REVIEW OF LITERATURE

In developing countries the morbidity and mortality due to the Reproductive tract infection (RTIs) or sexually transmitted infection are as high as those associated with other health problems. The consequences of RTIs which are numerous and potentially devastating includes: Post abortal and puerperal sepsis, Ectopic pregnancy, Fetal and prenatal death, Cervical cancer, Infertility, Chronic physical pain, Emotional distress, Social rejection in women, Infection in infants.

Normal and pathological flora of the Genital tract:

As there is an anastomosis between the lymphatic and blood vessels, the infection of one genital (pelvic) organ usually spread to others more frequently. Apart from the local defense mechanism present in vulva, vagina, cervix and fallopian tube, infection may involve upper genital tract and peritoneal cavity from normal flora of vagina and cervix (non-sexual transmitted infection).

A variety of species of commensal bacteria colonies the mucosal layer made up of transitional, columnar and Squamous epithelial cells. Mycobacterium smegmatis along with Gram Positive bacilli may present at vulva and penis. Women in reproductive age harbor large number of facultative bacteria Enterobacter, streptococci and staphylococci, in anaerobes such as Lactobacilli, anaerobic non-spore forming bacilli and cocci.

The flora of female genital tract varies with PH and estrogen concentration of mucosa, which depends on age of host. The bacterial pathogens involve in upper genital tract infection are principally derived from the normal flora of vagina & cervix. The exogenous source are sexually transmitted or following induced criminal abortion or during delivery in unhygienic surroundings.
Chlamydia trachomatis, Gardenerella vaginitis, Neisseria gonorrhoeae, Neisseria menigitidis, T.pallidum, Ureaplasma urealyticum, Mycoplasma hominis, other Mycoplasma, Human papilloma virus, Herpes virus, and other organism may acquired as sexual activity.

LIST OF GENITAL PATHOGENS RECOVERED IN CULTURE OF FEMALE GENITAL TRACT BACTERIA:

Chlamydia trachomatis: Non gonococcal urethritis, Cervicitis, Salpingitis, Infant pneumonia LGV

Gardnerella vaginalis: Gardnerella vagmiris ('Non-specific vaginitis)

Heamophilus ducreyi: Chancroid

Mycoplasma hominis: Non-specific urethritis.

Mycoplasma genitalium

Neisseria gonorrhoeae: Urethritis, Cervicitis, Pharyngitis, PID, Perihepatitis, Bartholmitis.

Neisseria meningitidis

Ureplasama urealyticum: Nongonococcal urethritis.

FUNGAL

Candida albicans: Vulvovaginitis, balanitis.

Other yeast

VIRUSES

Cytomegalovirus: Congenital birth defects.

Herpes simplex virus: Primary and recurrent genital herpes, Neonatal herpes

HIV: AIDS, AIDS-related complex (ARC), Prenatal and Congenital AIDS

Hepatitis B: HBsAg +ve hepatitis.

PROTOZOA;

Trichomonas vaginalis: Trichomonal vaginialis

ARTHROPOID:

Sarcoptes scabiei: Genital scabies
Phthirus pubis: Pediculosis pubis

Homosexual practice and increasing common heterosexual practice of anal-genital intercourse, numbers of gastrointestinal and systemic pathogens considered etiological agent of STDs.

In abnormal sex behavior reproductive tract infection may caused by intestinal pathogens and urinary tract pathogens.

Viruses e.g HIV, hepatitis, Cytomegalovirus shed in secretion or present in blood, are increasingly spread by sexual practice.

Sexually transmitted infections have been known to affected man since time immemorial. The terminology used to address it has changed over time. Initially it was known as:

- Veneral disease-named after “venus”. The goddess of love.
- Sexually transmitted disease- it was so named in order to reduce the stigma associated with veneral disease.
Sexually transmitted infections – so that it may include reproductive tract infections and also HIV. Most common complaints that bring females to gynecology clinic is reproductive tract infections, menstrual complaints, infertility. Out of these, reproductive tract infections are the most common. Reproductive tract infection (RTIs) can be Non Sexually Transmitted Infections and Sexually Transmitted Infections. Some prefer the term reproductive tract infection to designate the diagnosis and treatment of STIs and to encompass conditions such as bacterial vaginosis, whose designation as a STIs is no longer debated.

They may progress in the individual leading to serious complications and cause a high degree of morbidity during the sexually active period of life. Female with the reproductive tract infection present with the following complaints.

- Vaginal discharge (most common)
- Lower abdominal pain
- Urinary symptoms
- Genital warts and ulcers
- Infertility

**Vaginal discharge:** Apart from being a physical vaginal discharge is commonly caused by the Trichomonas vaginalis, anaerobic bacteria and the Candida albicans. Other more serious cause of vaginal discharge is Neisseria gonorrhoeae and Chlamydia trachomatis. Both agents cause the cervicitis which may lead to ascending infection resulting in the pelvic inflammatory diseases (PID), is a serious consequence of the infection.

It is know that the symptom of vaginal discharge is neither sensitive nor specific for any of the five causes at vaginitis or cervicitis. As mixed infection occurs
frequently it is not possible to exclude the cervical infection on clinical basis. Laboratory test are necessary to exclude, the causes of cervicitis confidently.

The speculum examination any foreign body in the vagina, such as retain tampon and or condom etc. should be removed. Antiseptic should not be used to vaginal speculum for vaginal examination.

The importance clinical criteria include the measurement of pH, amines test and microscopic examination of wet preparation and gram staining. The positive finding of microscopic examination of vaginal secretion is important step and is exclusion of cervical infection which is more serious than vaginal infection.

The cervix is inspected after the ectocervix is widening clean of vaginal material. Any ectopy edema warts ulcers etc. are noted and the quantity of the cervical secretion is assessed.

The term ectropian, sometime, interchanges with the term ectopy has also being used to described the patulous parous cervix which gapes and exposes the endo cervical columnar epithelium as blades of vaginal speculum are opened. Ectopy not associated with the congestion oedema and / or mucopurulent exudate are a normal finding and require no therapy.

Mucopurulent discharge in cervix may be associated with infection due to the Chlamydia trachomatis, Neisseria gonorrhoeae and Herpes Simplex Virus (HSV). The last may be addition, manifest as superficial erosions of ectocervical squamous epithelium. In some cases no cause is found.
A yellowish colour of the pus (presence of polymorphonuclear leucocytes) as compared with the white colour of the swab may sometime help.

**Lower Abdominal Pain in Women:** Lower abdominal pain in women is and may reflect the minor or serious illness such as PID. All the women presenting with lower abdominal pain conditions such as ectopic pregnancy, abdominal or any other cause of acute abdominal should be examine.

The clinically PID may be difficult to make and can only be confirmed by laproscopy and laprotomy. PID suspected all women who have a complains of lower abdominal pain and who have an irregular menstruation vaginal bleeding dyspareunia, pelvic pain, vaginal discharge and the fever and in whom the clinical examination revels, pelvic tenderness, cervical excitation, tenderness or tubo-overian mass.

**Genital Ulcer:** The common sexually acquired cause of genital ulcer includes Treponema pallidum (Syphilis) Haemophilus ducreyi (chancroid) and herpes simplex virus (HIV genital herpes). Other cause is Calymmatobacterium granulomatis (granuloma inguinale or Donovanosis) and Chlamydia trachomatis or serovars L1, L2, L3 (Lymphogranuloma venereum).

There are also other sexually and non-sexually acquired caused of genital ulceration such as Gonococcal, Candida, Trichomonas and aerobic infection, scabies, genital trauma, pyoderma, fixed drug eruption, Behcet’s diseases and the neoplasm etc.

If the patient has an ulcer and if syphilis and chancroid commonly occur, then he/she should be treated for both syphilis and chancroid for following reasons:
1) From the clinical examination, it is often difficult to tell whether the patient has syphilis or chancroid. The presence or absence of pain or indurations or the presence or absence of bubo are not reliable discrimination.

2) Mixed infections are not uncommon.

3) Both chancroid and syphilis are known to facilitate the acquisition and transmission of HIV.

4) Only laboratory test can help in making accurate a etiologic diagnosis.

**Ophthalmia neonatorum:** Ophthalmia neonatorum is a defined as a purulent conjunctivitis occurring in a baby less than 1 month’s age. The common STI related causes are Neisseria gonorrhoeae and Chlamydia trachomatis. The infection acquired, at the time of the birth during passing through birth canal.

Other causes includes E coli, staphylococcus aureus, streptococcus pneumonia, gonococal infection, which is usually superficial epithelium infection (conjunctivitis) and can lead the infection of the cornea (keratitis) if not treated effectively. This leads to blindness. The neonates first treated for gonorrhea. If in 48 hours the infection does not show sing of resolution then treatment for Chlamydial infection is given.

When obtaining a history from mother the following pointes should be noted;
Age of baby, method of delivery, place of delivery, whether ophthalmalmia prophylaxis was instituted and, whether the mother or the baby’s father has any symptoms. In some instant often new born babies have a block lacrimal ducts and tears production is excessive. Mother having any sing and symptom a
serological test TORCH for Toxoplasma, Rubella Cytomegalovirus and herpes simplex virus I & II should be carried out.

