

INDIAN JOURNAL OF TUBERCULOSIS

*Official organ of the
Tuberculosis Association of India*

Vol. XXXII : No. 2

April 1985

Emeritus Editor:
Dr. P.K. Sen

Editor:
Dr. S.P. Pamra

Editorial Board: Dr.
G.V.J. Baily Dr. M.D.
Deshmukh Dr. D.N.
Gupta Dr. S.K. Jain
Dr. S.C. Kapoor Dr.
K.V. Krishnaswami Dr.
D.D.S. Kulpati Dr.
A.M. Malaviya Dr.
M.L. Mehrotra Dr. N.
Naganathan Dr. D.R.
Nagpaul Dr. S.P.
Tripathy Dr. S.N. Tuli

Contents

Editorial : Tuberculosis case-finding and Camps	... 55
Bronchial Asthma and Environment — <i>M.S. Agnihotri</i>	... 57
Significance of Soluble Antigen Fluorescent Antibody Test in the Serodiagnosis of Tuberculosis — <i>Usha Kiran Shartna</i>	... 65
Ritampicin : Biological Values for Action and Intolerance — <i>P.K. Sen, B.N. Roy and R. Chatterjee</i>	... 81
Serum and Urinary Rifampicin and Hepatic Toxicity — <i>P.R. Gupta, S.D. Purohit, Y.R. Mehta, B.L. Jain, S. Konlwal and T.N. Sharma</i>	... 86
Short-Course Chemotherapy of Pulmonary Tuberculosis and its Applicability in Field Conditions — <i>K. Jagannath, S. Rajasekaran and R. Vasantha Kumari</i>	... 91
Excerpts from Dr. A.G. Patel's Presidential Address at the 39th National Conference on Tuberculosis and Chest Diseases	... 96
Summaries of the Papers presented at the 39th National Conference on Tuberculosis & Chest Diseases at Cuttack from 28th to 31st January 1985	... 97
Annual Meetings of the I.U.A.T.—1984	... 105
Report of the IUAT Scientific Committee on Treatment	... 107
A short review on 19th National Conference on TB & Chest Diseases held at Cuttack from 28th to 31st January 1985	... 109

NEWS & NOTES

Published quarterly in the months of January, April, July & October.
Annual Subscription Rs. 50/-, £ 5.00, \$ 12.00, Single copy Rs. 15/-.
Published on behalf of the Tuberculosis Association of India.
3, Red Cross Road, New Delhi-110001.

The Indian Journal of Tuberculosis

Vol. XXXII

New Delhi, April 1985

No. 2

TUBERCULOSIS CASE FINDING & CAMPS

It is indeed encouraging that attention is now being directed towards stepping-up of case-finding under the national tuberculosis programme (NTP). At the 39th national conference on tuberculosis and chest diseases, held recently in Cuttack, a panel discussion and several papers were devoted to case-finding, besides the usual large array of papers on chemotherapy. No doubt, chemotherapy is important. But, case-finding is equally important, if not more so. For too long, and perhaps to a disproportionate degree, the focus has been on chemotherapy : more on the newer drugs & drug regimens and less on improvements in case-holding.

That under the epidemiological situations in most developing countries, and with the given investment of efforts and resources there, any improvement in case-finding gives richer dividends than a commensurate improvement in case-holding and/or chemotherapy has been pointed out several times in the past. It has also been stressed that after more than two decades, the efficiency of case-finding under NTP is the poorest compared with that of case-holding and operational efficiency of chemotherapy. Commonsense would, therefore, demand that case-finding should at least be brought to the same level of efficiency as the other components in order to fully exploit the potentiality of chemotherapy, if nothing else. Yet, trends and fashions and not commonsense often rule the world, even of science and technology.

Programme administrators too have an apparent bias in favour of chemotherapy. For finding and treating 12.08 lakh new cases of tuberculosis under NTP during 1983-84, a sum of Rs. 4.69 crore was expended. But for 13.75 lakh cases targeted for 1984-85, the provision made is Rs. 10.5 crore : the sharp rise is for short-course chemotherapy in 18 districts. Seen in the context that only 359 out of 420 districts in the country have been brought under NTP since 1962, leaving sizeable chunks of population without organised tuberculosis services coupled with poor case-finding (around 30% of the potential), the generous provision made for short course chemotherapy does appear lopsided.

One of the reasons for the Cinderella like attitude towards case-finding is lack of innovative ideas and practices to fire the imagination of tuberculosis workers. The introduction of case-finding camps in the early nineteen seventies and, over ten years later, the decision to fix annual targets for sputum examinations as well as discovery of new cases under NTP are two recent innovations that come to mind. Both are reported to improve case-finding considerably. Unfortunately, the tools for case-finding have remained

unchanged for over half a century and the prospect of a breakthrough in that direction appears bleak. Rekindling of interest in case-finding research is necessary to break the shackles that bind NTP at present to the state of relative impotence.

The *modus operandi* of case-finding camps called Shibirs, held in Maharashtra countryside at first, negates almost every accepted case-finding principle under NTP. The advent for a day of a team of city super specialists to a "prepared" rural population, screening by fluroscopy of a motley crowd under questionable field conditions and leaving at the day's end a bunch of treatment cards at the area primary health centre (PHC) for treatment of the 'found' cases was neither here nor there. To Shibir organisers, it did not matter if a large proportion of the found cases were known patients who had been "attracted" to the camp by the fame of the specialists or to "oblige" the local volunteers. Nor, whether the discovered cases did start their treatment and, if so, how good was their case-holding. The often stressed health education value of Shibirs was also out of proportion. How could a one time effort succeed where the need for sustained long-term education is all too obvious?

The most pointed argument against camps is their sporadic character. Held only once or after intervals measured in years and not months, the camps could hardly have any programme value. Since case-finding and treatment are inseparable, it cannot even be a case of something being better than nothing.

Sputum case-finding camps advocated at Cuttack appear to have merit in as much as many of the above objections do not apply. These more frequent case-finding drives following NTP procedures are done by the microscopy centre staff helped by workers deputed from the district tuberculosis centre. The found cases are immediately put on treatment. Camps are organised by the district tuberculosis officer (DTO) taking care not to clash with the other programme health activities of the centre and the need to prepare the population through health education. The camps are held monthly or twice a month, at a different microscopy centre in the district. Attractive though the idea is, some of its aspects need careful assessment. For example, how far will direct involvement of DTO and his staff in camps lead to neglect of the all too important supervision responsibility which is already under some jeopardy on account of a poor attitude towards managerial activities. Will the PHC staff, already inclined to regard NTP activities as some kind of extra work, stop doing routine daily case-finding in between camps, thus distorting programme dynamics more than doing any good to case-finding. And, does the real cost of holding sputum case-finding camps justify the gains? If the gain is substantial, a way may have to be found to avoid sacrificing supervision. Carefully planned operational research, preferably at the National Tuberculosis Institute and not by DTOs in districts, is needed to evaluate sputum case-finding camps properly.

—D. R. Nagpaul

BRONCHIAL ASTHMA AND ENVIRONMENT**

M.S. AGNIHOTRI*

I am thankful to the Tuberculosis Association of India for giving me this opportunity to address you, the delegates of 39th National Conference on Tuberculosis and Chest Diseases. House of 'Wanders' deserves appreciation, as it is because of their assistance that the oration is a permanent feature of the conference.

Environment includes everything around us. Therefore, the scientists from various other fields are also contributing a lot in understanding the mysteries of environment. We medical men are interested in health and long life of mankind and freedom from illness and physical impairment. So my talk will be limited to the effect of environment on human health. Environmental diseases are closely related to the lung. Therefore, they concern us - the Chest Physicians.

Food, water and air are the basic raw materials required for normal living of mankind which he utilises from his environment. Man can live without food for 5 weeks, without water for 5 days but cannot live without air for more than 5 minutes. In one day, man requires about 1.3 Kg. of food, about 2 Kg. of water but requirement of air is 13 Kg. per day. Air is most vital for normal human life. Community is advised not to eat or drink unhygienic food or water but one has to inhale polluted air if present in the environment. Most of the air pollutants are invisible and affect the health. The air pollutants enter the body through exposed organs, commonest being the lungs. Inhaled substances include inorganic and organic dust, spores and pollen, microorganisms, fumes, vapours, gases and radio—active particles. Depending on their physical & chemical properties, concentration, duration of exposure, host factor and site of deposition, they are capable of producing various types of reaction in the lung such as mucosal inflammation, alveolitis, asthma, fibrosis, granuloma, emphysema and neoplasm (Comming et al, 1980)

Further, I shall limit myself to asthmatic reaction of lungs caused by the various environmental factor.

Definition of Asthma

Asthma is defined as a disease characterised

by wide variation over short period of time in resistance to flow in intrapulmonary airways (Scadding 1983). Increased resistance to airflow is due to environmental factors especially inhaled substances in concentrations that do not effect the majority of persons. Detectable factors include allergens, exercise, and physical and chemical stimuli. Diminution in increased resistance in response to bronchodilator drugs and steroids is usually demonstrable. Although asthma is characterised by paroxysms it may become continuous later on. In such instances diagnosis may be justified by the evidence that wide variability has been present in the past.

Epidemiology of Asthma

Epidemiological studies have thrown light on various aspects of bronchial asthma. Epidemiological studies in U.S.A. have revealed that 5% of adults and 10% of children suffer from asthma in U.S.A. (Gregg, 1977). Very few prevalence surveys of asthma have been conducted in India (I.C.M.R., 1961 and Viswanathan et al 1969).

A. Increase in Prevalence of Asthma

Various prevalence surveys confirm the clinical impression of increase in the frequency and severity of asthma in children and adults in the last 20 years. The interpretation and comparison of various prevalence surveys of asthma is difficult because of different definitions of asthma and type of prevalence (point/cumulative) studied (Tables I & II).

Cullen (1972) conducted prevalence surveys in Britain and Australia simultaneously with the same methodology and it was found that the prevalence of asthma was much higher in Australia as compared to Britain. The findings of this study disproved the notion that prevalence of respiratory diseases in general was higher in Britain because of its climate and also showed that asthma was less common in British children.

B. Importance of genetic and environmental factors

Morrison Smith (1971) compared prevalence

*Professor of Tuberculosis (Respiratory Allergy) K.G.'s Medical College, Lucknow.

**"Wander-TAI Oration" was delivered at the 39th National Conference on TB & Chest Diseases held in Cuttack in January 1985.

TABLE I
Prevalence of Asthma in Children

Author	Prevalence %	Author	Prevalence %
<i>Britain</i>			
Morrison Smith (1961) ⁶	1.8	Cullen (1972) ¹⁰	6.8C
<i>U.S.A.</i>			
Collins (1935) ⁷	0.5C	Hom & Gregg (1973) ¹¹	5.1 C
Broder et al (1962) ⁸	4.1 C	Peckham & Butler (1978) ¹²	3.5C
<i>Australia</i>			
Patrick (1962) ⁹	5.4C	Arbeiter (W) ¹³	9.4C
		Dodge & Burrows (1980) ¹⁴	8.5
		Cullen (1972)	10.2 C

C=Cumulative Prevalence

TABLE 2
Prevalence of Asthma in Adults

Author	Prevalence %	Author	Prevalence %
<i>Britain</i>			
Stocks (1949) ¹⁵	0.9	Buir et al (1975) ¹⁷	3.4 C
Williams (1951) ¹⁶	1.7	Buir et al(1979) ¹⁸	6.5 C
<i>U.S.A.</i>			
Collins (1935)	0.1-I C	Dodge & Burrows (1980)	6.2
Brodere (1962)	4.1 C		

C=Cumulative Prevalence

rate of asthma and wheeze in English and immigrant school children in U.K. Prevalence among Asian and West Indies children who had been born in England was similar to that of English children. Much lower prevalence rate was found in immigrant children who had been born in their native country.

