MED437
KING SAUD UNIVERSITY

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MICROBIOLOGY
437

Tuberculosis & Mycobacteria

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Revised by

Important!
Doctor's Notes
Only found in females' slides
Only found in males' slides
Extra Notes

" I'm not telling you it's going to be easy. I'm telling you it's going to be worth it."

Objectives

- Recognize that tuberculosis as a chronic disease mainly affecting the respiratory system.
- Know the epidemiology of tuberculosis worldwide and in the kingdom of Saudi Arabia
- Understand the methods of transmission of tuberculosis and the people at risk.
- Know the causative agents, their characteristic and staining methods.
- Understand the pathogenesis of tuberculosis.
- Differentiate between primary and secondary tuberculosis and the clinical features of each.
- Understand the method of tuberculin skin test and result interpretation.
- Know the laboratory diagnostic methods.
- Know the chemotherapeutic agents and other methods of prevention and control of tuberculosis.

Introduction

- Tuberculosis (TB) is an ancient, chronic disease affects humans, caused by Mycobacterium tuberculosis complex.
- A major cause of death worldwide.
- Usually affects the lungs, other organs can be affected in one third of cases.
- If properly treated is curable, but fatal if untreated in most cases.
- TB affects 1/3 of human race (2 billions) as a latent dormant tuberculosis.
- Incidence: a worldwide disease, more common in developing countries.
- Affects all age groups who are subject to get the infection.

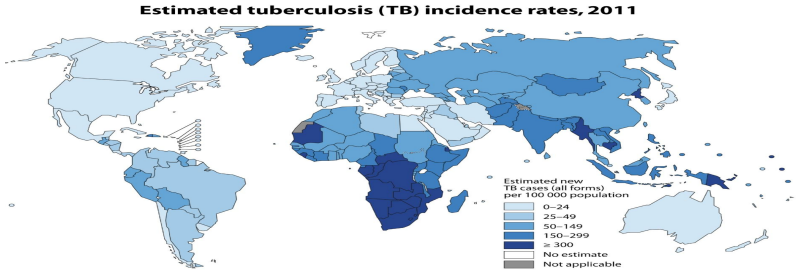
مو مهم نحفظ الارقام بس لازم نعرف كيف انه مرض منتشر بكل مكان بالعالم وبالذات بال low developing countries

- The WHO estimated 8.9 million new cases in 2004 & 2 - 4 million death(1 million in boys slides).
- Incidence :

  KSA : 32-64 cases /100,000. [(KSA 2011 Data): 0-24 cases /100,000 population in girls slides].

  USA : 5.2 cases/100,000

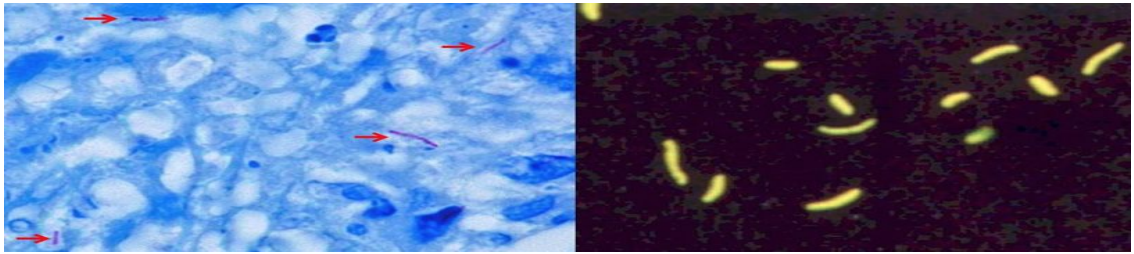
  Southeast Africa : 290 cases /10,000 due to coupling with HIV infection.