**Generalized Lymphadenopathy**: Generalized lymphadenopathy may be occurred in large number of infection and non-infection diseases. These include tuberculosis, syphilis, Cytomegalovirus infection, HIV infection, toxoplasmosis, Epstein Barr Virus infection, Cryptococcus, histoplasmosis, septic skin condition leukemias, lymphomas and Kaposis sarcoma.

The commonest clinical manifestation of HIV infection is symmetrical generalized lymph node enlargement which occurs fairly early during the course of the infection. The enlarge lymph nodes are most easily palpated in the groins, neck, axillae, and in the submental area and are generally painless, firm, mobile and rubbery.

On clinical examination lymphadenopathy of the HIV infection is not distinguish from that in secondary syphilis. The person with secondary syphilis may have a genital ulcer, Condylomata lata, moist papules or non-pruritic macular, papular or squamous rashes.

The patients with HIV infection may have fever, night sweats, weight loss, skin rash, cough, headache, oral hairy leukoplakia, hyper pigmented nails, oral and genital herpes.

If the patent with generalized lymphadenopathy is found to have genital ulcer or gives a history of having had a genital ulcer then secondary syphilis should be strongly suspected. Patient with genital ulcer should be treated for both syphilis
and chancroid. In asymptomatic enlargement of lymph nodes or in whom lymph node measure more than 3 cm in diameters indicated and in whom tuberculosis is suspected should be treated thoroughly.

**Genital Warts:** Genital wart caused by human papilloma virus HPV both overt and subclinical infection occurs. The infection is spread by direct sexual contact. HPV also cause the common skin warts and flat warts. The strain of HPV cause genital warts are known as a genital strains. Wart other than genital region is known as a non-genital strain.

**Vaginal Flora:** The commensal flora of the vagina varies with age. Before puberty, when the secretion is not acid (PH 6.5-7.5), the flora consists mainly of staphylococci, streptococci other than S. pyogenes, diptheroid bacilli and coliforms. From puberty to the menopause, the secretion is acid (e.g. PH 4.5) and lactobacilli predominate, though various other organisms are present in smaller numbers e.g. staphylococci, enterococci, streptococci other than S.pyogenes, Diptheroid bacilli, Coliform bacilli, Bacteroids, anaerobic cocci, anaerobic vibrios, yeasts, mycoplasma and ureaplasma after the menopause the secretion is again nonacid and lactobacilli no longer predominate in flora.

**Various natural defense mechanisms:**

1. Vulva and perineum: have inherent resistance due to two factors (1) apposition of labia and (2) apocrine glands which produce undecyclinic acids which acts as fungicide.
2. Vagina: is protected because of closure of anterior and posterior walls, unbroken squamous epithelium by glands, vaginal acidity and vaginal flora like Doderlein’s bacilli that keeps vaginal PH acidic.

3. Cervix: is protected by closure of mucous plug.

4. Uterus: is protected by periodical shedding of endometrium during menstruation.

5. Fallopian tubes: is protected due to downward beating cilia.

Women are biologically more vulnerable to diseases of the genital tract than are the men. This is because of lining of the vagina is a mucous membrane, more permeable to outside than the skin on the outside of the penis. Also, lack of lubrication during intercourse or changes in the cervix during cycle facilitate more efficient transmission of infection to women.
Factors affecting defense mechanisms:

Age: The vagina of the newborn child is under the influence of estrogen which has crossed the placenta from maternal side. By 10 to 14 days, the estrogen stimulus is lost and the epithelium atrophies and becomes devoid of glycogen until puberty, because of which younger females before puberty are more vulnerable. In older the age, epithelium is atrophied, glycogen content is decreased and hence, there is increase in PH and decrease in doderlein’s bacilli that predisposes them to infections.

Menstruation: Which cause loss of cervical mucus plug and increase in PH. Intercourse during this period increases chances of infection because of increased PH and menstrual blood provides good culture medium for the organisms to grow. Gonococcus is more virulent, can ascend following menstruation and Trichomonas vaginalis can relapse.

Puerperium: Defense mechanism is weakest due to raw placental site, cervix vagina is open and lochia provides a good culture medial for organism to grow. Coitus: Alkaline PH of semen predisposes for infection.

Immunology of female genital tract:
The genital tract has been considered a component of the common mucosal immune system (CMIs), and there is much evidence to sustain that concept, especially with respect to the female tract. The epithelium of the endocervix, fallopian tubes and uterus and ectocervical glands, express polymeric immunoglobulin receptor (PlgR) and underlying populations of plasma cells secrete predominantly polymeric Immunoglobulin A (PlgA), which is
transported into the luminal secretion by PIgR to form secretary immunoglobulin A (SIgA).

The secretion of both male and female systems contain significant levels of SIgA, but these may be exceeded by IgG levels, at least in human and other primates. Whereas SIgA is accepted as the distinct product of the CMIS, the source and means of delivery of IgG are less clear both local production and transudation form the circulation have been implicated.

It seems clear that both male and female tract lack true mucosal inductive sites, which are collectively known as mucosa associated lymphoid tissues (MALT) and are typified by intestinal payer’s patches and similar organized lymphoepithelial structures in the lower bowel and upper respiratory tract. Nevertheless, foci of lymphocytes and accessory cells, consisting of a core of B cells surrounded by T cells and an outer area of macrophage - like cells, have been predominantly CD8+ CD4- suggesting an immune-regulatory role. The same group has identified CD8+ cytotoxic T cells in the female genital tract. CD4+ HLA Class II+ langerhans cells occur in the cervicovaginal epithelium and might serve as antigen presenting cells. Thus, at least in the female tract, there is an obvious potential to induce immune responses by the local application of immunogens, and the direct instillation of antigens into the vagina or uterus has been examined experimentally.
Female genitalia: Anatomy

The vulva includes the mons pubis, the clitoris, the labia majora and minora, the vestibule and Bartholin's glands.

The mons pubis covered with hair at puberty, is a rounded eminence of fatty tissue in front of the pubic symphysis.

The clitoris, an analogue of penis is an erectile structure located, between and behind the anterior ends of the labia minora. The labia majora are two prominent folds of adipose tissue extending from the mons pubis to perineum. Their outer pigmented part is covered by pubic hair, whiles the inner smooth part, contain many sebaceous follicles.

The labia minora situated between the labia majora, are two small mucocutaneous folds, extending from and ensheathing the clitoris and uniting posteriorly to form forchette.

The vestibule is a cleft between the labia minora are the orifices of urethra vagina and bartholin’s ducts. Bartholin’s glands are the homologues of Cowper’s glands in male, are small mucus glands situated deep in posterior parts of the labia majora. Their ducts are 2 cm long and open on sides of the vestibule at the junction of middle and posterior third of the labia minora. They are lined by columnar epithelium.

Urethra:
The female urethra is about 4 cm long. Many small urethral glands open into the
urethra. Skene’s glands which are the homogenous of the prostate glands in the male are paraurethral glands situated near the lower end of urethra open either side of the external urethral orifice.

**Vagina:**
Vaginal canal extends from the vestibule to the uterus. The anterior wall is 7.5 cm long and posterior wall 9 cm long. It is situated behind bladder and in front of the rectum. It’s upper end surrounds the vaginal part of the cervix, forming anterior, posterior and two lateral fornices.

In the newborn infant the vagina has many layers of squamous epithelium due to the presence of mother’s oestrogen. After few weeks squamous epithelium is replaced by immature thin columnar or cuboidal epithelium.

The oestrogen level rises as puberty approaches and the vaginal mucous membrane in adult is lined by stratified squamous epithelium which is by then several layers thick. The estrogen levels falls after menopause and vaginal mucosa becomes thin and atrophic.

The glycogen content, the Doderlein bacilli, the oestrogen level and menstruation influence the pH of vagina. Normal pH of vagina is 4.5 or below in non pregnant women’s vagina at other than menstruation times. Acidity and the stratified squamous epithelium provide protection against pathogenic bacteria.

Uterus and Fallopian tubes: The uterus lies in the pelvic cavity between the urinary bladder in front and the rectum behind. It has two parts the body and cervix separated by the internal os. In the body of the uterus the endometrium is covered on it’s free surface with columnar epithelium.
The endocervix is lined by columnar epithelium while the ectocervix vaginal surface of the cervix is lined by stratified squamous epithelium.

Ectocervix in young girls may appear red (Ectopy) because of columnar epithelium. Pregnancy and oral contraceptive may also be associated with similar appearance.

On each side of the upper part of the uterus opens in the fallopian tubes. Each tube is about 10 cm long and terminates in fimbriated end close to each ovary. The fallopian tubes are lined by columnar epithelium.

The lymphatic drainage of the vulva, the lower part of the vagina and the perineal skin is into the inguinal lymph glands, and that of the upper part of the vagina and cervix into the external iliac, internal iliac and the sacral lymph glands. The lymphatic's of the body of the uterus, the fallopian tubes and the ovary drain mainly into the aortic group of lymph glands.

**Other organs which may be portals of entry of STI organisms:**
The anus or anal orifice is the lower opening of the anal canal. It is surrounded by folds of skin which coverage and continue into the lower part of the anal canal. The anal canal is 2-3 cm long. The upper part of the anal canal and the rectum is lined by columnar epithelium.