The prevalence of Asthma in Xhosa children living in African township of Capetown was determined by exercise challenge test and was

3.20% (Van Nieberk et al 1979). This is much higher than in the case of Xhosa children in Transkei using identical methods.

Cumulative prevalence of Asthma in Tokelauan children in Newzealand was found to be 25.3% (Waite et al 1980). This is greatly in excess of that found in Tokelau. These studies have shown that environmental factors are more important than genetic factors in pathogenesis of Asthma.

C. Identification of environmental factors

In 1971 the Americans in Japan suffered from asthma due to industrial chemical pollution and this epidemic of asthma is known as Tokyo Yokohama asthma (Salvaggio et al 1971). On the other hand in 1954 asthma epidemic was due to exposure of Neo Orleans to organic allergens in environment (Huber et al 1954). Prevalence rate of asthma with rhinitis found in a study in Sudan clearly demonstrates the importance of environmental agent—Green Nimmitti—as provocative factor (Kel et al, 1983). Thus, the epidemiological studies have proved that various environmental factors are responsible for precipitating attacks of asthma.

Various types of asthma caused by environmental factors may be:

1. Extrinsic asthma due to various detectable allergens present in the atmosphere.
2. Asthma due to various occupational agents present in the environment is called occupational asthma.
3. Asthma caused by food allergens is known as Alimentary Asthma.
4. Drug induced asthma is caused by injection, infusion and ingestion of drugs.
5. Asthma after exercise is known as Exercise induced Asthma.

Allergic diseases are confined to exposed organs of the body such as lungs, nose, skin and eyes. Asthma and rhinitis are allergic diseases related to lung and nose. Urticaria, rashes dermatitis, eczema are allergic skin diseases. Allergic conjunctivitis is characterised by redness and watering of the eyes which also is the usual feature in hay fever.

Recently, we analysed our cases of Allergy/Asthma clinic and observed that 69 % of cases attending our clinic had respiratory allergy and about 31 % could be diagnosed as suffering from skin and drug allergy (Mishra 1984). More cases of respiratory allergy attend our clinic because it is mainly for lung diseases.

About 10% of our population, suffer from allergic diseases involving various organs of body. Allergic diseases are on increase with industrialisation and domestication of life and are common in affluent society as compared to the poor. My Tuberculosis O.P.D. is full of poor patients and patients attending allergy /asthma clinic are better off.

Allergic bronchial asthma is characterised by early onset of symptoms, family history of allergic diseases and association of other allergic diseases. Patients are sometimes able to identify offending allergens and symptoms have a seasonal variation. There is no toxæmia but eosinophils are increased.

Allergens are classified as inhalants, ingestants and contactants according to their route of entry. The allergen responsible for symptoms in a particular patient can be recognised by history, skin tests (Shivpuri 1962) and bronchial provocation test (B.P.T.) (Hargreave et al 1972.)

Our experience in allergy/asthma clinic has shown that patients of asthma have a specific pattern of attacks. Patients having attacks at change of season think that symptoms are due to change in temperature, but it is not so. During change of season, e.g. Sept./Oct., and Feb./March the amount of pollens in the environment is maximum. Therefore the patient allergic to pollens gets into attack during the change of season. Ours was the first allergy clinic in the State of U.P. So patients of asthma from the hills of U.P. also reported at Lucknow. It was observed that patients from hills used to feel better as they came to plains because the environment of hills is different from that of plains. Another group of patients had attacks of asthma during winter nights. Some patients reported increase of symptoms in home and relief in outdoor environment, while others had symptoms during the day. For them occupational exposure is an important factor because patients are exposed to occupational agents during the day. So by taking careful history it is possible to find an offending allergen for a particular patient which is to be confirmed by skin test and B.P.T.

Skin is a mirror of immunological reactions of individual (Pepys et al 1975). Immediate, late and delayed reactions can be elicited in the skin by the use of proper antigenic extracts. Immediate reaction develops within minutes and resolves in one to two hours, and is mediated by skin sensitising anti-body. Tissue reaction is reversible and is characterised by wheal. Erythema response is produced by vasoactive amines released due to degranulation of mast cells. Cortisone has no effect on type one reaction. Late or Arthus type of reaction develops slowly within five to seven hours and resolves by 26 to 36 hours. Late reactions are mediated by precipitating type of anti-body. Tissue reaction is characterised by ill defined, extensive, soft swelling produced by toxic immune complexes which is tissue damaging. Delayed reaction develops after 72 hours and is mediated by T-lymphocytes due to release of lymphokines. Tissue reaction is characterised by a damaging

and firm induration. Bronchial provocation tests are performed by nebulizing the antigen in the bronchial tree and assessing the degree of spasm produced. Bronchial Provocation Tests are more specific than skin tests in identifying offending allergens. Dominant aero-allergens of Lucknow were reported for the first time in 1971 (Agnihotri et al 1971). In 1982 the allergenicity of pollens of various trees, grasses and weeds present in the atmosphere of Lucknow were reported (Verma 1982). 971 cases of respiratory allergy were skin tested and analysed. It was observed that *Holoptellia*, *Prosopis*, *Lantana*, *Ricinus*, *Brassica*, *Moringa*, *Azadirachata* *Phoenix*, *Albizia*, *Bombax*, *Eucalyptus*, *Althaea*, *Citrus*, *Ehretia*, *Melia* and *Morus* Pollens were important in the etiology of respiratory allergic diseases. Amongst weeds and grasses pollens of *cynodon*, *chenopodium*, *cannabis*, *amaranthus*, *ageratum*, *pennisetum*, *sorghum*, *saccharum*, *panicum*, *dichanthium*, *triticum*, *anthinm* and *aregmone* were found to be important allergens. It was further reported that majority of these plants pollinate during change of season, i.e. March, April, September, October. Thus this study demonstrated that maximum pollens are present in atmosphere during change of season and are important aero-allergens.

Physical inspection of the houses of patients of asthma giving history of attack at home revealed growth of fungi on the damp walls. We identified the fungi in the bedrooms of asthmatics and *aspergillus niger*, *A fumigatus* and *A. flavus* gave higher colony count in the bed-rooms as compared to the atmosphere (Agnihotri et al 1979). In the second study of 25 patients of allergic bronchial asthma having symptoms in their homes, the etiological significance of various species of *aspergillus* was studied by skin test, bronchial provocation tests and precipitation tests (Sharma 1980). It was observed that extracts of *Aspergillus niger* and *Fumigatus* gave 68% clinically significant reactions whereas 90% and 85.7% bronchial provocation tests were positive in these patients. Precipitin tests revealed precipitin line in 39.1 % and 52% tests done by *Aspergillus niger* and *Aspergillus fumigatus* respectively. These two studies have clearly demonstrated that the amount of fungal spores is higher in damp rooms and *Aspergillus* spores present in the damp rooms cause bronchial asthma. Thus pollution of indoor environment by fungal spores is a health hazard.

Lichens are composite plants having algal and fungal elements and they grow on barks of trees, rocks and ground in hills. In a study at Allergy/Asthma Clinic it was observed that the patients of various respiratory allergy coming from hills gave positive skin reactions in

27% cases, whereas 3.5% non-hilly patients of respiratory allergy gave positive reactions to Lichen extract. (Agnihotri et al 1978).

Asthma is often seen in patients using pillows filled with various type of organic material such as cotton, semal cotton clothes, furs, foam, maize husk and flowers. We know that inhalation of organic dust can cause allergic reaction in lung. This observation prompted us to study the allergenicity of semal cotton. *Bombex cejba* flox becomes powdery after use and dust which comes out of the pillows is inhaled by the patients. In this study patient of respiratory allergy having symptoms during winter night were studied. It was observed that 52.2% patients demonstrated positive skin reaction to extract of semal cotton and 54.5% patients were using semal cotton filled pillow? (Agnihotri et al 1976). This study has demonstrated that patients having symptoms in winter night should avoid using pillows stuffed with cotton specially semal cotton.

House Dust is a well-recognised cause of asthma. The allergenic component of house dust is 'Mite—an' organism which grows on mattresses and thrives on human scales. Mite allergy is not so common in India because of our habit of putting bedding in the sun. Mite on the mattress cannot survive in the Indian sun. Animal allergy is not very important in India because we do not live with animals as in the west. Animal allergy is often seen in patients involved in occupations dealing with animals.

Occupational Asthma

Occupational asthma is due to specific well-defined exposure of occupational agents and these patients get relieved by avoiding exposure. Asthmatic symptoms are produced either by reversible airway obstruction due to spasm of the bronchial muscles, swelling of the mucus membrane lining the bronchi and presence of secretions in the bronchial lumen, or due to reversible parenchymal injury leading to pulmonary oedema, inflammatory alveolitis and bronchiolitis. Occupational agents cause various types of reaction in the lung (Fish 1982 Lessof et al 1981 and Harrison et al 1976).

Toxic Reactions

Gases and fumes can cause direct tissue injury. The site of tissue, reaction depends on their solubility and concentration of gases. Highly soluble gases get dissolved in the bronchial secretions easily and cannot reach parenchyma and, therefore, symptoms of tracheo-bronchitis are produced whereas less soluble gases and fumes reach upto the parenchyma

and produce bronchiolitis or alveolitis. Gases in low concentration increase non-specific sensitivity of the bronchial mucosa due to epithelial injury. In high concentrations fumes cause parenchymal injury.

The clinical picture of toxic reactions appears with the first exposure to all exposed persons. There will be simultaneous evidence of irritation of nasal and conjunctival mucosa. Pulmonary function tests (PFT) may be normal or abnormal. Chest X-rays show infiltrates or evidence of pulmonary edema.

B. Immediate hypersensitivity reactions

Occupational agents have immunological potential to increase the production of specific IgE, and to trigger an attack of bronchial asthma similar to that of non-occupational asthma. The etiological significance of occupational agents can be demonstrated by direct skin test, passive cutaneous anaphylaxis and by demonstration of specific IgE. The clinical picture of immediate hypersensitivity reactions are wheeze and dyspnoea, chest-tightness, with normal X-ray and abnormal PFT during the attack.

The toxic and hypersensitivity reactions to occupational agents can be differentiated on the basis of history and routine examinations as shown in the following table:

rics, dal and timber mill workers. The worker is exposed to occupational agents during the day and therefore, one must consider occupational cause in patients having asthma during the day.

At the Allergy Asthma Clinic it was observed that various food articles can precipitate attack of asthma in an individual. 37.5% cases of asthma reported precipitation of attack by dal, wheat, nuts, milk, meat, fish, egg, banana, tomato and orange in 1979 (Agmhotri et al 1979). Number of factors both genetic and environmental predispose development of food allergy. Infants fed on breast milk and not given 'formula' foods are less likely to develop milk allergy whether there is genetic predisposition or not (Lessof et al 1981). Gastroenteritis increases the permeability of gastrointestinal mucosa and thus macromolecules of food allergy are absorbed from intestines which are capable of producing specific anti-bodies to foods (Harrison et al 1976).

