Khadija. K. Al-Dulaimy
B.Sc., M.Sc., Med. Microbial.

Microbiology /College Of Dentistry
Al- Anbar University
/3/2019

Mycobacteria

Mycobacteria are widespread both in the environment and in animals; they cause two major human diseases – **tuberculosis and leprosy**. They are aerobic, **acid-fast bacilli** (not stained by the Gram stain because of the high lipid component of the cell wall). The major medically important pathogens are:

• *Mycobacterium* tuberculosis**(TB)**, the agent of tuberculosis; one of the top three infectious diseases affecting humans globally.
• *M. bovis* causes tuberculosis in humans as well as in cattle
• *M. africanum,* which also causes human tuberculosis
• *M. leprae,* the agent of leprosy - a disease affecting millions in Asia and Africa
• mycobacteria other than tuberculosis bacilli (MOTT), such as *M. avium-intracellulare* complex and *M. kansasii,* which cause frequent disease in human immunodeficiency virus (HIV)-infected patients.

***Mycobacterium tuberculosis*(M.TB.)**
Tuberculosis is defined as an infectious disease caused by a bacterium; that most commonly affects the lungs.

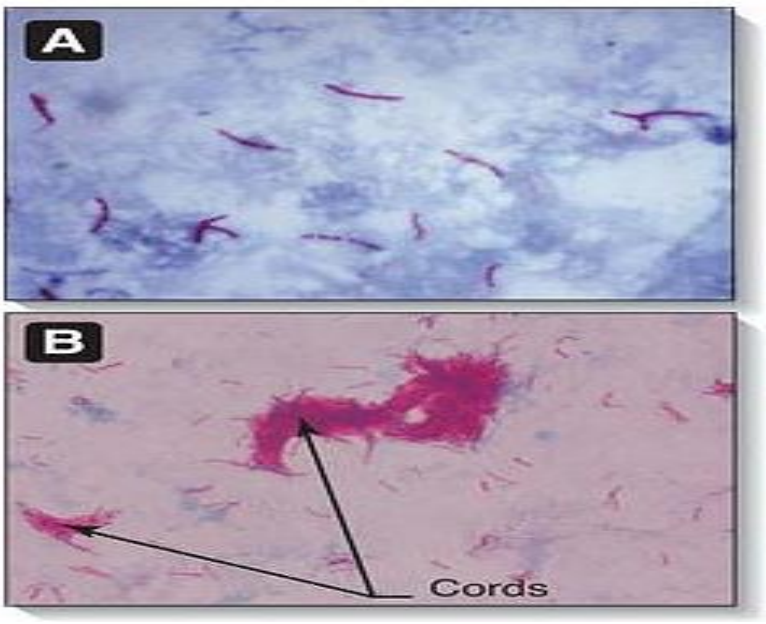
Habitat and transmission
Found in infected humans, mainly in the lungs; in the body it resides primarily in the cells of the reticuloendothelial system.

transmission is by coughing (droplet spread). TB is spread through the air from one person to another. Microscopic droplets that contain the bacteria may be expelled when a person who has infectious TB coughs or sneezes. They can remain suspended in the air for several hours, depending on the environment. When a person breathes in *M. tuberculosis*, the bacteria can settle in the lungs and begin to grow. From there, they can move through the blood to other parts of the body.

What are Mycobacteria?

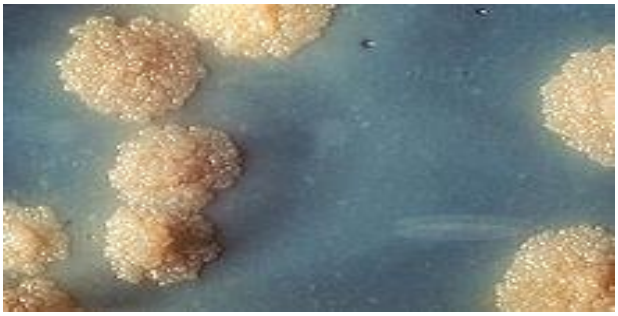
Tuberculosis complex organisms are:

- Obligate **aerobes** growing most successfully in tissues with a high oxygen content, such as the lungs.
- Facultative **intracellular pathogens** usually infecting **mononuclear phagocytes** (e.g. macrophages).
- **Slow-growing** with a generation time of 12 to 18 hours (c.f. 20-30 minutes for *Escherichia coli*).
- **Hydrophobic** with a high lipid content in the cell wall. Because the cells are hydrophobic and tend to clump together, they are impermeable to the usual stains, e.g. Gram's stain.
- Known as **"acid-fast bacilli"** because of their lipid-rich cell walls, which are relatively impermeable to various basic dyes unless the dyes are combined with phenol. Once stained, the cells resist decolorization with acidified organic solvents and are therefore called "acid-fast". (Other bacteria which also contain mycolic acids, such as *Nocardia*, can also exhibit this feature.)



Mycobacterium tuberculosis is an obligate aerobe. For this reason, in the classic case of tuberculosis, the M.TB. complexes are always found in the well-aerated upper lobes of the lungs. The bacterium is a facultative intracellular parasite, usually of macrophages, and has a slow generation time, 15-20 hours, a physiological characteristic that may contribute to its virulence.

Two media are used to grow M.TB. Middlebrook's medium which is an agar based medium and Lowenstein-Jensen medium which is an egg based medium. M.TB. colonies are small and buff colored when grown on either medium. Both types of media contain inhibitors to keep contaminants from out-growing M.TB. It takes 4-6 weeks to get visual colonies on either type of media.



Colonies of *Mycobacterium tuberculosis* on Lowenstein-Jensen medium

Cell structure and metabolism

M. tuberculosis has a tough cell wall that prevents passage of nutrients into and excreted from the cell, therefore giving it the characteristic of slow growth rate. The cell wall of the pathogen looks like a Gram-positive cell wall. The cell envelope contains a polypeptide layer, a peptidoglycan layer, and free lipids. In addition, there is also a complex structure of fatty acids such as mycolic acids that appear glossy. The *M. tuberculosis* cell wall contains three classes of mycolic acids: alpha-, keto- and methoxymycolates.

Virulence Mechanisms and Virulence Factors:

-M.TB. can grow intracellularly. This is an effective means of evading the immune system. In particular, antibodies and complement are ineffective. Once M.TB. is phagocytosed, it can inhibit phagosome-lysosome fusion. The bacterium may remain in the phagosome or escape from the phagosome, in either case finding a protected environment for growth in the macrophage.

-Antigen 85 complex. This complex is composed of a group of proteins secreted by M.TB. that are known to bind fibronectin. These proteins may aid in walling off the bacteria from the immune system and may facilitate tubercle formation although evidence of this is lacking.

-Slow generation time. Because of M.TB's slow generation time, the immune system may not readily recognize the bacteria or may not be triggered sufficiently

to eliminate them. Many other chronic disease are caused by bacteria with slow generation times, for example, slow-growing M. leprae causes leprosy, Treponema pallidum causes syphilis, and Borrelia burgdorferi causes Lyme disease.

-High lipid concentration in cell wall, accounts for impermeability and resistance to antimicrobial agents, resistance to killing by acidic and alkaline compounds in both the intracellular and extracellular environment, and resistance to osmotic lysis via complement deposition and attack by lysozyme.

Epidemiology:

Patients with active pulmonary tuberculosis shed large numbers of organisms by coughing, creating aerosol droplet nuclei. Because of resistance to dessication, the organisms can remain viable in the environment for a long time. The principal mode of contagion is person-to-person transmission by inhalation of the aerosol, and repeated or prolonged contact is usually required for transmission of infection. However, a single infected person can pass the organism to numerous people in an exposed group, such as a family, classroom, or hospital ward.

Risk Groups

Anyone can get TB. However, some groups are at higher risk to get active TB disease. People at high risk include those:

1. with HIV infection
2. in close contact with those known to be infectious with TB
3. with medical conditions that make the body less able to protect itself from disease (for example: diabetes, or people undergoing treatment with drugs that can suppress the immune system, such as long-term use of corticosteroids)
4. from countries with high TB rates
5. who work in or are residents of long-term care facilities (nursing homes, prisons, some hospitals)
6. who are malnourished
7. who are alcoholics or IV drug users

Disease progression depends on:

- Strain of M.TB.
- Prior exposure
- Vaccination
- Infectious dose
- Immune status of the host

Pathogenicity: After being inhaled, mycobacteria reach the alveoli, where they multiply in the pulmonary epithelium or macrophages. Within two to four weeks, many bacilli are destroyed by the immune system, but some survive and are spread by the blood to extrapulmonary sites. The virulence of M. tuberculosis rests with its ability to survive and grow within host cells. The organism produces no demonstrable toxins; however, when engulfed by macrophages, bacterial sulfo lipids inhibit the fusion of phagocytic vesicles with lysosomes.