The lymph vessels from the anus and the lower part of the anal canal drain into the inguinal lymph glands. The lymphatic's of the rest of the anal canal and the lower part of the rectum terminate in the internal iliac lymph glands, whereas
those of the upper part of the rectum drain into the pararectal and the preaortic lymph glands.

The nasal part of the pharynx is lined by ciliated columnar epithelium, the oral and laryngeal parts by stratified squamous epithelium while the part in between these regions is lined by cuboidal epithelium. The lymph vessels from the lower lip, the floor of the mouth and the tip of the tongue drain into the submental group of lymph glands. The lymphatic drainage of the rest of mouth, the tongue and the tonsils is into the submandibular and the deep cervical lymph glands.

The conjunctivae are the mucous membranes lining the eyelids which are then reflected on to the eyeball. The palpebral conjunctivae are lined by columnar epithelium and the ocular conjunctivae by stratified squamous epithelium. The lymph vessels draining the eyelids and conjunctivae end in the parotid and submandibular group of glands.
GONORRHOEA:

Gonorrhoea is an infection of mucosal surface, of the genitor-urinary tract, with bacterium. N. gonorrheae is mainly transmitted, by sexual intercourse.

In men, 90% are purulent urethritis, but organism may also, spread to epididymis and to prostate. In women, rate of infection are, 65-75% urethra, 85 to 90% cervix and rectal mucosal, in 25 -50%.

About, 10% infection may, extend to endometrium and fallopian tube from, cervix. In passive posterior, particularly rectal inter course, in homosexual men, rectal infection, also found.

In small percentage, systemic spread, gives rise to disseminated gonococcal infection characterized by arthritis with or without skin lesions.

ETIOLOGY:
The causative organism, Neisseria gonorrhoeae, commonly referred, as gonococcus, derives its generic name from, Albert Neisser who described it in 1879.

The bacteria are small Gram negative cocci, kidney shaped and arranged in pairs, (diplococci) with long axes in parallel and opposed surfaces slightly concave. The organisms are typically intracellular. By microscopy, it is impossible to differentiate the gonococcus from N.mengitidis, or from other pathogenic or potentially pathogenic Neisseriae or Bramhamella catarrhalis.
N. meningitidis and common sal Neisseriae can be found on the surface of the genitourinary tract and anal canal, particularly in women and homosexual men.

Gonococcus is a delicate organism with exacting nutritional and environmental requirements. Media require blood or serum enriched to 10% carbon dioxide, must be provided to ensure growth.

Gonococci are killed by drying, soap, and water and an antiseptic agent.

In nature N. gonorrhoeae is a strictly human pathogen, although infection has been induced experimentally in the urethra of the chimpanzee. (Lucas et al., 1971, Brown et al., 1972).

In males, the incubation period is 3-5 days (varies 2-10 days), while in females, incubation period is difficult to determine.

Because of the very delicate nature of gonococci, gonorrhea is ordinarily acquired by sexual intercourse with infected persons. So many variable factors make it difficult to assess the risk of acquiring an infection from a single exposure.

Gonorrhea infection from male to female is higher, 60-90% (Barlow et al. 1976, Chiepperfield and Catterall 1976, Evans 1976). While infection to male with an infected female, 20-50% (Holmes et al. 1970, Hopper et al. 1983).

Risk of transmission was for white American is 0.19, while 0.53 for black American men. Blackwell et al. 1985 noted, American patients with Blood group ‘B’, are more susceptible to Neisseria gonorrhoeae.
In studies increasing the challenge dose from $10^3$ to $10^5$ piloted gonococcal colony forming units (cfu) overcame, the efficiency of parenteral gonococcus pilus vaccine (Tramont et al 1985)

More than 70% are asymptomatic. Gonococci transmitted to cause pelvic inflammatory disease PID, where as 1% dissemination of gonococcus, is found.

Because of oro- genital contact, gonococci can be transmitted to pharynx. In one unpublished observation, by Young, prevalence of pharynx infection, in patient with Gonorrhea, in Edinburge 1985, was 9%, for heterosexual men, 15% for women, and 28 % for homosexual men. Oral to genital transmission, is rare although, it has been reported to, subjects of prostitute, in south East Asia, who specific in oral sex (Soendjojo 1983)

Tice and Rodriguez et al 1981 noted that kissing is rarely responsible for occurring pharyngeal gonococal infection.

During birth, when baby passing through, gonococcal infected cervix, may acquire gonococcal conjunctivitis, but because of journal improvement, in antenatal care, and antibiotic against infection, it is uncommon. Because of infected hand, contaminated, moist towel, infection may found, in older children, and adult.

PATHOGENESIS:

Commonly infection found, in columnar epithelium of urethra, and paraurethral duct and vestibular gland (Bartholins), cervix, conjunctiva, and rectum. Also
infection occurs in gland of penis, cornea and mouth is extremely rare.

By third day of infection gonococci penetrate mucosal lining of urethra and established in the superficial connective tissue according to Harkness (1948). There is an exudation of cells and serum because of dilated capillaries. Dense cellular infiltration consisting of polymorphonuclear leucocytes, plasma cells and mast cells, make their appearance beneath the columnar epithelium in the region of Litter's glands and ducks.

Inflammations of deep tissue found. Gonococci penetrate through cells rather than passing between cells intra cellular. Penetration of gonococci in mucosal cells desquamated from the cervix has been demonstrated by Watt et al (1978).

Watt et al, demonstrated sequence of event, in vitro with gonococci, by electron microscopy, of in human fallopian tube. First, gonococci adhere to non ciliated columnar epithelium cells of genital tract and become established. Ciliated columnar epithelial are second, to be infected by gonococci. Cell wall damage is due to gonococcal lipo-polysaccharide.

In nonciliated microvilli of host cells initial connect with gonococci engulf by the cell membrane and gonococci multiply intracellular. Numbers of gonococci are release in to the submucosal space after the lysis of cell by host defense localized; the gonococci occasionally may disseminate when defense is poor.

In 1977, Novotny et al suggest that, gonoccocal pathogenicity may partially base of disorganization of human macrophages.
Because of gonococci are appear in cluster surrounded by organelles and granules derived from host, in which they multiplies are call infectious units. The reason is 1) cocci multiply in them. 2) The whole complex makes contact with epithelial cells. 3) The cocci are not recognized by polymorphs as, there is coating of granules. 4) Cocci are probably protected, against humoral defense mechanism.

Normally, gonococcus phagocytes by the polymorphonuclear leucocytes are killed, but gonococci multiplying and surrounded by the granules are survives, in macrophages. In some cases host cell remnant is utilized gonococci become, less and less coated are re-phagocytes.

Mucosal outer surface is differing from the non invasive commensal neisseriae have ability to attack and subsequently invade. Outer membrane of gonococci infects, with host mucosal surface, are important for virulence and pathogenicity. The cell envelope of Gram negative bacteria also N.ganorrhoeae is composed of three macromolecular membrane and rigid peptidoglycone layer. The outer membrane composed of lipo-polysaccharied, phospholipids and protein. Filamentous protein pili, that extended several from gonococcal not isolated and characterized chemically.

The pili, outer membrane is lypo-polysaccharide (LPS), IgA protease, gives pathogenicity to gonococci in infection.

**PILI:** Filamentous protein pili extended, from several gonococci not isolated and characterized chemically.
In 1983 Kellaqq et al, described four, T1 to T4. Later on it has been observed, by Kellogg et al (1963 a), Jephcott et al (1971) found that, colony 1 and 2 type are virulent possesses pili, while colony 3 and 4 are a virulent, not possess pili. Freshly isolated strains possess pili.

Piliated gonococci attach more successfully to spermatozoa, erythrocytes, vaginal epithelial cells, fallopian epithelial cells, and buccal epithelium cells, found by Buchanan (1977).

Pilated, attachment, non specific and specifically, not yet fully characterized. Heckels et al (1976), a hydrophobic nature of the pilus protein, helps the negative charge bacterium, and host cells.

Protein II, outer protein involve, attach with host cell also.

**STRUCTURE AND FUNCTION:**

Pili are made up of pilin protein, each pilin molecule contains, 175 -180 amino acid, of them 53 amino acids are constant, 54 and 114 semi variable, while hyper variable, are from 115 to onward (carboxy amino acid). Constant region are weakly immunogenic, while variable carboxy region are immunodormiant.

**PHASE AND ANTIGENIC VARIATION:**

Phase and antigenic variation involved the generation of biochemical and antigenicity result from expression of alternative pilis genus. Frequency in changing antigenic structure of pilli may responsible in avoiding the last immune response. Zek et al (1984), noted that gonococcal isolated from the urethra of men, cervix and urethra from female partners, after differ in pilus
types. Viriji and Heckels (1984) found, that different attachment and virulence to last host is associated with variation in pili, in natural infection.


Protein I, also name as POMP (Principle Outer Membrane Protein) or MOPM; (Major Outer Membrane Protein), molecular weight, of 32000 to 39000 daltons. Sub divided in to IA subgroup lower molecular and IB subgroup higher molecular weight. Protin I is preliany a protein which forms chenals or ore. It can insert in to artificial lipid bilayar and in to membrane of RBC.

