MYCOBACTERIAL INFECTIONS : SIGNIFICANCE AND CONTROL

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DIABETES AND INFECTIONS

Individuals suffering from diabetes mellitus are often at a high risk for developing several types of infections. The infections that occur, in turn, often complicate the control of diabetes. A wide variety of pyogenic bacterial infections and specific infections such as tuberculosis and mycotic infections, are known to be associated with the diabetic state. Diabetes mellitus is identified as an independent risk factor for developing these infections. The reasons why diabetics are susceptible to infections are unclear and without any scientific proof. Although the components of humoral immunity appear intact, defective functioning of the polymorphonuclears results in a high susceptibility to bacterial infections. Another factor that may be responsible is insulin deficiency, which results in impairment of both leukocyte and lymphocyte mediated responses to infection. Malnutrition accompanies the state of diabetes and impairs the various components of the immune system including T lymphocyte function and cell mediated immunity. Another important contributory factor in these patients is protein under nutrition. These factors have been shown to improve with antidiabetic treatment. A good control of blood glucose in these patients is desirable goal for the prevention of certain infections and to ensure maintenance of normal host defences that determine resistance and response to infections [1]. Successful treatment of infections in the diabetics requires early and exact diagnosis, the execution of the correct antimicrobials and the treatment of the diabetic state and the associated disorders.

Among the several infectious diseases that complicate the diabetic state, are those caused by mycobacterium tuberculosis, mucor species, staphylococcus aureus and gram negative bacteria, which may occur with an increased frequency. The predisposition of these patients to lower respiratory tract infections probably represents an alteration in pulmonary host defences at several levels [2]. Morbidity and mortality from bacterial respiratory infection are worsened by low body weight and poor nutritional status.

TUIBERCULOSIS AND DIABETES

Tuberculosis is an important and common infection [3] and an association between diabetes and tuberculosis has long been established. It has been documented the diabetics with coexisting tuberculosis have a greater mortality rate than that in the general population. Though this association is a great clinical problem, epidemiologically it is rather insignificant [4]. The severity of disease appears to correlate with the degree of tuberculous activity. Factors that favour tuberculosis in a diabetic patient include the

- Age of the patient

- Duration and severity of diabetes (patients requiring more than 40 units of insulin per day have active tuberculosis with caseation).

- In patients with weight less than in normal controls, the incidence of tuberculosis is twice as much.

TUBERCULOSIS

Like many other scourges of mankind, tuberculosis is an infectious disease which has its own unique determinants. Tuberculosis is a widespread infection throughout the world. The tubercle bacillus does not respect borders and races. If however, the infection is correctly diagnosed and treated on time, it is curable. Effective treatment of infectious patients prevents spread of infection. It has been estimated that about 140 lakh people are suffering from tuberculosis is our country, of whom 35 lakh are sputum positive and highly infectious. The most important factor that determines the progress of the disease, new or old, is the adequacy of the host's immune response, especially the cell mediated immunity, to the presence of mycobacterium tuberculosis. Host defence mechanisms play an important role in every aspect of the disease, both in its acquisition as well as the progression of the clinical course. Progress of the disease occurs when the cell mediated immunity is inadequate. Any other associated compromising disease, alters the outcome [5]. Tuberculosis has been linked with putative impaired cellular immunity associated with age, malnutrition, genetic factors, the administration of immunosuppressive drugs, the presence of diseases like diabetes mellitus, lymphoreticular malignancies, chronic renal sufficiency, silicosis and notably infection with the human immunodeficiency virus. The diagnosis and management of tuberculosis in all these compromising states in the host, remains a serious clinical problem.

