I. INTRODUCTION

Tuberculosis (TB) is a chronic disease and associated with a high morbidity, consequent economic loss, and considerable mortality mainly among socially deprived class. In recent years, the disease is on a rise with the advent of HIV infection. In response to the seriousness of the problem, World Health Organisation declared TB as a global health emergency in 1993.

The causative organism of TB is Mycobacterium tuberculosis (MTB). Mycobacteria are Gram positive organisms, difficult to stain with routine bacteriological stains, with varying growth rates, some pathogenic mainly infecting the lungs, while others are mostly saprophytic, associated with multi-drug resistance. The time proven vaccine - BCG, has but limited value in prevention as shown in the Indian studies.

II. EPIDEMIOLOGY OF TUBERCULOSIS

Epidemiological progress of tuberculosis is presented in the accompanying flow chart (Fig: 1). The known characteristics of the disease opens up many important topics for discussion concerning the association between development of TB and characteristics of Mycobacteria.

A. Clinical manifestations

Adaptability of the organisms to different hosts, tissues and environments has also given opportunity to manifest with varying clinical manifestations of the disease. In addition, some of them have been able to present with atypical presentations.

Peculiarities in the cause and effect relation between the organism and tuberculosis as a disease:

1. It may not be possible to demonstrate the organisms either by direct microscopy or isolate them in all patients suffering from tuberculosis
2. The Mycobacteria are not easily detected by ordinary stains used for the identification of most other bacteria (Grams stain)
3. Presence of large number of environmental Mycobacteria further complicates the diagnosis in specimens due to the possibility of contamination
4. The organism is slow growing and causes problems in early confirmation of the disease
5. Special growth requirements make it more difficult to grow
6. There are more infected persons than those diseased, in the community
7. Disease is chronic requiring long term chemotherapy for cure
8. The organisms develop multi drug resistance
9. Fate of the organism after treatment, in terms of causation of relapse or reinfection, is not clear in most of the cases
10. At present there is no effective vaccine to prevent the disease

Quantification of the disease in the community is not accurate since there is more number of infected
individuals than those diseased. Added to this, problem of accurate diagnosis has been responsible for failure in introducing routine surveillance into the tuberculosis control programme.

B. Mycobacterial Diagnosis

Microscopy: Most important survival strategy of Mycobacteria is that it is not easily detected under a microscope since it does not easily stain with routine method of bacterial staining i.e., Gram’s stain. In the Gram’s stain, the organism can be seen as empty spaces. This is the basic difficulty, as encountered in identifying the causative organism of tuberculosis. Mycobacteria are Acid and alcohol fast. In other words, once stained, it is difficult to remove the stain by the use of acid or alcohol. Detection of Mycobacteria thus requires especially trained personnel. Further, it is not possible to demonstrate the organism from all those diseased.

The shortcomings of the smear examination has been well addressed and documented in the earlier work of Raj Narain et al in the longitudinal study of tuberculosis conducted by the National Tuberculosis Institute, Bangalore. As reported by him, all the declared positives could not be included as positives, due to the cases lacking collaborative evidences to clinch the diagnosis to be tuberculosis. Presently this point has been well addressed in the RNTCP where three smear examinations have been stressed to increase the specificity of the test and to reduce inter-personal bias in examination.

Alternative methods for diagnosis: Efforts have been put in to address some of the above issues. Acid / alcohol fast staining (Ziehl-Neelsen stain - fig: 2) remains as the mainstay even to this day. Fluorescent microscopy, serological tests and molecular techniques have been developed, but have not been able to totally replace the existing technique of simple microscopy, which of course, has limitations of sensitivity. However, all these newer techniques are still used as research tools. Molecular techniques, such as iso-enzyme pattern and use of genetic probes, have been useful as epidemiological tools. So far no serological tests, that are presently developed, have shown much promise for routine field application. The need of the hour is to develop methods to identify the infecting organism to the species level. This has become very important, since tuberculosis incidence has increased with immune deficiency states like HIV. Further, multi-drug resistance has been mostly associated with opportunistic Mycobacteria such as M. avium, etc involved in the infections, especially among those with lowered immunological states.