Food allergy can produce remote manifestations such as asthma, eczema or rhinitis possibly due to transmitted effect of mediators which are first liberated in gastrointestinal tract (Buisseret et al 1978). Recently we have observed that amongst our patients, milk was the most offending food. Therefore, we studied

	<i>Toxic Reactions</i>	<i>Hypersensitivity Reactions</i>
Symptoms	First exposure All are affected. Irritation in eyes & nose.	Subsequent exposure after a long period. Only a few are affected. Associated rhinitis and conjunctivitis.
Threshold Limit Values	Effective	Non-effective
PFT	Normal/abnormal	Abnormal
Chest X-ray	Patchy infiltrates. Pulmonary edema.	Normal

Same occupational agents can cause hypersensitivity reaction of ill-defined mechanism and other irritant reaction in lung. Late and delayed hypersensitivity reaction due to exposure of various avian and mammalian proteins, varieties of moulds and chemical agents is observed in Extrinsic allergic alveolitis.

Various types of dusty occupations which cause asthma in our society are Kirana shop workers, grain merchants, godown workers,

milk allergy in patients of bronchial asthma attending Allergy-Asthma Clinic. Correlation between skin test and elimination and oral challenge test revealed that in patients with positive skin reaction, elimination test were positive in 35 patients. Out of these 35 patients, 31 (88.57%) had positive oral challenge also. Thus 88.5% skin positive patients were also positive for elimination and oral challenge tests indicating that skin test by milk antigen is a good indication for diagnosis of milk

allergy in allergic patients (Arya 1983). It was further observed that chances of development of milk allergy were more in asthmatics who had milk other than the mother's milk in the first 3 months of life, thus emphasising that breastfeeding prevents development of milk allergy.

Drug Induced Asthma

Drug induced bronchospasm is a common problem. It may occur as pulmonary manifestation of anaphylaxis or after inhalation, ingestion or infusion of drugs. The drugs most commonly associated with induction of acute episodes of asthma are aspirin and tartarazine. (Fadden 1984).

Aspirin Asthma : It affects adults; starts with perennial vasomotor rhinitis followed by hyperplastic rhino sinusitis with nasal polyp and then progressive asthma. Symptoms appear 1-3 hrs. after administration of the drug. 10% of asthmatics have aspirin induced asthma. There is cross sensitivity with other non-steroidal anti-inflammatory drugs and desensitisation to aspirin is possible. The mechanism involved in aspirin asthma is unknown.

Exercise Induced Asthma (EIA)

Asthma can be induced or made worse by physical exertion. EIA is a feature of childhood asthma. Asthmatic attacks begin a short while after stopping the exercise and there is decreased response to further exercise after attack of EIA. This is known as refractory period. There is significant interaction between climatic environment in which exercise is performed and magnitude of post exertional airways obstruction. Inhalation of cold air during physical exertion markedly enhances the response whereas hot humid air can blunt or abolish it. (Welliver 1983 and Anderson et al 1982) Consequently, activities such as ice hockey, ice skating are more provocative than swimming in indoor heated, pools.

The principles of management of allergic bronchial asthma include avoiding offending allergens as shown by history, skin test or bronchial provocation test. But if it is not possible, prophylactic treatment is advocated. Prophylactic treatment includes specific prophylaxis by desensitisation treatment whereas non-specific prophylaxis is carried out with drugs like disodium chromoglycate. The desensitisation treatment aims at introducing a concentration of allergenic extract which the patient can tolerate. It produces a level of resistance which can cope with general concentration of allergens around the patients. This aim is achieved by production of blocking antibodies which interpose themselves between

the allergen and the mast cell. It has also been shown that by the process of desensitisation, IgA anti-bodies are produced which protect the mucus membrane.

Results of Immunotherapy

The results of immunotherapy of 468 patients followed for a period of 2 years were reported in 1984 (Mishra, 1984). It was observed that about 60 % of patients who continued immunotherapy for 6 months showed marked improvement and after 1 year 87% of them showed improvement. The percentage of improvement increased with passage of time. The results of immunotherapy depend upon proper selection of patients and allergens used in immunotherapy. Out of the various groups of allergens which can cause Respiratory allergy, immunotherapy is proved to be effective in pollen allergy, because standardised extracts of pollen are available and the active antigens have been recognised. In our country, pollens are very important aero-allergens and are present throughout the year. Therefore, immunotherapy has definite role in management of bronchial asthma in India. Immunotherapy is a prolonged treatment involving repeated injections. Immunotherapy is indicated only when the allergen is not avoidable and in severe asthma which requires intensive medication. Immunotherapy should always be used along with other medications.

In conclusion, most of the activities of man today are indoors, either during working or living. With urbanisation almost all the activities of mankind are indoors. Most of the hours of the day and most of the days of the years are spent in the same room of the same building in which he or she lives or works. Exposure to indoor air pollutants is prolonged and repeated. Indoor pollutants remain more concentrated as they have less chance of getting diluted as compared to outdoor air pollutants. These include virus and bacteria. All of us know that tuberculosis is more common amongst household contacts because of the presence of *M. tuberculosis* in indoor atmosphere. If a child suffers from measles, the other children living in same household have a possibility of suffering from the same illness because of indoor environmental pollution. Allergens like pollen, spores, dust, mites, insects, fumes and animal danders cause respiratory diseases in sensitive individuals. Combustion products and smoke produce indoor pollution. Consumer products such as paints, insecticides etc. can affect the inhabitants of that particular house. Thus, it is clear that so many things present in indoor environment can cause asthma. Out-door environmental factors of asthma can be checked by legislation on industries, but for indoor pollution, community is to be educated and it

is our duty. Every allergologist has to face the question—'Dr. I am allergic to what?' It is only the avoidable allergens that should be told to the patients and not the unavoidable one.

We must know about our environment not only for the sake of knowledge but also for healthy living, because environmental pollution causes asthma.

REFERENCES

- Agnihotri, M.S. and Singh, A.B.: Observation on pollinosis in Lucknow with special reference to offending factors, *Aspect of Allergy & Applied Immunology*; 1971, v. 135.
- Agnihotri, M.S., Misra V.K. and Misra P.K.: Study of fungi in bed rooms of Asthmatics: *Aspect of Allergy and Applied Immunology*; 1979, 13/14, 11.
- Agnihotri, M.S., Desh Deepak and Verma, H.: Observations on Allergenicity of some common Lichens of Uttar Pradesh, *Aspect of Allergy and Applied Immunology*; 1978, XT, 211.
- Agnihotri, M.S., Verma, H and Pandey, S.K.: Study on Bombax Ceiba, pollen and seed fiber in relation to Respiratory Allergy, IV International Palynological Conference; 1976; III, 442, 444.
- Agnihotri, M.S., Kaharia, P.C. and Udoyal, D.C.: Clinical Pattern of Allergic Diseases in Uttar Pradesh. *Allergy and Applied Immunology*; 1979; XII, 118.
- Anderson S.D., Schoeffel R.E. and Follet R. et al *Eur. J. Respir. Dis.*, 1982, 63, 459.
- Arbeiter, H.I.: How prevalent is allergy among United States school children? *Clin. Pediatr.* 1967, 6, 140-2.
- Arya A.K. : A study of Bronchial Asthma in children. Thesis for M.D. (Tuberculosis) Lucknow University; 1983.
- Broder, I. Berlow, P.P. and Horton, R.J.M: The epidemiology of asthma and hay fever in a total community, Tecumseh, Michigan, *F. Allergy*, 1962, 33, 513-23.
- Buisseret, P.D., Youlten, L.J.F., Heizelmann, D.I. and Lessof M.H.: Prostaglandin synthesis inhibitors in prophylaxis of food intolerance, *Lancet*, 1983j 1, 906.
- Burr, M.L., St. Leger, A.S., Devan, C. and Murrett, T.G.: A community survey of asthmatic characteristics. *Thorax* 1975, 30, 663-8.
- Burr, M.L., Charles, T.J., Roy, K., and Seaton A.; Asthma in the elderly: an epidemiological survey, *Br. Med. J.* 1979, 1, 1041-4.
- Collins, S.: Age incidence of specific causes of illness. Based on records for 9000 families in 18 states visited periodically for 12 months, 1928-1931. *Public Health Reports (Washington)* 1935, 50, 1404.
- Comming, G., Semple, J. Stephen: Disorders of the lung from occupation and environment. In *Disorders of the Respiratory system* edited by Gordon Comming & Stephen J. Semple (Blackwell scientific Publications II) 1980, pp. 493-520.
- Cullen, K.J.: Climate and chest disorders in school-children. *Br. Med. J.*, 1972, 4, 65-7.
- Dodge, R.R. and Burrows, B.: The prevalence and incidence of asthma and asthma-like symptoms in a general population sample. *Am. Rev. Resp. Dis.*, 1980, 122, 567-75.
- Fadden, M.C., E.R. Jr.: Pathogenesis of Asthma, *J. All Clin. Immunol*, 1984, 72, 413.
- Fish, E, James: Occupational Asthma —A spectrum of Acute Respiratory Disorders, *J. Occupational Medicine*, 1982, 24, 397.
- Gregg, Epidemiology of asthma. In Clark T.J.H., Godfrey, S., editors: *Asthma London*, 1977, Chapman and Hall, p. 214.
- Harrison M. Killey, A., Walter Smith J.A., Francis M.E. and Wood, B.S.: Cow milk protein intolerance—a possible association with gastro-enteritis, Lactose intolerance and 16A deficiency, *Br. Med. J.*, 1976, I, 1501.
- Hargreave, F.E. and Pepsy, J.; Allergic Respiratory reactions in bird fanciers provoked by allergen inhalation provocation test *J. Allergy, Clin. Immunol*, 1972, 50, 157.
- Horn, M.F.C. and Gregg, I.: The role of viral infection and host factors in asthma and chronic bronchitis. *Chest*, 1973, 32 (Suppl. 4) 44-8.
- Huber T.E., Joseph S.W., Knoblock E. et al *A.M.A. Arch. Ind. Hyg.* 1954, 10, 399.
- Indian Council of Medical Research, New Delhi; Morbidity survey of contributory health scheme beneficiaries; 1961.
- Kay, A.B., Maclean, C.M.U., Walkinson, A.H. and Gad Rab, M.O.: The prevalence of asthma and rhinitis in a Sudanese community seasonally exposed to a potent airborne allergen (the green nimitti midge, *cladotanytarsus lewisi*) 1983.
- Lessof M.H. and Buisseret, P.D.: Gastrointestinal Reactions. In M.H. Lessor (eds) *Immunological and clinical aspects of allergy*, 1981, pp. 141-177.

