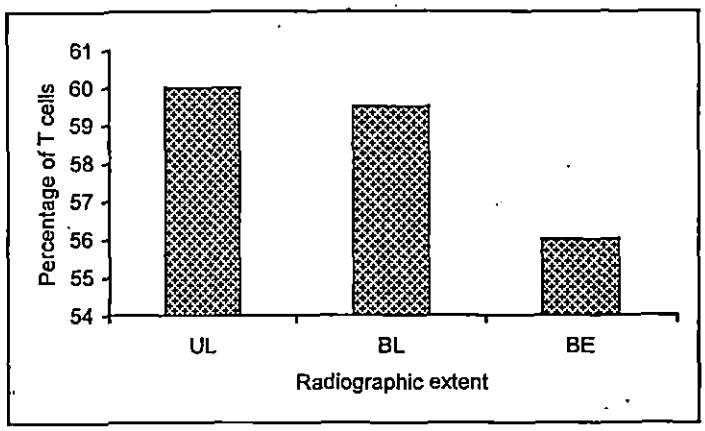


Fig. 14.T cell and the radiographic extent of the disease. UL, BL and BE stands for unilateral limited, bilateral limited and bilateral extensive respectively.

4.4.3 T cell and sputum status

T-cells were found to be lower in AFB positive (57%, AFB+ vs Control; t = 0.1%) and AFB negative (61%, AFB- vs Control; t = 5%) as compared to controls (64%). Again, when two patient groups were compared with each other no statisticallysignificant differences were observed.

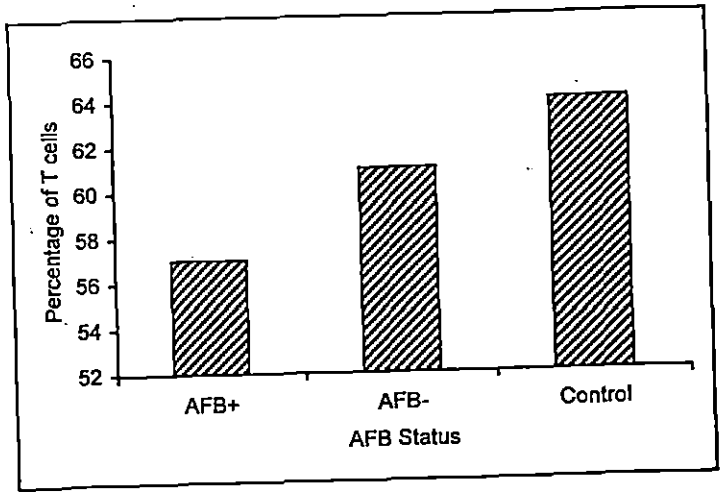


Fig. 15. T cell and the AFBstatus.

4.4.4 T cell and anti-tuberculosis chemotherapy

The T cells were also analyzed based on the response to antituberculosis chemotherapy. As compared to values in healthy controls (64%) the T cells were found to be lower in drug failure group of patients (57%, drug failure vs control; t = 0.1%). In drug responsive groups the number of T cells were 59% (drug responsive vs control, t = 1%) (Fig. 16).

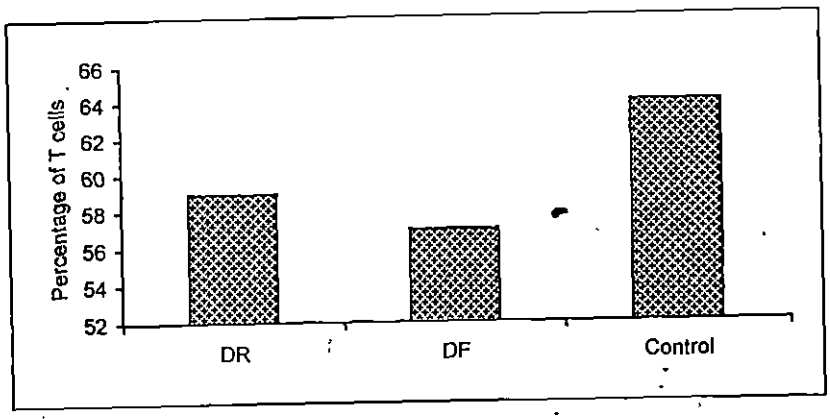


Fig. 16. T cells and anti-tuberculosis chemotherapy. DR and DF stands for drug responder and drug failure respectively.