Initial Infection and Primary Tuberculosis

The min infectious dose for lung infection is around 10 cells. The bacilli are phagocytosed by alveolar macrophages and multiply intracellularly. This period of hidden infection is asymptomatic or accompanied by mild fever, but some cells escape from the lungs into the blood and lymphatics. After 3 to 4 weeks, the immune system mounts a complex, cell-mediated assault against the bacilli. The large influx of mononuclear cells into the lungs plays a part in the formation of specifi c infection sites called **tubercles.** Tubercles are granulomas with a central core containing TB bacilli and enlarged macrophages, and an outer wall made of fibroblasts, lymphocytes ,and neutrophils**.** Although this response further checks spread of infection and helps prevent the disease, it also carries a potential for lung damage. Frequently, the centers of tubercles break down into necrotic, **caseous * lesions** that gradually heal by calcification when lung tissue is replaced by calcium deposits. The response of T cells to *M. tuberculosis* proteins also causes a cell mediated immune response evident in the **tuberculin reaction** used in diagnosis and epidemiology**.**

Latent and Recurrent Tuberculosis Although the majority of TB patients recover more or less completely from the primary infection or disease, live bacilli can remain latent and become reactivated weeks, months, or years later, especially in people with weakened immunity. In reactivated tuberculosis, tubercles filled with masses of bacilli expand and drain into the bronchial tubes and upper respiratory tract. Gradually, the patient experiences more severe symptoms, including violent coughing, greenish or bloody sputum, low-grade fever, anorexia, weight loss, extreme fatigue, night sweats, and chest pain. It is the gradual wasting of the body that accounts for an older name for tuberculosis—**-** *consumption.* Untreated reactivated disease has nearly a 60% mortality rate.

Extrapulmonary Tuberculosis

During the course of reactivated TB, the bacilli disseminate rapidly to sites other than the lungs. Organs most commonly involved in **extrapulmonary TB** are the regional lymph nodes, kidneys, long bones, genital tract, brain, and meninges. Because of the debilitation of the patient and the high load of tubercle bacilli, these complications are usually grave.

Renal tuberculosis results in necrosis and scarring of the renal medulla and the pelvis, ureters, and bladder. Genital TB often damages the reproductive organs in

both sexes. Tuberculosis of the bone and joints is a common complication. The spine is a frequent site of infection, though the hip, knee, wrist, and elbow can also be involved. Degenerative changes can collapse the vertebrae, resulting in abnormal curvature of the thoracic or lumbar regions. Neurological damage stemming from compression on nerves can cause extensive paralysis and sensory loss. Tubercular meningitis is the result of an active brain lesion seeding bacilli into the meninges. Over a period of several weeks, the infection of the cranial compartment can create mental deterioration, permanent retardation, blindness, and deafness. Untreated tubercular meningitis is invariably fatal, and even treated cases can have a 30% to 50% mortality rate.