- Mishra, D.P.: Management of Bronchial Asthma. Thesis submitted in Lucknow University for M.D. (Tuberculosis) 1984.
- Patrick P.R.: In annual report—Health and Medical Services, Queensland, 1962 (1961-62), 39.
- Peckham, C. and Butler, N.: A national study of asthma in childhood, *F. Epid. Comm. Health*, 1978, 32, 79-85.
- Peypys, J., Roth, A. and Carroll, K.B.: Rash, skin and nasal tests and the history in grass pollen allergy. *Cl. Allergy*. 1975, 5, 431.
- Salvaggio, J., Seabury, J., Schoenhardt, E.A., et al. *J. Allergy Clinical Immunol*, 1971, 49, 6.
- Sharma, I.D.: A study of offending factors in aetiology of Allergic Bronchial Asthma. Thesis for M.D. (Tuberculosis) Lucknow University, 1980.
- Shivpuri, D.N.: Comparative evaluation of the sensitivity of the common methods of diagnostic antigen tests in patients of respiratory allergy, *Ind. J. Chest Dis*: 1962, 9, 102.
- Scadding, F.G.: Definition and clinical categories of Asthma. *Asthma* edited by T.J.H. Clark and S. Godfrey, 1983.
- Smith, J. Morrison: Prevalence and natural history of asthma in schoolchildren. *Br. Med. J.*, 1981, 1, 711-3.
- Stocks, P.: Studies on Medical and Population subjects No. 2. Sickness in the population of England and Wales in 1944-47. H.M. Stationery Office, London, 1949.
- Smith, J. Morrison, Harding, L.K. and Comming, G. The changing prevalence of asthma in schoolchildren. *Clin. Allergy*, 1971, 1, 57-61.
- Van Mickerk, C.H., Weinberg, E.G., Shore, S.C., Heese, H., de V. and Van Schalkwyk, D.J.: Prevalence of asthma: a comparative study of urban and rural Xhosa children. *Clin. Allergy*, 1979, 9, 319-24.
- Viswanathan, R., Prasad, M., Thakur, A.K., Sinha, S.P., Prakash, N., Mody, R.K., Singh, T.R.B.P.N. and Prasad, S.N., Epidemiology of asthma in an urban population. A random morbidity survey. *F. Ind. Med. Assoc.*, 1969, 46, 480-3.
- Verma, H.: Pollen and spores in relation to Allergic diseases. Thesis for Ph. D. (Botany) Lucknow University, 1982.
- Waite, D.A. Eyles, E.F., Tonkin, S.I., and O'Donnell, T.V.: Asthma prevalence in Tokelauan children in two environments. *Clin. Allergy.*, 1980, 10, 71-5.
- Welliver, R.C.: *J. Allergy Clin. Immunol*, 1983, 72, 341.
- Williams, D.A.: Social importance of allergic diseases. *Proceedings of First International Congress on Allergy*. S. Karger, Basel, 1951, pp. 42-64.

SIGNIFICANCE OF SOLUBLE ANTIGEN FLUORESCENT ANTIBODY TEST IN THE SERODIAGNOSIS OF TUBERCULOSIS*

USHA KIRAN SHARMA

Summary: SAFA test detected significant levels of antibodies to Mycobacterial Saline Extract (MSE) antigen, in new cases of pulmonary tuberculosis (bacteriologically positive and negative.) alike It was also useful in prognosis of disease process as the antibody level in 1 year treated cases showed correlation with healing process. Statistical analyses established a significant difference in the antibody response of proved cases of pulmonary and extrapulmonary tuberculosis, versus the healthy control-group). IHA test was also found to detect anti-PPD antibodies. However, the false positive and false negative reactions were found to be much higher, as compared to those obtained by SAFA test. IHA test also revealed a significant difference in the antibody response of pulmonary and extrapulmonary tuberculosis versus the healthy control group. SAFA test was found to be negative in cases of mycobacterioses and also it was found negative in the post-BCG vaccinated cases.

Medical and social problems often alter the distribution and impact of a disease. There is perhaps no disease of which this is truer than tuberculosis. The 'white plague' of centuries past has become a condition that is today not only treatable and curable, but preventable as well. Medical scientists from times immemorial have battled against tuberculosis, the so-called 'white plague'. The disease spelt certain death and its cause was wrapped in mystery. It was in 1882 that Robert Koch, first identified the etiological agent of tuberculosis. Over the 100 years that have elapsed since the first description of the tubercle bacillus, the history of advancing knowledge of tuberculosis has been interwoven with the history of progress in many areas of medical knowledge.

Although morbidity and mortality from tuberculosis have declined steadily for several decades, the disease persists as an important health problem in the world and more so in India. As most of the world still lives in poverty, it is not surprising that we have an estimated 10 million new sputum positive cases arising every year. Even in a country like United States, almost 30,000 new cases of pulmonary tuberculosis were reported in 1980, with 3,000 deaths ascribable to this disease (Collins, 1982). While the annual incidence rates of tuberculosis in some developed countries are coming to the level of 10 per 1,00,000 or even below, the rates in many developing countries are often 20 to 30 times greater, exceeding in some cases 300 per 1,00,000.

In India, nearly half the population gets infected and 5-10% of those infected, are liable to develop an overt disease at some period in their lifetime (Nair, 1975). According to the 8th

Tuberculosis Prevention Trial (ICMR 1980); a prevalence of 1068 bacillary cases per 1,00,000 in a South Indian population has been reported. The prevalence of disease has been found to be lower among females than among males and increases with increase of age in both sexes. Thus, tuberculosis is by no means uniformly distributed in the population. Case rates vary markedly by age, sex, race and geographic location. Socio-economic factors are also important determinants of tuberculosis.

The control of the disease depends on the following:

1. Persons ill with tuberculosis must be discovered and rendered non-infectious;
2. Persons who are infected but not ill must be prevented from developing the disease; and
3. Persons not yet infected must be prevented from acquiring infection.

The diagnosis of tuberculous infection and disease is dependent upon a history of exposure, positive tuberculin skin test, bacteriologic culture of the organism and roentgenographic findings compatible with tuberculosis. Bacteriologic tests for culture, identification and differentiation of the tubercle bacillus from other mycobacteria, are slow and tedious procedures requiring specially trained personnel and special laboratory facilities. The tuberculin test is subject to false positive and false negative reactions and to operator differences in application and interpretation. A further complication is reflected in the possibility that an existing delayed type hypersensitivity response can be

Department of Microbiology, All-India Institute of Medical Sciences, New Delhi 110029.

*This paper won the Chanchal Singh Memorial Award for 1984 and was presented at the 39th National Conference on TB and Chest Diseases held in Cuttack in January, 1985.

augmented during serial skin testing of patient population. Thus, there is tremendous need for a rapid diagnostic assay, capable of differentiating patients with active and inactive tuberculous disease from each other and from healthy tuberculin positive and tuberculin negative persons.

A serologic test that would indicate active tuberculosis with reasonable precision would be of great value in the numerous conditions in which tuberculosis is a part of the differential diagnosis and in the diagnosis of diseases of internal organs that are difficult to assess, such as the spine or the gut. Such a test would also save time required for culture of specimens containing too few tubercle bacilli, to yield a positive direct smear examination.

Since the first description of a serological test for tuberculosis by Middlebrook and Dubos in 1948, several attempts have been made to develop a technique which would discriminate efficiently between patients with active disease and those who have inactive disease or have never been infected with *M. tuberculosis*. Attempts to employ various serologic techniques such as the complement fixation, haemagglutination, simple agglutination, precipitation, flocculation and agar gel tests for this purpose have been reported by various investigators, but with little success. Much of the confusion and controversy that surrounds the status of serological reactions in diagnosis of tuberculosis is perhaps the result of the fact that relatively few of the antigenic constituents of tubercle bacilli have been defined with certainty. High degree of false positive reactions have been encountered in most of the serological reactions to detect antibodies using PPD and related antigens. Numerous workers have attempted to isolate a specific mycobacterial antigen. It has been considered that the availability of such an antigen would offer a significant new impetus to use of serodiagnostic techniques in mycobacterial diseases (Daniel, 1978).

The Soluble Antigen Fluorescent Antibody (SAFA) test, for the serodiagnosis of tuberculosis was introduced by Toussaint *et al* (1969). Affronti *et al* (1973) found that the test was useful in serological diagnosis of active tuberculosis. Bhardwaj (1982) evaluated six of the commonly used mycobacterial antigens for their use in the SAFA test. Of the six antigenic preparations, Mycobacterial Saline Extract (MSB) antigen was found to give better results with SAFA test. In a trial conducted on cases of pulmonary tuberculosis, serological corroboration was obtained in over 82 per cent of cases. However, it was found to be of limited

utility in the sero-diagnosis of disseminated and extra-pulmonary tuberculosis.

The present study was conducted with the following aims and objectives:

1. To evaluate the modified technique of Soluble Antigen Fluorescent Antibody (SAFA) test using Mycobacterial Saline Extract (MSB) antigen, in the laboratory diagnosis of tuberculosis.
2. To compare the value of modified SAFA test with indirect Haemagglutination test (Hoyden, 1951) in the laboratory diagnosis of tuberculosis.

Material & Methods

The Study Groups: The sera were obtained from the following groups of cases:

- (a) Proved cases of tuberculosis;
- (b) Non-tuberculous control subjects

Proved cases of Tuberculosis

This group comprised 224 cases which included:—

- (a) *Pulmonary Tuberculosis:* A total of 126 sera were collected from cases of pulmonary tuberculosis. Of these:
 - (i) 51 serum samples were, obtained from cases of bacteriologically proved pulmonary tuberculosis. The serum was collected prior to the initiation of antitubercular therapy. The tuberculosis in these cases was proved on clinical, bacteriological and radiological grounds.
 - (ii) 30 serum samples were obtained from cases of pulmonary tuberculosis who were bacteriologically negative on the basis of direct smear examination but there was irrevocable evidence of tuberculosis on the basis of clinical and radiological findings. The serum was collected prior to the initiation of antitubercular therapy.
 - (iii) 25 serum samples were obtained from cases of clinically, bacteriologically and radiologically proved cases of pulmonary tuberculosis who had completed 3 months of antitubercular treatment.
 - (iv) 20 serum samples were obtained from cases of pulmonary tuberculosis who

had completed one year of antitubercular treatment. These were clinically, radiologically and bacteriologically proved cases of pulmonary tuberculosis at the onset of therapy.

- (b) *Tuberculous Lymphadenitis*: 45 serum samples were obtained from cases of tuberculous lymphadenitis. All of these were proved tuberculous on the basis of histological findings. Acid fast bacilli were demonstrated in smear and/or culture in 5 of these.
- (c) *Abdominal Tuberculosis*: 28 serum samples were collected from proved cases of abdominal tuberculosis.
- Of these
- (i) the tuberculous etiology was proved on the basis of histopathological findings in 21.
- (ii) In 7 cases the tuberculous etiology could be established by radiological findings although histopathology revealed non-tuberculous lesions.
- (iii) *Osteo-Articular Tuberculosis*: 25 serum samples were obtained from cases of osteo-articular tuberculosis. The etiology was established on the basis of clinical and radiological findings.

Non-tuberculous control subjects

This group comprised 235 cases which included :

- (a) *Leprosy* : 17 serum samples were obtained from patients suffering from leprosy.
- (b) *Post BCG Vaccinated children* : 13 serum samples were obtained from healthy children, 6 weeks post-BCG vaccination.
- (c) *Non-tuberculous intestinal lesions* : 15 serum samples were obtained from cases suffering from intestinal lesions initially suspected to be tuberculous but later confirmed to be non-tuberculous.
- (d) *Non-specific cervical lymphadenitis*: 125 serum samples were collected from children having cervical lymphadenitis, initially suspected to be tuberculous infection but histopathological findings revealed non-tuberculous infection.
- (e) *Tuberculin positive healthy adults* : 20 serum samples were obtained from medical personnel working in a large hospital.

The individuals in this group did not have any subjective symptoms nor showed objective evidence of any disease. Mantoux test was carried out with 5 T.U. PPD RT-23. Those showing a positive tuberculin reaction (≥ 8 mm) were included in the study.

- (f) *Tuberculin negative children* : 15 serum samples were obtained from tuberculin negative children. Tuberculin testing was done with 5 T.U. PPD RT-23 and blood was collected from those showing a negative reaction after 48 hours.
- (g) *Non-tuberculous sick* : 20 serum samples were obtained from patients attending the outpatient department due to diseases other than tuberculosis. Of these 10 samples were taken from patients with pneumonia, primary and metastatic carcinoma of the lung, sarcoidosis etc. The rest were cases of Amoebiasis, malaria, diarrhoea and pyrexia of unknown origin.
- (h) *Neonates* : Blood was collected from severed umbilic cords of 10 neonates born to healthy mothers.