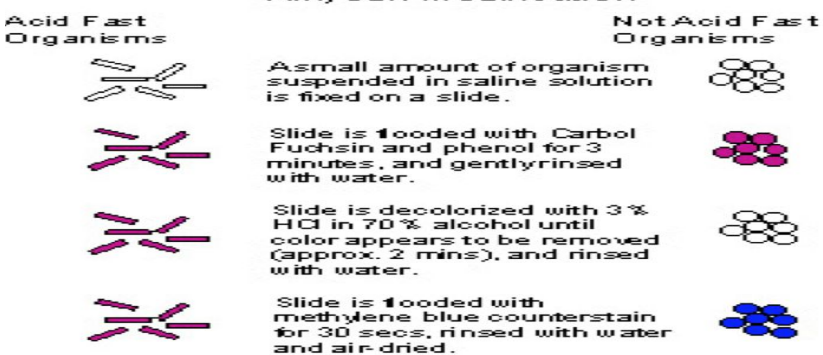


Acid-Fast Bacilli (AFB)

- Stains used : **Ziehl-Neelsen** stain (ZN stain) and **Auramine Rhodamine** stain.
- Strict aerobes (can't live with O2)
- Multiply intracellularly (inside the cells, macrophages, and other tissues)
- Cause delayed hypersensitivity reaction type 4 of immune response
- Slowly growing (between 2 - 8 weeks.) (due to high lipid that surround the cell wall preventing nutritions to reach the cell)

ZN Stain Vs. Auramine



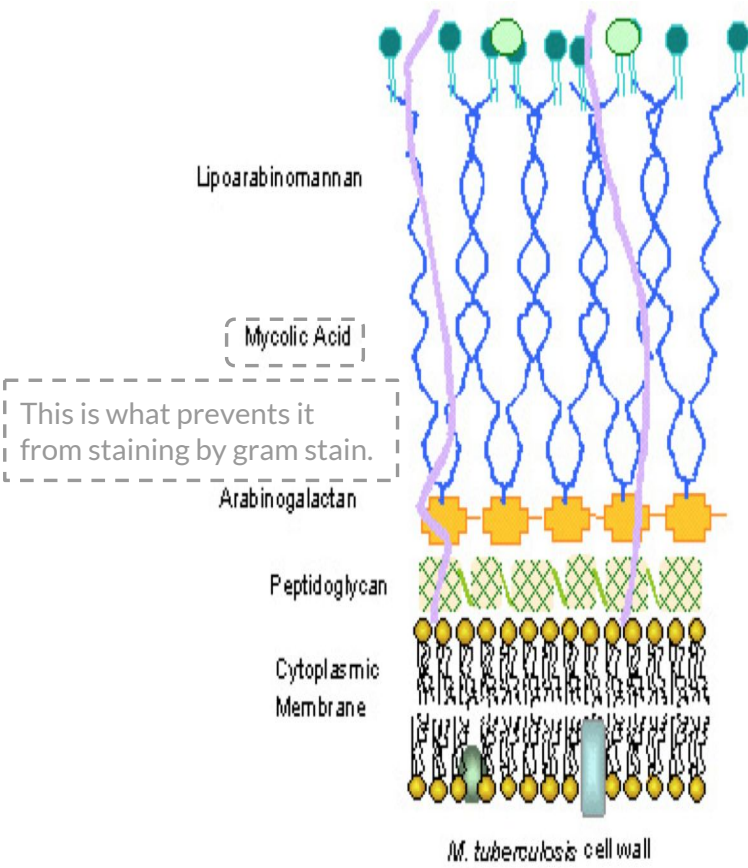


1. Staining by using carbon fusion stain (red color)
2. Fixation (using the heat to allow the dye to go inside the wall)
3. Decolorization by strong acid (methanol 3-5% or hypochloric acid)

نستخدم حمض قوي عشان نتأكد انها تتحمل الديكولارايزيشن بالحمض لذلك اسمها AFB

فمهمها استخدمنا حمض قوي في حاله التي بي it will not lose the dye

فلو كان المريض مصاب بالتي بي راح يبقى لونها احمر وما يتغير



Species of Mycobacteria

- ***Mycobacterium tuberculosis complex*** **cause <u>tuberculosis.</u>**
- *Mycobacterium leprae* causes **leprosy.**
- Atypical Mycobacteria / Mycobacteria other than tuberculosis (MOTT) cause infections in immunosuppressed patients.