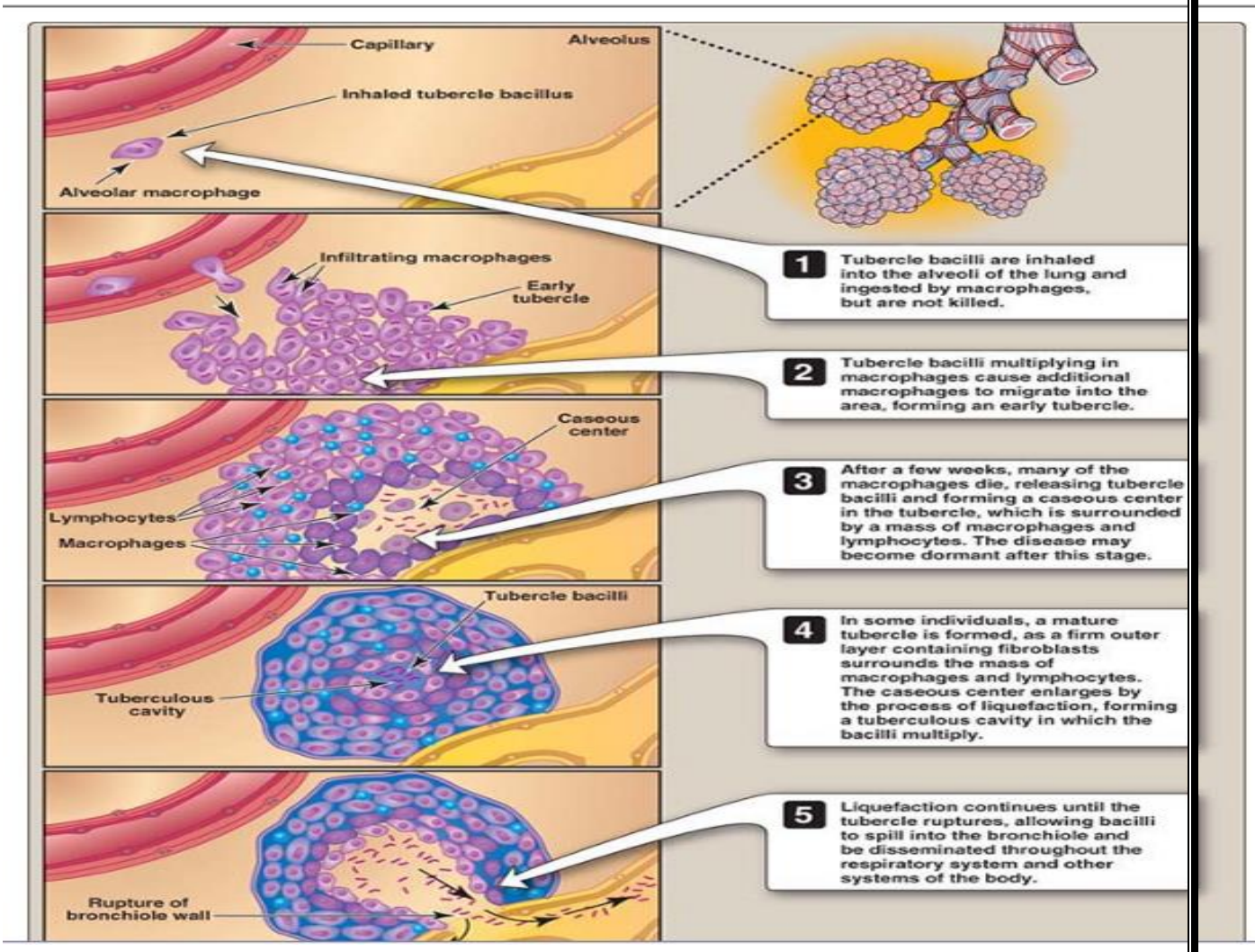


Figure 18.4 Progression of active tuberculosis infection

Clinical significance: Primary tuberculosis occurs in a person who has had no previous contact with the organism. For the majority of cases (about 90 percent), the infection becomes arrested, and most people are unaware of this initial encounter. The only evidence of tuberculosis may be a positive tuberculin test. A chest radiograph sometimes shows the initial pulmonary nodule (a healing tubercle), and some fibrosis. Approximately ten percent of those with an arrested primary infection develop clinical tuberculosis at some later time in their lives.