Soluble Antigen Fluorescent Antibody (SAFA) Test

Antigen : Saline extract of *M. tuberculosis* H₃₇ Ra was used as antigen (Cole et al, 1972). Prior to use, it was diluted to a final concentration of 500 µg/ml protein with 0.01 M Tris buffered saline (TBS) pH 8.0.

Test Procedure

Briefly, for every test serum two cellulose acetate paper discs (0.45 µm pore size) were taken. One of these (test disc) was impregnated with optimal dilution of mycobacterial antigen, whereas the other disc, without any antigen acted as the control. The discs were treated with fixative (Ethanol-HCl), washed and dried. Both the discs were moistened with 5 ml of 0.01 M TBS pH 8.0 then reacted with 40ml of test serum diluted 1:3. After incubation in a moist chamber for 45 mins. the discs were thoroughly washed in TBS. Next, the discs were treated with suitably diluted rabbit antihuman IgG for 45 minutes, washed and dried. Finally the discs were treated with optimal dilution of anti rabbit IgG (Goat) tagged with FITC, washed and dried. The fluorescence was measured using Aminco-Bowman's spectro-photofluorometer. The incident excitation of 495 nm emission at 550 nm and sensitivity X 10 were maintained constant all through the work.

One set of two discs (with and without mycobacterial antigen) were reacted with a standard

non-tuberculous serum sample. Results of all tests were compared against this standard serum and the fluorescence coefficient (F.C.) was calculated for each:

F.C. = A/B where

A = Reading of test serum disc with mycobacterial antigen *minus* reading of test serum disc without antigen. B = Reading of standard serum disc with mycobacterial antigen *minus* reading of standard serum disc without antigen.

A set of known positive and negative sera were included with each set. Test sera showing F.C. values more than $\text{mean} \pm 2\text{S.D.} (1.85 + 0.35)$ of the negative control group, were considered Strong Reactors. Thus the cases were classified as:

1. Strong Reactors - showing F.C. value 2.5 or more
2. Weak Reactors - showing F.C. value 1.5-2.4
3. Non - Reactors - showing F.C. value 1.4 or less.

Indirect Haemagglutination Test (IHA)

Anti PPD antibodies in the sera were estimated by IHA test of Boyden (1951) as modified to microlitre procedure by Sever (1962).

2.5% sheep red blood cells (SRBC) was prepared by adding 11.7 ml of physiological saline to 0.3 ml of packed sheep cells. The suspension was washed thrice in PBS pH 7.2. To 8 ml of 2.5% SRBC, 8 ml of 1:40,000 tannic acid was added and this was incubated at 37°C for 30 mins. To 3 ml of tanned cells, 9 ml of PPD (300 $\mu\text{g}/\text{ml}$) in PBS pH 6.4, was added and incubated for half an hour.

Serial two-fold dilutions of the inactivated test sera were prepared in the wells of microtitre plates. 1% Normal rabbit serum was used as the diluent. 0.023 ml of sensitized sheep cells (1.25%) were added to each well. Known positive and negative control sera were included in each lot of tests. The agglutination pattern was recorded after incubation at 37°C for 1 hour and again after overnight incubation at 4°C.

Results

Results of SAFA test

A total of 459 serum samples belonging to 224 proved cases of tuberculosis (pulmonary and extra pulmonary) and 235 controls were studied by the modified SAFA test.

The results of SAFA test in these groups as

obtained in the present study are described below:

Pulmonary Tuberculosis (untreated)

Of the 81 cases of pulmonary tuberculosis who had no history of any antitubercular therapy, 51 were bacteriologically positive for AFB. In 30 cases no AFB could be demonstrated on direct smear examination of sputum.

(a) AFB positive group

Thirty-five of the 51 AFB positive cases were suffering from far advanced disease while 16 were in moderately advanced state.

Thirty-two (91.4%) of the 35 cases suffering from far advanced tuberculosis were found to be strong reactors by SAFA test. The remaining 3 (8.6%) cases belonging to this group were weak SAFA reactors. Atypical mycobacteria were isolated from sputum culture of one of these (Table 1).

Of the 16 cases suffering from moderately advanced tuberculosis, 13 (81.2%) were found to be strong reactors, 2 (12.5%) gave a weak reaction while 1 (6.3%) was found to be non-reactor. The analysis of culture results showed that atypical mycobacteria were isolated from sputum culture of this patient. The mean F.C. value of this group was 3.61 ± 0.14 . The fluorescence coefficient (F.C.) values in the far advanced and moderately advanced cases did not show any difference in pattern. Also, no relationship was observed between the duration of symptomatic disease and F.C. values.

(b) A.F.B. negative group

Of the 30 cases where no AFB could be demonstrated on direct smear examination of the sputum, 13 had far advanced tuberculosis. All were found to be strong reactors by SAFA test. Of the 16 cases suffering from moderately advanced disease, 14 (86.5%) were strong reactors by SAFA test while the remaining 2 (13.5%) gave a weak reactor status. Only 1 case had minimal disease. However, this was found to be a strong reactor by SAFA test (Table 1).

The mean F.C. value obtained in this group (3.57 ± 0.18) was not found to be significantly different from that obtained in the AFB positive group (3.61 ± 0.14). No significant difference was observed in the F.C. value, when cases with minimal, moderately advanced or far advanced disease were compared.

Pulmonary Tuberculosis (Treated)

(a) Three months treatment

Of the 25 cases of pulmonary tuberculosis

TABLE I

Results of SAP A test Pulmonary Tuberculosis (Untreated)

Groups	No. of cases	Strong Reactors F.C. > 2.5		Weak Reactors F.C. between 1.5.-2.4.		Non-Reactors F.C. <1.4	
		No.	%	No.	%	No.	%
1. AFB Positive							
(a) Far advanced Tub.	35	32	91.4	2+1*	8.6	—	—
(b) Moderately advanced Tub.	16	13	81.2	2	12.5	1*	6.3
Total	51	45	88.24	5	9.8	1	1.96
2. AFB negative							
(a) Far advanced Tub.	13	13	100	—	—	—	—
(b) Moderately advanced Tub.	16	14	86.5	2	13.5	—	—
(c) Minimal Tub.	1	1	100	—	—	—	—
Total	30	28	93.33	2	6.66	—	—
Grand Total	81						

*Atypical Mycobacteria grown on sputum culture.

who had taken antitubercular therapy for 3 months, 13 had moderately advanced disease while 12 were suffering from far advanced tuberculosis (Table 2).

Twenty-one (84%) of the 25 were found to be strong reactors by SAFA test while 4 gave a weak SAFA reactor. None was found to be SAFA negative. The mean F.C. value (3.29 ± 0.18) was not significantly different from that obtained in the untreated group.

(b) One year treatment

Eleven (55 %) of the 20 cases of pulmonary tuberculosis who had completed one year of antitubercular therapy were found strong reactors by SAFA test. The remaining 45% belonging to this group were weak reactors and none was non-reactor by SAFA (Table 2).

The mean F.C. value (2.56 ± 0.19) was lower than the F.C. values of the preceding three

groups of cases. Lower F.C. values were recorded in cases showing signs of healing. Those with moderately advanced disease, showed higher F.C. value although it was lower than the untreated group.

Extrapulmonary Tuberculosis (Table 3)

(a) Tuberculous Lymphadenitis

Only 21 (46.6%) of the 45 cases of tuberculous lymphadenitis were strong reactors by SAFA test. Twenty (46.6%) were non-reactors giving F.C. value less than 1.5 and 4 (7.8%) were weak reactors (Table 3). The mean F.C. (2.0 ± 0.75) of this group was much lower than that obtained in cases of pulmonary tuberculosis.

(b) Abdominal Tuberculosis

A total of 28 cases of abdominal tuberculosis were studied which included 21 cases where

TABLE 2

Pulmonary Tuberculosis (Treated)

Groups	No of cases	Strong Reactors		Weak Reactors		Non Reactors		No. of
		No.	%	No.	%	No.	%	
1. 3 months treatment								
(a) Far advanced Tub.	14	12	85.7	2	14.3	—		
(b) Moderately advanced Tub.	11	9	81.8	2	18.2	—		
Total	25	21	84	4	16			
2. 1 year treatment								
(a) Moderately advanced Tub.	6	5	83.3	1	16.7	—		
Clearing	10	5	50	5	50	—		
change	4	1	25	3	75	—		
Total	20	11	55	9	45			
Grand Total	45							

TABLES

Extra Pulmonary Tuberculosis

Groups	Total No. of cases	Strong Reactors		Weak Reactors		Non-Reactors	
		No.	%	No.	%	No.	%
1. Tuberculous lymphadenitis	45	21	46.7	4	8.9	20	44.4
2. Abdominal Tuberculosis	28	23	82.1	4	14.3	1	3.6
3. Osteoarticular Tuberculosis	25	23	92.0	2	8.0		

the histopathological findings along with radiography, confirmed the presence of tuberculous lesions.

Seventeen (80.95%) of the 21 histopathologically proved cases, were strong reactors by SAFA test (Table 3). Only 1 (4.8%) was non-

reactor who also gave a negative Mantoux reaction and *M. fortuitum* was isolated from the biopsy on culture.

The mean F.C. value of the whole group (3.69 ± 0.30) was similar to that of pulmonary tuberculosis (untreated) group.

TABLE 4

Non-tuberculous Patients

Groups	Total No. of cases	Strong Reactors		Weak Reactors		Non-Reactors	
		No.	%	No.	%	No.	%
1. Leprosy	17	1	5.9	2	11.8	14	82.4
2. Non-TB intestinal lesions	15	2	13.3	7	46.7	6	40.0
3. Non-specific Cervical Lymphadenitis	126	8	6.3	16	12.7	102	81.0
4. Chest Infection other than TB	20	3	5.0	10	50.0	7	35.0

(c) Osteoarticular Tuberculosis

Of the 25 cases of osteoarticular tuberculosis, 23 (92%) were strong reactors by SAFA test, Two cases gave a weak reaction. All had a history of antitubercular treatment for over 1-2 years and had shown very little signs of healing.

The mean F.C. value of the group was (4.25 ±0.23) also comparable to that of pulmonary tuberculosis.

Nontuberculous Patients*(a) Leprosy*

Only one of the 17 cases of the Leprosy studied, was found to give strong reaction by SAFA (Table - 4). Two (11.77%) were weak reactors while 14 (82.36%) were non-reactors. The mean F.C. value was 1.8±0.19.

(f) Non T.B. intestinal lesions

Of the 15 cases of non-tuberculous intestinal lesions, two (13.3%) were strong reactors, 7(46.7%) were weak reactors, while 6(40.0%) were non-reactors. The mean F.C. value of the group was 1.61±0.21.

(c) Non-specific cervical lymphadenitis

One hundred and twenty-six cases of non-tuberculous cervical lymphadenitis were studied. Of these only 8 (6.4%) were strong reactors by SAFA test. 102 (81.6%) were non-reactors in this group. The mean F.C. value of the group was 1.81 ±0.13.

(d) Chest Infections other than tuberculosis

Only 3(15%) of the 20 cases of chest infection

studied, were found to give a strong reaction by the SAFA test. However, 10(50%) were weak reactors and only 7(35%) were nonreactors by this test. The mean F.C. (1.85±0.19) was slightly higher than the other non-tuberculous groups studied (Table 4).

