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## TOWARDS A SMOKELESS WORLD

From the increasing successes chalked up in fighting the smoke habit it is becoming obvious that a smokeless world is now a distinct possibility. It would be hard, however, to guess about a time frame for achieving it. In view of the high health risks at stake, the sooner it materializes the better. We need rededication and redoubled efforts on the part of everyone, and not health workers alone, to succeed. Besides, in the Eastern countries, tobacco is also consumed by sniffing and chewing with equal hazards, and attention should not be focussed on smoking alone. Some argue that they live in freedom and should have the choice to do what they want. True, but saving and not destroying life is the essence of living. There could be no justification for knowingly exposing populations to the risks of lung cancer, cardiovascular diseases, chronic bronchitis, etc. just to ensure individual and corporate freedom. If there is still doubt, the well-documented serious negative effects of passive smoking should settle the argument. Non-smokers have their rights too.

In Europe, a six point charter of the rights of non-smokers and a ten point programme of action to fight smoking has been prepared. Strong legislation, total ban on direct and indirect promotion of tobacco products, a 1% levy on tobacco sales for funding health programmes, buying out of tobacco industry's sponsorship of sports and cultural events, heavy taxation on tobacco products, protection given to non-smokers in offices and public places and mandatory health education in schools and colleges are the planks of this programme. A new tobacco administration is underway in WHO which may hopefully lead to a global programme on smoking. The IUATLD has been focusing on surveys and dissemination of information about the pernicious habit in a political perspective.

How prevalent is the habit ? In Czechoslovakia, a 1985 survey among factory workers showed upto 45.4% male and 25.8% female smokers; among the health workers, 34.2% male doctors, 29.2% female doctors and 36.8% nurses were current smokers. In Hungary, smoking is even more prevalent. As regards India, some more relevant information has become available recently. A Punjab survey among male college students in 1989 disclosed a prevalence rate of 8% smokers, almost equal among the urban and rural students. However, if Sikhs who are forbidden to smoke on account of religion are excluded, the prevalence rises to 14%. In 1982, in Himachal Pradesh, 32% of rural males and 16% of rural females were found to be current smokers, compared with 19% and 8% respectively in Himachal towns. Some

studies have however reported prevalence rates of upto 50% in comparatively more selected groups.

In Western countries, notably U.S.A., the smoking habit is declining. In Denmark, the proportion of smokers above 15 years of age has fallen from 57% in 1970 to 45%-more among men-in 1987. There is evidence that at least 5% of confirmed smokers and around 30% of potential victims would heed the advice given to them by their family doctor and give up smoking. Willingness to quit smoking has been measured recently in an European Economic Community survey : 35% of the general population and 36% of medical practitioners were smokers, of whom 69% and 65% respectively were willing to quit smoking.

A few studies have also shown some disturbing trends. Thus, a decrease in smoking among older ages is often not matched among the 20-40 years old, especially women. A rise in the smoking habit is taking place in developing countries, more among the young, rural folks and females. Taking advantage of the situation, some tobacco multinationals are marketing inferior types of cigarettes with high tar content, and often no filters, to such countries.

Very powerful social, economic, and political factors are mixed up with the smoking issue, with the result that tobacco growing countries may allocate over ten times the funds to subsidize tobacco farming and processing than what is budgeted for early detection of lung cancer or health education to discourage smoking. Media either ignores the topic of smoking or, even when advising against smoking, accepts advertisements that glamourize smoking. Banning of tobacco advertising is not even considered seriously by governments due to economic consequences, overlooking the fact that incomes from advertisements are far less than what the nation spends on services to ameliorate the ravages caused by smoking. Besides, families lose out on their earnings due to smoking related sicknesses, which adds to the grand national loss. In Czechoslovakia, the frequency of sickness-absence from work was significantly more and the frequency of visits to doctors higher among smokers compared with non-smokers. On our own television, smoking is not just tolerated but apparently accepted even when messages are sent out against smoking. Some health "spots" show cigarette or "hukka" smoking individuals advising on family planning, or whatever, suggesting that smoking is not just a trendy urban phenomenon but associated with rural wisdom as well.

Compared with what is being done in Western countries, our own anti-tobacco efforts appear incomplete as well as halting. There is no comprehensive legislation against smoking yet, perhaps because of the considerable revenue earned from tobacco sales. Some inconspicuous health warning written in English on cigarette packs, with nothing on bidies or pan masala, etc., is neither here nor there. True, some State governments have taken more stringent steps but elsewhere a few spots on electronic media and a few hoardings are all that is being done. The voluntary National Society on Tobacco and Health, other such agencies in the field of health, Central and State Governments and WHO should put their "heads" together to see what really should be done to make a smokeless World a reality, sooner than later.

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## MANAGEMENT OF CHEMOTHERAPY FAILURES

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**Summary:** A regimen of Kanamycin, Sodium PAS, Ethionamide and Isoniazid for the first 6 months followed by the oral drugs for a total period of 18 months has been investigated in the retreatment of drug-failure patients of pulmonary tuberculosis. All the drugs were given on domiciliary basis.

Out of 64 patients admitted in the study, 26 (41%) only were available for analysis, including four who died and 38 (59%) patients dropped out before completion of 18 months' therapy. At the end of therapy, 19 of 26 patients had a favourable bacteriological response. Drug toxicity with the regimen was not a problem.

### Introduction

Management of patients of pulmonary tuberculosis whose treatment with the commonly used anti-tuberculosis drugs has failed, presents many difficulties. In the treatment of such failures, many reserve regimens have been tried with variable results(1,2,3,4). The EA/BMRC investigation(3) with Streptomycin, PAS and Pyrazinamide in the retreatment of pulmonary tuberculosis reported favourable bacteriological response in 91 per cent of the patients. Tripathy(5) used Kanamycin, Ethionamide and Isoniazid daily for six months followed by the same regimen twice a week for 12 months and observed 89 per cent sputum conversion at the end of 18 months. We decided to investigate the therapeutic efficacy and acceptability of a four drug regimen consisting of PAS, INH, Ethionamide and Kanamycin in the treatment of drug failure patients of pulmonary tuberculosis.

### Material and Methods

Patients of pulmonary tuberculosis having two

positive sputum smears for acid fast bacilli, even after a minimum of six months' regular therapy with Streptomycin, Isoniazid, Ethambutol, Pyrazinamide and Rifampicin, in various combinations, were eligible for inclusion in the study. Patients with concomitant renal or hepatic insufficiency or diabetes mellitus were excluded.

The regimen prescribed for all eligible patients was Sodium PAS 200 mg/kg, Ethionamide 750 mg in two divided doses, given after meals, and Kanamycin in a single intramuscular injection of 1 gm daily for 5 days a week for the first 6 months followed by Ethionamide and PAS for the remaining 12 months. Isoniazid 300 mg daily was given throughout the period of chemotherapy.

The patients were kept on domiciliary therapy and were instructed to come for monthly follow up with an overnight sputum specimen which was examined for acid fast bacilli. Skiagram of chest (PA view) was taken at 0, 3, 6, 9, 12 and 18 months of therapy, or earlier, if required.

Criteria for a favourable response were that sputum smear should convert by six months and remain converted upto 18 months. Death, persistently positive sputum and reversion after conversion, were considered as unfavourable response.

### Observations

In all, 64, patients were included in the study. An attempt was made to find the possible factors contributing to the past treatment failure. It was found that 25 patients had failed because of irregular treatment, 12 due to inappropriate regimens and in 23 patients, both the factors were responsible. In the remaining four patients, no definite reason could be found.

The distribution of the pre-treatment factors (Table 1) showed that 77 per cent of the 64

**Table 1.** *Ages and sex distribution*

Age group	Male	Female	Total
< 20	3	1	4
	20	8	28
31-40	13	4	17
41-50	9	1	10
51- > 51	4	1	5
Total	49	15	64
Mean Age	34.3 ± 7.4		

patients were males and 50 per cent were less than 30 years of age; all the patients complained of cough, 58 had expectoration, 40 gave history of breathlessness, 36 had fever and 10 reported tinged sputum.

The duration of previous treatment was more than 3 years in about 47 per cent (Table 2). All the patients had moderate to far advanced radiographic disease and cavitation was present in 97 per cent of the cases.

**Table 2.** *Duration of previous treatment*

Duration (in years)	Number of patients
1-2	9
2-3	25
3-4	16
> 4	14
Total	64

Only patients receiving at least 80% of prescribed drugs were considered as continuing on treatment. The numbers dropping out or dying at the successive stages are shown in Table 3. At the end of 3rd month, out of 49 patients continuing on therapy, 47 (93%) had negative sputum smear for acid fast bacilli, while at 6th month, 37 out of 40 had negative smear. (Patients dying during treatment have been taken as sputum positive for this purpose). At 12 months, 22 out of 31 patients still on therapy had negative smear, 5 were sputum positive and 4 had died. By the end of therapy (18 months), 26 patients had completed therapy (including 4 who had died)

**Table 3.** *Treatment compliance and sputum status at successive stages*

Duration of therapy (in months)	Continuing treatment*		Death	Non-traceable	
	Total	Sputum converted		Total No.	Sputum converted before drop out
3	49	47	-	15	10
6	39	37	1	24	6
12	27	22	4	33	7
18	22	19	4	38	4

\* Those who received 80 per cent of drugs were considered as continuing treatment.

and 19 out of them had bacteriological conversion. Besides, at the end of 18 months, out of 26 patients available for assessment (including 4 deaths), 15 showed shrinkage in cavity size while cavity was completely obliterated in one patient, cavity size remained stationary in four patients out of which thickness of the cavity wall was reduced in three patients. The cavity size increased in three patients. Persistence of cavity in 18 patients with sputum conversion would tend to suggest open healing of such cavities.

One patient died of respiratory insufficiency between 3rd and 6th months of therapy while other three died between 6 and 12 months of therapy because of toxemia and respiratory failure.

Most of the side effects were observed during the first six months. Nausea and vomiting were major side effects observed in six patients, while 2 patients had giddiness and one had albuminuria (Table 4).

**Table 4.** *Side effects of drugs during treatment*

Side effect	Time Interval (in months)			Total
	0-6	7-12	13-16	
Nausea and vomiting	5		1	6
Dizziness	2			2
Albuminuria	1			1
Swelling over feet		1		1
Total	8	1	1	10

## Discussions

Rapid amelioration of symptoms because of the use of potent anti-tuberculosis drugs and consequent non-compliance in further treatment is quite common in India. This often leads to failure of treatment and poses a challenge to the treating physicians. One has to depend then on less potent and potentially more toxic drugs like Kanamycin, PAS, Ethionamide, Cycloserine, etc. in varying combinations. Their prohibitive cost and non-availability in India further worsen the situation. Besides, it is desirable to use four or more than four drug regimens for such patients(6) for at least two years(7). Non-availability of Capreomycin and Cycloserine in India has compelled us to evaluate the efficacy of an alternative four drug regimen consisting of Kanamycin, PAS, Ethionamide and Isoniazid.

The study has been done on the basis of smear positivity for acid fast bacilli in patients giving history of irregular chemotherapy in the preceding 6 months or more. We could not perform mycobacterial culture and sensitivity testing due to lack of facilities.

Khanna(4) observed two deaths and non-conversion of sputum in two out of 14 resistant pulmonary tuberculosis patients who were hospitalised throughout the 18 months' period and received Kanamycin, Ethionamide, Cycloserine and PAS. In the present study, four patients expired and 38 could not be followed up and sputum was converted in 19 out of 26 patients available for follow up at the end of 18 months of chemotherapy with Kanamycin, INH, PAS and Ethionamide. Three patients continued to excrete tubercle bacilli in their sputum. The results are considered satisfactory as it is very difficult to keep patients admitted in the TB Hospital for such a long period.

Although the ultimate fate of the 38 patients who left treatment prematurely is not known, it may be pointed out that in a majority of them, sputum was converted before they dropped out of the trial (Table 3).

Side effects with the regimen were not a problem and most of these were observed during the first six months. Two of our patients reported vertigo. Vestibular toxicity with Kanamycin has been reported to occur in less than 10 per cent cases and is generally reversible(8). Khanna(4) observed vomiting in seven per cent; EA/BMRC(3) study reported G.I.T. toxicity in

four per cent and in the present study nausea and vomiting were the main complaints observed in six (9%) patients.

In the present study, about 40 per cent of patients continued treatment with a regularity of above 80 per cent throughout the 1½ year study period. Tripathy *et al*(5) reported a treatment compliance of the order of 90 per cent, but in their study the patients were hospitalized during the initial phase of six months, whereas in our trial, the entire treatment was domiciliary.