4.4.5 T cell subsets

Using monoclonal antibodies T lymphocyte subsets were examined in patients with PTB and tuberculous pleurisy before and after *in vitro* culture with PHA (Table 13). Lymphocytes obtained from pleural fluid from patients with tuberculous pleurisy showed 44.1% CD4+ and 18.03% CD8+. The ratio was 2.2:1 whereas from PBMC of the same patients it was 44.8% of CD4+ and 16.1% CD8+. The ratio was 2.7:1. In healthy controls the percentage of CD4+ and CD8+ were 45.05 and 16.05 respectively. The ratio was 2.8:1.

Table 13. Percentage and ratio of CD4 and CD8 positive T cells

Subjects	CD4+ (%)	CD8+ (%)	CD4/CD8
PTB	33.00	16.50	2:1
Pleural Effusive			
PBMC	44.80	16.1	2.7:1
PFL	41.10	18.03	2.2:1
Healthy Control	45.05	16.05	2.8:1

B cells

4.4.6 B cells in PTB, Pleural Effusive patients and Controls

The percentage of B cells was higher in case of all the patient groups than control (16%). Highest percentage was observed in the pleural fluid (44%, vs control, t=0.1%). In the PBMC of pleural effusive and PTB patients the B cells were 39% (vs control, t=0.1%) and 34% (vs control, t = 0.1%) respectively (Table 12)

4.4.7 B cells and X-ray lung lesion

B cell percentage was increased significantly in all three groups (UL =33%, BL=35%, BE=36%) as compared to healthy controls (16%). No statistically significant deviation was observed among these patient groups (Fig.17).

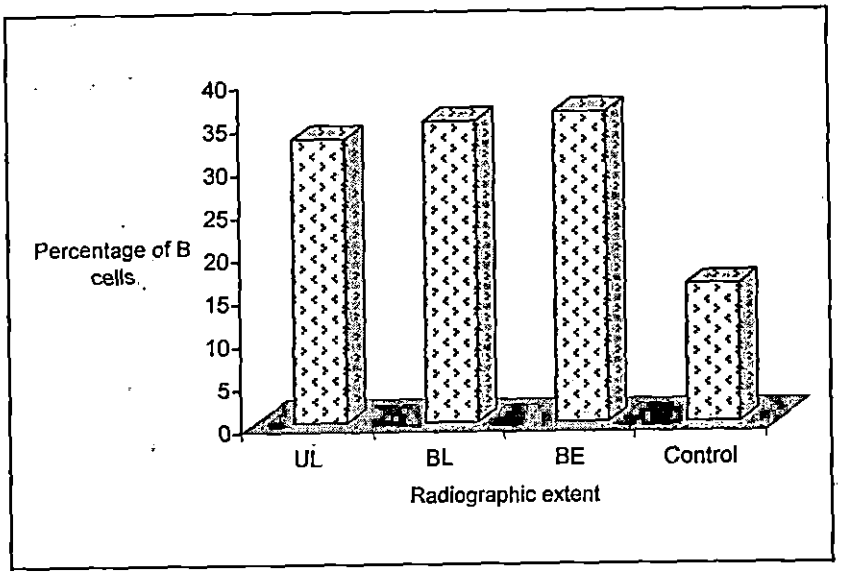


Fig. 17. B cell and the radiographic extent of the disease. UL, BL and BE stands for unilateral limited, bilateral limited and bilateral extensive respectively.

4.4.8 B cell and sputum status

B-cell was found to be increased in both AFB positive (31%, t = 0.1%) and negative (37%, t = 0.1%) as compared to controls. No statistically significant differences were observed when the two patient groups were compared to each other (Fig.18).