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Tuberculosis and diabetes mellitus frequently coexist in the population at risk for tuberculosis, eg. Those patients living in endemic areas. Diabetes mellitus has been a frequent underlying disease in chronic excretors of mycobacterium tuberculosis[6]. It is the most prevalent risk factor for active tuberculosis among PPD (purified protein derivative, tuberculin) positive patients (38.5%). This high prevalence of tuberculin positive patients amongst the diabetics increases when another reading is taken seven days after the first [7]. Hence, an aggressive screening and preventive therapy for tuberculosis is mandatory amongst the diabetics [8]. This also justifies the protocol use of PPD in diabetics [9]. The clinical presentation of tuberculosis in diabetics is different from that in non diabetics. Significant increase in cavitary and sputum positive disease and more of lower lobe involvement is often seen. The incidence of lower lung field tuberculosis is more frequently found in diabetes mellitus [10, 11]. Clinicians should suspect tuberculosis in the diabetic with an abnormal chest X-ray. In any febrile patient with a recent pulmonary pathology there should be a high index of suspicion of disseminated tuberculosis [12]. Miliary tuberculosis should be included in the differential diagnosis of ill defined, feverish disease in diabetes mellitus patients. Aggressive diagnostic measures and specific chemotherapy should be given and monitored to treat pulmonary tuberculosis [13]. Bacteriological relapses after chemotherpy is defined as a positive culture growing at least 20 or more colonies. In combination regimen that include Isoniazid and Rifampicin, the bacteriological negative conversion rates are similar in both the diabetic and the non diabetic patients. Relapses in the diabetic are due to resistant strains and is associated with a grave prognosis [14]. Drug resistance is defined as any strain of M. tuberculosis that is resistant to two or more of the five primary anti tuberculosis drugs used-isoniazid, rifampicin, pyrazinamide, ethmabutol and streptomyhcin. Many strains of M. tuberculosis are resistant to combination of various first line and second line drugs. A cavitary tuberculosis lesion and previous history of treatment for tuberculosis in a patient, are two important risk factors for developing drug resistant tuberculosis. The major factors associated with dissemination included immunosuppression, weight loss, old age and diabetes mellitus. Poorly controlled diabetes mellitus predisposes to reactivation of tuberculosis. Diabetes is the chief complication of pulmonary tuberculosis in 12.4% of patients [15]. In a diabetic patient, pulmonary tuberculosis may progress rapidly and remains a significant cause of mortality and morbidity in developing countries where tuberculosis is endemic. Those caring for patients with tuberculosis should also be aware of the increased prevalence of diabetes in these patients, since failure to diagnose the underlying compromising disease may adversely affect the prognosis [16]. The relative risk of developing pulmonary tuberculosis of all types and bacteriologically confirmed cases were 3.47 times and 5.15 times higher in the diabetes than in the matched non diabetic controls [17]. Diabetics and immunocompromised patients have a higher prevalence of multiple cavities within any given tuberculous lesion, which are often non segmental in distribution [18]. Most patients have a serious form of diabetes mellitus. The combined affection is characterized by a progressive course of tuberculosis with involvement of bronchi in the process. Specific features which comprise defective defence machanisms (alveolar macrophages, type II alveolar cysts and fibroblasts in the form of their dystrophy), generalised affection of the pulmonary vessels, intense fibrosis and disorganization of the forming tissue, have a bearing on the development of the pathological process [19]. Vigilance in the diagnosis of tuberculosis in the diabetic is suggested particularly where the X ray appearance of the anterior segment and upper lobe involvement would tend to favor an alternate diagnosis [20]. Tuberculosis should always be considered as a diagnostic possibility in patients with lower lung fields lesions and diabetes mellitus. Radiological changes are found most commonly in the right lung and bilateral involvement is infrequent [11]. Recurrences of tuberculosis are often found in patients with co existing diabetes mellitus [21]. Different forms of destructive tuberculosis run con-currently with diabetes mellitus [22].

A greater relative risk was observed in those at an age of 30-49 years than in those of 50 years or more [17]. The risk of tuberculosis in diabetes also increases with the age and year of evolution of disease. The incidence of tuberculosis among the insulin dependent diabetes mellitus patients is about 38 times more than in the general population under 40 years of age [23]. Also, the prevalence rates of tuberculosis are more in patients with insulin dependent diabetes mellitus than in patients with non insulin dependent diabetes mellitus (9% vs. 2.7%) [16]. Clinical symptoms and progress of tuberculosis in insulin dependent diabetes mellitus patients is characterized by an acute onset and manifest clinical symptoms. The lung involvement becomes rapidly advanced with exudation and multiple small size foci of destruction [24]. In the non insulin dependent diabetes mellitus, tuberculosis often occurs with less destructive lesions, that are solitary and large.

MICROBIOLOGY LABORATORY SERVICES

The resurgence of tuberculosis in recent years has stimulated a renewed interest in the microbiologic diagnosis of M. tuberculosis infection. As per the World Health Organization "Not only has tuberculosis returned, but it has upstaged its horrible legacy". Compounding this, is the challenge that is being faced by the increasing number of immunosuppressed patients, since many if these patients are infected with the multi drug resistant strains of M. tuberculosis.