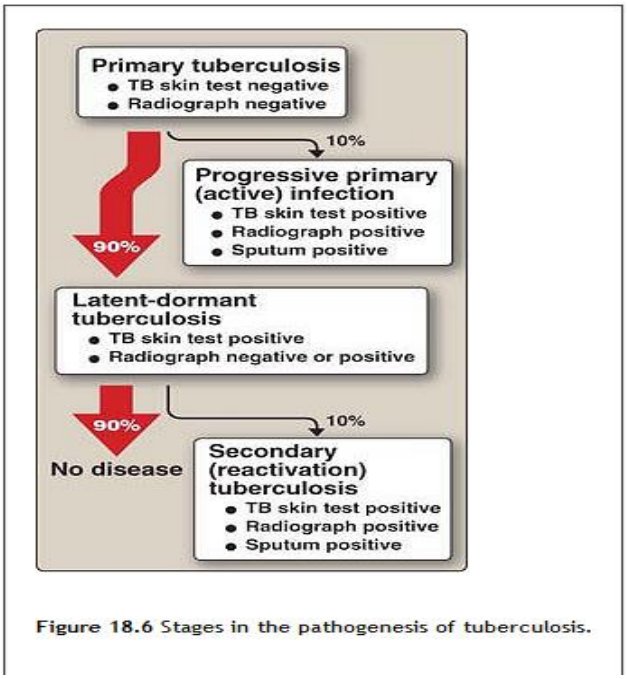


Figure 18.6 Stages in the pathogenesis of tuberculosis.

Tuberculin Skin Testing

What is it?

The **Mantoux tuberculin skin test** (TST) is the standard method of determining whether a person is infected with *Mycobacterium tuberculosis*. Reliable administration and reading of the TST requires standardization of procedures, training, supervision, and practice.

How is the TST Administered?

The TST is performed by injecting 0.1 ml of tuberculin purified protein derivative (PPD) into the inner surface of the forearm. The injection should be made with a tuberculin syringe, with the needle bevel facing upward. The TST is an intradermal injection. When placed correctly, the injection should produce a pale elevation of the skin (a wheal) 6 to 10 mm in diameter.

How is the TST Read?

The skin test reaction should be read between 48 and 72 hours after administration. A patient who does not return within 72 hours will need to be rescheduled for another skin test.

The reaction should be measured in millimeters of the induration (palpable, raised, hardened area or swelling). The reader should not measure erythema (redness). The diameter of the indurated area should be measured across the forearm (perpendicular to the long axis).

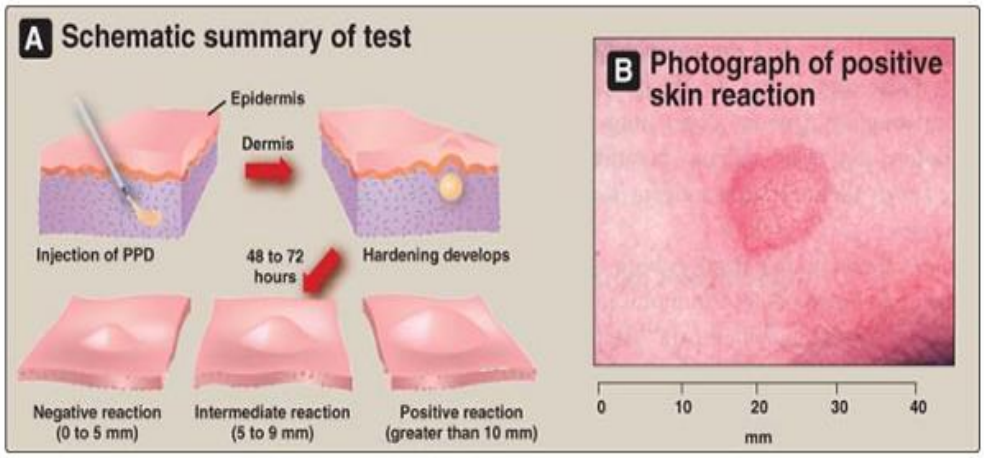


Figure 18.7 Mantoux skin test for tuberculosis. [Note: For some people, determination of a positive reaction may be interpreted more stringently (see Figure 18.8).]

Tuberculin reaction: The tuberculin reaction test is a manifestation of delayed hypersensitivity to protein antigens of M. tuberculosis. Whereas such tests can be used to document contact with the tubercle bacillus, they do not confirm that the patient currently has active disease. In the Mantoux test, purified protein derivative (PPD) is prepared from culture filtrates of the organism, and biologically standardized. Activity is expressed in tuberculin units (TU). In the routine procedure (Mantoux test), a measured amount of PPD is injected intradermally in the forearm. It is read 48 to 72 hours later for the presence and size of an area of induration (hardening) at the site of injection, which must be observed for the test to be positive (Figure 18.8). A positive reaction usually develops four to six weeks after initial contact with the organism. It remains positive for life, although it may wane after some years, or in the presence of immunosuppression by medications or disease.

What Are False-Positive Reactions?

