Mycobacteria

Mycobacteria are branching filamentous forms resembling fungal slender rods that sometimes show mycelium. In liquid cultures, they form a mould-like pellicle; hence the name mycobacteria, meaning fungus like bacteria. The genus includes obligate parasites, opportunistic pathogens, and saprophytes. There are more than 50 Mycobacterium species.

The mycobacteria that infect humans are:

Mycobacterium tuberculosis •

Non-tuberculous mycobacteria:Non-tuberculous mycobacteria also called as Atypical mycobacteria.

Mycobacterium leprae

MYCOBACTERIUM TUBERCULOSIS

A)Morphology

M. tuberculosis is a slender, straight or slightly curved bacillus with rounded ends, occurring singly, in pairs or in small clumps. It measures 1-4 um x 0.2 0.8 um (average 3 um x 0,3 µm) in size. These bacilli are acid fast, non-sporing, non-capsulated and non-motile. Zichl Neelsen staining is useful to study the morphology of these organisms. With this stain, tubercle bacilli are seen as bright red (acid-fast), while the tissue cells and organismothers are stained blue .Tubercle bacilli may also be stained with the fluorescent dyes (auramine O, rhodamine) and appear yellow luminous bacilli under the fluorescent microscope. Beaded or barred forms are frequently seen in M. tuberculosis. They are Gram positive but are difficult to stain with the Gram stain

B) CHARACTERISTICS GROWTH

Mycobacteria are obligate aerobes. The bacilli grow slowly the generation time in vitro is 20 hours. Colonies appear in about two weeks and may sometimes take up to 6-8 weeks. Optimum temperature is 37degree Celsius and growth does not occur below 25degree or above40 degree Celsius. Optimum pH is 6.4 to 70 Tubercle bacilli can grow on a wide range of enriched culture media but Lowenstein-Jensen (LJ) medium is the most commonly used. This medium consists of beaten eggs, asparagine, mineral salts, malachite green and glycerol or sodium pyruvate. It is solidified by heating (inspissation). Malachite green inhibits the growth of organisms other than mycobacteria and provides a colour to the medium. Colonies of M. tuberculosis are dry, rough, buff coloured, raised, with a wrinkled surface .M. tuberculosis has a luxuriant growth (eugonic growth).

In liquid media, the bacilli grow as surface pellicle Virulent strains tend to grow as serpentine cords in the liquid media, while avirulent strains grow in a more dispersed fashion,hence the

name mycobacteria, meaning fungus like bacteria.

C. Biochemical Reactions

Mycobacterial species can be identified by several biochemical tests. Niacin test and Nitrate reduction test are two important biochemical tests, which positive in Mycobacterium tuberculosis.

Niacin Test

A)Niacin test: Human tubercle bacilli form mad wher grown on an egg medium. When 10% ganger bromide and 4% aniline in 96% ethanol are added to a suspension of the culture, canary yellow colour indicates a positive reaction.

B)Sensitivity to paranitrobenzoic acid M titeralit and M. bovis are incapable of growing in the presence of paranitrobenzoic acid which differentiates them from other species of Mycobacterium

C) Catalase-peroxidase tests: These help in differentating tubercle bacilli from atypical mycobactets and provide an indication of the susceptibility of the strain as isoniazid Most atypical mycobacterial strains are strongly catalase positive, while tubercle bacilli are only weakly positive.

Aryl sulphatase test: This test is positive only with , atypical mycobacteria. A pink colour indicates a positive reaction.

PATHOGENESIS

pathogenesis of tuberculosis:

Tuberculosis is transmitted by aerosols from an infected individual to another. Inhaled bacteria penetrate to the alveoli and are ingested by alveolar macrophages .Bacteria grow intracellularly and slowly.

Tuberculosis is (TBS) is a potentially serious infectious disease that mainly affects lungs.The

bacteria that cause tuberculosis is Mycobacterium tuberculosis and are spread from

one person to another through tiny droplets released into the air via coughs

and sneezes, Many strains of tuberculosis resist the drugs most used to treat the

disease. People with active tuberculosis must take several types of medications for

many months to eradicate the infection and prevent development of antibiotic

resistance. Studies indicate that there exists a close association between TB and

AIDS. Therefore, spread of AIDS among the people is resulting in dramatic increases

TB.

Symptoms: Although our body may harbor the bacteria that cause tuberculosis Our immune system usually can prevent us from becoming sick. For this reason doctors make a distinction between:

Primary Tuberculosis:Primary tuberculosis is the initial infection

Primary tuberculosis is the initial infection by the tubercle bacilli where hilar lymph nodes are involved. In endemic countries like India, it usually occurs in young children: the bacilli engulfed by alveolar macrophages multiply and give rise to a subpleural focus of tuberculous pneumonia, usually in the lower lobe or the lower part of the upper lobe (Ghon focus).