Efficacy of insertion in lipid membrane is more in case of protein IA molecule, then protein IB molecule. Because of this in dissemination of gonococci are higher in preotein IA containing pilli then that of protein IB according to (Cannon et al 1983). Protein I also interact with natural IG and compliment in normal serum, in bacteria reactions.

Molecular weight of protein II ranges, from 24,000 to 30,000. There are number of different protein I. Gonococci may express one or more of these depends up on growth condition and anatomical site.

Major function of protein II is attachment to each other and to surface receptor of various cells types. Opaque colonies have more efficiency to attach eukaryotic cells then do gonococci from transparent colonies.

Different type of variation is found, in protein II from, different sites of infection in male and female partner (Zek et al 1984). Opaque colony forming gonococci
are tend to be isolated from patients with symptomatic genital infection, but rarely associated with invasive disease.

Transparent colonies of gonococci are usually isolated in sample, the cervix at the time of menstruation and from men, who are also consorts of women with salpingitis or disseminated infection.

A species specific Protein and is identically in all strains of gonococcal isolated, (molecular weight 30x10^3). Judd (1952), Swansan et al (1982), examined Protein II molecule, is exposed at surface in all gonococci, with surface labeling and monoclonal antibody studies.

Lipopolysaccharide (Lps) is composed of three parts a) The 'O' or somatic antigen, a polysaccharide, contain a series of monosaccharide b) 'O' Somatic antigen is linked monosaccharide, oligosaccharide, composed of different monosaccharide, such as heptanes, glucose, mannose, glucosamine and an eight carbon sugar acid 3-deoxy D mono octulanic acid (KDO) c) Lipid A Which bound to care usually, by KDO residue. LPS, Lipopolysaccharide composed of all there components known as smooth (S-LPS), where lack 'O' antigen is known as rough (R-LPS)

LPS similarly to, outer part of Gram negative membrane, and can lost spontaneously. In fallopian tube organ culture, Melly et al (1981) noted that bacteria bind to noncilited columnar epithelium cells release LPS responsible for sloughing off and death of ciliated epithelial cells. The LPS is virulent and antigenic antibody, produce against it and are bactericidal for the gonococci. Scheider et al (1982), Winstanley et al (1983), noted serum resistant of gonococci
associated with disseminated infection may be due to lack of LPS determinant. Bactericidal activity of human is due to the presence of gonococci with LPS.

IgA protease, produced by N.gonorrhoeae and also other N.menigitidis, Haemophilus influenzae and certain oral streptococci, (Kornfield and Plant 1981) IgA protease split antibodies, to IgA1 subclass at amino acid sites. Once IgA antibodies split, it loses its antibody activity. But the role of IgA protease, in gonococci are not clear, but presumably inactive IgA on mucosal surface. Other subclass IgA1 is unaffected.

HOST RESPONSE
Invasion of the genital tract, first contact mucosal surface, early in infection brings the gonococci into contact with plasma. IgG and complement is found in cervical secretion. Early complement component aids opsonization of bacteria, while terminal compliment component C5-C9 kills the bacteria.

Complement can be activated by classical path way (includeing antibody antigen complex and C1, C4 and C2 components) and/or alternative path. The alternative pathway misses out theC1, C4 and C2 comnents and starts at c3 step. The classical path way can be activated, by aggregated immunoglobulin and various polysaccharides, including the bacterial endotoxins.

Gonococcal infection results induce inflammation invading, gonococci with natural antibody and complement (Watt and Medlen 1979). These infections generate, chemotoxins, which attract phagocytes to the site of infection.

On examination of urethral exudates suggest that antibody-complement
mediated opsonization and PMN phagocytosis with intracellular killing of the gonococci, is important, in host defense mechanism. All such killing system are most important, in dissemination gonococcal infection.

The role complement, in serum is most important in preventing systemic Nesserial infection (Lim et al 1976, Peferson et al 1976). The terminal component C6, C7 and C8, in complement system may be deficits, in some hereditary deficient patient are susceptible to repeat the gonococcal infection.

In normal person, bacteraemic stage, generally not found, as normal antibody and complement system work. Disseminated gonococcal infection is commonly resistant to the bactericidal action of antibody and complement (Brooks et al 1976, Schoolnik et al 1976).

In some instant, the natural antibody block receptor for bactericidal antibody on the gonococcal surface, protecting gonococci from killing.

Natural gonococcal bacterial antibody is present in the serum, as most normal adults; arise without, obvious immunization or specific infection (Schoolink et al 1976). Natural antibody IgG, IgM and IgA arises due to pharyngeal carriage of non-gonococcal were detected, by Cohen (1976), with indirect immunoflourasce test.

Bactericidal activity of normal human serum (NHS) is due to natural IgM, conjugation with compliment. IgM natural bactericidal activity is develop at age from two year. Bactericidal activity of natural human serum, c ould be inhibited by endotoxin, release from gonococci, but not by gonococcal protein, showed by

McCutchan et al (1978) examined the mechanisms by which N. gonorrohea isolated from cases of DGI resisted the bacterial activity of normal human serum (NHS). They concluded, that natural IgG blocking, antibody binds, to gonococci possessing, the appropriate receptor, and protects them from killing by bactericidal antibody and compliment by preventing bactericidal antibody binding to the gonococcal cells.

Harriman et al, (1982), proposed that, serum resistance may be, due to, conformation changes, in LPS rendering, the complement membrane, attack complex C5b-9 ineffectual. Recent evidence suggests that, protein II, is a major antigenic determinant, for these IgG blocking antibodies. (Rice et al 1985)

Natural IgG also function as, heat stable opsonin, promoting phagocytosis of gonococci by PMN, thus natural IgG works, in early defense mechanism, prior to specific antibody response.

Following gonococcal infection serum IgG, IgM and IgA antibody level increased. Serum IgA is secretary in playing absorption, from the mucosal surface (Glynn and Ison 1978).

In response to different gonococcal antigen, human antibody response may vary. IgG and IgM antibody developed against gonococcal LPS. Antibody play important role in antibody complement mediated bactericidal action. IgG and IgA responses against pili and protein II.
Protein I can promote antibody response, but is not very immunogenic for human in term of antibody response (Buchanan et al 1980). Nevertheless antibody against protein I can protective against, the recurrence of complicated gonococcal infection. Women with pelvic inflammatory disease were produce d antibody protects sub sequent attack of PID with same protein I Serotypes.

In mucosal antibody response, McMillan et al (1979 b), demonstrate anti gonococcal antibody present in cervical exudates from women with gonorrhea, using whole cell gonococcal antigen in an immuneflorescent test. Mainly IgM and IgA predominantly found. IgM detected in approximately 40% of women and was associated with infection of less than 15 days duration, while IgA mainly of secretary.

Cell mediated immunity is important in protection from infection. Lymphocytes in wide range found more frequently in patient with history of multiple infections in both men and women.

Non-specific immunity may play role, but not everyone exposed, to an infection acquires the disease. This immunity is poorly understood but is likely to include urine pH osmolarity and urea concentration, as well as genital ph in women, by gonococcal infection. (Brade 1982)

Saigh et al 1978 shows that, certain component of endo-cervieal flora, are antigenic towards, gonococci and prevent, the establishment of gonococci infection.

Number of reason like a) variable immune response b) lack of protection against reinjection, c) very large number of gonococcal antigens, which makes difficulty
in selection of gonococci for vaccine, is responsible to prevent mucosal infection.

In gonococcal infection cocci are probably protected, against humeral defense mechanism, but in phagocytes coating of granules play role, in resistance.

EPIDEMIOLOGY:
Since man, is only natural host of N.gonorrhoeae, the epidemiological factor, that are important in gonococcal infection noted to human behavior factor and properties of organism.

The ganorrhoea is common in developed, as well as developing countries. On global scale there are 200 million cases per year. There was sharp increase rate of ganorrhoea in incidences of gonorrhea. In United Kingdom and United State, decrease slightly during late 1970s and early 1981s. Rate in gonorrhea is markedly decreased in homosexually probably resulting from concern over AIDS.

In United Kingdom, in Scotland, 5528 (106.39 new case of gonococci / 1, 00,000 population) and male female ratio was 1.7:1 in 1977. According Communicable Disease, Scotland, summary of sexually transmitted disease, from 1972-91 decrease, in both rates of infection 4938 (95.96) / 1, 00,000 population, and male: female ratio, at same number.

In 1982, in England, there are 55,166 cases of ganorrhea reported and male female ratio 1.7:1 and rate of incidence 111.39 /1,00,000 populations.(Extract from report of chief Medical officer 1985)
In the United States, data from 99,084 cases reported, (435/100,000 population), 92% of occurrence under the age of 35 years. The rate of male: female ratio decreased from 2.4:1 (1960) to 1.4:1 (1981), according to the Department of Health and Human Service, 1982.

Also, in Asia, Africa, Central and South America, the rate of infection is not known because of an inadequate reporting system. Gonorrhea is a major cause for high prevalence of infertility in part of Africa (WHO Scientific group 1978).