The current imperative is to bring the mycobacteriology laboratory forward into the modern era of clinical microbiology [25]. The contemporary diagnostic microbiology lab must respond to the change in the clinical spectrum of mycobacterial infection brought about by an increase in the number of patients who are immunocompromised. The mycobacteriology lab can best assist the physician in making a prompt and accurate diagnosis of disease by ensuring that a properly obtained specimen and sufficient clinical information reach the lab. The lab has a variety of staining techniques, media and methods for identifying the etiologic agent. An ongoing communication between the physician and the lab is essential. Sufficient clinical information will held dictate appropriate media selection, temperature and duration of incubation of cultures. The clinical microbiology laboratory thus plays an important pivotal role in the control of the spread of tuberculosis through timely detection, isolation, identification and the drug susceptibility testing of M. tuberculosis. Resurgence of tuberculosis and the outbreaks by multidrug resistant strains have brought in a new sense of urgency in the reporting of the several laboratory results to the clinician. The Centers for Disease Control (CDC), Atlanta, USA, has formulated recommendations for the role of microbiology lab in the control of tuberculosis (Table 1).

Table 1 : Recommendations of the Centres for Disease Control (CDC) to combat resurgence of tuberculosis [30]

1. Good communication between clinicians and laboratory
   *Proper sample collection
   *Rapid transport to lab within 24 hours.

2. Flourescence microscopy for AFB smear testing.

3. Rapid isolation techniques for mycobacteria.

4. Rapid identification of mycobacterial isolate.

5. Drug susceptibility testing against
   * First line drugs - Rifampicin
   - Streptomycin
   - Ethambutol
   - Pyrazinamide
   * Second line drugs – in a reference lab if no response to first line drugs.

6. Repear culture after 3 months and sensitivity if culture positive.

7. Immediate reporting of lab results to clinician.

8. - Maintainance of lab records
   - Review of lab procedures
   - Ensure safety precautions among lab personnel

The use of microscopy for acid fast bacilli to reach a preliminary diagnosis is of great importance. In addition, sputum microscopy is of significant value in the detection of open or infectious cases. It is well established that if no acid fast bacilli are seen in the sputum smear on a standard, yet competently performed examination, the patient is unlikely to be infectious. Thus the establishment of good sputum microscopy is of prime importance in developing countries where the first priority in tuberculosis control is to identify and treat the open cases [26]. The sputum examinatilon is carried out by direct sputum smear microscopy of specimen obtained from persons who present to the general health service for persistent cough of more than three weeks with or without associated fever and chest pain. Every such patient should have three sputum samples examined for acid fast bacilli, either by the conventional Zeihl Nielson’s method or by the Fluorochrome staining using the fluorescent microscope (available only in some laboratories). Detection of these bacilli in the sputum is the only reliable method for confirming pulmonary tuberculosis. Such patients are much more infectious than those with sputum negative for acid fast bacilli. The three sputum samples should be collected preferably within two days (spot-morning-spot) and examined by microscopy. The laboratory has to ensure that the collection and the testing of the sputum is accurate and the results of sputum microscopy are available to the treating physician at the earliest, preferably within 24 hours. The sputum is graded as shown in table 2 to indicate the load of infection and also to provide epidemiological information. A specimen with a count of more than 10,000 colony forming units per millilitre of specimen, is more likely to be smear positive. Whether by light or fluorescence microscopy, the acid fast smear examination is specific, rapid and simple to

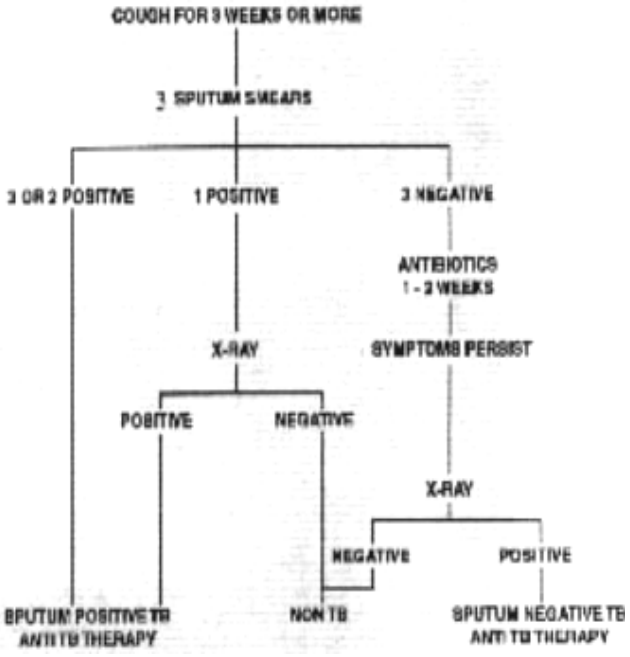
perform. While a single smear of a respiratory specimen has a sensitivity of 22-43%, the detection rate improves when multiple specimens from the patient are examined, and as many as 96% of the patients with pulmonary tuberculosis can be detected [25]. A person with at least two positive sputum samples is a confirmed case of smear positive pulmonary tuberculosis. The algorithm for the diagnosis of pulmonary tuberculosis in patients with respiratory symptoms is shown in figure1. The accurate and timely reporting of the results is essential for effective management of individual patients and for appropriate implementation of public health and infection control measures. The 24 hour turn around time for acid fast bacilli smear results presents a challenge to most clinical labs.