**To conclude, an oral domiciliary regimen of sodium PAS, Ethionamide and Isoniazid for 18 months supplemented with Kanamycin given for the first 6 months is an effective and safe chemotherapy schedule for drug failure patients who are excreting tubercle bacilli and who have previously received irregular chemotherapy with Streptomycin, Rifampicin, Isoniazid, Ethambutol and Pyrazinamide in different combinations.**

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## A CROSS-SECTIONAL STUDY OF IgG ANTIBODY TITRES TO ANTIGEN PPD-298 OF M. TUBERCULOSIS IN PATIENTS OF PULMONARY TUBERCULOSIS DURING THE COURSE OF TREATMENT

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**Summary:** In this study, 25 healthy Controls, 25 freshly diagnosed and untreated cases of active pulmonary tuberculosis (Group I) and 100 patients of pulmonary tuberculosis on chemotherapy (Group II) at various intervals, i.e., 12-14 weeks (subgroup IIa); 24-28 weeks (subgroup IIb); 48-52 weeks (subgroup IIc) and at completion of treatment (subgroup IId) were studied for IgG (Gamma chain specific) reaction to antigen PPD-298, by ELISA technique, to see the effect, if any, of chemotherapy on antibody titres. All the patients of group II were treated with SHT for the first three months followed by HT for the next one year. From the diagnostic point of view, the test had a sensitivity of 99% and specificity of 84%. The mean absorbance value in various subgroups of Group II were less as compared to group I, the difference being statistically significant only in subgroup IIb. Effective chemotherapy appears to reduce the titer of anti-tubercular antibodies but not in a uniform or predictable manner to enable the test being used to monitor the progress of treatment. All (except one) of the patients had absorbance values (antibody titres) higher than the used cut off titre while most (21 out of 25) of the controls, irrespective of their Mantoux status, had values less than the cut off point.

specific for the diagnosis of tuberculosis. However, quantitatively the results vary much depending upon the antigen used (1,2,3,4,5,6,7). Moreover, ability of the different antigens of Mycobacterium tuberculosis in raising antibody titres in different patients does not appear to be uniform (8,9,10,11) suggesting that all these areas need more careful study. Also, very little work has been done on levels of antibody titres against different antigens during the course of the disease and the chemotherapy.

In the present cross-sectional study, using PPD-298 as antigen, IgG (Gamma chain specific) antibodies were studied by ELISA technique in patients of tuberculosis at different stages of treatment. In our department, we have been concentrating on measuring IgG antibody titre against the same antigen in patients suffering from tuberculosis of different organs in a number of studies.

### Material and Methods

The following groups of subjects were studied:  
*Group-I* This group consisted of 25 freshly diagnosed, untreated patients with active pulmonary tuberculosis. In each case sputum was positive for AFB, Serum samples were taken before the patients were put on anti-tubercular treatment.

*Group-II* This group consisted of 100 patients who were sputum positive at the time of diagnosis and had been taking anti-tubercular treatment for varying durations. The patients in this group were subgrouped, as follows:

*Subgroup IIa* : 25 patients who had taken treatment for 12-14 weeks.

### Introduction

Recently, many workers have explored the use of the enzyme linked immunosorbent assay (ELISA) technique in the serodiagnosis of tuberculosis (see entire list of references). In general, the technique is quite sensitive as well as

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*Subgroup IIb* : 25 patients who had taken treatment for 24-28 weeks.

*Subgroup IIc* : 25 patients who had taken treatment for 48-52 weeks.

*Subgroup IId* : 25 patients who had completed their treatment.

All the patients were taking streptomycin, isoniazid and thioacetazone (SHT) daily, in standard doses, for the first three months followed by isoniazid and thioacetazone (HT) daily for one year (total duration of treatment being 15 months). Only such patients were included in group II who had shown sputum conversion from positive to negative for AFB and were also showing radiological and clinical improvement.

Another 25 healthy individuals from both the sexes taken at random served as controls.

All the subjects were investigated for anti-tubercular antibody titre by ELISA technique according to the principle of Engvall and Perlmann, using protein purified derivative 298 (heat killed, freeze dried mycobacteria) at the concentration of 10µg/ml obtained from Tuberculin Section, Central Veterinary Laboratory, Surrey, England).

24-wells U shaped polystyrene plates (Abbott Laboratories, U.S.A.) were used. Each well was coated with 100 ul of the antigen (1 µg) and incubated at 37° for 3 hours and the plates were covered with parafilm and stored at 4°C till used. Before use each plate was washed thrice with PBST (0.05M Phosphate buffer, P<sub>H</sub> 7.4, in normal saline with 0.5% Tween-20). After the plate was dried, 100 ul of the serum sample (1 : 100 dil, in PBST) was added to each well and the plate was incubated at 37°C for 45 minutes. The last row was used as the blank. The plate was again washed thrice with PBST and dried. 100 ul of the enzyme conjugate (Peroxidase conjugated rabbit immunoglobulin to human IgG, gamma-chain specific, obtained from Sigma Chemical Co, U.S.A. dil 1 : 1000 in PBST) was added to each well and the plate was incubated at 37°C for 45 min. After three washings with PBST, the plate was dried and 200µl of O-Phenylone diamine (40 mg/100 ml containing 200 ul of 3% H<sub>2</sub>O<sub>2</sub>) was added to each well. After incubation at room

temperature in dark for 15 min, 600 /d of IN H<sub>2</sub>SO<sub>4</sub> was added and the intensity of the colour was read at 492 mu in UV-VIS double beam spectrophotometer (Cecil, U.K.).

The reproducibility of the test was checked by using the same sera and same antigen on different days in repeat tests.

## Results

The mean absorbance values (antibody titre) for the control subjects, cases suffering from active disease (Group I) and cases at different periods of chemotherapy (Group II) are given in Table 1. Scattergram of the absorbance values of different groups is given in Fig. 1.

The mean absorbance values in active cases and cases on chemotherapy were significantly higher than the controls (P\* < .001 in each case).

The mean absorbance values in the various subgroups of group II were less as compared to group I, the difference, however, being statistically significant only in group lib (P\* < .002).

As shown in the scattergram, in the control group, the absorbance values of all except four cases were less than 0.08. All (except one) patients in the groups I and II had absorbance values more than 0.08.

## Discussion

Numerous antigens of Mycobacterium tuberculosis can lead to production of different types of antibodies in the patients(12). Effectivity of these different antigens in causing increase in

**Table 1.** Showing mean ELISA values in terms of absorbance of the final colour in different groups

Group	Mean ELISA value	Standard deviation	Standard error
Group I	0.284	0.127	± 0.025
Group Ha	0.253	0.178	±0.36
Group IIb	0.209	0.054	± 0.011
Group IIc	0.217	0.108	± 0.022
Group IId	0.254	0.078	± 0.016
Healthy controls	0.056	0.065	± 0.013

\*The statistical analysis was done using Student's t-test.

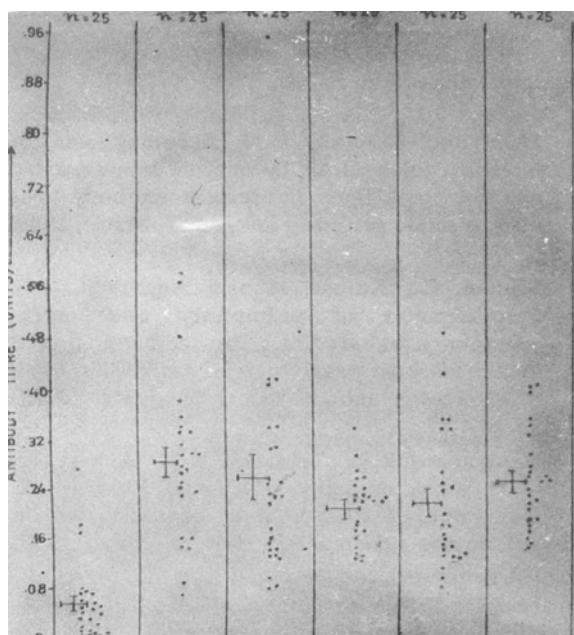


Fig. 1. Antibody titre in sera of control and experimental groups.

specific antibody titres, however, greatly varies from antigen to antigen and also from patients to patient for the same antigen.

In the present study, using PPD-298 as antigen, the cut off value of 0.08 absorbance was used to confirm the diagnosis of pulmonary tuberculosis. The same value had been used in all previous studies from our department. The sensitivity of serodiagnosis by ELISA in the present study using this cut off value was 99.2% and specificity 84%. However, sensitivity of serodiagnosis may not be 100% as Grange *et al*(13) have claimed that about 20% of tuberculosis patients may not have significantly elevated levels of antibodies to a number of antigens. The specificity of 84% implies the presence of cross reacting antibodies in certain persons not suffering from active tuberculosis. Although Grange(14) has studied this phenomenon in detail, further work is still required to put serodiagnosis of tuberculosis on a sound basis.

An equally important question relates to antibody titres to different antigens during the evolution of the disease as well as during the course of specific treatment. Only a few studies with different results are available on this aspect.

Kaplan *et al*(15) detected circulating

antibodies before and after treatment by microimmune diffusion test. They found that treatment not only led to a significant increase in the antibody titres but resulted in the appearance of antibodies in the sera of some who were antibody negative before the start of treatment. This serum conversion took 6-8 weeks whereas patients with pre-existing antibodies showed a rapid synthesis of new antibodies. These findings have been explained on the release of antigenic glycoproteins following death of mycobacterial cells. Similar results have been reported by Toussaint *et al*(12), Janicki *et al*(16) and Cole *et al*(17)

Kiran *et al*(18) using a modified SAFA (Soluble Antigen Fluorescent Antibody) test found a significant reduction in antibody titre after one year of treatment. Saline extract of mycobacterium H<sub>37</sub>R<sub>a</sub> was used as antigen. The decrease in antibody titre was explained on the basis of release of antigenic glycoproteins following death of mycobacterial cells. Singh *et al*(5) measured serum IgG levels by ELISA technique, using a specific protein fraction obtained from H<sub>37</sub>R<sub>v</sub> Mycobacterium tuberculosis as antigen and found a significant fall of antibody titre after four weeks of treatment. This decrease was explained on the basis of antigens released into the circulation by the killed mycobacteria binding with the antibodies and resulting in decreased antibody titre.

Daniel *et al*(19) using ELISA technique determined antibody (IgG type) titres to antigen-5 and purified protein derivative at monthly intervals following initiation of chemotherapy and found that the titres remained essentially stable till 16 months.

In the present study, titres of anti-tubercular antibodies were less in the treated groups IIa, IIb, IIc and IId (difference reaching significant level only in group IIb) compared to the untreated patients of group I. The results appear to indicate that effective chemotherapy tends to reduce the titres of anti-tubercular antibodies. It may not, however, be a uniform or predictable phenomenon since many factors leading to the enhancement as well as suppression of antibody formation appear to be operative during the treatment of tuberculosis (See references above).

The significant finding of the present study, however, was that in all the patients of groups IIa,

I<sub>1</sub>b, H<sub>e</sub> and I<sub>1</sub>d, the absorbance values were higher than the cut off value of 0.08 whereas in the case of most of the controls (21 out of 25), the values were less than 0.08. Keeping in view that most of the control group persons must have been exposed to infection with Mycobacterium tuberculosis (Mantoux test positive in 12 out of 21), **two important conclusions may be drawn :**

**(i) That antibody titres may be different in patients who simply get exposed to infection (without developing the disease) and those who develop the disease.**

**(ii) After exposure to tubercular antigens it takes a lot of time before the antibody titre reverts to the normal range.**

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## WESTERN BLOT ANALYSIS OF IMMUNE RESPONSE IN PULMONARY TUBERCULOSIS

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(Original received 16-11-1989; Received after revision 26-4-1990; Accepted 31-7-1990)

**Summary:** mycobacterial Saline Extract (MSE) was subjected to Sodium Dodecyl Sulfate-Poly Acrylamide Gel Electrophoresis (SDS-PAGE). The seven antigenic fractions obtained were Transblotted on to Nitrocellulose paper. These Fractions were then reacted against sera obtained from 20 bacteriologically confirmed pulmonary tuberculosis patients, 17 healthy adults and 10 neonates.

All the samples from pulmonary Tuberculosis cases reacted with 68-,45-,35-,22- and 20 kDa antigenic fractions but not with 18,4- or 14.3 kDa fractions. Ten of the 17 samples from healthy adults reacted with only the 20kDa protein while two other also reacted with the 68 kDa antigen. Six cord-blood samples also reacted with the 20 kDa protein.

This pilot study indicates that it is possible to identify patients suffering from tuberculosis by studying the immune response to MSE antigenic fragments.

### Introduction

The diagnosis of tuberculosis is based on the demonstration of mycobacteria in smear and/or isolation by culture. That is possible when bacteria are present in numbers detectable by these techniques, often in late stages of the disease. Satisfactory samples are often difficult to obtain, especially in extrapulmonary disease. Added to these constraints is the slow growth rate of the mycobacteria(1) with consequent delay in diagnosis.

Serological identification of disease is widely used in medicine and the problems encountered in making a bacteriological diagnosis in tuberculosis lead to the early application of such techniques(2). Numerous assay systems have been described, but the early encouraging results have frequently given way to frustration due to unacceptable occurrence of non-specific

reactions. The reasons for non-specificity have been elucidated by Stanford and Grange(3). They demonstrated four groups of soluble antigens in ultrasonically disintegrated preparations of mycobacteria : Group (i) antigens were reported to be common to all mycobacteria; Group (ii) antigens were found in slow growing species alone while Group (iii) antigens occurred in rapidly growing species only. Each species, however, was shown to contain antigens of its own-the Group (iv) species specific antigens.