The Ghon's focus together with the enlarged hilar lymph node constitutes the primary complex. Pulmonary tuberculosis is the most common presentation.

Latent TB: In this condition, one have a TB infection, but the bacteria remain in Our body in an inactive state and cause no symptoms. Latent TB, also called inactive TB or TB infection, isn't contagious. However, it can turn into active TB, so treatment s important for the person with latent TB and to help control the spread of TB in eneral. An estimated one-third of the world's population has latent TB

Active TB: This condition makes you sick and can spread to others. It can occur in

the first few weeks after infection with the TB bacteria, or it might occur years later

The post-primary (secondary or adult) type of tuberculosis is due to reactivation of latent infection or exogenous reinfection and differs from the primary type. It affects mainly the upper lobes of the lungs; the lesions undergo necrosis and tissue destruction, leading to cavitation.

The haematogenous spread of M. tuberculosis may result in extra pulmonary tuberculosis which can involve almost any anatomical site in the body. The common extra pulmonary sites affected are lymph nodes, bone and joints, genitourinary system, central nervous system, and the gastrointestinal system, including the peritoneum. It can disseminate to other parts including lung causing miliary TB.

SYMPTOMS OF ACTIVE TB INCLUDE

*Cough

*Fatigue

*Nght sweats

*Loss of appetite

*Chills

Tuberculosis usually attacks your lungs. Signs and symptoms of TB of the lungs include

*Coughing that lasts three or more weeks

*Coughing up blood or sputum

*Chest pain, or pain with breathing or coughing

Tuberculosis can also affect other parts of your body, including your kidneys. spine or brain. When TB occurs outside your lungs, signs and symptoms vary according to the organs involved. For example, tuberculosis of the spine may give you back pain, and tuberculosis in your kidneys might cause blood in your urine

Diagnosis: During the physical exam, doctor will check your lymph nodes for Selling and use a stethoscope to listen carefully to the sounds your lungs make Men you breathe. The most commonly used diagnostic tool for tuberculosis is a eskin test. A small amount of a substance called PPD tuberculin is injected below the skin of your inside forearm. Within 48 to 72 hours, a health care essional will check arm for swelling at the injection site. A hard, raised red means likely to have TB infection. The size of the bump determines whether best results are significant. The TB skin test isn't perfect, Sometimes, it suggests people have TIS when they really don't. It can also indicate that people don't Then they really do

Blood tests may be used to confirm or rule out latent or active ests use sophisticated technology to measure your immune en These tests may be useful if you're at high risk of tive response to the skin test, or if you received the are relatively new, many health departments don't

Epidemiology: Every year between 8-9 million new cases of tuberculosis appear and 3 million persons die from the disease. India is one of the worst affected countries. More than 40% of the population is infected and some 15 million suffer from tuberculosis in the country. RevisNational Tuberculosis Control Programme (RNTCP) is based on the DOTS strategy of 1993 and was adopted as an Indian National Programme in 1997.

Every day in India, under the RNTCP, more than 15,000 suspected cases are examined for TB, free of charge.

Laboratory diagnosis: Laboratory diagnosis is based on the demonstration of M. tuberculosis in clinical specimens

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: Sputum: well coughed out early morning sputum

Gastric juices

Gastric lavage/washings Broncho-alveolar washings

Cerebrospinal fluid

Tissue biopsies Pleural fluid and pleural biopsy

Laboratory tests: Diagnosis is based on a combination

of compatible clinical syndrome, supportive radiologic investigations, and detection of acid fast bacilli or culture of M. tuberculosis from clinical specimens.

Bacteriological diagnosis can be established by microscopy , culture examination or by animal inoculation test.

1. Specimen

Specimen collection depends on the site of involvement. Tuberculosis may involve lungs (pulmonary) or sites other than lungs (extrapulmonary).

(i) Pulmonary tuberculosis

Sputum is the most common specimen. It is collected in a clean wide-mouthed container. A morning specimen may be collected on three consecutive days. If sputum is scanty, a 24 hour specimen may be collected. When sputum is not available, laryngeal swab or bronchialwashings are collected. In children, gastric washings may be examined as they tend to swallow sputum.

(ii) Meningitis

Cerebrospinal fluid (CSF) from tuberculous meningitis (TBM) often forms a spider web clot on standing, examination of which may be more useful than of fluid.