Hopcraft et al 1973 noted 17.5% of women had gonorrhea, attending family planning clinics. Latif 1981, reported gonorrhea, 35% in men and 23% in women attending STD Clinic, in Zimbabwe.

Prostitutes are probably an important role in the speed of gonococcal infection in developing countries.

WHO estimated that, 62 million cases of gonorrhoea occurred worldwide in the adult population, in 1995 half them, in South and South East Asia (WHO 1995).

Overall, prevalence of N. gonorrhea, in African countries, is most likely the commonest STI, higher from Asia and Latin America.

**Epidemiology of Antibiotic Resistance:**

Antibiotic resistance in gonococcus results from mutation in chromosomal genes and/or from plasmid-mediated Beta lactamase production.

Chromosomally resistant Neisseria gonorrhoea (CMRMA): Minimum inhibitory concentration (MIC) of less than 0.06 mg/l of penicillin fully sensitive wild strain
of gonococci. Resistance of penicillin due to mutation of chromosome result in small additive increases in penicillin resistant at 1 mg/l. Penicillin resistant is found rate of penicillin resistant is 100 times more than those which prevailed when penicillin therapy was introduced in 1960s.

There are three loci PenA, PenB and mtr (according Sarubbi et al 1974 Sparling et al 1975) involved chromosomal resistance. Mutation at pen A, the mtr locus and PenB2 result in 8 fold, 4 fold and 4 fold respectively.

Penicillinase producing Neisseria ganorrhoea (PPNG): Plasmid is small circular piece of DNA that can replicate within a bacterial cell, independent of the chromosome. Gonococci totally resistant to penicillin, due to a plasmid coding for beta lactamase enzyme were first reported, in 1976 Asford et al.

EPIDEMIOLOGICAL TYPEING MARK:
Typing of gonococci is important in epidemiological studies. The ability to divide the gonococcus into groups and subgroups all the verity of organism types to determined as well as their distribution and prevalence in the specific geographical area.

Auxotyping was developed by Cotiln in 1973, based on nutritional profile of gonococci, isolates gonococci growing on, chemically defined media, containing amino acid, nucleic acid bases and certain vitamins.

Serotyping characterizing gonococci according to their reaction pattern (serovar) when tested against a panel of monoclonal antibodies reactive with epitopes on 34
protein I(Pe I). A panel of 6 protein IA and Six prIB reagents is normally used and can discriminate up to 25 IA serovars and 32 IB serovars.

Sandstrem and Danieclsson 1980, use Coagulation CoA system, for serological classification. CoA system based, up on attachment of antigonococcal IgG, by its Fc portion, to Protein A on whole Killed cells of staphylococcus aureus. Staphylococci have ability, to react specific IgG, in addition of gonococci, which is visible, to naked eye.

Sandstron and Doniclssan (1980) classified gonococci into 3 sub unit WI, WII and WIII, Serogrouping of clinical isolates can be performed by testing boil suspension of gonococci, can type by appropriate CoA reagent, within few minutes, on sub culture and morphological change, reaction with CoA reagent are stable and responsible.

WI, WII, and WIII subdivision was not possible, with polyclonal antibody, but later on, was overcome by monoclonal antibody.

1, 2 and 3 serotype protein I correspond to WI, serotype CoA reagent 4, 5, and 6 Serotype proteins I to WII serogroup, while WIII strain corresponds to serotype 9. Serotype 8 and 9 correspond to both, WII and WIII subgroup, according to Sandstram et al (1982b).

APPLICATION OF EPIDEMIOLOGICAL MARKER:
Extensive studies on auxotyping of isolated gonococci from different part of the world have shown that gonococcus is heterogenous and the distribution and prevalence of auxotypes is varied (Kupp 1985).
The AHU strain has received most attention as this auxotypes predominates in patient with disseminated gonococcus infection (DGI).

No association has been found between auxotype and PID although non AHU strain, more prevalent.

WII strain predominate account in 55% of isolated in larger cities where as WI stain account 80% of isolated from smaller cities In Europe 0-7% are serotype WIII association with acquired infection from abroad. In south East Asia WII strain predominately while serotype WI account only 0-25% accordingly work of Bygdeman (1977).

Reid and young (1984) noted, serotype WII were found, in rate of 95 to 100% in infection of homosexual, while WII and WIII were found in infection of heterosexual.

Morse et al (1962) found that, mtr phenotype strain, more prevalent among, isolated from homosexual men, than isolated heterosexual men and women.

In Europe, it has been found, that heterosexually acquire infection are serogroup WII and mtr phenotypes from Far East, as a result of general selective antibiotic usage (Reid et al 1985)

**CLINICAL CORELATION:**
Serogroup WI is correlated with disseminated gonococcal infection.

Sandstram et al reported, 84% of 101 gonococcus isolated from patient with DGI belonged to serogroup. WI compared with 40% from 168 isolated, from patient
with, uncomplicated gonorrhea. Clinical correlation, DGI and serogroup WI was better than, between DGI and auxotype.

Serogrouping with polyclonal antibody, gives much useful information, but has limitation in subgroup. With monoclonal antibody, fine subtyping of gonococcal strain is possible.

EPIDEMIOLOGICAL CHANGES:
The pattern of gonococcal strains of different types may change to the new strain or by genetic exchanges in locally existing strain. Both mechanisms are helpful at high risk of repeated and double infection.

The serovar analysis should also be use in development and assessment of gonococci vaccine.

DIAGNOSIS OF GONORRHEA:

LABORATORY AND CLINICAL PROCEDURE:
Microbiological test are mandatory in making a diagnosis of gonorrhea because of short incubation period, a high infectivity, rapid diagnosis followed by immediate treatment and contact racing are important in control of infection within the community.

As Neisseria gonorrhea is very fastidious organism, very careful technique are necessary for the collection of specimen and transport to laboratory, for culture and investigation.
Because of these patients, are seen at a clinic, with an adjacent or closely to laboratory, under this condition majority of the patient (about 90 - 95 % males and 50-60% of females) can receive appropriate treatment, after examination. Culture diagnosis and conformation of smear positive cases can be possible with 24 to 72 hr.

In female patient specimen for culture investigation should be taken from a) the urethra (Ur) (traditionally specimen taken from urethra after massaging from above downwards to expel any discharge from past-urethral glands), b) External cervical os and cervical canal (Cx) c) rectum and d) throat (T).

If pus is expressed from orifices of the duct vestibular gland, gram staining should be done Urethra and endo cervical culture should be done after gram staining. If the culture of the above sites, Ur, Cx, R, and T are negative then, test should be repeated after one week before. reassuring the patient that she does not have gonorrhea. Third set of tests is justified, additional precaution in the case of contact gonorrhea, who has given two negative cultures. The use of non selective medium should be considered for second and third test in gonorrhea contact.

TEST IN DISSEMINATED GONOCOCCAL INFECTION:
Routine test described and several blood cultures should be carried out before common therapy in suspected disseminated gonococcal infection. It is important to inform to laboratory, for blood culture, for disseminated gonococcal infection.

Although, immunoflorescent technique, may reveal gonococcal antigen, in skin lesion, but are not considered in routine diagnosis, it is of research interest.
Barr and Danielsson (1971) noted that there may be no genital symptom in case of DGI ano-genital (and pharyngeal) culture yield gonococci.

Fluid from joint should be examined for culture.

Repeated testing of multiple sites is necessary since not all infected woman, will detect infection, on first attempt. There are variable percentage of reports, of first attempts of detected gonorrhea, in culture varies, 66 % to 98 %. (66%, by Catterall 1970, 90%, Thin et al 1971, 91 % Chipperfield and Catterall 1976, and 98% Young at al 1979.)

Microbiological service constantly reaches a high standard, only two set of investigation, need to be taken to diagnosed or exclude gonorrhea, in woman (Barlow et al 1976, Young et al 1979)

The efficiency of detecting gonorrhea varies depending on factors such as culture medium used.90% culture are detected, from single endo-cervical swab, when modified New York City (MNYC) medium use, while 78% was when conventional Thayer Martin medium was used(Young et al 1974)

According to Bhattacharya et al (1973), a high vaginal swab is totally inadequate for diagnosis and exclude in gonorrhea as one out of three infected women to be missed. Poor results with vaginal specimen are to be expected, since this material detects only gonococci which have contaminated area, from the cervix, a site of infection, where gonococci are actively multiply.

Rectal cultures are important, in case of female about 25 -50 % of patient has ano-
rectal infection (Bhatachary and Jophcott 1974). Rectal infection is usually symptom less and may responsible, for treatment failure. Hence it is essential in screening of infection and treatment.

In general, detection of, endo-cervical infection, and urethral infection, in heterosexual women, men, and anorectal infection in homosexual men, is much more important than the detection of pharyngeal gonorrhea, in either sex.

TRANSPORTATION AND CULTURE SYSTEM:
Dry swabs should never use as gonococcal is very susceptible to drying. Direct plating gives best result. The worm plate (before plating, it should be 37°C temperature) inoculate directly, incubate at 37°C in moist atmosphere containing 5-10% carbon dioxide.