Six antigenic preparations obtained from *M. tuberculosis* H<sub>37</sub>R<sub>a</sub> were evaluated for serodiagnosis(4). The Mycobacterial Saline Extract (MSE) antigen was found to be the most suitable for use in the Soluble Antigen Fluorescent Antibody (SAFA) test. Similar results were reported(5) for the Enzyme Linked Immunosorbent Assay (ELISA). It was, however, felt that the specificity of the test could be increased if the MSE antigen could be characterised even further and the immunodominant epitopes identified.

In the recent past, a powerful tool in molecular genetics has been the blotting technique of Southern(6). Its adaptation for protein in the "Western Blot" method combines the separation power of Sodium Dodecyl Sulphate Poly Acrylamide Gel Electrophoresis (SDS-PAGE) with the specificity of immunological recognition with antibodies(7).

We undertook this pilot study to analyse the humoral response in pulmonary tuberculosis patients and to determine whether the immune recognition differed from that in healthy individuals and neonates.

### Material and Methods

*Study Group comprised :*

**Pulmonary tuberculosis cases :** Serum samples were collected from twenty

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bacteriologically proven cases of pulmonary tuberculosis before the initiation of therapy.

**Healthy Individuals :** Serum samples were collected from seventeen age and sex matched volunteers.

**Neonates :** Umbilical cord blood was collected from ten newborns.

**Mycobacterial Saline Extract (MSE) :** This was prepared as previously reported(S). Briefly, *M. tuberculosis* H<sub>37</sub>R<sub>a</sub> obtained from Research Institute for Microbial Disease, Osaka, Japan was grown on Sauton's medium as surface pellicle. Seven weeks old growth was killed by phenol treatment and the cells were recovered. The cells were then suspended in 0.15 M saline for 72 hours at 37°C. The supernate was precipitated with 70% Ammonium Sulphate and extensively dialysed. Protein concentration was adjusted to 4 mg protein/ml.

**Sodium Dodecyl Sulphate Poly Acrylamide Gel Electrophoresis (SDS-PAGE) :** MSE was subjected to SDS-PAGE separation using 10% acrylamide for resolution(9). The molecular weights of the 6 major and one minor bands were determined using SDS 70 (Sigma Chemicals Co.) molecular weight marker proteins(10).

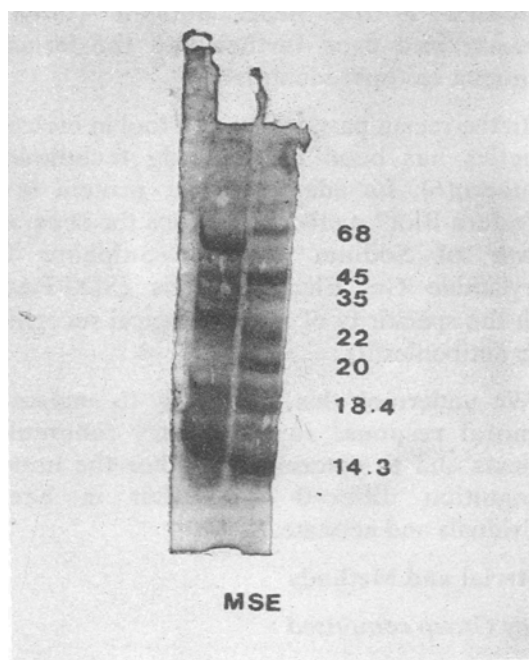


Fig L SDS-PAGE Profile of Mycobacterial Saline Extract (MSE) Molecular weight markers (SDS 70, Sigma) in left lane, MSE in right lane.

**Transfer of proteins :** These protein bands were transferred to nitrocellulose paper (NC) (Microdevices, Ambala, 0.45 um pore size) by electrophoresis in a locally fabricated transblot apparatus.

(a) **Evidence of efficient transfer of proteins :** After the transfer the gel was stained with Coomassie Blue for protein bands, the absence of bands in the gel provided evidence of efficient transfer.

(b) A strip of NC paper onto which one lane of SDS-PAGE separated proteins were transferred was stained with Amido Black to provide positive evidence of transfer of bands.

**Western Blot Test:** The NC paper was cut in strips of 12 cm X 0.75 cm and represented each lane of the SDS-PAGE. For blocking of the unsaturated sites, the NC paper was placed in a plastic plate which has slots for the strips. Each strip was immersed in 3% gelatine in Phosphate Buffer Saline (PBS) pH 7.2 in order to block all the unsaturated sites on the NC paper strip. For observing the **reaction with test serum**, after two hours' incubation at room temperature (RT) in the gelatine PBS, 20 ul of serum sample was added to each slot already containing 2ml of Gelatine PBS, thus diluting the sample to 1 in 100. The strips were then incubated in a shaking platform at RT for 30 minutes. The diluted sample was then sucked off from the slot and the strips were thoroughly washed with PBS. After three washings, the strips were blotted dry. Two millilitre of optimum dilution (1 in 5000) of Protein A horse radish peroxidase conjugated (Sigma Chemical Co) was then added to the slots and the strips were incubated at RT for a further 30 minutes. The conjugate was then sucked off and the strips were washed with PBS. After three washings the strips were again blotted dry. For colour development, two millilitre of freshly prepared 4 Cholo 1 Naphthol (Sigma Chemical Co) and Hydrogen peroxide was added to each slot and incubated for 15 minutes in the dark at RT. The reaction was stopped by pouring off the substrate followed by thorough washing of the strips in distilled water. A positive result was indicated by the development of blue lines on the NC paper strip.

## Results

All the twenty serum samples collected from

patients with bacteriologically confirmed pulmonary tuberculosis reacted with 68-, 45-, 35-, 22- and 20 kDa protein antigenic fractions of MSE. Six cord blood samples reacted with the 20 kDa protein and no other while four did not react with any of the antigenic fractions of MSE. Ten of the 17 samples obtained from healthy volunteers also reacted with the 20 kDa antigen while two reacted with the 68 kDa antigen as well.

### Discussion

Culture filtrates of mycobacteria are complex mixtures containing many antigens. There has been considerable interest in the purification of individual mycobacterial antigens so as to improve the specificity of the serum test(11,12). Purified by immunoabsorbant affinity chromatography, an antigen called "antigen 5" was used for serodiagnosis of tuberculosis by ELISA on the assumption that it was a species-specific antigen. However, after nearly a decade of work with this antigen, Daniel and Debanne(13) reported that "antigen 5" was not as species-specific as was originally believed and that this antigen contained both non-specific as well as specific epitopes.

The Western Blot analysis of the immune response in pulmonary tuberculosis, in our study, indicates that while there is a specific profile of reactions, healthy individuals also react with the 20 as well as 68 kDa antigens. Previous reports have indicated that the 68 kDa antigen is a group-

specific protein that is present in many mycobacterial species(14,15). Recently a 65 kDa protein has been reported which is a heat shock protein cross reacting with not only immunodominant antigens of other mycobacteria but also with *E.coli* and *Pseudomonas aeruginosa* antigens(16).

Six of the ten cord-blood samples reacted with a 20 kDa protein. This antigen, therefore, would also appear to be a cross-reacting antigen but further analysis is required to delineate its presence in other bacteria. A 46 kDa protein dimer consisting of two similar 23 kDa subunits has been reported to be secreted by BCG strains(17). Monoclonal antibodies raised against a 28 kDa protein have been shown to have a broad cross-reactivity on a panel of 12 species and strains of mycobacteria while monoclonal antibodies to 35 kDa protein react with *M. tuberculosis*, *M. bovis* BCG and *M. africanum* only(18,19).

In our study, pulmonary tuberculosis cases have been taken as the positive controls because the number of bacteriologically confirmed cases of extrapulmonary tuberculosis before initiation of chemotherapy are very few. Similarly, patients having atypical mycobacterial disease should have been included but we did not have any such subjects.

The Western Blot pattern obtained from the confirmed cases of pulmonary tuberculosis differed significantly from that obtained from healthy adults as well as neonates. A number of the samples reacted positively to one or more antigenic fractions of MSE as they expectedly did react to the MSE used in the ELISA test(20).

Coates *et al*(21) reacted serum pools obtained from patients with active pulmonary tuberculosis as well as apparently healthy adults against the SDS-PAGE fractions obtained from Seibert's antigens of *M. tuberculosis* and reported that patients reacted to antigens of A protein as well as polysaccharide II fractions while no antibody appeared to bind to any of the multiple silver staining bands observed in the B protein fraction. No antibody binding was observed in polysaccharide I antigens. With serum pool from normal individuals, weak antibody activity was seen in the 32 kDa antigen of A protein and the polysaccharide II fractions which ranged from 6 to 30 kDa.

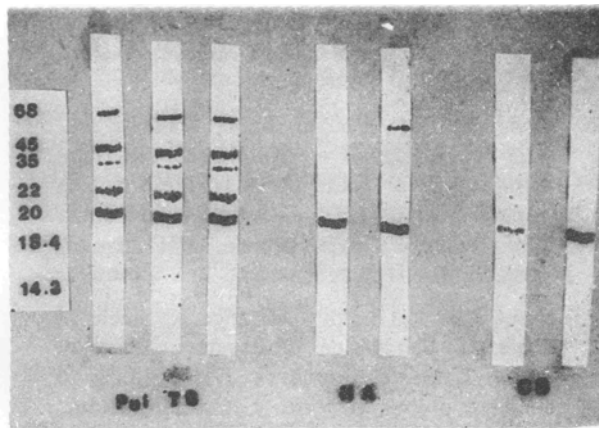


Fig. 2. Western Blot pattern obtained by reacting sera obtained from pulmonary tuberculosis (Pul TB), healthy adults (HA) and umbilical cord blood (CB) against MSE fractions.

Espita *et al*(2) subjected an alcohol concentrated filtrate of H<sub>37</sub>R<sub>a</sub> to SDS-PAGE and obtained 35 Coomassie blue stained bands ranging from 122 to 14 kDa. Forty five of the 47 sera obtained from pulmonary tuberculosis patients reacted with a total of 25 bands. Thirty of the 33 control sera reacted with as many as 14 bands though with considerably lesser intensity. They reported that a 31-33 kDa doublet band was found to react with 90% of pulmonary tuberculosis and 55.8% of normal sera. The 60 kDa protein was recognised by 82% of tubercular and 64.7% of normal sera but the 38 kDa band was unique in that it reacted only with 53% of sera obtained from patients with tuberculosis but with none of the healthy control sera.

**The analysis of serum antibody profile by Western Blot has suggested that the immune response of an individual is focused on particular antigens and that it is generally possible to distinguish discrete bands on Western Blotting indicating preferential recognition of a limited set of proteins (23). Our observations are in agreement with this view. The usefulness or otherwise of this test would, of course, lie in its ability to correctly pick up cases of extrapulmonary tuberculosis.**

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## A CLINICAL EVALUATION OF FINE NEEDLE ASPIRATION CYTOLOGY IN THE DIAGNOSIS OF LYMPHADENOPATHY

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**summary :** A total of 61 consecutive cases of lymphadenopathy were subjected to Fine Needle Aspiration cytology (FNAC) as well as concurrent surgical biopsy. Tubercular lymphnodes constitute the largest groups (39%). The overall sensitivity and specificity of FNAC using histopathological results as standard (for all lymphadenopathy) were 86.7% and 98.1% respectively. The sensitivity and specificity for tubercular lymphadenopathy by FNAC were 79.1% and 94.0% respectively. In 41.6% of the patients with tubercular lymphadenopathy, the aspirates were positive for Acid Fast Bacilli (AFB)

The aim of this study was to compare the results of FNAC with that of histopathology to judge the value of FNAC as a reliable and rapid method for the diagnosis of lymphadenopathies in general.

### Material and Methods

A total of 61 consecutive patients with lymphadenopathy presenting at the medicine OPD of MGIMS, Sevagram constituted the clinical material for this study. All the patients were subjected to FNA as well as immediate surgical biopsy. The technique of FNAC has been described by Engzell *et al*(2). All the slides were stained with the Papanicolaou staining method. Patients were diagnosed as having tubercular lymphadenopathy on FNAC when the aspirate had epithelioid cells with either caseation necrosis or AFB present. Culture for AFB and typing were not done due to lack of facilities and routine search for AFB was done both in FNA and concurrent slides.

Sensitivity and specificity of cytological diagnosis by means of FNA were calculated by comparison with the histopathological diagnosis.

### Results

Of the 61 cases subjected to FNAC, histological diagnosis revealed 24 tubercular glands, 9 reactive nodes, 19 secondaries in lymphnodes and 9 lymphomas.