Mycobacterium tuberculosis complex

- <u>Mycobacterium tuberculosis complex</u>:

1- M. tuberculosis (human type) very common

2- M. bovis (bovine type) (rare because of pasteurization of milk)

3- M. Africanum

4- BCG strains (used for vaccination but in rare cases it can cause TB in immunocompromised children)

All can cause TB

Epidemiology + Transmission:

mainly through inhalation of airborne (transported by air) droplet (tiny and wet) nuclei (< 5 µm) in pulmonary diseases case.
rarely through GIT & abraded skin

Reservoir:

Source

patients with open TB. (when a person has TB with chronic cough)

Age:

young children & adults

People at risk :

-lab. technicians
- workers in mines
-doctors
- nurses
- HIV patients
-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 because it Contain **high lipid conc. (Mycolic acid)** in the ***cell wall*** which resist staining. (prevent crystal violet to reach Peptidoglycan)

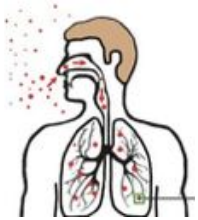
Called **Acid- alcohol fast bacilli (AFB)**, because it *resists decolorization* with up to **3% HCL, 5% ethanol or both.**
So, it is Stained by **Ziehl-Neelsen (Z-N)** and Auramine staining

Mycobacterium species appear tiny red bacilli acid fast bacilli (AFB) by Z-N stain

Pathogenesis of Tuberculosis

1. Mycobacteria acquired by airborne droplet reaches the alveolar macrophages, where they survive alveolar macrophages' main virulence factor. (when the bacteria survives intracellularly in the macrophages)
2. This starts cell mediated immune response; which controls the multiplication of the organism but does not kill it.
3. Granuloma is formed, organism lives in dormant state (latent tuberculosis infection).
4. Patient show evidence of delayed cell mediated immunity (CMI). (hypersensitivity reaction)
5. Disease results due to destructive effect of CMI.

- Clinically the disease is divided into primary or secondary



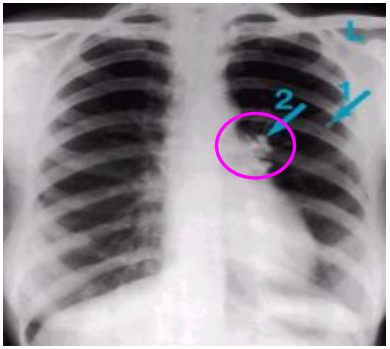
Primary Tuberculosis (Occurs in patients not previously infected)

Pathogenesis

Inhalation of bacilli → Phagocytosis → Lymph nodes calcify to produce GHON focus. GHON focus or "Primary Complex" occurs at the periphery of the mid zone of the lung.

★ **Microscopy** of lesion shows **Granuloma**.
★ **Clinically**, primary TB usually **asymptomatic** or minor illness. (rarely transmitted)

Ghon focus in chest x-ray



Other sites beside the lung

Non-pulmonary TB :

may spreads from pulmonary infections to other organs eg.:

- **TB of lymph nodes (cervical, mesenteric)**
- **TB meningitis** (specifically in children, immunocompromised and old people)
- **TB bone & joint**
- **TB of the genitourinary system.**
- **TB miliary (Blood and other organs.)**
- **TB of soft tissue (cold abscess): lacks inflammation with Caseation** (shows no signs of inflammation)

Caseation: due to delayed hypersensitivity reaction. Contains many bacilli ,enzymes, O2,N2 intermediates, necrotic center of granuloma with cheesy material. (characteristics of cold abscess or TB of soft tissue)

Secondary TB (reactivation)

- Occurs sometime at lifetime (Later in life).
- Lung more common site
- Immunocompromised patients. (and old people)
- Lesion localized in apices
- Infectious & symptomatic (most infectious)
- Microscopy: many bacilli, large area of caseous necrosis → cavity (with large number of bacteria) (open TB) with granuloma and caseation. (sometimes the cavity will spread into the bloodstream of the lung and once its spread the coughing of patient will contain blood which cause the cavity to be mixed with the blood)

- Clinically: fever, cough, hemoptysis ,weight loss & weakness. (chronic cough and fever that lasts for months **NOT DAYS!** and the patient isn't responding to treatment)
- Source of secondary TB :
  - Endogenous (reactivation of an old TB) (this bacteria will be activated by aging or any source of immunosuppression)

  OR
  - Exogenous (reinfection with new strain) (reinfection in a previously sensitized patient who has previous infection with the organism).

