

Lecture – 02

TUBERCULOSIS



Microbiology Team - 430

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Tuberculosis:

- ▶ Tuberculosis (TB) is a chronic disease affects humans (all age group), caused by *Mycobacterium tuberculosis complex*.
- ▶ Usually affects the lungs, other organs can be affected in one third of cases.
- ▶ Treatable but it could be fatal if untreated
- ▶ TB affects 1/3 of human race (2 billions) as latent dormant tuberculosis. The single most killing agent in the world, second to *Helicobacter pylori* which infects 50% of human race. And it's worldwide disease especially in developing countries.
- ▶ The WHO estimated 8.9 million new cases in 2004. And 2-4 million death. With the incidence of 32-64 cases/100.000 in KSA, 5.2/100.000 at the USA & 290/10.000 at south east of Africa (due to coupling with HIV infection)

Transmission of TB:

- **Transmission:** Mainly through inhalation of airborne droplet nuclei (< 5 μ m) in pulmonary diseases case , rarely through GIT & skin
- **Reservoir:** patients with open TB.
- **Age:** young children & adults
- **People at risk:** lab. Technicians, workers in mines, doctors, nurses. HIV pts., diabetics end stage renal failure, contacts with index case

Characteristics of the Genus Mycobacteria :

- Slim, rod shaped, non-motile, do not form spores.
- Do not stain by Gram stain. Why? Because it has a waxy coat on their cell wall
- Contain high lipid conc.(Mycolic acid) in the cell wall which resist staining so it is called Acid Fast Bacilli (AFB), why ? It resists decolorization with up to 3% HCL, 5% ethanol or both.

Acid-Fast Bacilli (AFB):

- Stain used : **Ziehl-Neelsen stain (ZN stain)**
- Strict aerobe
- **Multiply intracellularly (in the macrophages)**
- Delayed hypersensitivity reaction type of immune response
- **Slowly growing (2 - 8 wks)**

Mycobacterium tuberculosis complex:

- 1- **M.tuberculosis** (Human type)
- 2- M. bovis (Bovine type)
- 3- M. Africanum
- 4- BCG strains

All Mycobacterium tuberculosis Complex cause tuberculosis

And there is another mycobacterium cause leprosy: which is mycobacterium leprae
(don't grown on an artificial cell culture media)

Pathogenesis of TB:

- Mycobacteria acquired by airborne droplet, reaches the alveolar macrophages and able to survive their (main virulence factor).
- This **starts cell mediated immune** (CMI) response which controls the multiplication of the organism but does not kill it.
- Granuloma formed and organism lives in dormant state (latent TB infection)
- Patient show evidence of delayed CMI. And Disease results due to destructive effect of CMI.

❖ Clinically the disease is divided into :

1-Primary Tuberculosis:

- Occurs in patient's not previously infected (**initial infection**).
- Inhalations of bacilli \implies Phagocytosis \implies lymph nodes calcify to produce **GHON Focus** (or Primary Complex) at the periphery of mid zone of lung.
- Microscopy of lesion shows **Granuloma**.
- **Clinically:** primary TB usually asymptomatic or / minor illness.
- **Non-pulmonary TB:** may spreads from pulmonary infections to other organs e.g.:
 - 1- TB of lymph nodes (cervical, mesenteric) 2- TB meningitis
 - 3- TB bone & joint 4- Genitourinary TB 5- **Miliary TB** \implies **Blood**
 - 6- Soft tissue (**cold abscess**): lack of inflammation & caseation.
 (Caseation: due to delayed hypersensitivity reaction. Contains many bacilli, enzymes, O₂, N₂ intermediates \implies necrotic centre of granuloma \implies cheesy material).

2-Secondary Tuberculosis:

- Occurs later in life (**reactivation of previous infection or "reinfection"**)
- Lung more common site & **happens more with Immuno compromised patients.**
- Lesion localized in **apices (apex or the upper lobe)**
- Infectious & symptomatic
- Microscopy: many bacilli, large area of caseo~~l~~rosis cavity "open TB" (**open TB means that it's infectious**) with granuloma and caseation.
- **Clinically:** fever, cough, **hemoptysis** (coughing blood), **weight loss** & weakness.
- **Source of secondary TB :**
- **Endogenous** (**reactivation** of an old TB)
- **Exogenous** (**re-infection** in a previously sensitized patient who has previous infection with the organism).

PRIMARY

- Initial infection
- Microscopy: lesion shows **Granuloma**
- asymptomatic & may spread to other organs

SECONDARY

- Reactivation for the infection
- Happens if the patient immune system is depressed
- Infectious & symptomatic / occurs at the apex of the lungs

Immunity to Tuberculosis:

- Cell-mediated immunity associated with delayed hypersensitivity reaction.
- Detected by tuberculin test.
- Tuberculin test takes 2-10 weeks to react to tuberculin and becomes positive.

Tuberculin Test:

- Uses purified protein derivative (PPD).
- Activity expressed by Tuberculin unit.
- Activates synthesized lymphocytes to produce CMI which appear as skin indurations.
- **May not distinguish between active and past infection except in an individual with recent contact with infected case.**
(And cannot distinguish the BCG "vaccination ")
- Low level activity induced by environmental mycobacteria.

❖ Methods of Tuberculin Test:

- we do it intradermally

❖ Positive Tuberculin Test:

- **1- >5mm induration in the following:**
 - Recent contact with active TB.
 - HIV or high risk for HIV
 - Chest X-ray consistent with healed TB.
- **2- > 10mm induration:**
 - IV drugs user, HIV seronegative patient.
 - Medical conditions eg. diabetes , malignancy.
 - Residents & employee at high risk
 - Patients from country with high incidence.
 - Children < 4yrs or exposed to adult high risk group.
 - Mycobacteriology lab. personnel.
- **3- >15 mm induration: (positive with anyone)**
 - Positive in any persons including those with no risk factors for TB.