Table 1 shows the sensitivity and specificity of FNAC diagnosis. A definitive diagnosis of tuberculosis could be confirmed by AFB positivity in FNA material, in 41.6%. Out of the 24 tubercular cases, aspirate was cheesy in 17, purulent in 4, and mixed with blood in three. The purulent type of aspirates showed 75% positivity

### Introduction

Fine Needle Aspiration technique was described for the first time by Greig and Gray in 1904. Since the mid-1960's, it has been increasingly used and a high degree of accuracy has been achieved(1). Since this technique lends itself to outpatient diagnosis, it is eminently suited for use in peripheral medical centres. It is safe, nontraumatic, repeatable and inexpensive. With the recent advances in ultrasound and CT Scan technologies, focal lesions can be aspirated using these procedures.

Fine needle aspiration cytology (FNAC) has been used extensively for the diagnosis of primary and secondary malignant disorders involving lymphnodes, though the same does not hold true for non-neoplastic disorders. In malignant conditions of lymph nodes, FNAC enjoys a high sensitivity and specificity, the average being 95%(1,2,3,4,5). Comparatively fewer workers have used FNAC for diagnosing tubercular lymphadenitis(6,7,8,9,10).

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**Table 1.** Sensitivity and specificity of FNAC diagnosis

Histopathological diagnosis	No. of cases	Cytological diagnosis				
		Tuberculosis	Reactive cells	Secondaries	Lymphoma	Doubtful
Tuberculosis	249	19* 2	1	18	4	
Reactive hyperplasia	199		7		19	
Secondaries						
Lymphomas						
Total	61	21	8	18	9	5
-						
Suspected Tubercular						
Sensitivity (%)		79.1	77.0	94.7	100.0	
Specificity (%)		94.5	98.1	100.0	100.0	

\* Includes all AFB positive aspirates besides cytological results.

for AFB. While correlating the cytomorphologic features of the aspirate in relation to the presence of AFB, the organisms were mainly found in aspirates with necrotic material (Table 2).

### Discussion

In the present series, sensitivity of FNAC in the various pathologies of lymphnodes ranged from 77% to 100%. Tubercular lymphadenopathy constituted the largest number and we could confirm presence of AFB in 41.6% of these patients. Other authors have reported similar results : Rajwanshi, *et al*(6) reported 40.6%, Metre, *et al*(7) found AFB in 66% and Baily, *et al*(8) in 47.4% of their cases.

**Table 2.** Cytomorphologic features of aspirates correlated with presence of AFB in tubercular lymphadenopathy

Cytomorphologic feature	No. of cases	AFB present in
Epithelioid cells and caseation necrosis	8	3
Epithelioid and giant cells with caseation necrosis	8	4
Necrosis with polymorphs	2	-
Epithelioid cells only	2	-
Total	24	10

Cytomorphologic features of the aspirate are important for the detection of AFB in the tubercular lymphnodes. As was the observation of Rajwanshi, *et al*(6) we too found AFB mainly in smears containing necrotic material. This emphasises the need for AFB staining of all suspected tuberculous aspirates. Even if the aspirate suggests purulent lymphadenitis, the chances of finding AFB is maximum in such lesions and in the present study, 75% of the purulent aspirates were positive for AFB. Metre, *et al*(7) had reported 66% positivity for AFB in tubercular lymphnodes with purulent aspirates. FNAC does pose problems in diagnosing lymphomas(1,3) but in our series the sensitivity and specificity with regard to lymphomas was 100%, while that for secondaries were 94.7% and 100% respectively. These findings are similar to the results of Engzell, *et al*(2), Gupta, *et al*(4) and Ross, *et al*(3).

A diagnostic test is considered satisfactory if its sensitivity and specificity are around 90%. **We have found FNAC a satisfactory tool in the diagnosis of tubercular and malignant lymphadenopathy. Though the sensitivity in diagnosing reactive and tubercular glands was less than 90%, the high specificity (98% and 94.5% respectively) is regarded as an asset during bed-side evaluation in detecting pathological glands. The simplicity and cheapness of the procedure make it most suitable for use on outpatients basis even in peripheral hospitals and dispensaries.**

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## HYPERURICEMIC EFFECT OF ETHAMBUTOL AND PYRAZINAMIDE ADMINISTERED CONCOMITANTLY

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(Original received 2-1-1990; Received after revision 24-7-1990; Accepted 24-9-1990)

**Summary :** A prospective study involving 83 cases of pulmonary tuberculosis was conducted to observe the hyperuricemic effect of ethambutol and pyrazinamide administered concomitantly. There were three groups of patients : group E, (34 patients ) received regimens containing ethambutol (15 to 25mg / kg body weight / day ) but no pyrazinamide; group 'Z' (22patients) received regimens containing pyrazinamide (30 to 40mg/ kg bodyweight / day ) but no Ethambutol and group 'ZE' (27 patients) received regimens containing ethambutol and pyrazinamide administered concomitantly. A rise in serum uric acid level was observed in all the three groups. The hyperuricemic was higher in the 'ZE' group 91.34% compared to 'E' group (51.6%) but not much higher compared to 'Z' group (73.68%). Arthralgia occurred in 17.39% subjects of 'ZE' group, 15.79% of 'Z' group and 3.22% of 'E' group. Arthralgia was not severe enough to warrant the termination of the therapy. None developed arthritis. Hyperuricemia and Arthralgia responded well to low dosage of salicylates.

### Introduction

Hyperuricemia may manifest as articular pain or swelling or it may be asymptomatic. Many factors may lead to hyperuricemia. Impaired excretion of uric acid occurs in more than 75% of the subjects and excessive production of uric acid may be the cause in 20-25% of cases(1). It is well known that lactic acid, ergotamine, atropine, posterior pituitary extract, diuretics and alcohol decrease the excretion of uric acid(2). Ethambutol and Pyrazinamide are known to cause hyperuricemia and gouty arthritis(3,4,5,6,7). In this study we observed the

hyperuricemic effect of Ethambutol and Pyrazinamide administered concomitantly.

### Material and Methods

A total of 83 patients admitted to Kasturba Chest Hospital were inducted in this study. After discharge, 20 patients were also followed up in the out-patients department.

Untreated cases of pulmonary tuberculosis and those who had not received Ethambutol and Pyrazinamide during the preceding 6 months were eligible for the study. Patients with no history of joint pain, normal liver and renal function tests, and serum uric acid level less than 6.5 mg/100 ml were then selected. These patients were put on various regimens of anti-tuberculosis drugs keeping in view the previous therapy taken by them. The patients were then divided into the following three groups.

1. 'E' Group : (34 cases) Patients received regimens containing Ethambutol (15 to 20 mg/kg/day), but no Pyrazinamide *i.e.* INH + Ethambutol; Streptomycin + INH + Ethambutol; and Streptomycin + INH + Ethambutol 4- Rifampicin.

2. 'Z' Group : (22 cases) Patients received regimens containing Pyrazinamide (30 to 40 mg/kg/day) but no Ethambutol *i.e.* INH + Rifampicin + Pyrazinamide; and Streptomycin + INH + Pyrazinamide.

3. 'ZE' Group : (27 cases) Patients received regimens containing both Ethambutol and Pyrazinamide *i.e.* INH + Ethambutol + Pyrazinamide; Streptomycin + INH + Rifampicin + Ethambutol + Pyrazinamide; and INH + Rifampicin + Ethambutol + Pyrazinamide.

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For reasons beyond our control, allocation of patients to the three groups could not be made by a random process.

The patients were put on a vegetarian diet in the hospital and were asked to take vegetarian diet at home during the follow-up after discharge. Administration of ergotamine, atropine, posterior pituitary extract, diuretic and alcohol were forbidden.

Due to certain reasons 10 patients (3 each of 'E' and 'Z' group and 4 of \*ZE' group) could not be followed up. After excluding the 10 cases, 73 patients were left for analysis.

Blood was collected before the institution of therapy and serum uric acid was measured by arsenophos photungstate method(8). Serum creatinin level was measured by photometric method. After institution of therapy, serum uric acid and serum creatinine were measured at weekly interval for the next two months. Patients with serum uric acid level of more than 6.5 mg/ 100 ml were considered hyperuricemic. The normal serum creatinine level was taken as less than 1.5 mg/100 ml.

## Results

Table 1 shows that hyperuricemia was more common in 'ZE' group : 21 of 23 subjects developed hyperuricemia (91.34%). In 'Z' group, out of 19 subjects 14 developed hyperuricemia (73.68%); while in 'E' group hyperuricemia occurred only in 16 out of 31 subjects (51.61%).

Table 2 shows that out of 23 subjects of 'ZE' group, hyperuricemia developed in four after first week, in 20 after second week and in 21 after

**Table 1.** Distribution of cases with hyperuricemia during treatment

Group	No. of subjects	No. of cases with hyperuricemia	Percentage with hyperuricemia
E	31	16	51.61
Z	19	14	73.40
ZE	23	21	91.34

three weeks of therapy, while out of 19 subjects of 'Z' group, hyperuricemia developed in five after one week, in 13 after second week and in 14 after three weeks of therapy. And, out of 31 subjects of 'E' group, there was no hyperuricemia after first week of therapy but 6 subjects developed hyperuricemia after two weeks and 13 patients after three weeks of therapy.

Table 3 shows that among patients who developed hyperuricemia, the mean serum uric acid level rose from 4.55 mg/100 ml (pretreatment level) to 9.07 mg/100 ml after 9 weeks of Pyrazinamide plus Ethambutol therapy; to 8.63 mg/100 ml from 4.39 mg% level after 9 week of Pyrazinamide therapy and to 7.72 mgm% from 4.25 mg% level after 9 weeks of Ethambutol therapy.

The observed mean change in serum uric acid (difference between pretreatment and maximum serum uric acid level) was higher in 'ZE' (4.61 mg/100 ml) and 'Z' groups (3.82 mg/100 ml) compared with 'E' group (2.21 mg/100 ml).

## Side-Effects

Arthralgia occurred in a total of eight cases

**Table 2.** Frequency of hyperuricemia in relation to duration of therapy

Group	No. of subjects and (%)	Frequency of hyperuricemia according to duration of therapy			
		After 1st week	After 2nd week	After 3rd week	From 4th week onwards
E	31	-	6 (19.95)	13 (40.93)	16 (51.61)
Z	19	5 (26.31)	13 (68.42)	14 (73.60)	14 (73.68)
ZE	23	4 (17.39)	20 (86.95)	21 (91.34)	21 (91.34)

## HYPERURICEMIC EFFECT OF ETHAMBUTOL & PYRAZINAMIDE ADMINISTERED CONCOMITANTLY

**Table 3.** Mean level of serum uric acid among hyperuricemic patients according to duration of therapy

Duration of Therapy	Serum uric acid concentration mg/100 ml		
	E (16 pts.)	Z (14 pts.)	ZE (21 pts.)
Pretreatment	4.25	4.39	4.55
After 1st wk.	5.12	6.51	5.44
After 2nd wk	5.94	6.54	6.99
After 3rd wk.	6.49	7.49	7.40
After 4th wk.	6.57	7.93	7.51
After 5th wk.	6.76	8.02	8.64
After 6th wk.	7.25	8.19	8.65
After 7th wk.	7.25	8.36	8.81
After 8th wk.	7.27	8.62	8.81
After 9th wk.	7.72	8.63	9.07

(11.0%) : four in ZE group (17.4%), three in 'Z' group (15.8%) and only one in 'E' group (3.2%). Arthralgia was not severe enough to warrant termination of therapy. None developed arthritis. Of the eight cases, three were given salicylates in doses of 600 mg three times a day, one patient phenylbutazone 400 mg stat followed by 100 mg six hourly with good response.

### Discussion

It should be stressed at the outset that since patients could not be allocated to the various groups at random, the conclusions drawn from the present study cannot be generalised. The discussion is applicable only to the material under study.

In our study, 51 of 73 patients (69.9%) developed hyperuricemia. Hyperuricemia was observed in 73.7% subjects of 'Z' group. Hyperuricemia in 100% of the subjects treated with pyrazinamide has been reported(9,10,11). One of the causes of the difference between our study and other studies could be racial difference. However, hyperuricemia in only 33.3% of subjects put on a Pyrazinamide and Rifampicin regimen has been reported(6). Hyperuricemia in 'E' group of our study was 51.6% compared with 42% to 66% reported by other workers(4,7,12) among Ethambutol treated patients. As many as

21 out of 23 (91.3%) patients belonging to 'ZE' group developed hyperuricemia compared with 48.4% by others(6).

Most of the patients (68.4%) in our 'Z' group who developed hyperuricemia did so after the second week of therapy, and all such cases had developed hyperuricemia after 3 weeks of Pyrazinamide therapy. Thereafter, their serum uric acid continued to rise till the conclusion of the study. Hyperuricemia during Pyrazinamide therapy has been reported from within one week to within 2 months of starting Pyrazinamide therapy. Similarly, 40.9% of 'E' group patients who developed hyperuricemia did so after third week of therapy, and 51.6% of such patients had developed hyperuricemia after more than 4 weeks of Ethambutol therapy. Thereafter, serum uric acid continued to rise till the end of the study. On the other hand, 87.0% subjects of 'ZE' group who developed hyperuricemia did so after second week of therapy while 91.3% cases had it after 3 weeks of Pyrazinamide and Ethambutol therapy. Thereafter, serum uric acid continued to rise in these subjects till the end of study. It was noted that the onset of hyperuricemia in 'Z' and 'ZE' groups was earlier and the observed rate of increase was more rapid compared to 'E' group of case.