Healthy Controls (Table 5)

Of the 58 healthy control cases (13 post-BCG vaccinated children; 20 Tuberculin positive healthy adults, 15 Tuberculin negative children and 10 neonates), only 4 (6.9 %) were strong reactors by the SAFA test. None of the post-BCG vaccinated children and neonates were strong reactors. Two (10%) of the Tuberculin positive adults were strong reactors while 14 (70%) were weak reactors by SAFA test. Of the 15 tuberculin negative children, 33% were weak reactors while 54 % were nonreactors to SAFA. The mean F.C. value was least among the neonates(0.72±0.17). The tuberculin positive (1.85±0.17) and tuberculin negative (1.83±14) groups did not show any significant difference in the mean F.C.

Indirect Haemagglutination Test

In all the cases studied by SAFA test, anti-tuberculo-protein antibodies¹ were also estimated by the Indirect Haemagglutination reaction using PPD sensitized sheep erythrocytes. The sera showing antibody titre of more than mean ±2 SD of the healthy control group, were considered as positive. Thus an IHA titre of 1:40 or more was taken as strong reaction. Antibody titres of 1:20 was considered as weak reaction. In the proved cases of pulmonary and extra-pulmonary tuberculosis, the serum titre ranged from 1:640 to no reaction at all.

TABLE 5 *Healthy**Controls*

Groups	Total No. of cases	Strong Reactors		Weak Reactors		Non-Reactors	
		No.	%	No.	%	No.	%
1. Tuberculin positive adults	20	2	10.0	14	70.0	4	20.0
2. Tuberculin negative children	15	2	13.3	5	33.3	8	53.34
3. Post BCG children	13	—	—	2	15.4	11	84.6
4. Neonates	10	—	—	2	20.0	8	80.0
Total	58						

TABLE 6

Results of IHA test: Pulmonary Tuberculosis (untreated)

Groups	Total No of cases	Strong Reactors		Weak Reactors		Non-Reactors	
		No.	%	No.	%	No.	%
L AFB Positive							
(a) Far advanced Tub.	35	16	45.5	13	36.2	6	18.2
(b) Moderately advanced Tub.	16	10	62.5	5	31.2	1	6.3
(e) Minimal Tub.	—	—	—	—	—	—	—
Total	51	26		18		7	
2. AFB Negative							
(a) Far advanced Tub.	13	9	69.2	3	23.0	1	7.8
(b) Moderately advanced Tub.	16	4	25.0	7	43.8	5	31.2
(c) Minimal Tub.	1	—	—	—	—	—	—
Total	30	13		10		7	
Total	81						

Of the 51 AFB positive untreated cases of pulmonary tuberculosis, 26 (50.9%) showed a significant level of anti-tuberculo-protein antibodies (1:40). In 18 (35.3%) serum samples, low titre of antibodies was observed, while no antibodies were demonstrated in 7(13.7%) of the proved cases of pulmonary tuberculosis belonging to group I. The two cases of mycobacteriosis were weak reactors by IHA (Table 6).

Significant level of anti-PPD antibodies were also detected in 13 (43.3%) of the 30 cases where although no AFB were demonstrated in the direct smear examination of sputum, but there was irrevocable evidence of pulmonary tuberculosis on the basis of radiography and clinical findings. Low levels of antibodies were detected in 2 (6.66%) cases of this group while 7(23.3%) cases showed no anti-PPD antibodies (Table 6).

IHA test detected circulating antibodies to PPD in 40 % of the cases of pulmonary tuberculosis who had received antitubercular therapy for 3 months (group III). Low levels of anti-PPD

anti-bodies were detected in the remaining 60% (15 of the 25 cases) of the cases belonging to this group (Table 7).

High levels of anti-PPD antibodies were also detected in 19 (95%) of the 20 cases of pulmonary tuberculosis who had received anti-tubercular therapy for 1 year only. In one case the antibody titre was found to be low.

In 44.2% of the cases of tuberculous lymphadenitis, significantly high levels of circulating anti-PPD antibodies were detected. No antibodies were detected in 53 % of the cases studied while in 9 % of the cases low levels of anti-PPD antibodies were detected (Table 8).

Of the 28 cases of abdominal tuberculosis, significantly high levels of anti-PPD antibody were detected in 16 (57.1%) cases. Low levels of anti PPD antibodies were detected in 3 (10.7 %) of the cases while no antibodies were detected in 9 (32.2%) of the case.

IHA test detected high level of antibodies in 14 (56%) cases of osteoarticular tuberculosis.

TABLE 7

Results of IHA test : Pulmonary Tuberculosis (treated)

Groups	Total No. of cases	Strong Reactors		Weak Reactors		Non-Reactors	
		No.	%	No.	%	No.	%
1. 3 months treatment							
(a) Far advanced Tub.	14	9	64.2	5	35.8	—	—
(b) Moderately advanced Tub.	11	1	9.0	10	91.0	—	—
(c) Minimal Tub.	—	—	—	—	—	—	—
Total	25	10		15			
2. 1 year treatment							
(a) Moderately advanced Tub.	6	5	83.3	1	16.7	—	—
(b) Clearing	10	10	100.0	—	—	—	—
(c) No change.	4	4	100.0	—	—	—	—
Total	20	19		1			

TABLE 8

Results of IHA Test : Extrapulmonary Tuberculosis

Groups	Total No. of cases	Strong Reactors		Weak Reactors		Non-Reactors	
		No.	%	No.	%	No.	%
1. Tuberculous lymphadenitis	45	19	42.2	3	6.7	23	51.1
2. Abdominal tuberculosis	28	16	57.14	3	10.7	9	32.6
3. Osteoarticular tuberculosis	25	14	56.0	5	20.0	6	24.0

TABLE 9

Results of IHA Test : Non-Tuberculous Sick

Groups	Total No. of cases	Strong Reactors		Weak Reactors		Non-Reactors	
		No.	%	No.	%	No.	%
1. Leprosy	17	6	35.3	2	11.76	9	52.9
2. Non-TB intestinal lesions	15	5	33.3	2	13.3	7	40
3. Non-specific cervical lymphadenitis	126	20	16	8	5.6	98	78.4
4. Chest infection other than TB	3	15	9	45	8	40	

20% (5) of the cases were found to show a low antibody response while no antibodies were detected in 24% (6) of the cases.

Significant level of anti-PPD antibodies were also detected in 6 (35 %) cases of leprosy, 5(33.3%) cases suffering from intestinal lesions of non-tuberculous etiology, 20(16%) of the cases of non-specific cervical lymphadenitis and 3 (15%) of the cases of chest infections other than tuberculosis (Table 9).

Demonstrable levels of circulating anti-PPD antibodies were also detected in 11 (23 %) of the healthy control subjects. High levels of antibodies were detected in 3 (23%) 6 weeks post BCG vaccinated children, 5 (25 %) healthy tuberculin positive cases and 3 (20 %) tuberculin negative children. However, none of the 10 neonatal sera tested showed anti-PPD antibodies (Table 10).

SAFA vs. IHA

Of the 51 bacteriologically proved, untreated pulmonary tuberculosis belonging to group I, anti-PPD antibodies were detected only in 50.9% by the IHA test whereas SAFA test detected antibodies in 88.2% of the cases belonging to this group (Table 10).

IHA detected significant level of antibodies in 43.3% of the 30 AFB negative, untreated cases of pulmonary tuberculosis (group II). On the other hand, significantly high level of antibodies was detected in 93.3% of the cases belonging to this group by SAFA test.

Among the cases of pulmonary tuberculosis (group III) who had completed three months of antitubercular therapy, circulating antibodies to PPD were detected in 40% of the cases whereas after 1 year of antitubercular therapy (group IV) anti-PPD anti-bodies were detected in 95 %

of the cases studied. In contrast, the results of SAFA test indicated a high level of antibodies in 84 % of the cases after 3 months treatment and only 55 % of the cases demonstrated a significant level of antibodies by SAFA test, at the end of the year of treatment (Table 12).

Considering the cases of extrapulmonary tuberculosis, IHA test detected a significant level of antibodies in 44.2 % cases of tuberculous lymphadenitis, 57 % cases of abdominal tuberculosis and 56% cases of osteoarticular tuberculosis (Table 13). On the other hand, SAFA

TABLE 10

Healthy Controls

Groups	Total No. cases	Strong Reactors		Weak Reactors		Non- Reactors of	
		No.	%	No.	%	No.	%
1. Tuberculin positive adults	20	5	25.0	8	40.0	7.0	35
2. Tuberculin negative children	15	3	20.0	5	33.3	7	46.6
3. Post BCG vaccinated children	13	3	23.1	2	15.4	8	61.5
4. Neonates	10	—	—	4	40.0	6	60.0

TABLE.11

*SAFA Vs IHA**Pulmonary Tuberculosis (untreated)*

Groups	Total No. of cases	Strong Reactors		Weak Reactors		Non-Reactois	
		SAFA	IHA	SAFA	IHA	SAFA	IHA
1. AFB positive	51	88.2%	50.9%	9.8%	35.3%	2.0%	13.7%
2. AFB negative	30	93.3%	43.3%	6.7%	33.3%	—	21.7%

TABLE 12

Pulmonary Tuberculosis (treated)

Groups	Strong Reactors		Weak Reactors		Non Reactors	
	SAFA	IHA	SAFA	HAI	SAFA	IHA
1. 3 months treatment	84%	40%	16%	60%	—	—
1 year treatment	55%	95%	45%	5%	—	—

test detected high levels of antibodies in 46.6% of the cases of tuberculosis lymphadenitis, 82% of the cases of abdominal tuberculosis and 84 % cases of ortheoarticular tuberculosis.

Significant levels of anti-PPD antibodies were detected by the IHA test in 15-35 % of the cases of leprosy, non-tuberculous intestinal lesions and non-specific cervical lymphadenitis. The SAFA test detected antibodies in 6-15% of these cases only (Table 14).

IHA test detected antibodies in 23 % of the healthy controls including tuberculin positive healthy adults, tuberculin negative and 6 weeks post-BCG vaccinated children. SAFA test detected antibodies in only 8% of the healthy controls studied (Table 15).

No antitubercular antibodies were detected

in the 10 neonatal sera tested both by SAFA test as well as IHA.

Sensitivity and Specificity of SAFA versus IHA

The sensitivity and specificity of SAFA and IHA were calculated as follows:

$$\text{Sensitivity} = \frac{a}{a + b}$$

$$\text{Specificity} = \frac{d}{c + d} \text{ where,}$$

a=No. of tuberculous cases positive by the test.

b=No. of tuberculous cases negative by the test.

c=No. of healthy controls positive by the test.

d=No. of healthy controls negative by the test.

TABLE 13
SAFA Vs IHA
Extrapulmonary Tuberculosis

Groups	Strong Reactors		Weak Reactors		Non-Reactors	
	SAFA	IHA	SAFA	IHA	SAFA	IHA
1. TB lymphadenitis	46.6%	44.2%	9.3%	6.7%	46.5%	49.1%
2. Abdominal Tuberculosis	82.1%	57.1%	14.3%	10.7%	4.8%	32.6%
3. Osteoarticular tuberculosis	84.0%	56.0%	12.0%	20.0%	4.0%	24.0%

TABLE 14 SAFA Vs IHA
Non*tuberculous Disease Controls

Groups	Strong Reactors		Weak Reactors		Non	-Reactors
	SAFA	IHA	SAFA	IHA	SAFA	IHA
1, Leprosy	5.9	35.3	11.8	11.8	82.34	52.9
2. Non-TB Intestinal lesions	13.3	33.3	46.7	13.3	40.0	40.0
3. Nonspecific cervical lymphadenitis	6.4	16.0	12.8	5.6	81.6	78.4
4. Chest Infections other than tuberculosis	15.0	15.0	50.0	45.0	35.0	40.0

The figures indicate percentages

TABLE 15

Healthy Conrail

Groups	Strong Reactors		Weak Reactors		Non -Reactors	
	SAFA	IHA	SAFA	IHA	SAFA	IHA
1. Tuberculin positive adults	10.0	25.0	70.0	40.0	20.0	35.0
2. Tuberculin negative children	13.3	20.0	33.3	33.3	53.3	46.6
3. Post BCG vaccinated children		23.1	15.4	15.4	84.6	61.5
4. Neonates		—	20.0	40.0	80.0	60.0

The figures indicate percentage.