Some persons may react to the TST even though they are not infected with M. tuberculosis. The causes of these false-positive reactions may include, but are not limited to, the following:

- Infection with nontuberculosis mycobacteria
- Previous BCG vaccination
- Incorrect method of TST administration
- Incorrect interpretation of reaction
- Incorrect bottle of antigen used

What Are False-Negative Reactions?

Some persons may not react to the TST even though they are infected with M. tuberculosis. The reasons for these false-negative reactions may include, but are not limited to, the following:

- Cutaneous anergy (anergy is the inability to react to skin tests because of a weakened immune system)
- Recent TB infection (within 8-10 weeks of exposure)
- Very old TB infection (many years)
- Very young age (less than 6 months old)
- Recent live-virus vaccination (e.g., measles and smallpox)
- Incorrect method of TST administration
- Incorrect interpretation of reaction

What are the differences between latent tuberculosis infection and tuberculosis disease?

Person with LTBI (Infected)	Person with TB Disease (Infectious)
Has a small amount of TB bacteria in his/her body that are alive, but inactive	Has a large amount of active TB bacteria in his/her body
Cannot spread TB bacteria to others	May spread TB bacteria to others
Does **not** feel sick, but may become sick if the bacteria become active in his/her body	May feel sick and may have symptoms such as a cough, fever, and/or weight loss
Usually has a TB skin test or TB blood test reaction indicating TB infection	Usually has a TB skin test or TB blood test reaction indicating TB infection
Radiograph is typically normal	Radiograph may be abnormal
Sputum smears and cultures are negative	Sputum smears and cultures may be positive
Should consider treatment for LTBI to prevent TB disease	Needs treatment for TB disease
Does **not** require respiratory isolation	May require respiratory isolation
Not a TB case	A TB case

How is TB diagnosed?

Symptoms of tuberculosis include:

- Fever
- Night-time sweating
- Loss of weight
- Persistent cough
- Constant tiredness
- Loss of appetite

Diagnosis of tuberculosis is made by a positive **tuberculin skin test**, an immune reaction to a small quantity of tuberculosis antigens. It can be confirmed by **X rays of the chest** and microscopic examination of sputum. Detection of significant numbers of acid-fast bacilli (**using the Ziehl-Neelsen stain**) in sputum or tissue samples is considered a positive diagnosis, although disease may confirmed by laboratory **culture of the bacterium** (difficult, dangerous and slow - takes at least 4 weeks).

Treatment

Long-term therapy (6-9 months) with antituberculous drugs (isoniazid, rifampicin, pyrazinamide, ethambutol). As drug resistance is growing and a persistent problem, combination therapy should always be given. Tubercle bacilli resistant to a number of antituberculous drugs (multidrug-resistant tuberculosis, MDR-TB) is a growing problem. Hence regimentation of drug delivery is a cornerstone of managing the disease, which is achieved by a global programme termed directly observed therapy (DOT).

Prevention

Prevention is by BCG (bacille Calmette-Guerin) vaccination containing live attenuated organisms, in childhood.

Pasteurization of milk and general improvement of living standards have played a valuable role in prevention.

Oral tuberculosis

Oral lesions of tuberculosis are usually secondary to primary infection elsewhere, commonly the lung. Primary infections of the oral mucosa by *Mycobacterium tuberculosis* are rare. In the case of secondary infection the sources of infection are contaminated sputum or blood- born bacilli. Lesions are found more commonly in the posterior area of the mouth and it has been suggested that this may be due to the relative propensity of lymphoid tissue in this region. The major oral lesions are: oral ulceration , tuberculous lymphadenitis, periapical granulomas and bone infections.