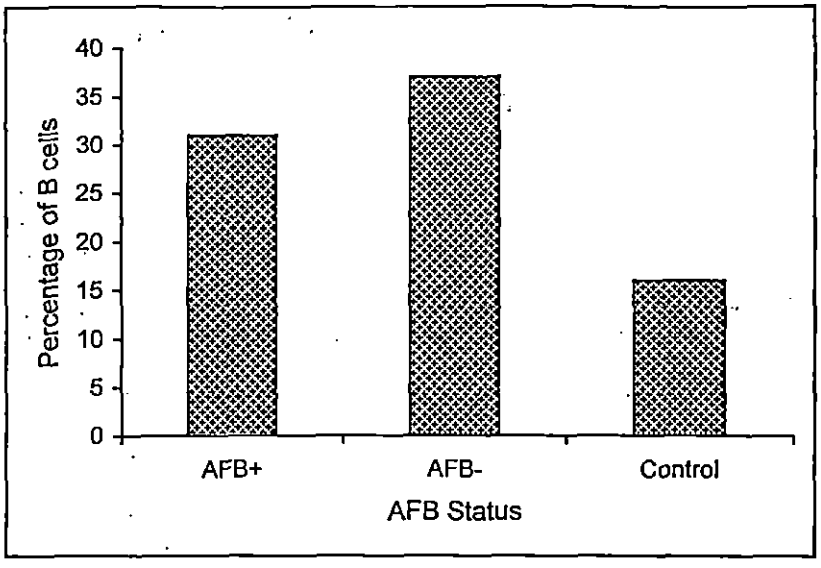


Fig. 18. B cell and the AFB status.

4.4.9 B cell and anti-tuberculosis chemotherapy

The percentage of B cells in PTB patients was also anlysed based on the response to antituberculosis chemotherapy. As compared to values in healthy controls (16%) B cells were found to be significantly increased in both drug responsive (30%, t = 0.1%) as well as drug failure group of patients (38%, t = 0.1%). No significant deviation was found between the groups (Fig.19)

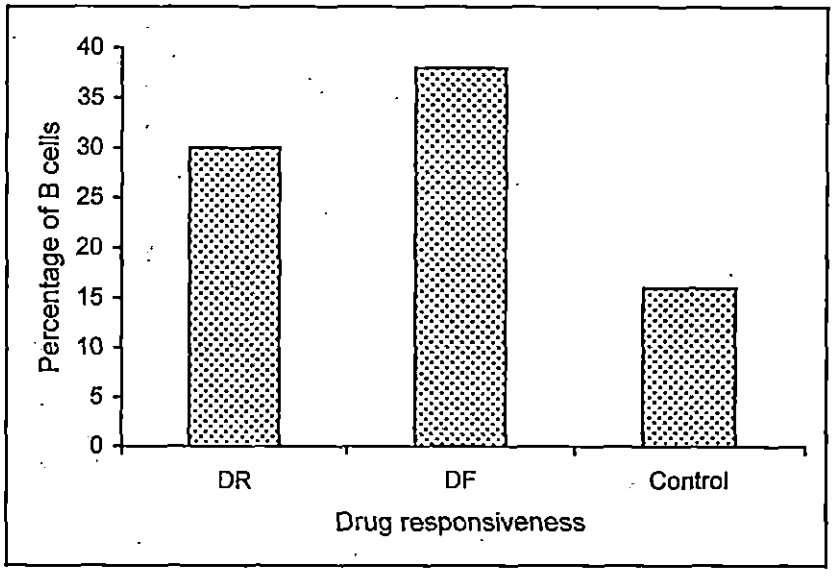


Fig. 19. B cells and anti-tuberculosis chemotherapy. DR and DF stands for drug responder and drug failure respectively.

4.4.10 Blasts

Both T and B blasts were higher in patients when stimulated with PHA (Table 12).

4.4.11 Discussion

The present study reveals that there was an overall diminution in the percentage of T cells in PTB patients than the healthy controls. The result support the view of Tsuyuguchi in case of tuberculosis (Tsuyuguchi, 1996). The reactive T cells were concentrated in PFL compared to peripheral blood in pleurisy patients. The density of T cells in tuberculous PFL could represent selective accumulation or *in situ* expansion of this cell population, thereby causing peripheral depletion. In contrast PBMC of patients with PTB were more responsive than the PBMC of tuberculous pleurisy patients and shows a higher percentage of T cells hence, they have a more chance to be PPD positive.