TABLE 16

Sensitivity & Specificity

Group	Sensitivity		Specificity	
	SAFA	IHA	SAFA	IHA
1. Pulmonary Tuberculosis	0.83	0.54	0.90	0.77
2. Extrapulmonary Tuberculosis	0.65	0.51	0.92	0.80

The SAFA test was found to be much more sensitive than IHA when cases of pulmonary tuberculosis were considered (SAFA - 0.83, IHA - 0.54). In the extrapulmonary cases also, SAFA test was found more sensitive than IHA (SAFA-0.65; IHA - 0.51) although the sensitivity of SAFA in this case was found to be less than that obtained in cases of pulmonary tuberculosis. The sensitivity of IHA test did not show variation when cases of pulmonary and extra pulmonary tuberculosis were compared. The specificity of SAFA test was also found to be higher than that of IHA test (Table 16).

Discussion

In applying any serodiagnostic test to a clinical problem, most frequently the purpose is to detect in the serum of the patient antibodies, reasonably assumed to be present as a result of sufficient exposure to a specific disease agent. Beyond this, it is desirable, if possible, to deter-

mine an approximate quantitative measure of this antibody.

Ideally, a diagnostic test should be simple to perform, sensitive, specific and indicative of the active disease (Glenchur and Kettel, 1965); Snider D.E. 1982). Stability of reagents, reproducibility of results (Cole *et al* 1972) and correlation of the degree of results with the clinical progress of disease constitute additional features of a good diagnostic test. In tuberculosis, a serological test is required to detect the active disease rather than a state of past infection i.e. it should differentiate between antibodies of active infection by *M. tuberculosis* and those resulting from vaccination or induced by infection with atypical mycobacteria (Diena, 1971)) Almost all the standard serological techniques like complement fixation, indirect, haemagglutination, precipitation, primary antibody binding radioimmunoassay, ELISA, fluorescent antibody test have been tried so far. The

present study was designed to compare the modified SAFA test (Bhardwaj, 1978) with IHA (Boyden, 1951) test.

Modified SAFA test as a serodiagnostic test in Tuberculosis

It has been already emphasised in many studies that no serological test can be expected to give 100 % positivity in any disease as some individuals do not respond to the antigenic stimulus. The significance of antibodies has been debated to mycobacteria in human tuberculosis. In tuberculosis, because of irregular and unpredictable proliferation of tubercle bacilli, the level of humoral immune response is not related to the degree of infection and the duration of disease (Parlett, 1950). The rise of antibody to a maximum may have occurred by the time the activation of disease is demonstrable by roentgenography and the patient is checked in for serological study. The results of SAFA test obtained in this study appear to be well in conformity with the acceptable criteria for a diagnostic test.

SAFA in pulmonary Tuberculosis (Untreated)

The results of SAFA test in cases of sputum positive untreated, pulmonary tuberculosis, reveal that this test picked up cases with minimal disease as well as those having moderately to far advanced disease process, with equal reliability. Also the test was discriminative of infection with *M. tuberculosis* and that caused by atypical mycobacteria. The two cases where atypical mycobacteria were isolated on culture, one was found to be weak reactor and the other non-reactor by SAFA test. However, this needs to be further evaluated.

In the absence of demonstrable causal mycobacteria, a definite diagnosis on the basis of results obtained by SAFA test could be made in 28 out of 30 cases. The results obtained in this group were found to be similar to those obtained in the sputum positive cases, as shown by little difference in the mean F.C. value of the two groups. These results further prove the reliability of SAFA test as a serodiagnostic test in tuberculosis.

SAFA in pulmonary tuberculosis (Treated)

The results of SAFA test in cases of pulmonary tuberculosis prior to the initiation of chemotherapy, 3 months after chemotherapy and 1 year after chemotherapy, reveal that demonstrable levels of antibodies to Mycobacterial saline extract antigen could be detected by SAFA test in most of the patients with new tuberculosis prior to the initiation of chemotherapy. After 3 months of chemotherapy signi-

ficant levels of antibodies could be detected in all the cases of TB. The level of circulating antibodies was high in most of the cases (21/25) while in some (4/25) the level of antibodies was slightly lower. After completion of 1 year of chemotherapy also, varying levels of antibodies could be detected in all the cases studied. However, the number of cases with high level of antibodies (strong reactors) had considerably decreased at this time although low levels (weak reactors) of antibodies were still demonstrable in all the remaining cases. This decrease in antibody level after chemotherapy could possibly be explained by the recent finding of Elmer and Daniel (1979) reporting that mycobacterial arabinomannan has demonstrable immunosuppressive properties. These polysaccharides are released into the system after the cell death following chemotherapy. This suppressor cell activity may also be responsible for the depressed antibody response following chemotherapy.

SAFA in Extrapulmonary Tuberculosis

(a) Tuberculous lymphadenitis

In India, tuberculous cervical lymphadenitis is a common disease entity and is frequently seen in the paediatric age group (Pamra *et al* 1977). The disease carries high morbidity, laboratory diagnosis of tuberculous lymphadenitis is difficult since it is not easy to demonstrate Mycobacteria in the lymph node either by smear and/or culture or by modified Ziehl-Neelson staining of tissue sections.

The modified SAFA test which showed promising results in the diagnosis of pulmonary tuberculosis was extended for the serodiagnosis of tuberculous lymphadenitis. An analysis of results of SAFA test revealed high level of antibodies in 45 % of the cases of tuberculous lymphadenitis. The low antibody response in the remaining cases could be attributed to the poor nutritional status and hence the poor immune response in these cases. It was found that in two cases atypical mycobacteria were isolated on culture of the lymph node. Sera from these cases were negative by SAFA test. This provides additional evidence that SAFA test detected antibodies specifically against *M. tuberculosis*. Since lymph node culture is not very rewarding in establishing the etiology of infection due to *M. tuberculosis*, SAFA could be used as an additional test along with histopathological findings to confirm the diagnosis of disease.

(b) Abdominal Tuberculosis

The results of SAFA test were highly promising when cases of abdominal tuberculosis

were studied. In all the cases of abdominal tuberculosis investigated by SAFA test, the diagnosis was serologically corroborated in 82% (23/28) cases. In addition 14.3% weak reactor status pointed to *M. tuberculosis* as being the possible etiological agent of the disease. Negative results were encountered in one case only. The lowered immune response in this patient was also confirmed by a negative tuberculin reaction. *M. fortuitum* was isolated on culture of infected tissue from this patient.

(c) Osteoarticular Tuberculosis

On the 25 cases of osteoarticular tuberculosis studied, the diagnosis was serologically confirmed by SAFA test, in 92% (23/25) cases. In the remaining 8% or 2 cases, low level of antibodies was detected by this test. Negative results were not recorded in any of the cases studied.

All the cases of osteoarticular disease due to *M. tuberculosis* included in this study were old cases of tuberculosis with a history of at least 6-8 months of chemotherapy. Increased ESR (> 50 mm) was observed in all the cases. The increased titre of antibodies in these cases, could not have resulted from prolonged treatment in these cases, since no difference in F.C. value was observed when cases who had taken chemotherapy for 20-22 months were compared to those who had taken antitubercular treatment for 6-8 months only.

SAFA test in Non-tuberculous sick

The results of SAFA test, in respect of cases suffering from diseases other than tuberculosis, were found to vary in the various groups of cases studied. Overall false positive reactions were observed in 5-15% of the cases. This is consistent with the known fallacies encountered as inherent in any serological procedure.

SAFA in Healthy Control Subjects

Exposure to saprophytic mycobacteria in the environment may account for some of the antibodies observed in normal healthy subjects (Bardana *et al* 1973). The presence of such organisms in tap water, natural water and soil would facilitate such a continuous and universal exposure. The role of these antibodies in normal subjects is speculative. 2/20 (10%) healthy tuberculin positive subjects were found to be strong reactors by SAFA test while a majority 14/20 (70%) were weak reactors. All the cases included were employed in microbiology laboratory and the two strong reactors in this group were handling mycobacteria routinely although there were no clinical signs of any disease.

Among the tuberculin negative individuals also 13.3% (2/15) were found to be strong reactors. Antibodies in sera from the tuberculin negative group are in accord with some reports of antibody production following exposure to PPD (Daniel, 1965; Bardana *et al* 1973).

Parlett and Youmans (1959) and Matsumara *et al* (1968) have reported that BCG vaccination may be associated with a high frequency of persistent humoral antibodies as well as with cellular immunity. In contrast, in our study no antibodies were detected in 85% (11/13) of the cases 6 weeks post-BCG vaccination. Weak reactor status could be detected in 15% (2/13) only. Low false positive reaction after BCG, is one of the criteria for a good serological test in tuberculosis. However, the number of cases included in this study was too small for a categorical inference.

In the ten cord blood samples from neonates, there were no false positive reactions. In two cases where the tuberculin reaction of mother was more than 10 mm, a weak reactor status was observed, while in all the others (8/10) no antibodies were detected.

Thus it appears reasonable to conclude that the results of modified SAFA test were more specifically related to the existence of active disease and infection with *M. tuberculosis* as compared to those obtained with indirect haemagglutination test.

REFERENCES

- Affronti, L.F.; Fife, E.H. and Grow, I.: Serodiagnostic test for tuberculosis. *American Review of Respiratory Disease*; 1973, 107:822-825.
- Bardana, E.R. Jr.; McClatchy, J.K. and Farr, R.S. Universal occurrence of antibodies to tubercle bacilli in sera from non-tuberculous and tuberculous individuals. *Clinical and Experimental Immunology*; 1973, 13:65-77.
- Bhardwaj, O.P. & Shrinivas: Soluble Antigen Fluorescent Antibody test in the serodiagnosis of tuberculosis: Selection of antigenic preparation. *Indian Journal of Medical Research*; 1982, 76: 5-9.
- Boyden, S.V.: The adsorption of Proteins on erythrocytes treated with tannic acid and subsequent haemagglutination by antiprotein sera. *Journal of Experimental Medicine*; 1951, 93: 107-120.
- Cole, R.V.; Lazarus, A.W. and Hedrick, H.G.: Development and evaluation of simple latex agglutination test for diagnosis of tuberculosis. *Applied Microbiology*; 1972, 24: 525-534.

