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- Title of the Unit: Unit 3 – Bacterial diseases
- Module Name: Genito -Urinary Tract Infections : Syphilis (*Treponema pallidum*)
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NOTES

Introduction

Syphilis is a chronic, multistage sexually transmitted disease caused by the spirochete *Treponema pallidum*. It is one of the ancient sexually transmitted or venereal diseases (derived from the name Venus, the Roman goddess of love). The name was derived from a famous poem written by an Italian physician and poet Girolamo Fracastoro in the year 1530 which described a legend of a shepherd boy named Syphilis, who had been punished for being disrespectful to the Gods by being cursed with the disease. Syphilis has been called “The Great Pretender” as its clinical manifestations mimic many other diseases.

History

Year	Name	Microbiological history
1500	The Vikings/Christopher Columbus and his crew	Introduced syphilis in Europe
1838	Philippe Ricord	Recognition of the different stages of syphilis
1905	Fritz Schaudinn and Erich Hoffmann	Discovered <i>Treponema pallidum</i> as the etiologic agent of syphilis

1906	August von Wassermann	Introduced the diagnostic test for syphilis
1909	Paul Ehrlich	Introduced an arsenic derivative Salvarsan as therapeutic agent for syphilis

Morphological Characteristics

It is a slender, flexible bacterium twisted spirally around long-axis, with tapering ends. *Treponema* meaning 'turn' and *nema* meaning 'thread'. Size: 5-20 μ long \times 0.2 μ wide. It has 10-20 fine spirals at regular distance of 1 μ . It is actively motile and shows three types of movements, viz. Flexion and extension, Translatory and Corkscrew-like motility. *Treponema pallidum* is known colloquially as "The Stealth Pathogen" because of its denuded outer membrane, which is comprised of mostly non-immunogenic transmembrane proteins.

T. pallidum is a very delicate bacterium. It is readily inactivated by heating and drying (41-42°C for 1 hour), therefore fomites are of little importance in transmission. It is killed at 0-4°C in 1-3 days, hence transfusion syphilis can be prevented by storing blood for 4 days in refrigerator before transfusion. It can be inactivated by common soaps and antiseptics

Antigenic structure of *T. pallidum*.

Antigens of *T. pallidum* are of following two types.

1. Nonspecific Ag
 - Cardiolipin Ag induces reagin Ab. It is not known whether the Ab is induced by cardiolipin in spirochaetes or is released from damaged host tissue
2. Specific Ags
 - Group specific Ag: Protein Ag- shared by pathogenic and non-pathogenic treponemes
 - Species specific Ag: Polysaccharide- used for detection of species specific Ag

Mode of Transmission

- The disease caused by *T. pallidum* is syphilis
- It is a sexually transmitted disease
- It enters through minute abrasions on mucosa or skin

Pathogenesis

- After contact, *T. pallidum* rapidly penetrates through minute abrasions on the skin or mucosa.
- It binds avidly by its hooked tip to the epithelium.

- Within a few hours, it enters the lymphatics and blood to produce systemic infection and metastatic foci long before the appearance of a primary lesion.

Incubation period ranges from 10 to 90 days and is inversely proportional to the number of organisms inoculated. Key to the capacity of the syphilis spirochete for immune evasion and thus 'stealth pathogenicity' is its unusual outer membrane, which lacks lipopolysaccharide and contains an extremely low density of integral membrane proteins and a paucity of surface-exposed lipoproteins

Clinical features of syphilis.