Cultivation: Growth requirements and slow growth of the pathogenic Mycobacteria have been addressed through newer methods of cultivation using Bactec, etc. In spite of these developments, a battery of tests is to be performed even to this day in order to classify the infecting species of Mycobacteria in question. On the whole, these have not been cost effective.

C. Species identification of Mycobacteria

Some important tests required for classification of Mycobacteria to identify infecting species are:

- Broadly classify into pathogenic and non pathogenic
- Growth rate in culture (> 7 days or < 7 days)
- Pigment production
- Tween 80 hydrolysis (potentially pathogenic are negative)
- Tween opacity test (separates some pigmented slow growers)
- Urease test
- Arylsulfatase test (separates potentially pathogenic M. fortuitum complex)
- Catalase test at pH 7 / 68: C (M. tuberculosis, M. bovis, M. gastri, M. malmoenses and M. haemophilum loose catalase activity at 68: C)
- Semi quantitative catalase test
- Nitrate reduction test
- Iron uptake test (helpful mainly in determining the virulence)
- Growth on 5% sodium chloride
- Growth on citrate or mannitol
- Growth on MacConky agar

* Specific for some pathogenic group of organisms

- Niacin test (for M. tuberculosis)
- Pyrazinamidase test (to distinguish M. bovis from M. avium, M. marinum from M. kansasii)
- Tellurite reduction test (for M. avium complex)
- Growth on Thiophen-2 carboxylic acid hydrazide (TCH) (unique for M. bovis)

Apart from these, there are other biochemical tests, diffusion tests and phage tests to specifically speciate the infecting organisms. Some advancement in morphological studies in relation to speciation has been done. Molecular diagnostic tests using DNA, RNA analysis using different PCR techniques are being tried for early detection and characterization of infecting agent.

III. PROBLEMS ASSOCIATED WITH MYCOBACTERIAL, DISEASE THERAPY

Practice of mono-therapy in the earlier days, irrational use of anti tubercular drugs, long course
chemotherapy associated with patient’s noncompliance have all contributed to the change in bacterial behavior. Multi-drug resistance to therapy no doubt, is a bacterial adaptation for its own survival. This problem has been addressed well in the tuberculosis programme with combination therapy, short course chemotherapy and the latest in the list, the development of DOT under RNTCP.

Due to a long drawn therapy, treatment given can not be equated with the treatment consumed. Factors which have contributed to the development of drug resistance include slow growth of the organism and the adaptation of the organism to occupy different compartments of the body with varying pH environments. These have given the organisms ample opportunity of survival, due to denaturing of different antimicrobial agents, possibly from use of mono therapy in the early period of tuberculosis management. Cell wall has given ample support in the entire process of development of drug resistance. Methods of diagnosing antimicrobial resistance are not available at all centers. Even when available, they are cumbersome and time consuming.

Another dimension of tuberculosis treatment is the lack of understanding about the end point. As soon as treatment is instituted, usually within a month’s time, a patient becomes negative for Mycobacteria. However disease in some patients tends to recur. Relapse, re-infection and resistance are arbitrarily defined. Bacilli are known to be persistent in spite of adequate therapy, though they exhibit changes in morphology and staining. This raises certain questions:

- Is cure equivalent to bacillary elimination?
- Is dormancy of bacilli achieved by intermittent treatment in tuberculosis which has been questioned in Leprosy programme (Pulse therapy)?(3,4)
- Are quiescent bacilli aroused due to immunological deficient states at a subsequent time?

If these are borne out by facts, are we leaving a large size of a previously treated population of infected persons harboring dormant bacilli in the community? Is it too large for comfort? Could the problem be compounded due to the comparative socio economic vulnerability of the group, increasing longevity and ageing of the general population, including the previously treated group in them?