Table 2: Grading of sputum smears for Acid Fast Bacilli (AFB) [33]

Examination	Result	Grading	No of fields
More than 10 AFB/ oil immersion field	Positive	3+	20
1 – 9 AFB per oil immersion field	Positive	2+	50
10 – 99 AFB per 100 oil immersion field	Positive	1+	100
1 – 9 AFB per 100 oil immersion fields	Scanty	Record exact number seen	200
No AFB in 100 oil immersion fields	Negative	0	100

The laboratory diagnosis of tuberculosis depends not only on the detection but also on the recovery and identification of the mycrobacteria from clinical specimens. While smears are an important adjunct to the detection of M. tuberculosis, they are not adequate criteria alone and must be followed by culture for mycobacteria. The microbiologist must be prepared of process and culture a variety of both pulmonary and extra pulmonary specimens for recovery of the mycobacteria. Efficient procedures that allow for an early diagnosis or detection of tuberculosis, especially pulmonary, should be used [25]. Today, many new techniques are available for the detection and identification of M. tuberculosis. The currently available culture techniques, like the automated systems, the BACTEC 460 TB, are capable of making a diagnosis in 80-90% of the cases, but the usually only after a substantial delay. However, they involve prohibitive expenditure in terms of instrumentation, expenditure and reagents. Hence, they are beyond the means of public health laboratories in the developing countries [27].

Figure 1 : Diagnosis of tuberculosis in symptomatic patients [33]



The inherent limitations and the delay in the detection of the presence of mycobacteria by the culture based diagnostic tests specific for M. tuberculosis, pose problems both for the microbiologist and the clinician, thereby limiting or delaying the implementation of appropriate treatment and control measures. These problems have been partly overcome by the development and introduction of molecular techniques like the polymerase chain reaction (PCR) and the nucleic acid amplification assays. These tests have been evaluated particularly for the diagnosis of tuberculosis and leprosy. They offer the promise of same day detection and identification of M. tuberculosis. The performance characteristics of these assays are quite good for smear positive respiratory specimen. More impressive is the ability of the PCR to detect a substantial proportion of smear negative, culture positive cases as in the case of paucibacillary pulmonary or extra pulmonary tuberculosis. This finding could have a significant impact on clinical practice and could reduce the need for more invasive procedures like fibreoptic bronchoscopy and transbronchial biopsy. But in addition to the promise shown by these techniques, several problematic issues

have arisen from several evaluation studies. PCR, although conceptually straight forward, is technically complex to perform. The quality control, reproducibility of the results and the variations between laboratory to laboratory remain significant problems. Furthermore, the cost of the technique is a great limiting factor for its application in the developing countries [28].

At present, if technical and cost issues are not a problem, PCR may be a useful technology for the rapid diagnosis of smear negative case of active tuberculosis. However, it should be remembered that, these new techniques only supplement the culture methods rather than replace them totally. They should not be used for initial screen, given the cost and the labor requirements of the assay. Also, culture for M. tuberculosis will still be required to obtain the organism for drug susceptibility testing and to detect non tuberculous mycobactgeria.

When PCR is applied to a clinical specimen in an attempt to pulmonary tuberculosis, the characteristics of the patient for whom the assay is being performed must be a part of the interpretation of the final test result. Since these assays do not depend on the viability of the organisms for detection, in a patient who has a known history of tuberculosis, a positive PCR result must be interpreted with extreme caution, because it may not be indicative of an active disease. In patients receiving anti tuberculosis therapy, PCR should not be used as an indicator of infectivity, as the available data suggest that this assay remains positive for a far longer time than the smears and cultures [29].