Clinical features can be divided into following three stages:

1. Primary
2. Secondary
3. Tertiary

▪ **Primary**

- This stage is characterized by the presence of chancre at the site of entry of spirochaetes; usually it is genital, rarely extragenital- seen on lips or cheeks and nipples
- The chancre is painless, avascular, indurated circumscribed, superficial ulcer, hence called hard chancre
- Chancre is covered with thick glairy exudates rich in spirochaetes
- Regional lymph nodes are enlarged, rubbery, nontender and discrete
- Chancre heals leaving a thin scar
- The infection is rarely contracted by doctors and nurses nonvenereally, in whom chancre appears on fingers

▪ **Secondary**

- This stage occurs due to widespread multiplication and dissemination of treponemes through blood
- It manifests as mucocutaneous eruptions in the form of roseopapular rash and condylomatas at mucocutaneous junctions
- Osseus, ophthalmic, meningeal involvement may occur
- After secondary lesion disappears, there is a period called "latent syphilis" during which the diagnosis can be made only by serological tests

▪ **Tertiary**

- This stage manifests as
 - Aneurysms
 - Chronic granulomata (gummata)
 - Meningovascular involvement
 - General paralysis of the insane

- Congenital syphilis- *T. pallidum* can cross-placental barrier after 4 months of gestation
- Obstetric history of untreated female is typical one of abortion and stillbirth, followed by live births with stigmata of syphilis and finally healthy infant

Congenital Syphilis

- Treponemes cross the placenta from mother to baby.

Symptoms:

- Notched incisors or Hutchinson's teeth
- Perforated palate
- Saber shins
- Aged-looking face with saddle-nose
- Nasal discharge

Women should be tested for syphilis as part of their prenatal testing to prevent such congenital infections.

Laboratory diagnosis of syphilis.

Specimens

- Blood, exudates from lesions, lymph node aspirate

Collection

- Exudate and aspirate are collected for microscopy
- Exudate- with all precautions, the lesion is cleaned with warm saline and margins scraped and pressure applied to base of lesion. Exudate is collected without admixture of blood
- Lymph node aspirate- It can be aspirated with a syringe
- Blood- is collected for serological test by venepuncture in a plain bulb

Transport

- Samples for microscopy should reach laboratory immediately after collection

Processing of Specimen

Microscopic Examination

- Dark ground microscopy
 - Material collected should be placed directly on slide to observe motile slender, spirals with pointed ends
- Direct fluorescent antibody technique

- Smear is prepared from specimen and then stained with fluorescein-labelled monoclonal Ab to the bacteria. If they are present in smear, fluorescence is observed. It has more sensitivity than dark ground microscopy
- Treponemes in tissue
- These can be demonstrated by immunofluorescence or silver impregnation technique such as Levaditi stain

Serological Tests

- Standard tests (Nonspecific)
 - . VDRL
 - . Kahn
 - . Wasserman
- Treponemal tests (specific)
 - . By using Reiter's strain - RPCFT
 - . By using Nichol's strain
 - Live strain - TPI
 - Killed Treponema – TPA, TPTA, FTA
 - Extract of treponemes - EIA

- Standard Tests
- These are also called reagin Ab tests
- Standard tests include the following tests:
 - a. VDRL (venereal disease laboratory research) test
 - b. Kahn test
 - c. Wasserman test

VDRL (Venereal Disease Research Laboratory) Test

- The test was developed in venereal disease research laboratory, New York

Principle

- It is a slide flocculation test, a type of precipitation test

Procedure

Qualitative test

- It is carried out on VDRL tile (Tile with multiple concavities)
- Inactivated drop of serum is added to concavity
- A drop of VDRL Ag is then added
- Mixed with stick
- Rotated manually or mechanically on VDRL rotator fixed at 180 rpm/minute for 4-8 minutes
- Result read under microscope

Qualitative test

- Serial doubling dilutions of serum are prepared and used for the test

- Highest dilution found reactive is taken as titre

Interpretation

- Presence of floccules – Reactive
- Absence of floccules – Nonreactive
- A titre of 1:8 or more is considered as significant

Modifications of VDRL

- Rapid plasma reagin test (RPR)
- Tolidine red unheated serum reagin test (TRUST)
- Automated reagin test (ART)
- Unheated serum reagin test (USR)
- VDRL – ELISA

Advantages of RPR over VDRL

	VDRL	RPR
Specimen	Serum	Serum/plasma
	Serum needs inactivation	No heating of serum needed
Ag	Must be freshly prepared each day before use	Ready and stable for use
Test Reading	Needs microscope	Can be read with naked eyes
Technique	Complicated	Easy and quick