But when we categorised the PTB patients under standard radiographic criteria, the patients with bilateral extensive disease had lower number of T cells. Advanced TB patients showed low level of IL-2 (Ellner *et al*, 1986). This report corroborates the finding of the present study. This may be a cause for poor differentiation of T cells. Though the PPD positiveness is comparatively higher in pulmonary cases than pleural effusion but there was a report of the trend toward smaller skin test reaction in the group with advanced PTB patients in Karachi, Pakistan (Hussain *et al*,). The lower number of T cells in bilaterally extensive PTB patients in this study may account for this.

The absence of a difference in T cell percentage between AFB positive and AFB negative patients indicates that exposure to pathogen rather than the bacterial load is responsible for the alteration of the number of T cells.

Lymphocytes obtained from PTB patients showed lowest CD4/CD8 ratio compared to either PFL and PBMC of TB pleurisy or PBMC from healthy controls. Mantoux test is always a first attempt to find out whether the patient is infected with *M. tuberculosis* antigen or not. Tuberculin skin test is a T cell mediated phenomenon and it shows the positive reactions when the number of CD4 cells doubles the number of CD8 cells and infiltrate at the site. Patients with tuberculous

pleurisy usually show an impaired tuberculin skin test in most of the cases. The CD4/CD8 ratio was lower in pleural fluid (2.2:1) than the healthy control (2.8:1). This finding recently confirmed by flow cytometric analysis as well (Kleinhenz *et al*, 1987). Result obtained in the present study suggest that there may be different reasons other than CD4/CD8 ratio for the impairment of tuberculin skin test in pleural effusive patients as the ratio was not significantly changed in the cases of PPD negative.

PFL from pleurisy patients were highly responsive to antigens show more activated T lymphocytes. But the investigation with T cell subsets revealed that CD8 cells were more in number than CD4 cells which may have a major role in protective immunity because these CD4 T cells when activated express Ia antigens on their cell surfaces. The existence of suppressor macrophage in low tuberculin response was shown by Ellner *et al*, 1978. The suppressor T cells in addition to suppressor macrophages are responsible for impaired immune response in tuberculosis. In all the three samples (PBMC of PTB and pleural effusive and PFL of pleural effusive) lower number of CD4 cells were obtained which was most in PBMC of PTB patients in comparison to healthy controls. This CD4 deficiency provide nonsurveillance of latent foci of infection (Ellner, 1996) causing the reactivation of tuberculosis which is most common in the area under study, because being a prone area more or less all the people possibly were already infected with the pathogen. CD4 T-cells are known to produce IL-2, GMCSF, IFNγ and TNFα which are the key cytokines for mediating protection. These cytokines activates macrophages to kill intracellular pathogens. As CD4+γ/δ T cells were higher in healthy contacts (Tsuyuguchi, 1996), it seems that these cells might be playing a role in preventing the host from developing PTB following frequent mycobacterial infection. The decrease in CD4+ cell might drive the individual into the disease. So, the present study suggests that activation of CD4+ cells may be a novel approach for the protection of the disease.

The present investigation revealed a high immunoglobulin level which accounts for the higher percentage of B cell. It is possible that certain lymphokines like BCGF, BCDF, TGFB overexpressed during microbial infection and promote differentiation of B cell. The increased activity of (ab')2 γ in patients with PTB (Rajlingam, *et. al*,1996)

which were involved in the immunoregulatory processes and as B cell mitogens (Parker, 1980) also suggests a substantial degree of B cell activation in patients. It has been shown that the ideotypic antibodies react with surface immunoglobulin receptor on B lymphocyte (Birdsall *et al*, 1984) and the elevated activity of heterologous anti-immunoglobulins (LCA) (Rajlingam, 1997) denotes a substantial degree of B cell activation in patients.

The linear increase of B cell percentage from UL group to BE group according to the radiographic extent of the disease, showed a parallel correlation with disease advancement. Though the relevance is not clear, but the stimulus for B cell activation is abundant in patients with bilateral extensive disease.

The absence of significant difference in B cell percentage between AFB positive and AFB negative indicates that the exact mechanism for B cell proliferation lies somewhere else rather than the bacterial load.

Though the B cell percentage is more profound in drug failure patients there is no significant decrease following successful treatment with anti-tuberculosis chemotherapy.