Immunity to Tuberculosis

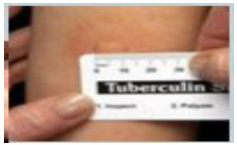
- Cell-mediated immunity associated with delayed hypersensitivity reaction.
- Detected by tuberculin skin test.
- Tuberculin test takes **2-10 weeks** to react to tuberculin and becomes positive.

Tuberculin Skin Test

- Uses purified protein derivative (PPD).
- Activity expressed by Tuberculin unit.
- Activates synthesized lymphocytes to produce CMI which appear as skin induration.
- May not distinguish between active and past infection except in an individual with recent contact with infected case.
- Low level activity induced by environmental mycobacteria, previous vaccination. (cross reaction with the mycobacteria in the environment might cause the TB skin test to be false positive)

- **Methods of Tuberculin Skin Test:**

1. Mantoux Test
   - Intradermal inoculation of 0.1 ml of PPD, (purified protein derivative) 5 tuberculin units.
   - Read after 48-72hrs.
2. Heaf test (screening).



Tuberculin Skin Test

POSITIVE (the patient has or had TB)

1. >5mm induration positive in:

- Recent contact with active TB
- HIV or high risk of HIV
- Chest X-ray consistent with healed TB

2. >10mm induration positive in:

- IV drugs user
- HIV seronegative* patient
- medical conditions eg: diabetes, malignancy
- Residents & employee at high risk, and also patients from country with high incidence
- Children < 4 years or exposed to adult high risk group

*The lab result is negative for HIV although the person have it. If the patient has TB we have to suspect HIV because it's always associated with it

3. >15mm induration positive in:

- Any persons including those with no risk of TB

NEGATIVE

- 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

IMPORTANT!

1) Specimens	2) Direct microscopy of specimen	3) Culture
❖ **Pulmonary TB:** 3 *early morning*(1) sputum samples or *bronchial lavage*(2) or *gastric washing*(3) (infants) ❖ **TB Meningitis:** Cerebrospinal fluid (CSF). ❖ **TB of the genitourinary system:** 3 early morning urine. ❖ **TB bone & joint:**Bone, joint aspirate. ❖ **TB of lymph nodes:** pus or tissues, NOT swab. ❖ Repeat the sample.(4)	Z-N or (Auramine) stain. *eggs for nutrition - asparagine: amino acid source one tube with glycerol: to improve the growth of M. human type other tube with pyruvate: to improve the growth of M.bovis malachite green: inhibits other bacteria and select just M. TB 1-because the number of bacteria is higher. 2- for patient who cannot give us sputum.(المنومين) 3-because children swallow their sputum. 4-if the sample isn't good and we still think he/she has TB.	**Culture:** the gold standard. Important for identification and sensitivity. ❖ **Media used:** Lowenstein-Jensen media (LJ). ❖ **Media 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) -enhanced by pyruvate: (M.bovis).

Laboratory Diagnosis of TB

IMPORTANT!

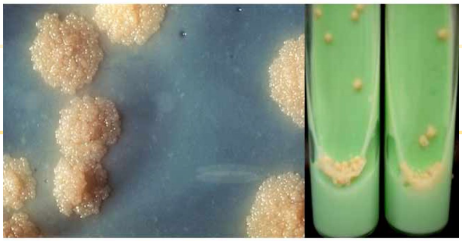


Fig: Cultural Characteristics of *Mycobacterium tuberculosis*

4)Other media plus LJ media may be used:

- Fluid media (middle Brook).
- Automated methods : eg. Bactec MGIT (Mycobacteria Growth Indicator Test).
- Measurement Interferon –gamma release assay (IGARAs): positive in latent TB.(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 skin test. (increased risk of developing TB).
- Molecular method : to detects the DNA of the bacteria

1-ProbTech ;detects nucleic acid directly from respiratory samples.
2-Xpert MTB/RIF detect nucleic acid and resistance to rifampicin .
3-PCR(polymerase chain reaction): molecular test directly from specimen (CSF).