Biological false positive (BFP) reactions

- Cardiolipin Ag is present in *T. pallidum* and in mammalian tissue so both may induce Ab response. This is a reason for biological false positive VDRL test and it is not a technical error
- Positive reaction is obtained in cardiolipin test with negative results in specific treponemal test in the absence of Treponemal infection
- Types-acute and chronic
 - Acute BFP reactions last for a few weeks to a month and chronic for more than 6 months
 - Acute BFP-are observed in infections, inflammations, injuries
 - Chronic BFP-are observed in
 - i. Autoimmune diseases, e.g. systemic lupus erythematosus (SLE)
 - ii. Lepromatous leprosy
 - iii. Malaria

- iv. Infectious mononucleosis
- v. Hepatitis
- vi. Topical eosinophilia

Kahn Test

It is tube flocculation test, rarely used now.

Wasserman Test

This is complement fixation test, rarely used now.

Treponemal Tests

These include the following tests:

- Test using Reiter's strain Ag
- Tests using Nichol's strain as Ag

Test Using Reiter's Strain Ag

- This test is also called Reiter's protein complement fixation test (RPCFT)
- It uses Ag from cultivable treponemes. They are not in use now

Test Using Nichol's Strain as Ag

i. Test using live strain

- Treponema pallidum immobilization (TPI) test
 - Test uses live treponemes (Nichol's strain)
 - Serum incubated along with complement and treponemes
 - In presence of Ab in sera treponemes are immobilized
 - Most specific, but complex so done in very few laboratories

ii. Tests using killed treponemes

- Fluorescent treponemal antibody (FTA) test
 - It is indirect immunofluorescence test using treponemal Ag
 - Serum is allowed to react with the smear prepared with Nichol's strain
 - Excess serum is then washed and smear treated with antihuman Ig fluorescent conjugate
 - Smear is examined under fluorescent microscope
 - Treponemes are seen as fluorescent objects if test is reactive
 - Test has good sensitivity 80%, 100%, 95%, in primary, secondary and tertiary syphilis
 - Can be modified as fluorescent treponemal antibody absorption (FTA-ABS) test and IgM-FTA

- Treponema pallidum agglutination (TPA) test
 - Killed suspension of treponemes is mixed with test sera and incubated
 - In presence of Ab, treponemes are found agglutinated under dark ground microscope
 - Not very specific and gives false positive, hence not used now for diagnosis
- Treponema pallidum immune adherence (TPIA) test
 - Killed suspension of treponemes is mixed with serum, complement, and fresh whole blood and incubated
 - In presence of Ab treponemes adhere to erythrocytes
 - Not used now for diagnosis

iii. Tests using extracts of treponemes

- Treponema pallidum haemagglutination (TPHA) test
 - Ag is absorbed on surface of RBC
 - When Ag is mixed with sera, in presence of Ab, haemagglutination occurs.

Epidemiology

According to the World Health Organization, 11 million new cases of venereal syphilis occur globally each year.

- 1.5 million pregnant women are estimated to be infected each year; approximately one third of these infections will result in stillbirths or other adverse outcomes of pregnancy.
- In developing countries, transmission is largely heterosexual, while transmission among homosexuals predominates in industrialized nations.
- Genital ulcers caused by syphilis are a major cofactor for bidirectional transmission of HIV.

Prophylaxis

- Avoiding sexual contact with an infected individual
- Refrain from having multiple sex partners
- Treatment of cases and contacts (sexual partners)
- Education about safe practices
- Prophylactic use of barrier contraceptive methods

Treatment

- 2.4 million units of **Benzathine Penicillin G** is adequate in early cases.

- For late syphilis, above repeated weekly for 3 weeks
- Doxycycline given to patients allergic to penicillin
- Ceftriaxone given for Neurosyphilis
- Sometimes, after treatment with penicillin, patients experience shaking chills, a rise in temperature, and intensification of skin rashes known as the **Jarisch–Herxheimer reaction** (JHR) with symptoms resolving a few hours later.
- This reaction was named after European dermatologists who described it in patients with syphilis.