- Collins, P.M.: The Immunology of Tuberculosis. American Review of Respiratory Diseases; 1982, 125 (3): 42-49.
- Daniel, T.M.: Observations on antibody response to Mycobacterial antigen. Journal of Immunology; 1965, 95: 100-108.
- Daniel, T.M. and Janicki, B.W.: Mycobacterial antigens. A review of their isolation, chemistry and immunological properties. Microbiological Reviews; 1978, 42: 84-113.
- Diena, B.N.: Problems in sero-diagnosis of tuberculosis. Annals of Internal Medicine; 1971, 75: 132-133.
- Glenchur, H. and Kettel, I.J.: A study of Agar gel double diffusion test in human tuberculosis. American Review of Respiratory Diseases; 1965, 91: 89-96.
- ICMR "Tuberculosis Prevention Trial, Madras-Trial of BCG vaccines in South India for Tuberculosis Prevention IJMR; 1980, 72 (Suppl.) 1-74.
- Pamra, S.P.; Goyal, S.S. and Mathur, G.P.: Extent and pattern of tuberculosis amongst children. Indian Journal of Tuberculosis; 1977, 24: 75-77.
- Parlett, R.C. and Youmans, G.P.: An evaluation of the specificity and sensitivity of a gel double diffusion test for tuberculosis. A double blind study. American Review of Respiratory Disease; 1959, 80: 153-166.
- Snider, D.E. Jr.: The Tuberculin test. American Review of Respiratory Diseases; 1982, 125 (3): 108-118.
- Toussaint, A.J.; Fife, E.H.; Parlett, R.C.; Affront!, L.F.; Wright, G.L.; Reich, M. and Morse, W.C.; A soluble Antigen fluorescent Antibody test for serodiagnosis of *M. tuberculosis* infection. American Journal of Clinical Pathology; 1969, 52: 708-713.
- Ellner J.J.; Daniel T.M.: Immunosuppression by Mycobacterial arabinomannan Clinical and Experimental Immunology; 1979, 35: 250-7.
- Nair, A.: Progress in control of tuberculosis—Drug therapy: a key role. Indian Journal of Tuberculosis; 1975, 22 73-75.

RIFAMPICIN: BIOLOGICAL VALUES FOR ACTION AND INTOLERANCE*

P.K. SEN, B.N. ROY and R. CHATTERJEE

Summary: Biological factors including "Peak Concentration (PC)" Peak Hour of Concentration (PH), Half Life (HL) and Effective Concentration Coverage (E CC) of Rifampicin were estimated. "Minimum Inhibitory Concentration (MTC)", was taken as 1.00 meg/ml. Rifampicin attains not only a higher concentration in the Eastern region than in the others but also its effectiveness or strength of Peak Concentration (PC/MIC) comes close to that of 1NH and single dose of each drug administered at the interval of two hours will also help in attaining synchronised Peak Concentration.

It appeared to the authors that intolerance to rifampicin appear to be more frequent in the Eastern region than what has been recorded or experienced in other places, even though no controlled clinical comparative study has been made with" regimens containing rifampicin and without it. But hundreds of cases where rifampicin had been introduced in a drug regimen, reactions, mostly gastro-intestinal like nausea, vomiting, anorexia, etc. appeared and when this drug was withdrawn (retaining others) symptoms abated. On reintroduction of the drug after a few weeks, similar reactions reappeared. Sometimes such trials were made introducing and withdrawing the drug several times and each time re-introduction of the drug was followed by re-appearance of similar reactions.

The cause of these unwanted reactions may be inherent in the preparation of the drug. It also seemed reasonable to assume that the biological factors may also be one of the reasons for such reactions. With this idea, this biological study was carried out.

Material and Methods

Nineteen hospitalised tuberculous patients, all of them having rifampicin, were accepted for this study. Their age ranged from 19 to 74 years. All but one were males. The weight of all the patients was under 50 Kg.

All the drugs were withheld from patients for 3 days. To lessen the number of venipunctures, which are generally disagreeable to the patients, and at the same time to include larger number of specimens at different times the subjects were divided into two groups of 9 and 10 each.

On the day of experiment a sample of blood was drawn at 7.00 a.m. for use as the "Blank" and then the patients were given orally on empty stomach 450 mg. rifampicin. Thereafter the blood was drawn from one group at 8. a.m., 10 a.m. and 2 p.m. that is, at 1,3, and 7 hours

after drug administration and from the other group at 9 a.m., 11 a.m. and 3 p.m. that is 2, 4, and 8 hours after drug administration.

Serum was separated from the blood samples and serum level of rifampicin was estimated by the method of Sunahara and Nakagawa (1972).

Mixture of 1 ml. of serum, 1 ml. distilled water and 1 ml. of 0.15 M phosphate buffer at PH 7, was extracted with 2 ml. isoamyl alcohol. Then the intensity of colour was measured at 475 mμ wave length in a spectrophotometer. Concentration of rifampicin was estimated from a standard curve previously drawn for this purpose.

Ten mg. powdered rifampicin taken from a rifampicin capsule was dissolved in a minimum quantity of methanol in 10 ml. volumetric flask. The volume was made up to the mark with distilled water. From this 10, 20, 30,40 and 50 mg/ml of rifampicin concentration were made with distilled water. The procedure of Sunahara and Nakagawa was followed with 1 ml. of each of these solutions in the same way as it was carried out with 1 ml. of serum. The reading was taken in the spectrophotometer at 475 mm wave length and was plotted against concentrations in a graph paper.

Half-life was determined by placing the log. concentrations of the drug of 19 patients at different hours in a graph paper and calculated according to the following formula.

$$\text{Half-life (t}_{1/2}\text{)} = \frac{0.693}{K} \text{ where } K \text{ is } \frac{2.303}{t_1 - t_0} \times \log \frac{C_0}{C_1}$$

The pattern of fall of the concentration of the drug in the blood from peak concentration (P.C) to minimum inhibitory concentration (MIC) was taken into account to determine the duration of the effectiveness of the drug or effective concentration coverage (ECG).

(B.C. Roy Tuberculosis and Chest Diseases Research Institute, Calcutta)

*Paper presented at the 39th National Conference on TB & Chest Diseases held at Cuttack in January 1985.

Results

The serum concentration of rifampicin of 19 tuberculous patients in two separate groups of 9 and 10 patients in each, is presented in Table I.

The findings show that the concentration

Ranged from 7.25 to 22.7 mcg/ml at 1st hour 8.00 to 25.75mcg/ml at 3rd hour, and 0 to 14.00 mcg/ml at 7th hour in the 1st group. The average serum concentrations of rifampicin in 9 tuberculous patients in 1st group at 1, 3 and 7 hour after drug administration were 12.5, 15.7 and 9.0 mcg/ml respectively

TABLE I

Serum concentration (mcg/ml) of rifampicin of 17 tuberculous patients

No. of Patients.	Hours after drug aciministiation						
	1	3	7	2	4	8	
1st GROUP	1.	8.5	14.0	3.25			
	2.	8.5	14.0	9.25			
	3.	13.25	8.0	0			
	4.	x	19.0	9.5			
	5.	7.25	11.5	7.5			
	6.	16.0	19.5	14.0			
	7.	16.0	12.0	7.0			
	8.	22.75	25.75	13.5			
	9.	10.0	18.0	8.5			
2nd GROUP	1.				15.0	20.0	4.0
	2.				15.5	15.5	10.0
	3.				12.0	13.75	9.25
	4.				x	12.5	8.0
	5.				18.0	12.5	7.0
	6.				18.5	18.7	13.0
	7.				7.0	10.0	5.5
	8.				15.0	18.0	14.0
	9.				15.5	12.5	8.0
	10.				14.0	14.7	8.5
MEAN	12.5	15.7	9.0	14.0	14.7	8.5	

The concentrations varied from 7.0 to 18.5 meg/ml at 2nd hour, 10.0 to 20.0 meg/ml at 4th hour, and 4.0 to 14.0 meg/ml at 8th hour in the 2nd group. The average serum concentrations of rifampicin in 10 tuberculous subjects in the 2nd group were 14.0, 14.7 and 8.5 meg/ml at 2, 4 and 8 hours respectively. Thus the average serum concentration of rifampicin taking 2 groups together was found to be 12.5, 14.0, 15.7, 14.7, 9.0 and 8.5 meg/ml at 1, 2, 3, 4, 7 and 8 hours respectively.

The average half-life was calculated to be 5.41 hours. Taking 1 meg/ml as minimum inhibitory concentration (MIC) *in vitro*, the effective concentration coverage (ECC) of rifampicin is about 24 hours. Table II shows the average biological values of rifampicin in this area.

Discussion

On a comparative study with such findings on 13 patients in Tokyo and on 6 patients in London, the findings presented hereunder in Table III, it appeared to us that the Peak Concentration of the drug is higher and duration of effective concentration is longer in this area.

Further, a comparative study has also been made with the finding of a number of such studies from abroad (Table IV). There are marked differences in MIC values in different studies. Without going into detailed discussion,

it may be accepted that, by and large, most of the values are higher in this area.

Effectiveness of a drug depends on its presence in blood and the higher its concentration, the greater should be its antibacterial action. However, the drug becomes less effective by its elimination till it reaches such a low concentration that it is no longer effective. Because of this fact, the authors think that the real biological value of a drug should be the measure or ratio of these values i.e. PC divided by MIC. In this respect, both rifampicin and INH record highest values, viz. 15.7 and 14.5 respectively, more than the other anti-TB drugs.

It may also be specially significant that, according to our findings, rifampicin takes 2 hours more than INH to reach the 'Peak Concentration'. Therefore, to get synchronised effect of "Peak Concentration" of these two drugs it may be profitable to administer rifampicin before break-fast and INH after break-fast or around a gap of two hours (Sen *et al*, 1975, 1981). Table V shows the comparative biological values of these two drugs and those of pyrazinamide and thiacetazote as found in Dr. B.C. Roy Reaseroh Insitute in support of this statement.

In conclusion, the authors, feel that higher concentration and longer duration of the drug

TABLE II
Average biological values of rifampicin of 19 tuberculous patients

No. of Patients.	Peak concentration (meg/ml)	Peak Hour (Hours)	Half-Life (Hours)	Effective concentration coverage (Hours)
19	15.7	3	5.41	24

TABLE III

Biological values of rifampicin in different places studied by different group of workers

	No. of subjects	PC (mcg/ml)	PH (hour)	HL(hour)	ECC (hour)
Present Study (Calcutta)	19 subjects	15.7	3	5.4	about 24
Sunahara <% Nakagawa (Tokvo)	13 "	12.5	2	4	17.0
Emmerson et al (London)	6 "	5.5	1	1.5	4.7

TABLE IV

Serial No.	No. of days of treatment	PC mcg/ml	PH (hours)	HL (hours)	MIC mcg/ml	ECC (Approx Reference hours)
1.	About 365	15.7	3	5.4	1	24 Present Study
2.	0	12.5	2	6		28
	30	10.0	2	4		18 Sunahara & Nakagawa (1972)
	60	9.0	2	4		18
	90	11.0	2	4		18
3.	1	5.9	2	2.7		10 Emmerson et al
	8	5.5	1	1.5		5.5 (1978)
4.	1	10.5	2			Acocella et al (1978)
	7	9.4	2	x		x
5.	0	20.0+				Boman et al (1975)
		6.9	1	x		x
6.	0	6.88	1	3	2.5 or 5	x Verbistetal(1968)
7.		7	2—4	x	less than 1	x Yoninans (1979)
8. 9.		7	2—4	1.5—5	.005-0-2	x Goodman & Gillman(1980)
		8	2—4	3	.005—20	Lyka Laboratories

TABLE V

Average biological values of Rifampicin Isoniazid, Pyrazinamide and Thiacetuzone

Drugs	Peak concentration (mcg/ml)	Peak hour (Houis)	Peak cone/minimum inhibitory cone, (ratio)	Effective concentration coverage (hours)
Rifampicin	15.7	3	15.7	24
Isoniazid	2.9	1	14.5	13.5
Pyrazinainide	86.0	2	3.4	19.5
Thiacetazone	2.9	5	1.4	5.5