Tuberculosis

TEAM 439
MICROBIOLOGY

VERSION 1

Objectives

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

Colour index:

Red: Important & Doctor's notes.

Grey: Extra info & explanation.

Purple: Only in girl's slides.

Orange: Only in boy's slides.

Green: Lecture notes

Any future corrections will be in the editing file, so please check it frequently.

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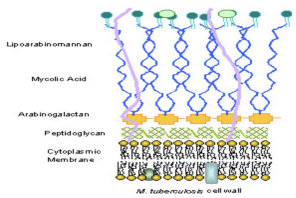
SCAN ME

Characteristics of the Genus Mycobacteria

- Slim, rod shaped, non-motile, do not form spores, strict aerobes
- Multiply intracellularly
  We can't detect antibodies to mycobacteria because it multiplies intracellularly inside the phagocyte
- Cause delayed hypersensitivity reaction type of immune response to mycobacterium protein
- Slowly growing (2-8 weeks) because the mycolic acid (fatty acid) prevents food from reaching the bacteria
- Do not stain by Gram stain because the cell wall contains high lipid concentration (Mycolic acid) found above peptidoglycan which resists staining by Gram stain.
  Gram stain usually stains the peptidoglycan but in this case it can't reach it

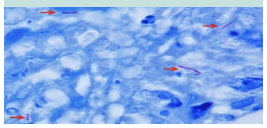


Acid- Alcohol Fast Bacilli (AFB)

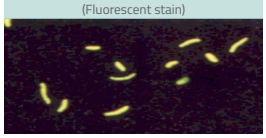
Resist decolorization with up to 3% HCL, 5% ethanol or both.
It's another method for staining (alcohol based staining) and when decolorization is done the the stain will still be present and that is because of the mycolic acid and because the mycobacteria tolerates high acid and alcohol. A positive AFB stains the mycobacteria with red and a blue counterstain (background)

➢ **Stains used :** Can penetrate the mycolic acid
- **Ziehl-Neelsen stain (ZN stain)**
- **Auramine** Rhodamine stain Needs to use fluorescent microscope.

Ziehl-Neelsen stain (ZN stain)



Auramine Rhodamine stain
(Fluorescent stain)



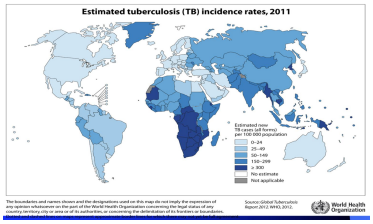
Species of Mycobacteria

Mycobacterium tuberculosis complex Pathogen causes disease even in immunocompetent There are 8 types but these are the most important ones	**Include:** - M.tuberculosis (Human type) Most common - M. bovis (Bovine type) الدرن البقري، تسبب درن بالحيوانات Very rare nowadays because milk is being pasteurized - M. Africanum Common in African countries - BCG strains ضعيف جدًا جدًا لذلك نستعمله في اللقاح **Cause:** Tuberculosis in human
Mycobacterium leprae Pathogen causes disease even in immunocompetent	**Causes** leprosy الجذام Nearly became extinct from the world, existed previously.
Atypical Mycobacteria / Mycobacteria other than tuberculosis (MOTT)	**Cause** infections in immunosuppressed patients Doesn't cause disease in immunocompetent (healthy) person

About 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.

Epidemiology



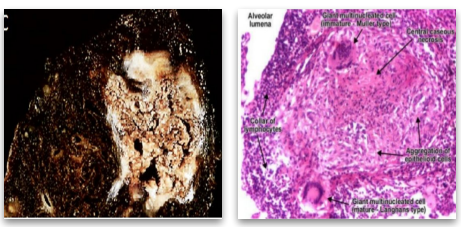
Incidence	❖ A world wide disease, more common in developing countries (see diagram).في الدول النامية يكون مستوطن ❖ WHO estimated 8.9 million new cases and 2-4 million death in 2014 ❖ TB affects 1/3 of human race as a latent dormant tuberculosis. Latent dormant TB: the body contains the TB but the immune system is keeping it under control, so it stays latent (كامن/خامل) for years and when the person gets older and his immunity is weakened he develops secondary TB.
Age	❖ Affects all age groups who are subject to get the infection. ❖ Young **children** & adults higher risk in children and elderly
Transmission	Mainly through inhalation of **airborne droplet nuclei** (< 5 µm) in pulmonary diseases cases, rarely through GIT & skin. Due to the small size of the airborne droplet nuclei it stays in the air for a longer time and travels a longer distance which makes the area infectious for a longer time but on the other hand the virus with a bigger size tend to fall to the ground so after a period of time the area isn't infectious. People who are suspected to be infected with TB are placed in an airborne isolation, which is a room with negative pressure that traps the air inside.
Reservoir	Patients with open TB.
People at risk	❖ Lab technicians ★ Immunosuppressed patients (Note: mainly patients with **HIV** & Children) + Patients with diabetes, renal failure, on steroids or chemotherapy etc.. ❖ Workers in mines لان المناجم ما فيها تهوية كويسة ❖ Contacts with index case. E.g. family members (Very common)