Hence they cannot be used to monitor patients receiving anti tuberculosis therapy, unlike the smear and culture methods. Also, the extreme analytical sensitivity of the molecular assays gives a higher percentage of false positive results. In populations with a large number of persons with previous tuberculosis but clinically inactive disease and who present with abnormal chest radiography, a substantial number would be positive by PCR , due to its high degree of sensitivity to detect the organisms (even dead bacilli). Sample contamination, it appropriate laboratory practices and methods are not adopted while performing the assay, may also give rise to false positive result. Further, the results of PCR in the case of tuberculin positive patients who are otherwise healthy and with normal chest radiographs have to be evaluated extensively [28]. Much effort has been devoted to the development of serologic tests for tuberculosis but no test has found widespread clinical use. Though the host's cell mediated immune responses play a major role in the pathogenesis of tuberculosis, humoral or antibody, mediated responses also occur simultaneously in these patients. However, the antibody response appears to be week and the antibodies induced do not have any role in the host's immune defence [26]. The presence of these antibodies in the infected patients has been widely exploited as an aid to the serodiagnosis of tuberculosis. However, with the complex and diversified antigenic structure of the mycobacteria and the cross reactivity with related groups or organisms, the serology of these infections poses an interpretative challenge. The specificity of serologic tests that use crude antigen preparations are too low for clinical application. Specificity can be enhanced by using purified antigens, but since not all patients respond to the same antigens, the increased specificity results in decreased sensitivity. As with any test, other than smear and culture, for the diagnosis of tuberculosis, the performance characters of the presently available serology tests have to be widely evaluated, especially in endemic countries along with populations based studies for an appropriate cutoff limit of the assays. At present, in any given situation, a positive serology test may only help to further support the diagnosis of tuberculosis. In such cases, every attempt should be made to pursue the diagnosis. However, when the test is negative, it does not rule out the possibility of tuberculosis. In an already confirmed case of tuberculosis, the test has no role to play. Thus it suffices to say that in a problematic situation, by choosing the appropriate cutoff limits for a high specificity and correlating the results with available clinical and other laboratory data, the serodiagnosis of tuberculosis can be used as an additional supportive evidence [30].

In essence, inspite of the availability of several sophisticated systems and procedures for the detection and diagnosis of tuberculosis, the detection of acid fast bacilli by direct microscopy and identification of the mycobacterial isolates by conventional methods are still the recommended procedures, especially in the developing countries. An important fact that remains is, with facilities for diagnosis and treatment at the primary health care centers and clinics, and the requisite knowledge and motivation among the staff, it is staff, it is possible to diagnose and treat a substantial proportion of tuberculosis patients right at the periphery. Most infectious cases can be diagnosed even in outlying areas by imparting simple training to technicians with adequate facilities for microscopy and staining.

TUBERCULOSIS CONTROL PROGRAMS

In developing countries where 80% of the world's cases of tuberculosis are found, emphasis has been

correctly placed on the identification by passive finding of patients who are the source of transmission of M. tuberculosis [31]. Establishment of tuberculosis survelliance system is essential to watch the trend of tuberculosis carefully and to keep its diagnosis and treatment at a high technical level among these high risk patients.

With these perspectives, the National Tuberculosis Program was implemented in India, by the Government of India, in 1962 [32]. Since inception, the program is integrated with primary health care delivery systems and implemented through the district tuberculosis centers which are manned by trained medical and paramedical personnel and have laboratory facilities for diagnosis. At these centers, free of cost diagnostic treatment facilities, including free supply of anti tuberculosis drugs, are provided to the tuberculosis patients. In 1992, a Revised National Tuberculosis Control Program (RNTCP), was envolved with the objective of laying emphasis on cure of infections cases through administration of Directly Observed Therapy of short course chemotherapy (DOTS). RNTCP in India, was perhaps the most important public health intervention of the last decade of the 20th century., As the name implies, DOTS means that the patients swallow the drugs in the presence of the health care worker under direct observation. This ensures the intake of a complete course of the therapy by the patients and therapy complete cure. The main aims of the DOTS are that the responsibility for the patient cure is on the health care worker and not on the patient [33]. This indirectly would help to reduce the incidence of drug resistance. National screening and control programs, ie. The Revised National Tuberculosis Control Program (RNTCP) could be used in any diabetic clinic and would considerably improve the health of the diabetic patients. Screening programs in any diabetic clinic with investigative facilities would considerably help to identify infected patients.

These enhanced tuberculosis control programs with an emphasis on preventive treatment for patients at risk of developing active disease, especially those with diabetes mellitus and chronic renal failure, could decrease the incidence and eventually control tuberculosis [34]. Thus an organized concentration of public health effort aimed at those areas of population identified as high risk for tuberculosis may control this infection in the near future.

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