(iii) Renal tuberculosis

Three consecutive days morning samples of urine are examined

(iv) (iv) Bone and joints tuberculosis: Aspirated fluid

(v) (v) Tissue:Biopsy of tissue.

Direct Microscopy

Smear is made from the specimen and stained by the Ziehl-Neelsen technique (refer to chapter 2 for method of Ziehl-Neelsen staining). It is examined under oil immersion lens. The acid-fast bacilli (AFB) appear as bright red bacilli against a blue background. A negative report should not be given till at least 300 fields have been examined. Grading of smears is done according to number of bacilli seen .

ACID FAST STAINING

1)The smear is stained with a solution of carbol fuchsin

with the application of heat.

2. It is then decolourised with 20% sulphuric acid. 3. Lastly, it is counterstained with a contrasting dye such as methylene blue.

3)The acid fast bacteria retain the fuchsin (red) colour, while the others take the counterstain (blue). Acid fastness is thought to be due to the high content of mycolic acid in Mycobacterium tuberculosis.

Table 32.1 : Grading of Ziehl-Neelsen (ZN) Smear

No. of AFB seen in oil immersion field	Report
0 / 300 fields	AFB not seen
1-2 / 300 fields	Doubtful, repeat the smear
1-9 / 100 fields	1+
1-9 / 10 fields	2+
1-9 / field	3+
10 or more / field	4+

If a large number of smears are to be examined, fluorescent microscopy is more convenient. Smears are stained with fluorescent dyes such as auramine 'O' or auramine rhodamine and examined under ultraviolet light. The bacilli appear as bright bacilli against dark background.

However, grading of smears is done differently under Revised National Tuberculosis Programme

.concentration of Specimens

a,)homogenisation of the specimen.

b) Decontamination to kill the microorganisms present the specimen

c) Concentrate the bacilli in a small volume without inactivated

Such concentrate is used for smear and culture and animal inoculation.
Petroff'smethod

It is a simple and widely sad the p mixed with equal volume of 87% sodium hydroxide is incubated at 37°C with frequent shaking fo 30 minutes. It is then centrifuged at 300 rpm for minutes The supernatant fluid is poured and deposit is neutralised by adding / hydrochlon presence of a drop of phenol red indicator. The d is used for smear, cultors and animal insulation

Other methods

mucolytic agents such as N-acetyl-1-cysteine with sodi hydroxide and pancreatin are used for concentration of specimens. In urine and CSF specimens centrifugation s done to concentrate the specimen. Centrifuged deg is used for smear and culture examination

4. Culture

Culture is a very sensitive method for detection of tubercle bacill. It may detect as few as 10 to 100 baci per ml. The concentrated material is inoculated on two bottles of Lowenstein-Jensen medium. The taberle bacilli usually grow in 2 to 3 weeks

In a positive culture, characteristic colonies appear on culture mediufn. Smear is prepared from isolated colony and stained with Ziehl-Necksen technique. When acid-fast bacillus (AFB) is slow growing, non-pigmented and niacin positive, it is regarded as M. tuberculosis Confirmation is done by biochemical reactions

In radiometric method such as BACTEC, the growth may be detected in about a week by using MC labelled substrates. Culture media contains "C-labelled palmitic acid. Mycobacteria metabolise the C-labelled substrates and release radioactively labelled "CO, The instrument measures "CO, This significant method has now been discontinued due to radioactivity

5. Serology

Serology includes detection of anti mycobacterial antibodies in patient serum. Various methods such as enzyme linked immunosorbent assay (ELISA), radio immunoassay (RIA), latex agglutination assay have been employed. Diagnostic utility of these antibodies is equivocal. WHO has banned the use of these tests for diagnosis of active tuberculosis

Molecular methods:PCR

Koch's Phenomenon

The response of a tuberculous animal to reinfection was best explained by Robert Koch. When a healthy guinea pig is inoculated subcutaneously with virulent tubercle bacilli, the puncture site heals quickly and there is no immediate visible reaction. After 10-14 days, a nodule appears at the site of injection which ulcerates and the ulcer persists till the animal dies of progressive tuberculosis. The regional lymph nodes are enlarged and caseous. If on the other hand, virulent tubercle bacilli are injected in a guinea pig, which had received a prior injection of tubercle bacilli 4-6 weeks earlier, an indurated lesion appears at the site of injection in a day or two which undergoes necrosis in another day or so to form a shallow ulcer. This ulcer heals rapidly without involvement of the regional lymph nodes or tissues. This is called Koch's phenomenon. This phenomenon is a combination of hypersensitivity and immunity.

Tuberculin Skin Test

Principle

Tuberculin skin test (TST) is delayed or type IV hypersensitivity reaction.