Mycobacterium

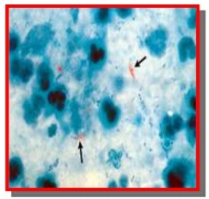


Fig. 5.3: Myc. TB in Sputum, Z.N stain
(few thin pink bacilli with blue background)

Fig. 5.1: Selective media for Myc. T.B.

Fig. 5.2: Culture of Myc. TB on L.J. media
- Grow after 6-8 week

Identification

- Morphology , growth at 37c + 5 -10 % CO2
- Biochemical tests: Niacin production & Nitrate test.
- Susceptibility testing to detect resistance to anti-TB agents
- Guinea pig inoculation: rarely done.

Management of a TB case

- Isolation for 10-14 days (for smear positive cases i.e. > 1000 organisms / ml of sputum considered infectious case).
- Triple regimen of therapy. Why? (combination of 3 or 4 drugs)
  1. *To prevent resistant mutants*
  2. *To cover strains located at different sites of the lung*
  3. *To prevent relapse*
- Treatment must be guided by sensitivity testing.

Treatment of TB

VERY IMPORTANT!

First Line Treatment

- Isoniazid (INH)
- Rifampicin (RIF)
- Ethambutol (E)
- Pyrazinamide (P)
- Streptomycin (S)
- Directly Observed Therapy (DOT).

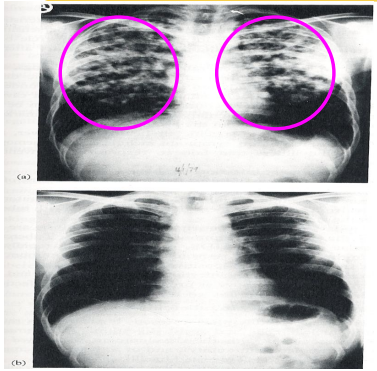
Combination of 3 drugs initially (4 drugs if resistance is suspected)
For the first 2 months → INH + RIF + P or E +S For the next 4--6 months → INH + RIF

-Why do we use this large amount of drugs? Because some "multidrug resistant TB" is resistant to INH and RIF.

Second Line Treatment

- PASA (Para-Aminosalicylic acid).
- Ethionamide.
- Cycloserine.
- Kanamycin.
- Fluoroquinolones.

-Used if the bacteria was **resistant** to first line drugs.
-More **toxic** than the first line drugs.



Tuberculosis: (notice how it became more clear after the treatment)

(a) Chest X-ray of a patient with tuberculous bronchopneumonia.