Date stamp are advisable for plate, when stored in refrigerator. There is no difference in result of gonococci culture, at room-temperature or moist plate, from directly stick from refrigerator. As most identification methods for use with primary culture work best with young 18-24 hour culture rapid growth is an important advantage of pre worm in plates to 37°C.

Adler et al (1978), reported that half of the STD clinics, sent specimen, to laboratory, in non nutrient transport media, in England and Wales. Stuart transport medium, are use majority, 87% while Amies medium (Modified Stuart medium) use in 13% of clinics. As charcoal is present, in modified Amies medium, which neutralize toxic ions produced, by a) irritation of commercial swabs sticks b) cotton wool c) agar and d) by products of bacterial metabolism, more widely use, compared to stuart transport medium. Charcoal coat swabs,
used in Amines medium, is journal not acceptable, by the patient. Less problem of contamination is found, in Aimes medium, use of glycerol phosphate acts as, energy source.

The nutrient transport and culture media includes transgrow (Martin and Leser 1971) having chamber facilities to provide co₂ while JEMBEC (Martin and Jackson 1975) a system of generating co₂ available in market. Condensation on inside of the Transgrow bottle, it is difficult or impossible to examine culture satisfactory in order to identify growth of gonococci while such problem is overcome in JEMBEC.

JEMBEC system is rectangular culture plate contain selective medium and also recess in to which, co₂ generating tablet placed at the time of inoculation. Co₂ generate as a reaction between citric acid and sodium bicarbonate in tablet. The close plate placed in plastic bags and sealed to gas tight. After incubation, suspected colonies, on the surface of the medium can be, easily examined and identified further.

Ebright et al (1982), Sneg et al (1982), Spence et al (1983), suggest that, significant loss of viability will occur, when specimens are in transit for longer than three hour. In some area when ambient temperature, is high above to 26°C like Singapore Seg et al (1982), suggest that, loss of viability of gonococci increases.

Ebright et al noted that, time of transportation for 24 hr and 48 hr, can reduce isolation rate, as much as 40% and 63% respectively. When more prolonged transport is anticipated, it is best to use transport system and to incubate it over night, in clinic prior to sending the laboratory. The transport system and nutrient
transport are expensive and little advantage when transit time is about 3 to 4 hr.

In cultivation, gonococci viability during transit, particularly when the inoculums is low, non culture, antigen detection methods, are more under development and evaluation.

Smear prepare by rotating the high vaginal swab over clean glass slide Allow it to air dry, fix with methanol, as heat destroy the delicate gram negative cocci especially

a) MICROSCOPY:
This is important as it enables as presumptive diagnosis to be made in clinic , so that, appropriate treatment can be given immediately. Diagnosis and treatment facilities prevent spread of infection and progression of disease and to its more serious sequel in that patient likely to default.

Gram Stain:
A smear of secretion or discharge is prepared and Gram stained, by standard bacteriological technique, (Callee et al 1988) but, 1% neutral red is preferred, as counter stain. After staining, dried slide is examined, under oil immersion objective lens.

Goodhart et al (1982), found, that sensitivity of Gram staining of cervical smear ranges, from 23 to 65% and specificity, from 88 to 100%.Gram staining of urethral smear, have no significant important, to diagnosis gonorrhea in women, according to, Barlow and Philips (1978).In routine, rectal smear are not examining microscopically, in female patient.
In infection, without symptoms is, commonly seen in women. Number of gonococci is less in number compared, to male. Bacterial flora, qualitative and quantitative of female genitor-urinary tract, is greater than, in the male.

A typical, positive Gram stained smear, of urethral or cervical discharge from a male and female patient with gonorrhea, usually shows, large number of characteristic kidney shaped, gram negative diplococcic (GNDC), laying within the polymorphonuclear leucocytes, with few extra cellular organism.

If pleomorphic, extra cellular Gram negative diplococci and bacilli, with rare intra cellular GNDC, with morphologically typical of N.ganorrhea are seen. The smear examination is equivocal and not diagnostic. If no GNDC are seen, result is reported as, negative.

The organism visualize, are small gram negative coci and coco bacilli, some time appearing, in pair intracellular. Interpretation of smear is difficult, when Gram negative gonococci are extra cellular considered as positive. The sensitivity of smear (gram staining) increased from 31% to 51 % while the specificity decreased from 99 to 86%.

Performance of gram staining against, isolation of gonococci, the quality of culture system, will influence the observed sensitivity of microscopy. A poor culture system fails to detect infection, when low number of gonococci, will result, in falsely microscopic examination, when patient taken an antibiotic.

Small percentage of both male and female shows positive gram staining smear,
but gonococci will not be isolated on culture. These could be also found, when patient have taken an antibiotic before coming to the clinic or due to particular strain of gonococci are sensitive to vancomycin used in certain selective media. The sensitivity of vancomycine varies from 2% to 30% (Mirret et al 1981, Windall et al 1980).

**Immunoflorescence Stain:**
Immunoflorescence antibody staining of secretions, from patient is time consuming and technically demanding, not recommending other vice, it provide similar result to those obtain by Gram staining. Yet Immunoflorescence antibody technique is valuable tool, in studying pathogenesis and in rapid diagnosis of disseminated gonococcal infection. Also useful, in identification of gonococci from sites, which normally give poor culture result e.g. Skin lesion or joint aspiration.

b) **CULTURE:**
Immediate diagnosis must be supplemented, as well as conformed by culture maximum number of positive result is to be obtained. Culture is essential in a) Rectal b) Oral C) Disseminated and d) A Symptomatic infection, in both sexes, and are also essential, in order to determine antibiotic sensitivities, and to know its effectiveness.

Number of culture media to be use, are contain blood as nutrient base supplement lysed partially, by direct heat or completely by chemical saponin. For culture various selective media or their modification are routinely use.

Theyer Martin™ selective Media, is selective medium formulated, by Thayer and
Mertin (1966), allow growth of gonococci and manigococci from all site (Genital, Rectal, Disseminated etc). Such medium is provide to be best in rectal and pharyngeal site as. Antibiotics, such as vancomycine, colistin and nystatin present in medium, prevent other flora which overgrowing any gonococci present. But spreading growth of proteus spp. may found, because of resistant to all three antibiotics.

Martin et al (1974) modified the medium by using double concentration agar (2.0%), glucose (0.25%) and trimethoprim (5.0 mg/ml) used for isolation of gonococci and complete inhibition of proteus spp.

A modified TM Medium have limitation, vancomycin sensitivity is variable, in the gonococci population, certain strains AHU, are more sensitive than others, Mirret et al 1981. Windall et al reported, up to 30% auxotropes strains are sensitive to vencomycine.

Martin Lewis Media is similar to modified TM Medium, but contains anisomycin, is more stable then nystatin, provide larger shelf life of medium.

Modified New York City medium is simple modification of original New York City (NYC) medium, described, by Faur et al (1973), provide luxuriant growth of pathogenic Neissriae after 24 hr. Medium contain a) lincomycin less inhibitory to gonococci, than vancomycin, b) colistin and c) amphotericine are more effective to yeast, than nystatin and trimethoprime. Medium contains, yeast dialysate and glucose, and so that growth, is more rapid and colonies are larger.

Young (1981) suggest, that MNYC medium improves, the overall isolation rate,
and enable a larger percentage of isolation, to be identified with 24 hr, compare to Thayer Martin Medium.

If rectal cultures are not examined, until 48 hr of incubation contaminant could mark small numbers of gonococcal colonies.

A combination of a selective and non selective medium has been recommended to ensure the detection of gonococci with markedly increased susceptibility; to antibiotic as well as vancomycin sensitive gonococci. A non selective medium can always be used in sampling site, such as joint fluid and blood, which are normally sterile.

**Identification:**
After 24 hr of incubation, plates are examined and any colonies suspected, as being gonococci are tested by cytochrome oxidase test. Incubation is continued for 48hr, plate reexamined before culture can be reported as negative.

The cytochrome oxidase test is useful in screening test since all neisseriae are positive, while coliform, staphylococci and lactobacilli are negative.

The test is carried out by touching to colony with (Cotton swab) filter paper soaked in a 1%(W/V) solution of tetramethyl-p-phenylenendiamine diahydrochloride. A positive reaction, is indicated by purple colour of filter paper, when touch with colonies, within 5 to 15 second.

The test is simple and quick. When selective medium is used minimizing the risk of missing gonococci is less due to atypical colonial appearance. Colonies are of
same appearance and oxidase positive, should be examined, by gram staining, as other organism may be oxidase positive e.g. Kingella, Morexiella and Heamophilus.

In gram stain smear from colony, normally members of Neisseria are indistinguishable by microscopy, as all appear as, Gram negative cocci usually in pair but some time in clump or singly.

Presumptive diagnosis of gonococci are on basis of, oxidase positive GNDC growing on selective medium, in sample of urethral specimen from men and ano-genital specimen from women.(Department of Genitourinary Medicine, Edinburgh Royal Infirmary1978-85) Smelter et al 1980 found 98.51% diagnosis from endo-cervical specimen, from low prevalence.

Carbohydrate utilization test and certain new carbohydrate test are widely used for definitive diagnosis. In Carbohydrate utilization test, Neisseria spp are classified on basis of various sugar fermentation (Biochemical test), although widely used.