At the end of the study, the observed mean level of serum uric acid was higher in 'ZE' and 'Z' groups than in 'E' group.

In our study, only a small number of patients developed arthralgia more in 'ZE' and 'Z' groups. The patients with arthralgia were treated with small doses of salicylates without termination of therapy. A similar response to salicylates was observed by others as well(6,10).

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## DIAGNOSIS OF TUBERCULAR CERVICAL LYMPHADENITIS BY FNAC, MICROSCOPY AND CULTURE

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*Summary* : fine needle aspiration cytology (FNAC) is a simple out-patient diagnostic procedure used for the diagnosis of tubercular lymphadenitis. The efficacy of FNAC as compared to biopsy has already been established, particularly in the diagnosis of tubercular lymphadenitis. In this study, FNAC was complemented with smear examination and culture for AFB and it was observed that 54/100 cases were diagnosed as tubercular lymphadenitis by FNAC alone but when combined with culture of AFB, 60 out of 100 cases were found to be of tubercular etiology. Thus, FNAC, when combined with microscopy and culture, improved the diagnostic efficacy.

### Introduction

Tubercular lymphadenitis is a common manifestation of extrapulmonary tuberculosis. Fine needle aspiration cytology (FNAC) being a simple outpatient diagnostic procedure is well accepted by patients and has practically no complications(1). The efficacy of FNAC as a diagnostic procedure is already established and it has been found to be as efficient as biopsy, particularly in cases of tubercular lymphadenitis(2). In the present study, FNAC along with smear examination and culture for acid fast bacilli (AFB) has been undertaken for the diagnosis of tubercular lymphadenitis in suspected cases of chronic cervical lymphadenitis.

### Material and Methods

One hundred cases of chronic lymphadenitis of more than 4 weeks duration and clinically diagnosed as tubercular lymphadenitis were included in the study.

At the outset, X-ray chest and Mantoux test

were done. Patients of all groups were included in the study.

### Procedure

I. FAMC-The lymph nodes were subjected to fine needle aspiration, and aspirates were stained by Giemsa stain for cytology and by Ziehl Neelsen's method for acid fast bacilli.

The criteria for diagnosis of tuberculosis were taken as:

(a) presence of epithelioid cell granuloma with or without necrosis.

(b) presence of AFB in necrotic smears stained positive for AFB by Ziehl Neelsen's stain.

II. *Culture*-The cultures were put up on Lowenstein and Jensen's media and examined every week for presence of growth, upto 8 weeks(3). The positive cultures were examined for their rate of growth, type of growth, pigment production and were subjected to biochemical tests for species identification(4).

### Results

The patients' age varied from 1 year to 60 years. There were 52 males and 48 females. The X-ray chest revealed parenchymatous lesions in 14% cases. There was evidence of active pulmonary tuberculosis in only 2 cases. The results of the Mantoux test and cytopathological diagnosis are shown in Table 1. On the basis of cytopathology, cervical lymphadenitis could be divided into two groups, tubercular (54) and non tubercular (46). The Mantoux test was +ve in 24 tubercular and 8 non tubercular cases. Among the 24 tubercular cases 10 were children below 5 years. Thus, Mantoux test was +ve in 10 out of total 15 children below five years. This shows that Mantoux test has significance in the diagnosis of tuberculosis in children below 5 years.

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**Table 1.** Results of Mantoux test and types of lymphadenitis by FNAC

Mantoux test (with 5 TU/read after 72 hours)	Cytopathological diagnosis	
	Tubercular lymphadenitis	Non-tubercular lymphadenitis
Positive (> 10mm)	24	8
Negative	30	38
Total (100)	54	46

Tubercle bacilli were demonstrated by smear and/or culture in the two etiological groups, as shown in Table 2. According to Table 2, six cases diagnosed as non-tubercular lymphadenitis by cytopathology were AFB culture +ve. Thus, a total of 60 out of 100 cases clinically diagnosed as tubercular lymphadenitis were found to be of tubercular etiology by FNAC, microscopy and culture together.

All the positive cultures were identified as described in Table 3. The cultures were identified as *M. Tuberculosis* (30), *M. Kansassii* (1), *M. Scrofulaceum* (3) and *M. Fortuitum* (4).

### Discussion

Although histopathology is most rewarding for diagnosis of cervical lymphadenitis, its feasibility is limited due to lack of facilities and non-acceptability, being an invasive procedure(S). Previously, biopsy was used for diagnosis of tubercular lymphadenitis; now it has been greatly replaced by FNAC. The correlation of cytopathology and histopathology has already been discussed by several workers(6,7,8,9). In literature, so far, there are only a few reports

**Table 2.** Demonstration of tubercle bacilli by smear and/or culture

Smear/culture for AFB	Cytopathological diagnosis	
	Tubercular group (54 cases)	Non-tubercular group (46 cases)
Smear +ve alone	2	
Culture +ve alone	14	6
Smear and culture+ve	18	
Smear and culture -ve	20	40
Total (100)	54	46

Available for simultaneous FNAC and culture used for the diagnosis of tuberculosis. Purohit *et al* 07(10), have used Mantoux test, skiagram chest, histopathology and culture of mycobacteria as diagnostic parameters. In our study, we have complemented FNAC with microscopy and culture in addition to conventional X-ray chest and Mantoux test. **We have found that FNAC when combined with microscopy and culture improved the diagnostic accuracy, 54 cases out of 100 were diagnosed tubercular by FNAC alone, as against 60 when combined with microscopy and culture.** Out of 38 positive culture, the species were identified as *M. tuberculosis* (30). *M. Kansassii* (1), *M. Scrofulaceum* (3) and *M. Fortuitum* (4). The incidence of atypical mycobacteria in tubercular lymphadenitis has been reported earlier(11,12,13,14). The identification of species of mycobacteria would help to study various biological properties of mycobacteria including drug sensitivity and therapeutic approach.

**Table 3.** Species identification of Mycobacteria

Species	Number	Growth rate	Type of growth and pigment		Niacin test	Nitrate reduction	Catalase 68°C	Aryl sulfate
<i>M. Tuberculosis</i>	30	Slow	R	N	+	+		
<i>M. Kansassii</i>	1	Slow	SR/S	P		+	+	
<i>M. Scrofulaceum</i>	3	Slow	S	S	-	-	+	
<i>M. Fortuitum</i>	4	Rapid	SF/RF	N		+	+	+

R = Rough; S = Smooth; SR = Intermediate in roughness; F = Filamentous extensions;

N = Nonphotochromogenic; P = photochromogenic; S = Scotochromogenic

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## MYELODYSPLASTIC SYNDROME AND HYPERCOAGULABLE STATE WITH TUBERCULOSIS--A CASE REPORT

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(Original received 25-11-1989; Received after revision 19-3-1990; Accepted 15-5-1990)

*Summary* : A 60 year old male presented with pulmonary tuberculosis and moderate anaemia. He was put on antitubercular treatment and haematinics. The anaemia remained refractor to treatment and the patient developed recurrent deep vein thrombosis and pancytopenia, which subsequently evolved into acute myeloid leukemia (AML). The development of AML suggested that the haematological aberrations observed earlier were actually states of myelodysplastic syndrome (MDS). The significance of concurrently developing and MDS is discussed and reported for the first time.

### Case Report

A 60 year old male presented with complaints of cough and expectoration of six months' duration and a history of fever, off and on, malaise, fatigue and breathlessness for two months. He was a non-smoker and non-alcoholic. Physical examination revealed marked pallor. There was no jaundice, lymphadenopathy, purpuric spots or bony tenderness. Chest examination revealed an impaired percussion note in the left infraclavicular region with a slight increase in vocal fremitus and vocal resonance in the same area. Auscultation revealed bilateral vesicular breathing with minimal crepitations in the left upper zone. Examination of cardiovascular and central nervous systems was normal.

The progress of the disease including clinico-radiological and haematological profiles is depicted in the accompanying charts.

Based on the clinical and investigation findings shown in the progress chart, a diagnosis of pulmonary tuberculosis was made and the anaemia was ascribed to tuberculosis. The patient was, therefore, put on anti-tubercular therapy and haematinics. After about two months, while the chest symptoms were slightly relieved, malaise, fatigue, breathlessness persisted. There was an added complaint of painful swelling of both the feet which was diagnosed to be deep venous thrombosis. At this stage, as shown in the progress chart, the anaemia had progressed to pancytopenia. An occasional myeloblast was seen in peripheral blood and bone marrow biopsy revealed a border line increase in the number of myeloblasts. These changes indicated that the refractory nature of anaemia could have been

### Introduction

A variety of haematological alterations ranging from various cytopenias to leukaemoid reaction(1) and even frank leukemia in association with tuberculosis have been reported(2). The development of acute leukaemia from a state of pancytopenia in a case of pulmonary tuberculosis has not been reported so far. While pancytopenia in tuberculosis has been attributed to necrosis, mechanical replacement of bone marrow and products of bacillary metabolism(3). Hyper-sensitivity to tuberculo-proteins has been held responsible for the development of leukaemoid blood picture(4).

We are presenting a case which developed tuberculosis and myelodysplastic syndrome (MDS) almost simultaneously. The MDS subsequently evolved into acute myeloid leukemia (AML) A state of hypercoagulability in the form of recurrent deep vein thrombosis was also observed.

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syndrome (MDS). The deep venous thrombosis, associated with slightly enhanced coagulation activity, observed at this stage responded to low dose aspirin therapy, as shown in the chart.

Six months later, however, the deep venous thrombosis reappeared and the patient was found to have developed full blown features of acute leukemia including the involvement of bones and lymphnodes. The clinico-radiological and bacteriological evidences of active pulmonary disease were still there.

### Discussion

The initial presentation of this case was that of active pulmonary tuberculosis with microcytic hypochromic anaemia as if manifestation of a chronic disorder and a common accompaniment of tuberculosis. The anaemia, however, was not only refractory to treatment but progressed to pancytopenia and finally developed into acute myeloid leukemia while tuberculosis could not be fully controlled even after eight months of treatment.

Retrospectively, it appears that even at the initial presentation, when the haematological profile could not be considered adequate for the diagnosis of MDS, the patient probably had an impaired immune response which caused reactivation of a latent focus of tuberculosis. Subsequently, the anaemia was found to be refractory in nature and the development of pancytopenia with the appearance of myeloblasts in peripheral blood and their increased number in bone marrow suggested MDS. That state developed later into frank leukemia.

The MDS represents a pre-leukemic state in which a clonal abnormality of haemopoietic stem cell is characterised by a variety of phenotypic manifestations with varying degrees of ineffective haemopoiesis(5) and where cells have undergone only a proportion of the changes required for acquisition of the leukaemic phenotype(6). Depending upon the presence of immature cells and blasts in peripheral blood and bone marrow, Bennett *et al*(7) have described 5 types of primary MDS which are:

- (i) Refractory anaemia
- (ii) Refractory anaemia with ring sideroblasts
- (iii) Refractory anaemia with excessive blasts

(iv) Refractory anaemia with excessive blasts in transformation.

(v) Chronic myelomonocytic leukaemia.

The possible relationship between tuberculosis and blood dyscrasias can be :

- (i) Activation and dissemination of a latent tuberculosis focus due to loss of immune mechanism, particularly, cell mediated immunity in bone marrow failure and leukemia,
- (ii) Blood dyscrasias might be an unusual immunologic response to tubercle bacilli(8,9), or :
- (iii) the dyscrasias might be related to anti-tubercular drugs.

The present case, initially, had only refractory anaemia and the blast count in bone marrow was within the normal limits. A variable number of patients with MDS may progress to frank leukemia state depending upon the increase in the percentage of blasts, but the progression of a refractory anaemia with a normal blast count into leukaemia is unlikely(6). The progression of refractory anaemia to the state of leukaemia in the present case might have been accelerated by the tubercular infection. On the other hand, the existence of MDS might not have allowed the anti-tubercular drugs to be effective. Aplastic anaemia and pure red cell aplasia have been reported in patients due to antitubercular drugs(10,11). The anaemia in the present case, which subsequently proved to be refractory, was present even before the anti-tubercular therapy was started.

Pancytopenia and a leukaemoid blood picture simulating acute myeloblastic leukemia have been described in association with disseminated tuberculosis(4). Development of leukaemia in the present case was confirmed and the possibility of a leukaemoid picture was excluded as the patient developed hepatosplenomegaly, lymphadenopathy and punched out skeletal lesions. Further, the myeloblast count in bone marrow increased to 40 percent and there was pathologic evidence of tissue invasion (myeloblasts in lymph node aspiration smears), thus satisfying the criteria described by FAB group(6,12). Development of AML in this case confirmed the pre-existing myelodysplastic state which presented as deep vein thrombosis, refractory anaemia, and pancytopenia.