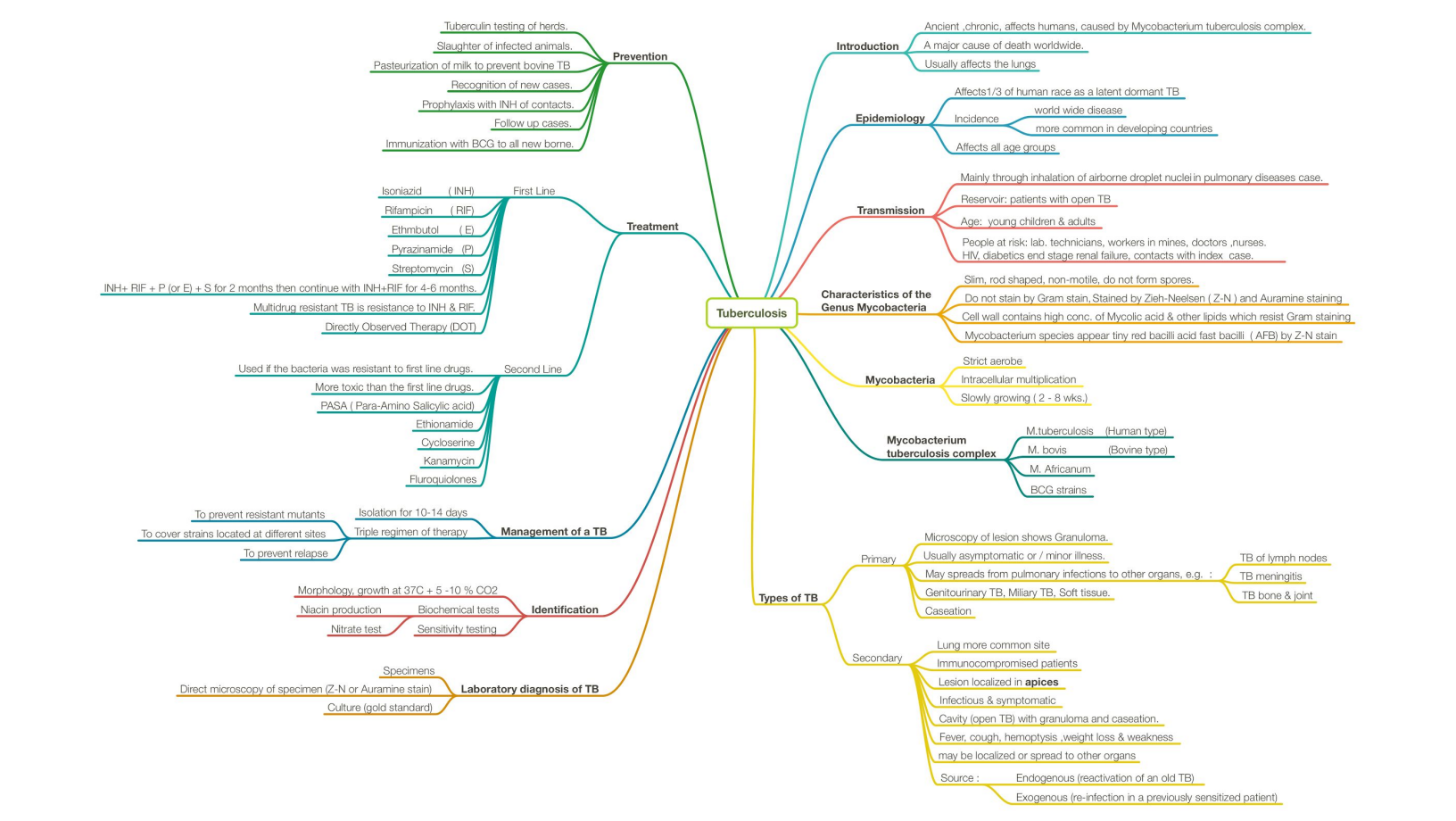
(b) Chest X-ray of the same patient 10 months after antituberculous therapy.

Prevention of TB

- Tuberculin testing of herds.
- Slaughter of infected animals.
- Pasteurization of milk to prevent bovine TB.
- Recognition of new cases.
- Prophylaxis with INH of contacts.
- Follow up cases.
- Immunization with BCG (live attenuated) to all newborns. (The baby will get a vaccine(BCG) on the second day of delivery)

Important notes from Dr. Ali Alsomaily

- Tb is the most infectious mortality disease.
- Tb is transmitted by inhaling Aerosols not droplets
- Tb can be hard to diagnose.
- Mycolic Acid is the waxy substance that prevents the Tb from being stained by gram stain.
- BCG vaccine is given to prevent from Tb.
- Not taking the BCG vaccine will increase the risk of Tb Meningitis *very important
- INH can be given as an anaphylactic
- He told as about the different between the sample sent to the histology lab and the microbiology lab:
- The sample sent to the histopathology lab has "Formalin- Formaldehyde" to keep the integrity of the sample while the sample sent to the micro lab does not have Formalin because we do not want to kill the microorganism
- The following is very important and Dr. Ali directly mentioned that there will be a question about:
- Mantoux test Vs Quantiferon test.
- Dr. Ali asked this question:
- Which test can be used to predict the future of Tb? Quantiferon because it's specific