Oral ulceration

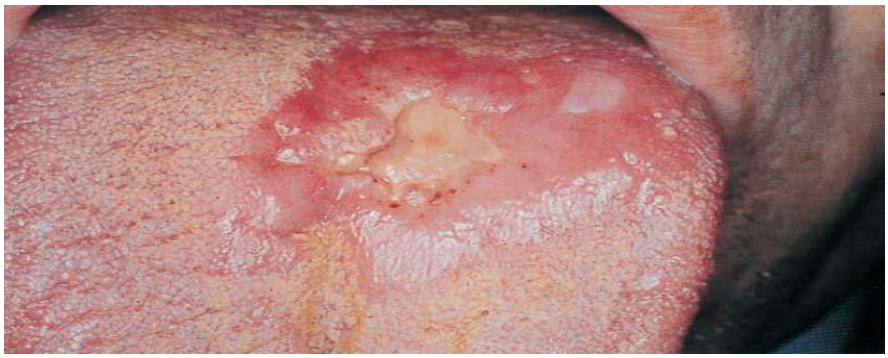
There is a wide spectrum of tuberculous lesions of the oral mucosa, including indolent ulcers, diffuse inflammatory lesions, granulomas and fissures; pain may be mild or absent. The tongue is most commonly affected but lesions have been noted on the buccal mucosa, gingivae, floor of the mouth, lips, and the hard and soft palates. Primary tuberculosis of the oral mucosa is more common in children and adolescents than in adults and usually presents as a single, painless indolent ulcer, commonly on the gingiva, with enlarged cervical lymph nodes, or as a white patch

. **Oral Tuberculosis**

- **Clinically**, the ulcer is painless and irregular, with a thin undermined border and a vegetating surface, usually covered by a gray-yellowish exudate.

- The dorsum of the tongue is the most commonly affected site, followed by the lip, buccal mucosa, and palate.

- **Differential diagnosis:** carcinomas, syphilis, malignant granuloma, major aphthous ulcer.-**Treatment:** Antituberculous drugs.



typical ulcer on the dorsal surface of the tong

Diagnosis of Oral Tuberculosis

Oral lesions: Identified by biopsy and microscopic examination
Chronic granulomatous lesions with areas of necrosis surrounded by macrophages, multinucleated giant cells, and lymphocytes
Tissue : May be stained to reveal the organisms

Mycobacterium leprae

Habitat and transmission

Humans are the only known hosts of *M. leprae,* which resides mainly in the skin and nerves. Prolonged contact is thought to be the mode of transmission.

Characteristics

Aerobic, acid-fast bacilli; no known toxins.

Culture and identification

Cannot be cultured in vitro but grows on the footpads of mice or armadillos, yielding chronic granulomas at the inoculation site.

Pathogenicity

The leprosy bacillus causes a slow, progressive, chronic disease which mainly affects the skin and the nerves; the lesions are predominantly seen in the cooler parts of the body. Two forms of leprosy are recognized.

Lepromatous leprosy. The cell-mediated immune response is depressed or absent; *M. leprae* bacilli are usually seen in large numbers in the lesions and in blood; commonly involves mucosae, especially the nose; leads to much disfigurement.

Tuberculoid leprosy. Associated with an intense cell mediated immune response to the organisms; principally involves the nerves, with resultant anaesthesia and paraesthesia. Hence damage to extremities is caused, with resultant loss of fingers and toes .

What are the complications of leprosy?

If left untreated, leprosy can cause permanent damage to the nerves in the fingers, toes, hands and feet. This may affect a person's ability to feel pain and temperature in these areas of the body. When you can't feel your fingers or toes, you may accidentally burn, cut or hurt yourself. Repeated injuries and nerve damage can cause muscle weakness, deformities and even the loss of fingers and toes. Untreated leprosy can also cause swelling, and skin sores and lesions that are more severe.

Laboratory identification: *M. leprae* is an acid-fast bacillus. It has not been successfully maintained in artificial culture, but can be grown in the footpads of mice and in the armadillo, which may also be a natural host although playing no role in human disease. Laboratory diagnosis of lepromatous leprosy, where organisms are numerous, involves acid-fast stains of specimens from nasal mucosa or other infected areas. In tuberculoid leprosy, organisms are extremely rare, and diagnosis depends on clinical findings and the histology of biopsy material.

Treatment and prevention: Several drugs are effective in the treatment of leprosy, including sulfones such as dapsone, rifampin, and clofazamine Treatment is prolonged, and combined therapy is necessary to ensure the suppression of resistant mutants. The fact that vaccination with BCG) has shown some protective effect in leprosy has encouraged further interest in vaccine development. The best way to prevent leprosy is to avoid contact with body fluids and the rashes of people who have leprosy.

References:-

1-Medical microbiology.4th edition.
2-Oral microbiology.5th edition.
3- Lippincott's Illustrated Reviews: Microbiology, 2nd Edition