Gonococci produce acid from glucose only, where as menigococci produce acid from both, glucose and maltose. N lactamica organism found by readily grow and to similar morphology the menigococci, produces acid from lactose, in addition to glucose and maltose

Gonococci can be differentiated by their ability to utilize glucose and maltose usually in combination with sucrose and/or fructose.(Collee et al 1988)
N. cinerea is asaccharolytic in most sugar utilization tests. Brahmmella catarrhalis is always asaccharolytic.

Carbohydrate utilization is of two types a) Conventional test b) Rapid test.

Conventional test for carbohydrate utilities a solid medium containing appropriate carbohydrate and pH indicator and test organism is to inoculate.

But problem arises in finding medium that grow all gonococci and at sometime give reproducible and clear cut indicator change. Now days Cystine trypticase Agar (CTA) widely used, as commercial medium in USA, but problem with such medium, culture sent to CDC Atlanta for conformation.

In Rapid Carbohydrate Utilization Test performed enzyme, is measured, by adding suspension of, over night growth of suspected organism, to the buffered, in non-nutrient solution containing, the sugar, to be tested and pH indicator.(Younget al 1976). It’s rapidly, compared to identification of Neisseria, after growth, it require only 1 to 3 hr, incubation at 37°C. The reagent are properly quality controlled, the 18-24 hr young culture, require for test, are grow on glucose containing media. RCUT are more accurate than conventional test. (Tapsall and Cheng 1981)

In other carbohydrate utilization methods, includes BACTEC, for Neisseria differentiation kits are available commercially, N.ganorrh oea, N menigitis and N lactamica are identified, on bases of 14°C labeled Co2, resulting from the metabolism, at 14 C labeled sugar. The main disadvantage, of this method, is radio labeled material used, and expense of maintaining equipment system, is
over sensitive, and gives positive result with N cinerea (Boyce et al 1985).

A number of Nesseria identification system (Biochemical) are available include RapID NH System (innovative Diagnostic system, USA) and Gonocheck (E-Y Laboratory, USA).

Rapid NH System is, four hour, test designated, to differentiate, Nesseria spp; Branhalmella, Moraxilla and Heamophilus based on both, sugar utilization test and single substrate chromogenic biochemical tests. A suspension of test organism, is added to reconstitute dehydrated reagent, in the appropriate wells of plastic tray, beta galactosidase, amino-peptidase, Indole production, urea hydrolysis and phosphate and nitrate degradation, are included.

Robinson and Oberhofer (1983) found that, there were considerable problem were associated, with the use of this system. B catarrhalis could not be distinguished, from Morexella and Kingella. System has problem, with isolates N menigitidis and also fail to differentiate between N.ganorrhaeae and N.cinera (Boyee and Mitchell 1985).

The GONOCHECK system, is differentiate, N.gonorrhoeae, N.meningitidis, N.lactamica and B. catarrhalis. The enzyme produced by N. gonorrhoeae, N.menigitidis and N.lactamica are praline aminopeptidase, gammaglutamyl amino peptidase, and beta galactomidase, respective. B catarrhalis dose not produced, any of these enzymes. Oxidase positive GNDC are added, to the reconstitute tube, after 30 min 37°C change of tube color, designate species. A blue colour designates N lactamica and yellow colur N.menigitidis. If no colour development, diazonium salt derivateive (EY20) is added. If the tube is turn red,
the isolate is gonococcus, if no colour changes then B catarrhalis.

Immunological confirmation of the identity of gonococci can be obtained, rapidly and with very small amounts, of bacterial growth.

Both, Coagulation (CoA) and Immunoflourescence (IF) methods, uses widely but the disadvantage failure to, detect a small proportion gonococcal isolates and the give a clear cut negative reaction, with a similar proportion of non gonococcal neisseria (Young and McMillan 1982), cross reaction occur frequently, with N.lactamica (Anand and Kadis 1980). Co$_2$ method is simple to perform, easy to interpret and not require expensive fluorescence equipment.

The advent of new monoclonal antibody reagent has increased the sensitivity and removed the problem of cross reaction with other neisseria thus working the test valuable.

The phadebact monoclonal GC test contain 2 separate reagents WI and WII/WIII with different pools of monoclonal antibodies reactive with epitopes on prolein IA and Prolein IB, respectively. A light suspension of suspecte oxidase positive GNDC from primary isolated plate prepared in 0.9% and boiled for 5 minutes when test suspension is mixed with the reagent monoclonal antibodies in appropriate bind any specific gonococcal antigen coagulation is visible to naked eye

In preliminary but extensive evaluation this testy was shown to be 100% sensitive and specific when tested against 550 gonococcal and 197 non gonococcal GNDC including N.menigitis, N.lactamica and B catarrhalis
specificity and sensitivity is 100% and 99.7% respectively.

**Miscellanous Identification Methods:**

Lactins and peroxal test is miscellaneous test Lactins are plant protein which react with carbohydrates certain lactins agglutinate gonococci and can be used in slide test.

The superoxal test was described by Saginur et al (1982) as a simple rapid low cost test for identification of N gonorrhoeae. Catalase test performed with 20 to 30% hydrogen peroxide in placed of 3% as used in the conventional method As number of menigococci neisseriae and B catarrhlis give a positive value.

Odogbemi and Arko (1983), found a negative superoxal test valuable in differentiating kingella denitrificans from N gonorrhoeae. K.dentrificans is oxidase positive and has a colonial morphology similar to that of a gonococcus.

**ANTIBIOTIC SENSITIVITY TEST:**

**Detection of penicillinase, producing Neisseria gonorrhoeae (PPNG):** Most important aspect of sensitivity testing, screening can be performed by inoculating a suitable non selective medium with growth from colonies and placing 6 μg disc on the well. After overnight incubation, any isolate showing zone of inhibition of < 20 mm is likely to be PPNG and should be confirmed, by are of the specific penicillinase detection methods.

One of the most sensitive and convenient method, uses commercially available is paper strips impregnated, with chromogenic cephalosporin
When few PPNG colonies, are rubbed on to a test strip, moistened with the water, the resulting hydrolysis of Beta lactamase ring carries, a purple color to form around, the area where organism were added. Bacteria break down penicillin to penicilloic acid, to dissociate starch iodine complex or by a Ph indictor system. Using this method results for penicillinase production may be available before disc sensitivity (Young 1978)

**Minimum Inhibitory Concentration (MIC):**

Majority of the patient with gonococcal infection has been treated on the basis of positive smear. Antibiotic tests are little help the in initial management of patient.

Nevertheless, they are important, for epidemiological purposes and in planning rational therapy for use in geographic area concerned.

Minimum inhibitory Concentration (MIC) testing is desirable, most laboratory use disc or agar dilution producers, with media and inocula, to suit their individual need and preferences.

Disc susceptibility testing, has been widely used, give rapid guide to the sensitivity or resistance, of on isolate and simple method.

More accurate result is obtained, with on agar dilution method. In agar dilution method antibiotic to be tested is serially diluted and each dilution, incorporated into one agar plate. A series of different antibiotic, each at several concentrations can use. Accurate amount of antibiotic are available commercially, in tablet form.
Normally two fold dilution of penicillin, from 1.0 to 0.015 mg/L are used. Other antibiotic, such as tetracycline, erythromycin, Spectinomycine and Cefuroxime, can also included. Standard suspension of each strain is inoculated on agar plates using the multi pointer indicator. After incubation of 24hr, lowest concentration of antibiotic, which inhibits growth, is taken as MIC.

Majority of reports define, a less sensitive strain, as one requiring 0.125 mg/l or more of penicillin, for inhibition. Most of trains (approximately 80% are fully sensitive to penicillin and many are sensitive to very low level(less than 0.015 mg/l)

In one large clinic, in London (Sng et al 1984) the prevalence of strains, with a penicillin, MIC of >1 mg/L was 8.5%. According to Rice et al 1986, in USA chromosomally mediated resistant gonococci, have been reported in 23 states. Antibiotics, other than penicillin, tetracycline or erythromycin, should be used, in treating gonococci with chromosomally mediated resistance to penicillin (MIC>1 mg/l)

**Non Cultural Diagnosis:**

Non cultural diagnosis of gonorrhea, is based upon, the detection of gonococcal components, in secretion. Non culture diagnosis is evident from the number of clinic, which has to rely on culture results, from specimen which have been transported some time for prolonged period. Non culture methods are suited to large centralized laboratories, serving peripheral population. The immunological detection of antigen, shows the most immediate promise
Detection of Antigen:
The Gonozyme test is currently the only commercial, antigen detection system. The test is performed, with urethral and cervical exudates, collected on swabs and transported to laboratory transport tube containing preservative. Polystyrene beads are used, as the solid phase to capture antigen, which is detected by polyclonal antigonococcal antibodies, in as ELISA.

Gonozyme giving, sensitivity range of 94-100% and specificity 96-100 %( Young and Reid, 1987) with, specimen of urethritis in men. In specimen of Urethritis, Gram stain, a simple highly specific and sensitive. Gonozyme test has limited value.