Tuberculosis
Introduction
Ancient ,chronic, affects humans, caused by Mycobacterium tuberculosis complex.
A major cause of death worldwide.
Usually affects the lungs
Epidemiology
Affects1/3 of human race as a latent dormant TB
Incidence
world wide disease
more common in developing countries
Affects all age groups
Transmission
Mainly through inhalation of airborne droplet nuclei in pulmonary diseases case.
Reservoir: patients with open TB
Age: young children & adults
People at risk: lab. technicians, workers in mines, doctors ,nurses.
HIV, 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, Stained by Zieh-Neelsen (Z-N) and Auramine staining
Cell wall contains high conc. of Mycolic acid & other lipids which resist Gram staining
Mycobacterium species appear tiny red bacilli acid fast bacilli (AFB) by Z-N stain
Mycobacteria
Strict aerobe
Intracellular multiplication
Slowly growing (2 - 8 wks.)
Mycobacterium tuberculosis complex
M.tuberculosis (Human type)
M. bovis (Bovine type)
M. Africanum
BCG strains
Types of TB
Primary
Microscopy of lesion shows Granuloma.
Usually asymptomatic or / minor illness.
May spreads from pulmonary infections to other organs, e.g. :
TB of lymph nodes
TB meningitis
TB bone & joint
Genitourinary TB, Miliary TB, Soft tissue.
Caseation
Secondary
Lung more common site
Immunocompromised patients
Lesion localized in apices
Infectious & symptomatic
Cavity (open TB) with granuloma and caseation.
Fever, cough, hemoptysis ,weight loss & weakness
may be localized or spread to other organs
Source :
Endogenous (reactivation of an old TB)
Exogenous (re-infection in a previously sensitized patient)
Prevention
Tuberculin testing of herds.
Slaughter of infected animals.
Pasteurization of milk to prevent bovine TB
Recognition of new cases.
Prophylaxis with INH of contacts.
Follow up cases.
Immunization with BCG to all new borne.
Treatment
First Line
Isoniazid (INH)
Rifampicin (RIF)
Ethmbutol (E)
Pyrazinamide (P)
Streptomycin (S)
INH+ RIF + P (or E) + S 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 was resistant to first line drugs.
More toxic than the first line drugs.
PASA (Para-Amino Salicylic acid)
Ethionamide
Cycloserine
Kanamycin
Fluroquiolones
Management of a TB
Isolation for 10-14 days
Triple regimen of therapy
To prevent resistant mutants
To cover strains located at different sites
To prevent relapse
Identification
Morphology, growth at 37C + 5 -10 % CO2
Biochemical tests
Niacin production
Nitrate test
Sensitivity testing
Laboratory diagnosis of TB
Specimens
Direct microscopy of specimen (Z-N or Auramine stain)
Culture (gold standard)

Quiz

1- Which of the following diagnostics tests can be used to diagnose drug resistant tuberculosis?

A. Sputum smear microscopy
B. Liquid culture
C. Interferon-gamma release assay (IGRA)
D. Chest x-ray

2- The ideal clinical specimen for pulmonary TB diagnosis is

A. Blood
B. Sputum
C. Urine
D. Tissue

3- The correct transmitting of tuberculosis is :

A. Cough
B. Sexual transmission
C. Water
D. Touch

4- Which of the following ATT drugs has no hepatotoxicity potential?

A. Rifampicin
B. Pyrazinamide
C. Isoniazid
D. Ethambutol

5- Which of the following drugs has the highest potential to produce exfoliative dermatitis?

A. Isoniazid
B. Rifampicin
C. Pyrazinamide
D. Ethambutol

6- Which one is refer to tuberculosis?

A. Patholosis
B. White plague
C. Granulomatis
D. Sinusitis

SAQs

1. **Describe the correct drug regimen for a newly diagnosed patient with pulmonary tuberculosis.**

2. **List the characteristics of the Genus Mycobacteria, does it stained by gram? Why & why not?**

3. **What is the name of the bacteria which causes tuberculosis ?**

ANSWERS

- Q1. 2 months of Rifampicin, Isoniazid, Pyrazinamide and Ethambutol followed by 4 months of Rifampicin and Isoniazid
- Q2. Slim, rod shaped, non-motile, do not form spores. Do not stain by Gram stain . Because the cell wall contains high conc. of Mycolic acid & other lipids which resist Gram staining .
- Q3. Mycobacterium tuberculosis (M. TB)

ANSWERS

1. B
2. B
3. A
4. D
5. C
6. B

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