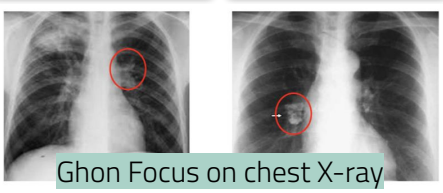
Pathogenesis of Tuberculosis

- Mycobacteria is acquired by airborne droplet which reach the alveolar macrophage by inhalation and are able to survive there because they're not recognized by antibodies yet (main virulence factor).
- Stimulates cell mediated immune response which controls the multiplication of the organism by surrounding it but does not kill it.
- Granuloma is formed and organism lives in dormant state. (Latent tuberculosis infection).
  ماراح يعدي أحد
- Patient show evidence of delayed cell mediated immunity (CMI)
- Disease results due to destructive effect of CMI.
- Clinically the disease is **divided into primary or secondary**

	Primary TB	Secondary TB
Overview	Occurs in patients not previously infected. Inhalation of bacilli>phagocytosis by macrophages>lymph nodes calcify to produce **ghon focus** (primary complex) at the periphery of mid zone of the lung.	Occurs later in life (After reactivation) (Immunocompromised patients)
Clinically	95% of the cases are usually asymptomatic or minor illness (mild symptoms like fever) لأن الاميون سستم حقهم شغال تمام وخلاه يهجد However, 5% of the patients will develop a symptomatic primary disease (if they are children or immunocompromised)	Infectious and symptomatic. Symptoms like fever, cough (Bloody), hemoptysis, weight loss and weakness
Microscopy	Lesion shows Granuloma	Lesion localized in apices of the lung قلنا فوق أن MTB is strictly aerobic والـApex of the lung تعتبر أكثر مكان فيه اوكسجين Many bacilli, large area of caseous necrosis (cavity، open tb) with granuloma and caseation.
	Non pulmonary TB: may spread from pulmonary infections to other organs (severe progression of primary or secondary disease will cause the bacteria to get out of the lungs and spread to other organs) • Lymph nodes (cervical, mesenteric) • CNS, meningitis (fatal) • Bone and joint e.g. pott's disease • Genitourinary TB • Miliary TB (blood) • Soft tissue (cold abscess): lack of inflammation with caseation.	Sources of secondary TB: (Lung is the most common site) 1- Endogenous (reactivation of an old TB). (in most of the cases) كان عنده تي بي من زمان بس الاميون سستم تعامل معه، لكن بعدين ضعفت مناعته وصار له reactivation 2- Exogenous (re-infection with new strain).

Caseation: due to delayed hypersensitivity reaction. Contains many bacilli, enzymes, O_2, N_2 intermediates which will lead to necrotic center of granuloma (cheesy material)





Ghon Focus on chest X-ray

Immunity to tuberculosis (Check immunology team)

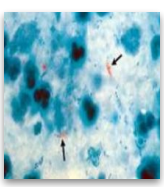
- CMI associated with delayed hypersensitivity reaction.
- Detected by tuberculin skin test.test.
- Tuberculin test takes 2-10 weeks to react to tuberculin and becomes positive