❖ Negative Tuberculin Test:

- **No induration , either due to:**
 - No previous infection
 - Pre-hypersensitivity stage
 - Lost TB sensitivity with loss of antigen
- **AIDS patients are anergic and susceptible to infection.**

Laboratory Diagnosis of TB:

● Specimens:

How to collect specimens :

- **In case of Pulmonary TB:** take 3 early morning sputum samples ,or bronchial lavage, or gastric washing (infants) ,...etc.
- In case of TB meningitis : Cerebrospinal fluid (CSF)
- We can also use 3 early morning urine
- Or Bone , joint aspirate
- And in case of Lymph nodes, pus or tissues but **we don't use** swab.
- Repeat sample.

● Direct microscopy of specimen :

- **Z-N** or (Auramine) stain.to identify infectious cases[open cases]

● Culture: gold standard for identification and sensitivity.

- **Media used:** Lowenstein-Jensen media (L J).
- **Contains:** eggs, asparagine, glycerol, pyruvate/ malachite green.
- Colonies appear in LJ media after 2-8 weeks as eugenic, raised,buff,adherent growth enhanced by glycerol (*MTB*) or by pyruvate (*M.bovis*).
- Other media **plus** LJ media may be used :
 - Fluid media (middle Brook)
 - MGIT(**Mycobacterium Growth Indicator Tube**)
 - Automated methods:- eg.Bactec MGIT.
 - Measurement of interferon –gamma (IF-γ) secreted from sensitized lymphocytes challenged by the same mycobacterial proteins in a patient previously exposed to disease, will produce interferon gamma. **Has a specific significance than tuberculin test.**
 - **PCR:** directly from specimen (CSF).

● Identification:

- Morphology , growth at 37C + 5-10 CO₂
- Biochemical tests :Niacin production & Nitrate. (To elect enviromental mycobacterium)
- Sensitivity testing
- Guinea pig inoculation: rarely used.

❖ In General :

- you take specimen (sputum or CSF or urin ... etc)
- Then you stain it (Z/N) and you look for AFB
- Then after that you culture the specimen in either fluid media (middle Brook) or solid media(LJ)
- Then you identify the organism (by Biochemical tests)

Management of a TB case:

- **Isolation: for 10-14 days (for smear positive cases i.e. > 1000 organisms / ml, considered infectious case).**
 - We use Triple regimen of therapy (3 drugs) .Why ?
 - To prevent resistant mutants
 - To cover strains located at different sites of the lung .
 - To prevent relapse
- **Treatment must be guided by sensitivity testing.**

❖ First Line Treatment:

- **Isoniazide (INH)**
- **Rifampicin (RIF)**
- **Ethambutol (E)**
- **Pyrazinamide (P)**
- **Streptomycin (S)**
- INH+ RIF +P for 2 months then continue with INH+RIF for **4-6 months**.
Multidrug resistant TB is resistance to INH & RIF.
- Directly Observed Therapy (DOT).

❖ Second Line:

- Used if the bacteria resist the 1st line drugs (More toxic than the 1st line drugs).
- 1. PAS (Para-Amino Salicylic acid)
- 2. Ethionamide
- 3. Cycloserine,
- 4. Kanamycin,
- 5. Fluroquiolones

❖ Prevention of TB:

- **Prevent bovine TB By :**
 - 1. Tuberculin testing of herds
 - 2. Slaughter of infected animals
 - 3. Pasteurization of milk
- **Recognition of new cases.**
- **Prophylaxis with INH of contacts.**
- **Follow up cases .**
- **Immunization with BCG to all new borne**

- The best way is to Isolate the patient
- Use of BCG " vaccination "
- Pasteurization of milk

Summary:

- ❖ TB is a chronic infectious disease which mostly caused by Mycobacterium Tuberculosis M.TB.
- ❖ TB could be Latent infection (which affect 1/3 of human race) or Active disease (usually devolved after patient immunity is compromised).
- ❖ In Africa it is common that TB couple with HIV infection.
- ❖ Immuno compromise patients (e.g. Diabetic patient's) are the most likely to have TB infection.
- ❖ The immunity in TB infection is Cell Mediated Immunity (CMI).
- ❖ Primary infection is initial infection " first time to be infected "
- ❖ Secondary infection it's either Re-activation or Re-infection.
- ❖ Most common site for TB is lungs the pulmonary TB is the most infectious (because you can cough it or sneeze it ...).
- ❖ Pulmonary TB my spreads from Lungs to Lymph Nodes, Bones & tissue.
- ❖ The organism is very slow growing, it take almost 6 to 8 week to grow in the laboratory.
- ❖ The best confirmatory test to diagnose TB is isolation of the organism in the specimen at the laboratory.
- ❖ The quickest test is when you stain the specimen and look for the organism under the microscope.
- ❖ Isolation of the patient for 10-14 days for smear positive cases i.e. > 1000 organisms / ml, because it's considered infectious case.
- ❖ To Treat TB we use :Isoniazide (INH), Rifamoicin (RIF), Ethambutol (E), Pyrazinamid (P), Streptomycin (S)
- ❖ For the prevention of TB we use BCG " vaccination "

Quiz

1) Direct microscopy is used in the diagnosis of:

M tuberculosis

2) In treatment of tuberculosis:

a) Ethambutol

b) Pyrazinamide

c) Isoniazide

d) Rifampicin

3) BCG is :

Live attenuated vaccine

4) Which one of these mycobacterium cause AIDS:

M tuberculosis

5) The time estimated for inoculation of TB is:

8 weeks