Clinical Chart	
Clinico-radiologic profile	Haematologic profile
<b>At the time of initial presentation</b>	
<p><b>X-ray chest:</b> Infiltration in left upper zone</p> <div style="border: 1px solid black; padding: 5px; margin-top: 10px;"> <p><b>Treatment: Rifampicin, INH, Ethambutol, folic acid, Pyridoxin and iron</b></p> </div>	<p>&lt;Hb = 6.5 gm. %</p> <p><b>Anaemia :</b> Microcytic hypochromic</p> <p>TLC: 8600/Cumm,</p> <p><b>DLC :</b> P<sub>64</sub>, L<sub>29</sub>, M<sub>5</sub>, E<sub>2</sub>, B<sub>0</sub>,</p> <p><b>Platelets:</b> 357,000/Cumm</p> <p><b>Bone marrow:</b> Normocellular</p> <p><b>M : E Ratio</b> 5 : 1 , normal maturation of myeloid and megakaryocytic series. Inadequate haemoglobinisation in normoblasts and normal iron stores.</p>
<b>At 2 months</b>	
<p><b>X-ray chest:</b> Infiltration in left upper zone</p> <p><b>Sputum :</b> Positive for AFB (++)</p> <p>Painful swelling of both feet (Deep vein thrombosis)</p> <p><b>Sputum culture :</b> Mycobacterium tuberculosis, Klebiella aeroginosa and Staph. aureus</p> <p><b>ESR :</b> 128 mm in 1st hour (Westergren)</p> <div style="border: 1px solid black; padding: 5px; margin-top: 10px;"> <p><b>Treatment: Rifampicin, INH, Ethambutol, B.complex, Blood transfusion and low dose aspirin</b></p> </div>	<p><b>Hb = 5 gm. %,</b></p> <p><b>Anemia :</b> Normocytic, hypochromic</p> <p><b>TLC:</b> 3500/Cumm</p> <p><b>DLC :</b> P<sub>40</sub> L<sub>54</sub> M<sub>4</sub> E<sub>2</sub> B<sub>0</sub> occasional myeloblast.</p> <p><b>Platelets:</b> 76000/Cumm.</p> <p><b>BT = 3', CT = 3'-50",</b></p> <p><b>PTI = 75%, PTTK = 90%,</b> Urine and blood-negative for fibrine degradation products (FDP)</p> <p><b>Bone marrow:</b> Hypercelluler M</p> <p><b>: E Ratio 3 : 1</b></p> <p><b>Myloblasts = 7%,</b> ↓ megakaryocytes, megaloblastoid erythropoiesis, ringed sideroblasts and ↑ iron stores.</p>
<b>In 20 days</b>	
Disappearance of Deep Vein Thrombosis (DVT)	
<b>At 6 months</b>	
<p>Bone pains +, Generalised lymphadenopathy, Hepatosplenomegaly, Painful swelling of both lower limbs (DVT), Skeletal survey—punched out lesions in the skull</p> <p><b>X-ray chest:</b> Infiltration in left upper zone</p> <p><b>Sputum :</b> Positive for AFB (++)</p> <p>Lymph node aspiration cytology : 30% blasts, positive</p>	<p><b>Hb = 4 gm. %,</b></p> <p><b>Anaemia:</b> Normocytic, Hypochromic,</p> <p><b>TLC:</b> 3800/Cmm,</p> <p><b>DLC :</b> P<sub>38</sub>, L<sub>51</sub>, M<sub>5</sub>, E<sub>1</sub> B<sub>0</sub>, myeloblast 5.</p> <p><b>Platelets:</b> 60000/Cumm,</p> <p><b>BT = 10', CT = 10'-20",</b></p> <p><b>PTI = 60%, PTTK = 55%,</b> Urine and blood negative for FOD,</p> <p><b>Bone marrow:</b> Hypercelluler,</p> <p>Myeloblasts = 40%, ↓ megakaryocytes, megaloblastoid erythropoiesis, ↑ iron stores.</p>

To the best of our knowledge, pancytopenia evolving into frank leukaemia in association with tuberculosis has not been reported earlier, though, it is possible that cases of pancytopenia resulting in fatality, described in the earlier literature(13), might have been instances of myelodysplasia and death might have been caused by complications prior to the development of leukaemia. Furthermore, recurrent deep vein thrombosis due to hypercoagulable state or chronic DIG could be another manifestation of myelodysplastic state.

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## PULMONARY TUBERCULOSIS MIMICKING METASTATIC NODULES

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*Summary* : A case of pulmonary tuberculosis presenting as multiple nodules with fuzzy margins on chest roentgenogram is presented. Routine examination of blood, urine, sputum, Mantoux test, chest X-ray and bronchoscopy could not help in reaching the diagnosis. Computed Tomography (ct-scan) and guided Fine Needle Aspiration Cytology (FNAC) helped in making diagnosis of pulmonary tuberculosis.

dl, total leucocyte count 14000/Cu mm differential leucocyte count P<sub>70</sub> L<sub>20</sub> M<sub>8</sub> E<sub>2</sub>, ESR 20 mm 1st hour and urine routine and microscopic examination, normal.

Sputum examined by smear and culture (three specimens) : negative for *M. tuberculosis*.

Sputum for malignant cells (three specimens) : all negative.

Mantoux test with I TU of PPD : zero mm at 72 hours.

Blood sugar fasting : 100 mg/dl, post prandial 144 mg/dl.

Blood examined for rheumatoid factor, antinuclear factor and L.E. cells was negative.

Plain X-ray chest PA view (Fig. 1) showed bilateral nodular opacities with fuzzy margins in mid and lower lung zones, without any pleural reaction.

Ultrasound of Abdomen : normal liver, spleen, kidneys, gall bladder, pancreas. No lymphadenopathy.

Bronchoscopy: normal findings.

- (a) Bronchial aspirate and brushings for AFB : smear and culture negative.
- (b) Bronchial aspirate for malignant cells : negative.
- (c) Transbronchial biopsy necrotic tissue with macrophages : No epitheloid cells or giant cells were seen.

C.T. scan of thorax (Fig. 2) showed multiple nodular opacities in lung parenchyma without any mediastinal node enlargement.

C.T. scan of abdomen and pelvis : normal liver, spleen, gall bladder, pancreas, kidneys, bladder and uterus. No pelvic mass was seen.

C.T. guided fine needle aspiration from lung

### Introduction

Pulmonary tuberculosis presenting as multiple nodular opacities is very infrequent(4). Also, a patient with increasing exertional dyspnoea and multiple nodular opacities precludes pulmonary tuberculosis since exertional dyspnoea is not an early feature of pulmonary tuberculosis.

### Case Report

A fifty two year old post-menopausal female was admitted with complaints of cough with white, scanty mucoid sputum for two months, exertional dyspnoea for one month, low grade continuous fever with intermittent chills for one month and dull aching pain in the left chest anteriorly for one month. She had lost four kg of weight in the last one months. There was no history of haemoptysis, wheeze, dysphagia or altered voice. There were no bowel, bladder or menstrual complaints.

Examination revealed tachycardia and tachypnoea. There was no clubbing, icterus or glandular enlargement. Chest examination showed occasional fine inspiratory crackles at the bases. Both breasts were normal. Other systemic examinations did not reveal any abnormality.

On investigation, haemoglobin was 10.9 gm/

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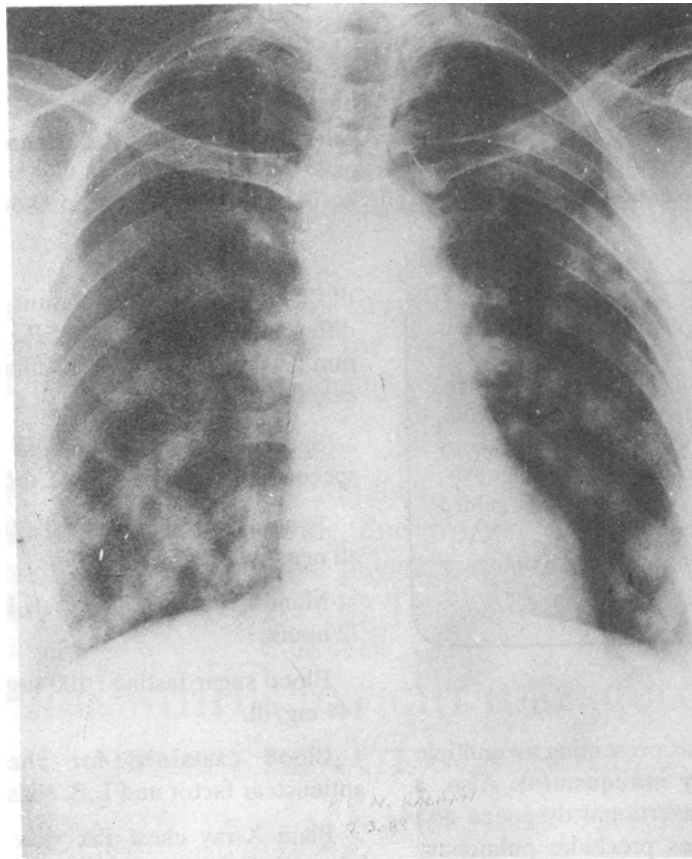


Fig. 1. Plain X-ray Chest PA view at initial presentation.

Fig. 2. CT scan of thorax showing multiple nodular opacities.

nodule revealed granuloma showing histiocytes, multinucleated giant cells of both foreign body and Langhan's type. Anthracotic macrophages were also seen. No AFB were seen on smear examination.

The patient was treated with isoniazid, rifampicin, ethambutol and pyrazinamide. The patient responded to treatment well and chest skiagram after 12 weeks of treatment showed marked clearance of opacities (Fig. 3).

### Discussion

The differential diagnosis of multiple nodular opacities in elderly patients includes metastatic tumors, infectious granulomas (tubercular, pyogenic or fungal), non-infectious granulomas (sarcoidosis, rheumatoid nodules, Wegner's disease, lymphomatoid granulomatosis) alveolar cell carcinoma, lymphoma, multiple primary neoplasms, benign tumours, septic emboli and arterio-venous malformations(1).

A thorough physical examination often helps in detecting unsuspected primary neoplasm. Roentgenological characteristics of nodules may help sometimes. Fuzzy margins of nodules suggest septic emboli, alveolar cell carcinoma and non-infectious granuloma. Infectious granulomas often calcify, sometimes with a diagnostic laminar (onion skin) pattern. Afferent and efferent vessels are often detectable in cases of arterio-venous fistulae. Sometimes, culture and cytologic studies of sputum are diagnostic. If nodules are densely packed, trans-bronchial lung biopsy is occasionally helpful.

C.T. scan may reveal the diagnostic information about the nodule, mediastinum and often the primary site also(2). CT guided fine needle aspiration biopsy is often diagnostic, particularly with malignant lesions(4).

In the present case, metastatic malignant disease was considered to be the first possibility followed by infectious granuloma and non-

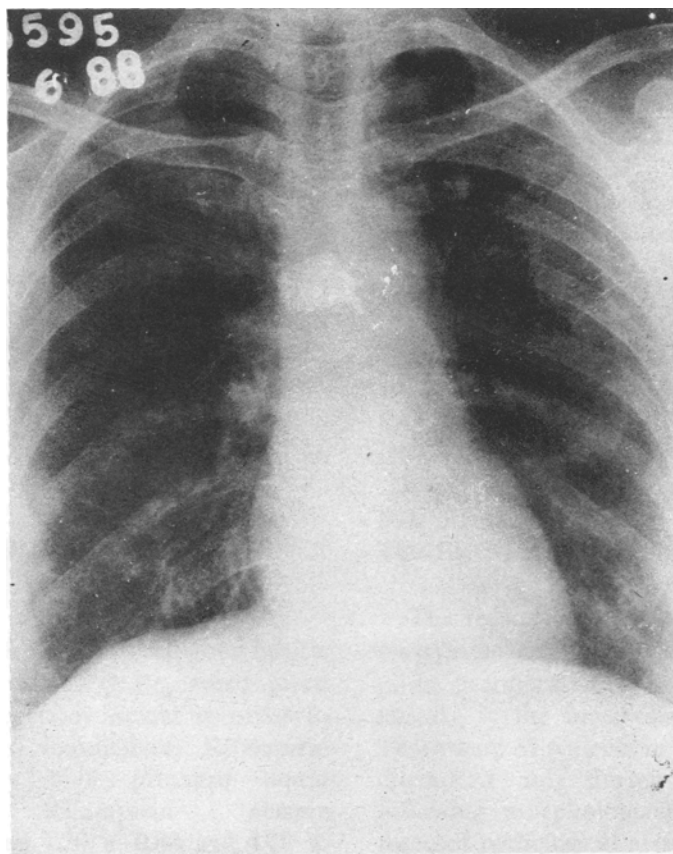


Fig. 3. Plain X-ray Chest PA view after Treatment.

infectious granuloma. Pulmonary tuberculosis presenting as multiple nodular opacities is very infrequently observed(3). However, as is well known, tuberculosis can mimic any lung condition and it should always be considered in patients with multiple nodular opacities.