Some of the research carried out in the field of chemotherapy for tuberculosis is the search for compounds which can make the drug release to be sustained and long acting. Success in this direction would definitely bring down the duration of treatment and improve patient compliance.

**IV. VACCINE**

Presently vaccine is not a solution towards tuberculosis control. It is mainly used as a preventive tool against development of hematogenous forms of the disease, not necessarily responsible for causing dissemination themselves. BCG has been found to be useful only in preventing the so called childhood forms and complications. Failure of BCG vaccine to protect individuals from the disease has thrown open many questions about the earlier understanding of the disease. Questions to be asked in this connection include -

* Has long standing cultivation of vaccine strains of Mycobacteria brought in changes in the bacterial antigenic presentations, or
* Has increased exposure to environmental Mycobacteria brought down the host response to the pathogenic strains, or
* Has there been any change in host response, or
* Is it associated with changes in the bacteria?

These points require a fresh look into the understanding of the biology of the Mycobacteria and host parasite relation. This is important in the development of newer vaccine for tuberculosis infection.

Consequently, newer thoughts towards vaccine development and delivery are being nurtured today. Some of the newer vaccines that are under scrutiny include - use of other environmental Mycobacteria as vaccine candidates, use of sub-unit vaccine (post office vaccine) and DNA vaccines. All these are still in experimental stages.

More people remain infected in the community than those who are diseased. Though immunity, nutritional states, infection with saprophytic Mycobacteria from environment, etc. have been attributed for this situation, understanding of exact mechanisms involved could help in finding out newer and most effective control methods of the disease and the vaccine development is depended on these.

On the whole, the infecting organism is a parasite on the infected host. Good parasitism is one, where the parasite lives with the utmost harmony with
the host causing very little damage to the host. Understanding of the host parasite relation is thus important. The parasite has undergone lot of changes during its evolution for its survival and has adapted to its new environment. No denying the fact that the Mycobacteria, being a disease agent, causes disease, but how best has it adapted for its own survival? Many of the peculiar structural and physico-chemical acquisitions of the infective agent in relation to disease causation and survival are yet to be understood. It is important to understand the disease agent in the light of parasite biology and host responses.

A. Issues of bacterial survival:

1. Mycobacteria have a wide geographical distribution which favors better survival.

2. Adaptation to infect many species of animals is a positive parasitic adaptation for survival.

3. The organisms have adapted to living in various tissues of the body. They have adapted to varying environmental conditions for survival. For example, they are well adapted to both extra and intra cellular environments. Similarly, they are well adapted for acidic, alkaline and neutral environments. This is again a better survival strategy of the organism, giving it an opportunity to adapt and survive in different organs of the body.

4. Slow growth of most of the pathogenic members of Mycobacteria point to the fact that it is one of the strategies adapted by the organism for survival.

Most of the pathogenic Mycobacteria belong to the group of slow growers. Slow growth of the organism helps it to reduce its metabolic rate and adapt to different environments. Secondly, most of the bactericidal drugs act mainly on rapidly multiplying organisms. Pathogenic Mycobacteria to some extent appear to have used the slow growth habit to its advantage for survival. Differential growth of different component members of the group, along with their capabilities to produce pigment in culture, has been used to classify and identify clinically important Mycobacteria into different groups. Classification of Mycobacteria on growth is given below (Table).

Cell wall changes: Cell wall is elastic and forms the most vital part of the organism. Complex composition of the superficial layer plays a vital role in bacterial survival and multiplication. Molecular and structural composition of the cell wall is related to the cytopathology as well as immunology of the disease. It is a bi-layered structure with a rigid inner basal layer and a diffuse, unstainable outer layer. The basal membrane maintains the Gram positive character of the organism. However, the outer layer is made up of lipopolysaccharides and peptidolipids. These give the pathogenic members the ability to react to the changes in the environment. Lipopolysaccharides are almost identical in all pathogenic members of the Mycobacteria (group specific). Lipopolysaccharides are not highly immunogenic. This helps the organism in overcoming the immunity of the host. This fact has been well demonstrated in Leishmania, a haemoparasite (S). The lipopolysaccharide cell wall prevents the detection of surface antigens by the host antibodies.