Laboratory Diagnosis of TB

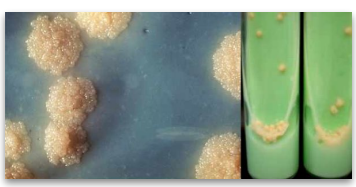
Specimens (Depends on affected organ)	★ Pulmonary TB (Three sputum samples (at least one early morning) or bronchial lavage, or gastric washing (used on infants since they can't release a sputum sample) • Concentration of the bacteria is higher in the morning before consumption of any food. ❖ TB meningitis Cerebrospinal fluid (CSF) ❖ Urinary tract tuberculosis three early morning urine ❖ TB of Bone. Join aspirate ❖ TB of Lymph node Pus or tissues, not swab ❖ Repeat sample as required
Direct microscopy of specimen	**After taking the specimen,** we do direct microscopy. **Use Z-N or (auramine) stain** مو دقيق بنسبة ١٠٠٪ لأن فيه احتمالية يطلع لك False negative لو كان عدد البكتيريا قليل بالعينة لكن بنفس الوقت مستحيل نطلع المريض البيت ونخليه يعدي الكرة الأرضية كلها على بال ماتنمو الكلتشر لو هو مصاب الزبدة اذا طلع Positive وشفنا AFB في الـsmears نعزل المريض ونسوي الـMolecular methods و نبدأ العلاج بشكل عاجل، اما لو طلع nagative وماشفنا AFB يصير فيه احتمالين ١- ان المريض فعلا سليم ٢- انه مصاب لكن عدد البكتيريا عنده قليل مره لدرجة انه Not infectious . وبكل الحالتين نطلعه عادي وننتظر نتيجة الكلتشر
Culture	★ Culture is the **gold standard**, Important for identification and sensitivity لازم نسويها بكل الحالات سواء لقينا AFB في الـsmears أو مالقينا **Morphological characteristics:** growth at 37 C + 5 -10 % CO2 الكلتشر عشان تنمو لازم تنحط في incubator درجة حرارته ٣٧ وفيه CO2 **Media used:** we use 2 medias; 1 solid (LJ) + 1 liquid (MGIT) **1- Lowenstein-Jensen media (LJ)** **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) الميديا فيه منها نوعين، نوع مع جلسرول وهذي بتنمو فيها Human type الـMycobacterium tuberculosis ونوع ثاني فيها بايروفيت، تنمو فيها Mycobacterium bovis **2- Bactec MGIT** (Mycobacterium Growth Indicator Test) هذي الميديا Liquid & Fast growing لكنها تستعمل مع LJ وتحت مع حطوا مليون خط. الزبدة يعني LJ شيء اسااااااسي والميديا هذي للمعلومية يعني ماتطلع لك colonies زي LJ إنما بس تبين لك كمية الCO2 اللي استهلكتها البكتيريا عشان كذا ما تكفي لوحدها
Other Methods <u>in addition</u> to LJ media	❖ **Automated methods:** **Bactec MGIT** (explained above) We say automated because it is a fully automatic system that exploits the fluorescence of an oxygen sensor to detect growth of mycobacteria in culture. ❖ **Molecular methods:** Fast method, takes about **1 hour** - **ProbTech** detects nucleic acid directly from respiratory samples - **Xpert MTB/RIF** detects nucleic acid and resistance to rifampicin هنولي متى نسويهم؟ اذا طلعت الـsmear بوسئف ولقينا فيها AFB وفايدتهم يعلمونا قاعدين نتعامل مع اي Mycobacterium وهل هي مقاومة ولالا؟ نفترض سوينا smear وطلعت positive for AFB وجينا وسوينا Xpert MTB/RIF وطلع Negative ايش يعني؟ أنه عنده Atypical mycobacteria غير MTB ولازم ننتظر الكلتشر

After weeks, the culture showed growth. Now what?

Biochemical tests	Niacin production & Nitrate test. If the bacteria produced niacin & nitrate then we know it is mycobacterium tuberculosis. If not, then it is another mycobacterium. E.g. mycobacterium leprae
Antimicrobial Susceptibility Testing	Detect resistance to anti-tuberculosis agents (antibiotics)



Mycobacterium TB in sputum ZN stain



Culture mycobacterium TB On LJ media

Management of a TB case

Isolation	Isolation of the patient for 10-14 days (until sputum is -ve). Smear positive cases contain more than **1000 organisms / ml** of sputum and considered infectious باختصار هنا يقولك متى يعزل المريض؟ اذا لقينا اكثر من ١٠٠٠ اورقانزم في ١ مل من الـSputum.
Triple regimen of therapy	❖ To prevent resistant mutants ❖ To prevent relapse ❖ Treatment must be guided by sensitivity testing.

Mycobacterium TB treatment

First line	**Isoniazid**
	Rifampicin أهم، أقوى، وأفضل واحد
	Ethambutol
	Pyrazinamide
Second line	PAS (para-aminosalicylic acid)
	Ethionamide
	Cycloserine
	Kanamycin
	Fluoroquinolones

4 for 2 then 2 for 4

- For the treatment of Active TB Combination therapy is used: where **All 4 drugs or (INH + RIF + P) are used for 2 months** then only INH & RIF are continued for the next 4-6 months
- **Directly Observed Therapy (DOT):** A method of drug administration in which a healthcare professional watches as a person takes each dose of a medication
- Second line drugs are used **when the bacteria is resistant to 1st line** drugs. They are more toxic and less effective than first line drugs.