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## ACUTE ISONIAZID POISONING

Maulana M. Ansari\*, Mohd. Hanif Beg\*\* and Shahla Haleem\*\*\*

(Received 6-2-1990; Accepted 15-2-1990)

**Summary :** A case of acute isoniazid poisoning secondary to a simple overdose is presented with excellent outcome following intensive care including pyridoxine therapy.

### Introduction

Isoniazid is widely used in the treatment of all forms of tuberculosis. However, acute isoniazid poisoning is a rare adverse reaction except in the French people(1). Acute isoniazid poisoning is rather uncommon in India too. This case report is presented to emphasize that an overdose may result in frank poisoning with severe clinical manifestations.

### Case Report

A young man of 30 years was being followed up as a case of chronic pyopneumothorax with intercostal tube drainage and anti-tuberculosis therapy (Rifampicin, Isoniazid and Ethambutol). One day, he missed his isoniazid and took a double dose (600 mg) the next day. Within one hour, he developed dizziness, slurring of speech and mild generalised tonicidity of the body. On re-admission to hospital, he was found to be semi-conscious with irrelevant talk; pulse was 130/min., regular; BP-120/70 torr and respiratory rate was 28/min. There was increased tone in all the muscles of the body and pupils were semidilated and sluggish. He vomited twice just after admission. The breathing was acidotic and he became unconscious.

He was put on intravenous (I/v) infusion, frusemide 40 mg I/v, Inj. Neurobion (R) (containing 100 mg of pyridoxine per ml) 2 ampoules I/v B.I.D. and Inj. sodabcarb 50 mg I/v repeated 6 hourly. After 6 hours, he

developed generalised convulsions for which diazepam and phenytoin were given I/v. Lumbar puncture followed by CSF examination (cytology and biochemistry) proved to be normal. Fundoscopy did not reveal papilloedema. Other investigations were Hb, 9.6 gms %; TLC 10500/Cu.mm.; polymorphs 70%; lymphocytes 28%; eosinophils 2%; blood urea 35 mg %; blood sugar (random) 134 mg %; serum albumin 3 gms %; serum globulins 1.6 gms %; serum bilirubin 0.9 mg %; SCOT 54 I.U.; SGPT 50 I.U.; S. alkaline phosphatase 11.5 K.A.; urine analysis and X-ray of chest were normal. It was not practicable to measure levels of INH in blood and urine due to lack of facilities.

Patient remained afebrile and comatose with neck stiffness and diminished deep tendon reflexes associated with occasional episodes of clonic seizures for three days. On the fourth day, he started to show signs of clinical improvement and regained consciousness on the 6th day. He recovered completely in another week's time without any residual deficit. The patient is being followed up regularly for the last 4 months and is on the same anti-tuberculous therapy as before.

### Discussion

After ingestion of high doses (5-6 gms.) of isoniazid, clinical symptoms appear within ½ to 3 hours(1,2,3). Such mode of ingestion is usually suicidal or accidental(4). Manifestations of acute intoxication are acidosis, coma and tonic-clonic seizures in adults and children(2,5).

Kelso and associates(6) emphasized that metabolic acidosis in conditions of acute isoniazid poisoning results in cerebral oedema and coma. Inhibition of activity of an active form of pyridoxine, pyridoxal 5-phosphate, results decreased levels of GABA (Gamma ammo

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butyric acid) which is responsible for seizures(7,4).

The present case is unique in the sense that a mere double dose (600 mg) of the routine isoniazid daily dosage given during anti-tuberculous therapy and equal to the high dose of biweekly regimen resulted in intoxication with severe clinical manifestations. This lends support to the recent case report of Prabakaran(8) regarding a fatal case after the ingestion of only 500 mg of isoniazid. Therefore, it cannot be over-emphasized that all patients on anti-tuberculous therapy should be instructed not to take a double dose of isoniazid if a dose was missed the previous day. Also, to always take 7-10 mg pyridoxine along with high dose of isoniazid in the interrupted regimens.

Recovery from acute isoniazid poisoning is usually complete, without any residual neurologic deficits following intensive care including pyridoxine therapy(1), as was confirmed by the excellent outcome in the present case.

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**ISONIAZID INDUCED ANAPHYLACTIC REACTION****S.K. Gupta\*, R.C. Gupta\*\* and R.R. Agrawal\*\*\***

(Received 24-4-1990; Accepted 25-5-1990)

**Introduction**

Allergic reactions have commonly been reported with Streptomycin, Thioacetazone and Rifampicin(1). These reactions are less often seen with Isoniazid. Recently, we came across a case of Isoniazid induced anaphylactic reaction and are reporting it for its rarity.

**Case-Report**

A 17 year old female suffering from pulmonary tuberculosis was put on treatment with Rifampicin, Isoniazid and Pyrazinamide on 11-9-89. There was no previous history of anti-tubercular treatment nor any episodes of allergic manifestations or drug allergy in the patient or her family. After two months of initial intensive phase therapy, Pyrazinamide was omitted. On 28-11-89 (77th day), she was brought to the hospital in a state of shock. History revealed that one and a half hours after taking the usual morning dose of Rifampicin and Isoniazid, she developed itching all over the body, giddiness, dryness of mouth and shortness of breath. There was no history of taking any other drug. Patient was treated with Inj. adrenaline, antihistaminics and parenteral fluids. She recovered. All the medicines were stopped and the patient was called after a week. She was then given a test dose of 30 mg of Isoniazid (one tenth of the standard dose considering the severity of the reported reaction(2). After an hour, patient developed urticaria and was immediately given treatment for anaphylaxis. Next day, 150 mg of Rifampicin was given as a test dose and the full dose was given on the following day on getting no untoward reaction with the test dose. Subsequently, Ethambutol was

added without any side effects and the treatment was continued with Rifampicin and Ethambutol in the usual doses.

The patient experienced one more attack of anaphylaxis on 8-1-90, when she consumed a tablet of Isoniazid accidentally at night mistaking it for aspirin. She had to be hospitalised for treatment of anaphylaxis once again.

**Discussion**

Hypersensitivity reactions to Isoniazid are uncommon. Reactions like fever, skin eruptions, arthritic symptoms have all been attributed to this drug. Extensive search of literature did not reveal any anaphylactic reactions reported with Isoniazid as seen in our case.

Further, most reactions with Isoniazid occur in the first 4 weeks after start of treatment(3). In the present case the reaction occurred on the 77th day which is rare. Isoniazid is considered safe, least toxic, and most important drug for the treatment of all types of tuberculosis, but it is important to recognise that severe anaphylactic reactions may occur even with the drug considered least offending, as in the case reported.

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## FORUM

Sir,

We read with great interest the article "Tuberculosis of the Breast" by Tiwari *et al* (Ind. J. Tub., 1990,37,149). In this connection our own experience may be of interest to the readers.

We have reviewed 100 histologically proved granulomatous lesions of the breast from 1983 to 1989 with special reference to tuberculosis. We have found that pain, as an early symptom in tuberculosis of breast, is fairly uncommon. It appears quite late when the lump has attained a larger size or the skin has been involved. Many other workers have also described pain as an infrequent and late symptom(1,2). Persistent or recurring abscess, after adequate drainage on previous occasions, is more characteristic of tuberculous mastitis rather than the mere presence of a sinus which could also be seen in non-tuberculous granulomatous mastitis such as plasma cell mastitis, fat necrosis and duct ectasis.

For diagnosis, histomorphological features are the mainstay as AFB demonstration and culture positivity are uncommon. The mere presence of granulomas with or without areas of necrosis are not diagnostic of tuberculosis of breast. Idiopathic granulomatous mastitis (granulomatous lobular mastitis) is a strong contender in differential diagnosis(3). We have noted that the following features are typical of tuberculosis.

- (i) Predominant lobular distribution of granulomas (in all the cases).
- (ii) associated granulomas within the fat (in 90 per cent of the cases).
- (iii) endarteritis and caseation necrosis.

Similar features have also been described recently by some workers.<sup>3</sup> In idiopathic granulomatous mastitis, the granulomas are discrete, non-caseating and strictly lobular in distribution with normal blood vessels.

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**S.S. Yadav and R. Sen**

*Deptt. of Pathology,  
Medical College, Rohtak.*

Sir,

With regard to paper "Sample Survey of awareness of symptoms and utilisation of health facilities by chest symptomatics" by T. Subramaniam, published in the April 1990 issue of the Journal, a brief description of the sampling design elucidating the sampling frame, method of measurement, method of sample selection, sampling estimator, period of study and field work is needed to enable the reader to guess the amount of sampling and non-sampling biases and errors.

**K.D. Gautam**

*TB Demonstration, Training Centre  
and Chest Institute, Agra.*

### The author replies briefly

The sampling frame consisted of villages in Cheput block, municipal wards in Thiruvannamalai town, and Corporation divisions in the first stage and streets in the second stage in Madras City. Simple random samples were selected, and all the households in the selected samples were investigated. The period of field work was approximately 2 years. Full details will be given in the comprehensive report which is in the process of preparation for publication.

## NEWS AND NOTES

### TAI AWARDS

The Tuberculosis Association of India has decided that its prestigious *Khushi Ram Shield* for outstanding work done during the year 1989 be awarded to the Andhra Pradesh TB Association, and the *Bhai Mohan Singh Cup* for outstanding general activities and achievements during 1989 be awarded to the Maharashtra State Anti-TB Association. The Association has also decided, from this year, to award a *Cup with a Citation* for the all-round activities during 1989 to the Delhi TB Association.

The Association's TB Seal Trophy for highest collections from the 39th Seal Campaign has been won by the Anti-TB Association of Tamil Nadu, and the Runner-up Cup for the next highest collections by the Kerala Tuberculosis Association. The Cup for highest collections by smaller States has been won by the Tuberculosis Association of Goa. The Association also decided to award a Cup and a Citation for the second best collections by a smaller State and this was won by the Tripura Tuberculosis Association. The Association has also decided to award Certificate of Merit to the TB Associations of Delhi and Karnataka, for the improved activities and Seal Collections for the year 1989.

### HEALTH VISITORS' COURSE

The 1991-92 TB Health Visitors' Course will commence in July, 1991. The Course will be of nine months' duration and will be held at the New Delhi TB Centre. The minimum qualification for admission to this course is 10 + 2 with Science or Hygiene and Physiology in Matriculation, (equivalent to 10th standard). Application forms for admission can be had from the Secretary-General, Tuberculosis Association of India, 3, Red Cross Road, New Delhi-110 001. The last date for receipt of applications is 30th April, 1991.

### CHANCHAL SINGH MEMORIAL AWARD-1991

The Tuberculosis Association of India awards a cash prize of Rs. 1,000/- to a medical graduate (non-medical scientists working as bacterio-

logists, biochemists, etc. in the field of Tuberculosis will be eligible) below 45 years of age and working in Tuberculosis, for an original article not exceeding 30 double spaced foolscap typed pages (approximately 6,000 words) excluding charts and diagrams on a subject relating to TB. Articles or papers already published or based on work of more than one author will not be considered for this award. Papers may be sent, in quadruplicate, to reach the Secretary-General, Tuberculosis Association of India, 3, Red Cross Road, New Delhi-110 001, before the 31st July, 1991.

### ESSAY COMPETITION, 1991

The Tuberculosis Association of India awards every year a cash prize of Rs. 500/- to a final year medical student in India for an original essay on Tuberculosis, adjudged best by a Special Committee of the Association. The subject selected for the 1991 competition is "Short Course Chemotherapy in Extra-Pulmonary TB." The essay should be written in English, typed in foolscap size, double spaced and should not exceed 15 pages (approximately 3,000 words excluding tables, diagrams, etc.). Four copies of the manuscript should be forwarded through the Dean or Principal of College/University to reach the Secretary-General, Tuberculosis Association of India, 3, Red Cross Road, New Delhi-110 001, before the 31st July, 1991.

### BROCHURE : LECTURES ON TUBERCULOSIS

The Association is bringing out the second edition of Five Lectures on Tuberculosis for General Practitioners revised by Dr. S.P. Pamra, former Honorary Technical Adviser of the Tuberculosis Association of India. Those who wish to have the copies of the same may kindly contact the Secretary-General, Tuberculosis Association of India, 3, Red Cross Road, New Delhi-110 001. This is a priced publication.

### U.P. STATE CONFERENCE

The XIth U.P. State Conference on TB and

Chest Diseases sponsored by the U.P. Tuberculosis Association, will be held at Mathura from 12th to 14th January, 1991. The Conference will discuss scientific papers on 'Early Diagnosis and Prompt Treatment' and 'Non-Tubercular Chest Diseases'.