Thick lipid moiety of the cell wall helps the organism in becoming intracellular. The cell wall is the main organelle of the bacteria even in the antibiotic resistance. Thus the outer cell wall layer plays an important role in the biology of Mycobacteria.

The next most important factor is the cell adhesion factor, which could be different in pathogenic and non-pathogenic Mycobacteria. This aspect requires elaborate studies. Some of these factors also play an important role in the immunological response of the host. Studies in this direction are required to be taken up.

Virulence: Doubts about the action of BCG vaccination brings in yet another point for discussion, namely virulence. Virulence of the organism has been associated with certain genetic markers associated with the infecting organism. Some of these have been identified by genetic studies comparing between M. tuberculosis, M. bovis and different strains involved in BCG vaccine. An important aspect meriting attention at this juncture is that some genetic material is missing in the open frames of the strains used in BCG vaccine production. Nearly, 38 open frames have been found to be missing when BCG strains are compared with M. bovis. Morphologically, it is interesting to note the changes noticed in H37Rv and H37Ra, in comparison to the wild strains of M. tuberculosis from patients. These strains have been maintained in the laboratories as standard strains for long time to study the virulence of the wild strains. But due to long term cultivation, the organisms have become smaller in size and are in chain formation, unlike the wild strains. Many factors such as antigen presentations, orientation of the proteins, etc., however, come into discussion in this regard.

V. SUMMARY

No ready made solutions are available for TB control, though it is one of the oldest diseases known to mankind. An Understanding of the dynamics of the
disease and the attendant bacteriological peculiarities brings to light some of the shortcomings in the diagnosis and effective treatment delivery under the TB Control Programme. These issues may have important implications for the integration of TB control programme with the general health. These also need to be raised for the development of a suitable TB Programme. Some of the bottlenecks in the understanding of the disease are summarized below.

- Tuberculosis despite being associated with human infection for a long time has many problems of diagnosis. Trained man power is required for proper diagnosis. RNTCP stresses the need for three sputum smear examination. Newer tests that have been developed are not yet available for routine field application.

- There are difficulties in cultivation and characterization of the organism.

- Definitions of various states such as disease, relapse, re-infection, etc. lack clarity, since there are more number of infected individuals than diseased in the population.

- It has not been possible to establish effective surveillance system in the tuberculosis control programme.

- There is always a tendency to over treat since one is not sure when the treatment is complete and bacteriological elimination is achieved in the host.

- Long infected states and drug resistance add on to the problem.

- Vaccine can not guarantee cure. It is only a preventive tool suitable for preventing specific manifestation only. The available vaccine has been found to be ineffective in limiting transmission of infection. Newer vaccines are not available as yet.

- It is not clear whether the increase in incidence in TB in recent years is the result of (i) HIV infection, which brings down the immunity of the individual (fresh infections) or (ii) due to activation of tubercular organisms in infected individuals as a result of lowered immunological states due to ageing, as in Japan, (iii) or for reasons of other supervening infections, as in the case of HIV infection (relapses / reactivation) or, (iv) Whether multiple mechanisms are operative.

VI. CONCLUSION

A host of developments have taken place during the recent times, regarding diagnosis, treatment, vaccines, etc in the field of TB. The major factors which are still to be addressed include insight into bacterial biology, host bacillary interactions in relation to easier and rapid diagnostics, role of immunity especially in the wake of recent advent of HIV/AIDS, better chemotherapeutic agents and better and more effective vaccines. An attempt has been made to highlight the issues which are important to the health administrators when the TB programme has to be planned or reviewed.

ACKNOWLEDGEMENT

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FURTHER READING


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<table>
<thead>
<tr>
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<th>Complex designation</th>
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Fig 1: EPIDEMIOLOGICAL PROGRESS OF TUBERCULOSIS

FIG 2. MYCOBACTERIA AS SEEN INZIEHL-NEELSSEN STAIN