Diagnosis of Latent TB

- Measurement of interferon -gamma release (IGRA)
  Measures the release of a substance called gamma interferon by white blood cells in a sample of blood when the cells are exposed to specific TB antigens.
- Tuberculin Skin testing

Spoiler

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 infected contacts
Follow up cases
Immunization with **BCG** (live attenuated) to all newborns (the BCG vaccine doesn't completely remove the chance of developing TB it just reduces its severity)

Summary

Check our summary by clicking here.

SAQ

SAQ1: A 40 year old patient is admitted to the ER with fever, night sweats & a worsening productive cough for the past 2 months. Chest examination showed a cavity lesion in the left upper lobe.. A) What is the most probable diagnosis? B) The physician decides to take a specimen, where should he take it from & when & how many? C) Most likely causative agent?

SAQ2: A 65 year old came to the hospital complaining of blood when coughing, malaise, and severe weight loss. After doing some tests the doctor diagnosed him with TB
. A) What drugs should be used for treatment? B) what is the management of this case?

SAQ3: A 37-year-old man presents to his primary care physician with subjective fever, malaise, and cough. He reports a few episodes of night sweats and has noted an unintentional 15-pound loss over the course of 2 months. Yesterday, he noted bloody sputum. He recently immigrated from Central Africa and currently lives with many family members in a small apartment. Chest radiograph demonstrates a cavitary lesion in the right upper lobe of the lung. A) What is you diagnosis? b) What are the test that you can do to confirm your diagnosis?

SAQ1: (A) Pulmonary Tuberculosis (B) sputum - at least 3 samples (1 in morning) (C) Mycobacterium Tuberculosis

SAQ2: (A) INH + RIF + P + E (B) Isolation for 2 weeks

SAQ3: Pulmonary tuberculosis (B) Direct microscopy AFB / Culture LJ + Bactec MGIT / Probtech (Molecular)

MCQs

Q1: A 26-year-old man comes to your clinic because of a persistent cough. He has episodes on and off for the past 7 years. The cough is usually non-productive, but he has seen blood a few times. He has also noticed his sheets are sometimes wet in the mornings. He works as a dentist in the Southeastern US, but is considering moving out West because dry air improves his symptoms. His mother also had a long-standing cough and passed away when he was fifteen. His temperature is 37.8°C (100.2°F), pulse is 85/min, respirations are 16/min, and blood pressure is 118/70 mm Hg. His BMI is 23 kg/m2. Physical examination shows a thin, young male in no acute distress. Regular rate and rhythm are heard on auscultation of the heart. The lungs are clear to auscultation. Which of the following histological findings are most closely associated with his underlying disease?			
A- Acid-fast & aerobic	B- Acid-fast & anaerobic	C- Gram-negative & aerobic	D- Gram +ve streptococci
Q2: What is used in the prevention of TB			
A- BCG vaccine	B- Isonizol	C- EJK vaccine	D- None of the above
Q3: Which of the following is not a drug used to treat TB			
A- Ethambutol	B- Pyrazinamide	C- Rifampicin	D- Levofloxacin
Q4: We say a person is infectious when his positive smear contain more than ..... Organisms / ml of sputum			
A-100	B-1000	C-10000	D-100000
Q5: Diagnosis of latent TB includes			
A- Xpert MTB/RIF	B- IGRA	C- Tuberculin Skin testing	D- B & C
Q6: Niacin & Nitrate production is measured by			
A- ProbTech	B- IGRA	C- Biochemical tests	D- Bactec MGIT
Q7: All true about secondary TB except:			
A- Occurs later in life (After reactivation)	B- Infectious and asymptomatic.	C- Lesion localized in apex of the lung	D- Shoes granuloma and caseation.

Q1	Q2	Q3	Q4	Q5	Q6	Q7
A	A	D	B	D	C	B

Team Leaders

- Duaa Alhumoudi
- Manee Alkhalifah

Team Members

- Renad Alhomaidi
- Sadem Alzayed
- Shahad Almezel
- Raghad Albarrak
- Noura Alsalem
- Ghadah Alsuwailem
- Noura Alshathri
- Mayasem Alhazmi
- Rand AlRefaei
- Muneerah Alsadhan
- Sarah AlAidaroos
- Sarah AlQuwayz
- Sadeem Alhazmi
- Abdulaziz Alderaywsh
- Faisal Alomri
- Abdulaziz Alomar
- Meshal Alhamed
- Homoud Algadheb
- Abdulaziz Alsuhaim
- Bassam Alasmari