2. Reagents

(i) Old tuberculin (OT)

It was originally described by Robert Koch. This crude product may lead to serious complications in some patients, it is now rarely used.

(ii) Purified protein derivative (PPD) A purified preparation of the active tuberculoprotein (PPD-S) was by growing M. tuberculosis in a semisynthetic medium.

It's called purified proteins derivative

It is called purified protein derivative (PPD). The dosage of PPD is expressed in tuberculin unit (TU). Another PPD is PPD RT-23. I TU of PPD RT-23 is equivalent of STU of PPD-S.

3. Method

Mantoux test

0.1 ml of PPD containing 5 TU of PPD-S is injected intradermally into flexor aspect of forearm. A PPD-S dose of 1 TU is used when extreme hypersensitivity is suspected. In India 1 TU of PPD RT -23 is recommended and not PPD-S.

. Result

In the Mantoux test the site of injection is examined after 48-72 hours and interpreted as follows:

Positive test

In a positive reaction, there is induration (local oedema of 10 mm diameter or more surrounded by erythema at the site of inoculation. Positive test only confirm past infection with tubercle bacilli but does not indicat presence of active state of the disease..

TREATMENT

The antitubercular drugs include bactericidal agents such as rifampicin (R), isoniazid (H), pyrazinamide (Z), streptomycin and bacteriostatic agents include ethambutol (E), thiacetazone, ethionamide, paraamino salicylic acid (PAS) and cycloserine. Short course regimens of 6-7 months are used. As resistant strains emerge readily by mutation and selection, combinations of two or more drugs are used.

A serious consequence of unchecked drug resistance has been the emergence of multidrug resistance tuberculosis (MDR-TB). The term multidrug resistance refers to resistance to rifampicin and isoniazid, with or without resistance to one or more other drugs. MDR-TB is a global problem especially in HIV infected persons. The directly observed therapy under supervision (DOTS) is being used to prevent deterioration of resistance problem by ensuring the patient's compliance.

Another serious condition extensively drug resistant tuberculosis (XDR-TB) has emerged recently. XDR-TB is due to M. tuberculosis strains which are resistant to any fluoroquinolone

and at least one of three injectable second line drugs (capreomycin, kanamycin and amikacin), in addition to isoniazid and rifampicin.

PROPHYLAXIS

BCG Vaccine:

Protection from tuberculosis may be done by public health measures and BCG vaccination. General measures such as nutrition and health education are also important.

Calmette and Guerin (1921) prepared an attenuated strain of M. bovis. The strain was attenuated by repeated subcultures. When the strain became incapable of producing tuberculosis in the susceptible guinea pig, it was named Bacille Calmette Guerin.

(i) Dose and administration

Vaccine is given intradermally in a dose of 0.1 ml. BCG vaccine should be given soon after birth failing which it may be administered at any time during the first year of life

(ii) Protective efficacy

A number of BCG vaccine trials were undertaken and the results varied from 0 to 80%. The immunity has been reported to last for about 10 years. The general opinion at present is that BCG does protect from tuberculosis. Even if disease occurs, it runs a milder course in vaccinated children.

Revised National Tuberculosis Control Programme (RNTCP)

Under RNTCP, any patient with cough for 2 weeks or more is included for diagnosis of pulmonary tuberculosis. Diagnosis is mainly based on good quality microscopy. Two sputum samples are collected from the patient. One early morning specimen and other is collected on spot when patient visits the chest clinic.

Both the sputum specimens are stained with Zichl Neelsen staining and observed for acid-fast bacilli (AFB). If one or both smears are positive then patient is diagnosed as sputum positive pulmonary tuberculosis. and antitubercular treatment is started. If both the sputum smears are negative for AFB, a course of antibiotic is given for 10-14 days. If cough persists after antibiotic treatment, again two sputum specimens are collected and examined for AFB by ZN staining. Antitubercular treatment is started if one or both smears are positive and it is declared as smear positive pulmonary tuberculosis.

If both smears are negative, diagnosis is done on X-ray chest findings. In case of X-ray chest suggestive of tuberculosis, patient is diagnosed as sputum negative pulmonary tuberculosis.

Antitubercular treatment is started. Patient is declared not suffering from tuberculosis when there is no finding suggestive of tuberculosis on X-ray chest.

DOTS treatment is given for tuberculosis as described earlier. However in MDR-TB cases, treatment is given with second line drugs under DOTS plus. There are five categories (category I to V) of patients under RNTCP, from newly diagnosed cases to MDR-TB and XDR-TB patients. MDR-TB patients are included in Category IV and XDR-TB patients in Category V.