#### ANDHRA PRADESH TB CONFERENCE

The Tuberculosis Association of Andhra Pradesh, in collaboration with the Dist. TB Association, Guntur and the Guntur Medical College, Guntur, organised the 17th Andhra Pradesh TB and Chest Disease Workers' Conference at Sri Venkateswara Vignana Mandir, Guntur, on 8th and 9th September, 1990. Hon'ble Sir Mohd. Jani, Minister for Exports, Commerce and Seri-culture, inaugurated the Conference. Sri R.P. Watal, IAS, Collector and District Magistrate and President, Dist. TB Association, Guntur, released the Souvenir published on this occasion. The Conference was attended by about 200 delegates and was jointly sponsored by M/s Lupin Laboratories Ltd., Bombay, M/s Malini Chit Fund Pvt. Ltd., Guntur, Sri K. Markandeya Raju, ML/S Model Bottling Co., Guntur, M/s Apple Diagnostics Pvt. Ltd., Guntur and M/s Natco Fine Pharmaceuticals Pvt. Ltd., Hyderabad.

#### REFRESHER COURSE IN TB

A Refresher Course in Tuberculosis organised by the Western Railway was held on 23rd September, 1990 at Officers' Club, Bhavnagar Para, Gujarat. This course was inaugurated by Sri S.R. Bahadur, Divisional Railway Manager, Bhavnagar Para and Dr. J.R. Parekh, Civil

Surgeon, presided over the function. The course was attended by about 70 doctors from all over the State.

#### TUBERCLE

The Churchill Livingstone Medical Journals has offered to Indian Tuberculosis Workers the Tubercle sponsored by the International Union Against Tuberculosis and Lung Disease, Paris, at half price (£ 41 sterling in 1991). This is the only International Journal devoted entirely to tuberculosis and mycobacterial disease. Anyone wishing to subscribe to the Tubercle may write to M/s Churchill Livingstone Medical Journals, Robert Stevenson House, 1-3, Baxter's Place, Leith Walk, Edinburgh EH 1 3AF, United Kingdom.

#### MEMBERSHIP OF THE IUAT-LD, PARIS

The Tuberculosis Association of India is enrolling individual members of the International Union Against Tuberculosis and Lung Diseases, Paris, for the year 1991. By virtue of this membership, members will receive, free of cost, copies of the IUAT-LD Bulletins, Circulars, Newsletters, World Health Organisation Publications dealing with tuberculosis and chest diseases, etc. The annual subscription per member is 500 French Francs (Rs. 2,000/- at current exchange rate). Those who wish to enrol themselves as members of the International Union Against Tuberculosis and Lung Diseases may kindly remit a sum of Rs. 12,000/- to the Secretary-General, Tuberculosis Association of India, 3, Red Cross Road, New Delhi-HG 001, before 31st March, 1991.

#### ERRATA, OCTOBER 1990, Vol. XXXVII

In the paper entitled "Initial drug resistance to anti-tuberculosis drugs in patients attending an urban District Tuberculosis Centre" by Sujatha Chandrasekaran, *et al* (pages 215-216), kindly read as follows :

	Page 215, Para 5	Printed as	Read as
1.	Line 11	$\leq 20$	$\geq 20$
2.	Line 14	$\leq 8$	$\geq 8$
3.	Line 16	$\leq 64$ ug/ml	$\geq 64$ ug/ml
4.	Line 17	$\leq 8$ ug/ml	$\geq 8$ ug/ml

Indian Journal of Tuberculosis

ABSTRACTS

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Vol. XXXVIII

January 1991

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**EFFECTS OF NALOXONE IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE AND ACUTE BRONCHIAL ASTHMA**

C. LOMBARDI ET AL; ITALIAN JOURNAL OF CHEST DISEASES; 1989, 43, 87.

An increased activity of opiate-like peptides may be relevant for COPD and asthma and a possible therapeutical role of opiate antagonists in respiratory disease has been reported. The present study was undertaken to determine whether IV naloxone administration would have a significant action against patients with stable COPD and acute bronchial nonatopic asthma. This study has shown that naloxone causes an improvement in the respiratory function parameters in patients with COPD and asthma. The data obtained suggests two possible distinct mechanisms of action of naloxone. In fact, we suggest that, in COPD, naloxone acts primarily at the central level modifying breathing pattern, while, in acute asthma, this drug seems to act at the peripheral level modifying the bronchial tone of the airways.

**BIODISPONIBILIDAD DE LA RIFAMPICINA EN LOS NIÑOS**

JORGE A. PILHEU ET AL; REVISTA ARGENTINA DEL TORAX; 1988, 49, 221.

A group of 20 children in overall good health, aged between 4 and 14 years, were given Rifampicin 10 mg/kg body weight in order to determine the drug's bioavailability. Rifampicin was measured by high pressure liquid chromatography. Rifampicin plasma concentration attained  $7.20 \pm 0.88$  and  $4.65 \pm 0.65$  meg/ml at 2 and 4 hs. respectively; half-life was  $4.94 \pm 2.35$  hs. All values were similar to those obtained in adults with the same dose. These results suggest that for Rifampicin, either

as treatment or prophylaxis, the same dose should be used in children that is normally used in adults.

**ALLERGIC ALVEOLITIS IN A RURAL POPULATION**

PNEUMONOLOGIA POLSKA; 1989; LVII, 15.

Twenty-seven rural inhabitants with allergic alveolitis were evaluated. Diagnosis was based on clinical and radiological features, pulmonary function tests, broncho-alveolar lavage, lung biopsy, presence of serum antibodies against environmental allergens, etc. The results of treatment with corticosteroids were assessed in terms of lung function, improvement of vital capacity and symptoms and radiological assessment. Small dose continuous administration of corticosteroids proved to be beneficial and prevented occurrence of acute exacerbations as well as decrease of respiratory functional parameters.

**IS PREOPERATIVE CHEMOTHERAPY OF PATIENTS WITH LUNG TUBERCULOMA OBLIGATORY ?**

M.I. PERELMAN, ET AL; PROBLEMS IN TUBERCULOSIS, MOSCOW; 1989, 11, 22.

The results of surgical operations performed on patients with lung tuberculoma who were not given preoperative chemotherapy because of mistake in the initial diagnosis were analyzed. There were 87 patients in whom tuberculosis was diagnosed only during or after operations following morphological examination of the resected pathological materials. High efficacy of surgical treatment was observed in patients with tuberculoma having no obvious signs of active tuberculosis and not subjected to preoperative anti-tuberculosis chemotherapy. Early surgical

treatment of such patients without preoperative chemotherapy is considered to be advisable.

### **TUBERCULIN-TRANSAMINIDASE TEST, A NEW PROCEDURE FOR DIAGNOSIS OF NEPHROPHTHISIS**

T.P. MOCHALOVA, ET AL; PROBLEMS IN TUBERCULOSIS, MOSCOW; 1989,11, 6.

The possible use of the provocative standard test, with subcutaneous administration of tuberculin combined with determination of the activity of transaminidase (a renal specific enzyme) in serum and urine before and 24, 48, 72 and 96 hours after administration of tuberculin, was studied in 63 patients with the purpose of diagnosing nephrophthsis. It was shown that a two fold or higher increase in the transaminidase activity in response to subcutaneous administration of 50 TU during the period from 24 to 72 hours after doing the test could serve as a sign of nephrophthsis.

### **SLIDE CULTURE SENSITIVITY TESTS**

J.M. DICKINSON ET AL; TUBERCLE; 1989, 70,115.

A new method for slide culture sensitivity tests of Mycobacterium Tuberculosis is described in which smear-positive sputum spread on slides is incubated without prior decontamination in a selective lysed human blood medium. Results are available 7 days after setting up the tests and are particularly useful for guiding the treatment of smear-positive patients with a long history of unsuccessful chemotherapy. Drug concentrations and definitions of resistance are suggested for tests against Isoniazid, Streptomycin, PAS, Rifampicin, Ethambutol and Ethionamide. A good correlation was seen between the results of these tests and those of standard indirect sensitivity tests.

### **ABDOMINAL TUBERCULOSIS IN PREGNANCY**

O. FREEMAN; TUBERCLE; 1989, 70,143.

Two patients are described who presented

with abdominal tuberculous ascites and hepatomegaly during pregnancy. One of them aborted during the second trimester of pregnancy and the other was diagnosed in the post partum period following a normal delivery. Both patients responded to anti-tuberculous chemotherapy. The diagnosis of abdominal tuberculosis was made on the basis of isolation of tubercle bacilli from the ascitic fluid in one case and the other case had cavitory tuberculous lesion in the lung, though tubercle bacilli could not be isolated from the ascitic fluid.

### **A CONTACT STUDY TO EVALUATE THE BCG VACCINATION PROGRAMME IN SEOUL**

B.W. JIN ET AL; TUBERCLE; 1989, 70, 241.

A contact study was undertaken in Seoul to determine the protective effect of the BCG programme in children up to 5 years of age. There were 1993 contact children to 4484 smear-positive patients with pulmonary tuberculosis. 1223 completed the examination, 806 had evidence of BCG vaccination, 417 had not. In total, 129 or 126 children were considered cases of tuberculosis according to radiological/clinical classification or scoring system, respectively. For the unvaccinated, the respective numbers of cases were 84 and 80 and for the vaccinated 45 and 46. The data were stratified for factors that could have distorted comparability : age and sex, relationship of index case, feeding habits, room occupancy, treatment history of index case and health centre that diagnosed the index case. Only age was found to have a small effect. Correcting for tins, the observed level of protection was 74% with 95% confidence limits of 62% and 82%. It appeared to be the same for all types of disease observed.

### **PULMONARY TUBERCULOSIS AND MYCOTIC INFECTION-CLINICAL AND SEROLOGICAL DIAGNOSIS**

HITOSHI IWATA ET AL; KEKKAKU; 1989, 64(1), 7.

The antibody activities against Aspergillus Fumigatus and Candida albicans by indirect hemagglutination (IHA) and counter

immunoelectrophoresis (CIE) were examined twice at a month's interval in 251 sera from 169 male and 82 female patients admitted to the Higashi Nagoya National Hospital for pulmonary diseases. The patient population was composed of 226 patients with active or cured pulmonary tuberculosis including 25 patients complicated with pulmonary aspergillosis and 25 other lung diseases. The antibody activity against *Aspergillus* by IHA was positive in 2.9% of the sera in the first and in 0.9% in the second test and was positive against *Candida* by IHA in 44.9% in the first and in 44.0% in the second test. As regards patients with pulmonary tuberculosis, the population whose serological reaction was positive against *Candida* did not increase with age. Two results of the antibody activities against *Aspergillus* and *Candida* by CIE were the same. The CIE results were positive in 19.5% against *Aspergillus* and in 16.3% against *Candida*. In CIE test, 4.3% were positive against both *Aspergillus* and *Candida*. The causes of high positive rate in IHA against *Candida* may be due to (1) difference in the antibody, namely, antibody measured by IHA mainly composed of IgM and that by CIE composed of IgG and (2) difference in the virulence between *Candida* and *Aspergillus*. The antibody activity against *Aspergillus* detected by IHA is expected to be low as *Aspergillus* is not found among the normal flora in the airway and our cases of pulmonary aspergilloma were not acute. 24 cases were diagnosed to be false positive clinically. IHA could be a method suitable for diagnosing acute pulmonary aspergillosis and CIE for chronic pulmonary aspergillosis.

#### **SEROLOGICAL DIAGNOSIS OF PULMONARY ASPERGILLOSIS BY ELISA**

SETSUKO YAMAMOTO ET AL; KEKKAKU;  
1989, 64 (1), 15.

Pulmonary aspergillosis usually develops on

the basis of systemic immunosuppression and/or local impairment of respiratory system. Diagnosis of pulmonary aspergillosis has many difficulties. Chest X-ray findings of most cases are complicated with pre-existing changes due to the underlying diseases, and the detection rate of the pathogenic fungi from clinical specimens is unsatisfactorily low. Therefore, immunological or serological diagnosis is urgently required and precipitation-in-gel method has been widely applied. Determination of anti-*aspergillus* antibodies by ELISA and precipitation-in-gel method were confirmed.

About two-thirds of 45 healthy adults (control) did not show any detectable IgG anti-*aspergillus* antibody and mean of IgG anti-*aspergillus* antibody titer of the control groups was 28.97. Patients who had shown positive culture of fungus or were clinically diagnosed or strongly suspected as pulmonary aspergillosis, showed significantly high anti-*aspergillus* IgG antibody titer in comparison with the control group. Further, patients who were positive in precipitation-in-gel tests showed significantly higher IgG antibody titers than those who were negative in that test.

IgG antibody titer determined by ELISA corresponded with clinical diagnosis much more than the precipitation-in-gel test. Further, the results obtained by ELISA were objective and quantitative in comparison with the latter test.

We concluded that ELISA was much superior to precipitation-in-gel test and that ELISA IgG antibody titers 2500 or more were confirmative and those between 570 and 2500 were strongly suggestive for the diagnosis of aspergillosis.

IgM anti-*aspergillus* antibody titers were not different among healthy control group and patient groups, and could not be used for the diagnosis.

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