In women Gonozyme test, is less reliable then men, sensitivity and specificities are 60-95% and 70-98% respectively. In practice the Gonozyme, is more reliable then, culture following transport, for excluding gonorrhea, in women.

A positive Gonozyme test, in patient from a population, with a low prevalence for gonorrhoea, should be confirmed, by culture as around half, are likely to be false positive Culture. Conformation is advisable as, it will provide an opportunity to perform antibiotic susceptibility testing.

Miscellaneous detection method includes, endotoxin genetic transformer DNA hybridization

The Lymulus amoebocyte lysate assay is a test, to detect endotoxin. It can be used to detect, gonococcal endotoxin, in urethral and cervical exudates. The test is not specific, for gonococcal endotoxin, but absence of other bacteria, producing
endotoxin in amount, that would give as, positive result.

According to prior and Spagna 1981, used the test to differentiate, between gonococcal and non gonococcal urethritis. Spagna et al used test, in diagnosis of gonococcal cervicitis.

Gonococcal DNA can be detected, in clinical specimen, by transforming auxotrophic indicator gonococci to prototrophy. (Janik et al 1976) This assay is extremely sensitive, and can detect as far as, 50 colony forming units (CFU) of donor gonococci. Disadvantage of this method, is that clinical isolates of N.gonorrhoeae, may themselves be auxotrophic, for the some marker, as indicator strain and thus unable to transform it.

Gonococcal DNA has been detected, in urethral exudates, by hybridization with the gonococcal cryptic plasmid, as radio-labelled probe (Totten et al 1983).Test is very sensitive detecting around 100cfu of gonococci or 0.1g of gonococcal cryptic plasmid DNA.The gonococci are common in certain geographic area especially Canada..

Detection antibody includes humoral and local immunity.

The gonococcal complement fixation test is the only test, which has been used to routine diagnosis. The test has fallen in to disrepute, over the year and it should not be relied upon, either to detect or exclude uncomplicated infection. There is real need, for a sensitive and specific serological test, in order to screen large groups of individuals at risk, as it is difficult to carry out, genital examination in, such situation.
Modern approaches using radioimmunoassay (RIA) and ELISA technique, with highly purified antigen, such as gonococcal pilus protein or outer membrane protein, have failed to provide a suitable test (Donegan 1985).

The main problem is that antibody levels, in infected and noninfected individuals overlap, to such an extent that, it is impossible to define, an antibody level that gives, a reliable indication of infection.

An approach to the diagnosis of gonococcal infection is depending up on laboratory resources available. Possible alternative approaches, to the diagnosis of gonococcal infection, in places where laboratory resources, are absent or limited, and have been usefully define in WHO technical report. Where adequate facilities exists, suitable culture and identification, depends on factor, such as source of the secretion (hospital, clinic, family planning clinic, general practioner etc) and the proximity of the microbiological laboratory.

Laboratory and clinic are situated, close to each other, than direct plating should be used. Fully identification and antimicrobial susceptibility should also be undertaken. Differentiation of gonococcus from the menigococcus and other neisserae and Bramhmella is important not only in, a) medicolegel cases but also in b) oral infection, in c) cases disseminated gonococcal infection in which the gonococcus and menigococcus may identical clinical syndrome and in d) examination of conjunctival specimen.

Unfortunately because of fragment problem with carbohydrate utilization, particularly where using CTA sugar N.menigitid is and B.catarrhalis are the two
organisms, most often confused with N.gonorroeae (Arko et al 1982)

When examining throat culture, accurate differentiation is even more important, as then rest majorities of GNDC isolated will be menigococci. Simple, rapid and accurate differentiation of gonococci, can also be obtained by phadebact Monoclonal GC Test.

All non gonococcal Neisseriae from further, an ano-genital sites, should be identify to conform, that they are not gonococci which lack the usual protein I epitopes.
NON GONOCOCCAL URETHRITIS, CHLAMYDIA INFECTION AND OTHER CONDITION:

Chlamydiae:
Chlamydiae are obligate intracellular parasites. C. trachomatis are divided into 5 serotypes based on antigenic differences in the outer membrane protein (MOMP).

Of these types D to K are associated with non-gonococcal urethritis and mucopurulent cervicities in adults.

Types L1, L2 and L3 are associated with Lymphogranuloma venerum (LGV).

Morphology and Growth Cycle:
Chlamydiae occur in two forms, the elementary body and the reticulate body. The elementary body is the extra cellular, infective form. It is a spherical particle, 200-300 nm in diameter, with a rigid trilaminar cell wall similar to the cell walls of gram negative bacteria, and an electron dense nucleoid. The reticulate body is the intracellular growing and replicative form, 500-1000 nm in size. Its cell was wall is frangible and pliable, leading to pleomorphism.

Infection is initiated by the attachment of the elementary body to the surface of a susceptible epithelial cell, followed by its endocytosis. Inside the host cell, the elementary body lies within the endosome, being separated from the host cell cytoplasm by the endosomal membrane through out its active growth cycle. By about 8 hours, the elementary body within the endosome undergoes spheroplast like transformation to the large reticulate body, which begins to divide by binary
fission by 12 hours. By 20-24 hours, the pleomorphic progeny show central condensation and are converted to elementary bodies. Binary fission continues till about 40 hours. The developing micro colony within the host cell is called inclusion body. The mature inclusion body contains 100-500 elementary bodies which are ultimately released from the host cell.

During the active intracellular growth of the organism, the Chlamydiae specific lipopoly saccharides accumulate on the host cell surface. This highly antigenic material includes inflammatory and immunological response which contributes to the pathogenesis of chlamydial diseases.

CLINICAL FEATURES:
Almost 70-80% of women and 50% of men affected with Chlamydia do not show any symptoms.

Non-gonococcal urethritis:
It is characterized by a scanty discharge, which may be clear, milky, serous or mucoid.

Mucopurulent cervicitis:
It is characterized by mucopurulent cervical discharge inflammation of the cervix and greater than 30 polymorphonuclear cells per high power field on microscopy.

A) Non gonococcal urethritis (NGU) synonym or Non specific Urethritis(NSU):
Non specific Urethritis (NSU) is very term described common condition, seen in men and presenting clinically, as purulent or mucopurulent urethral discharge,
associated often with, the symptom of dysuria, few day to few weeks, after intercourse.

The purulent urethral discharge contains pus and may not detect, the gram negative cocci Nesseria gonorrhea in gram stain microscopy and as well as culture. Asymptomatic infection is common.

**Etiology:** The experimental evidence shows that two organism a) Chlamydia trachomatis and b) to lesser extent, Ureaplasma urealyticum, have role infection in aetiology. Role of infection of Mycoplasma hominis and Mycoplasma genitalium, may have not yet clear.

**Epidemiology:** Chlamydia infection of genitals is common worldwide. Mainly most male and females are asymptomatic. Because of no symptoms and not attending clinics, infection may spread one to other.

Application of newer test e.g. PCR and LCR revels high rate of infection 89 million new cases of STI in 1995 shows Chlamydia trachomatis infection.

Clinical symptoms are similar to that of Nesseria ganorrh oea. The prevalence rate was 7% in some area, 20% in Africa, among cervix infectio n, presenting family planning antenatal or gynecology clinic.

According Pham Kantur et al 1996, the mixed infection of Chlamydia trachomatis and Nesseria gonorrhoeae was 44-95%, in their study in a area of South Africa,
Clinical Features: In the male NGU present clinically a mucopurulent urethral discharge associated with dysuria. Dysuria with discomfort or pain localized, to the shaft of penis or to the region of metus. Urethral discharge may be in small quantity mucoid or copous and frank purulent. It may be more copious, in the morning sample compared to other times. Occasionally in severe cases, where there is urethrocystitis, frank haematuria may occur, throughout urination.

Pathogenesis and Immunity: Chlamydia replication may cause tissue damage and an inflammation response and possibly hypersensitive reaction.

There is infiltration polymorphonuclear leucocytes (PMN), followed by localized lymphoproliferative response leads, follicular urethritis, follicular cervicitis and follicular conjunctivitis. Fibrosis and scarring of affects mucus membrane.

As results, significant immunity do not present but repeated infection as, it is common, only low level immunity develop.

Diagnosis: Chlamydia trachomatis is implicated, in the aetiology of many cases of NGU, in male and related to female microbiological test are described.

In case of urethritis in men, it is duty to exclude the presence of gonococcus by careful microscopy and also culture. If laboratory is far distance, the transport medium is use e.g. Amines transport media.

Examination of good pus from urethra shows numerous WBC but no GNDC diagnosis of nongonococcal urethritis may be made. The NGU shows haziness with/without thread in 1st glass then 2nd and 3rd urine (Clear). In case of
urethrocystitis all specimen of urine are crude-hazy, due to pus cells. Hazy in urine due to precipitated phosphate is differentiated by adding 10% acetic acid to dissolve the phosphate.

The role of Trichomonas vaginalis and Candida albicans are not important, in case of NGU.

In NGU, Chlamydia infection diagnosis is essential with monoclonal antibody technique and should be possible where tissue culture method is not possible. Single serum sample have no diagnostic value in Chlamydia infection of urethra but in acute pelvic inflammatory, perihepatitis, high titer or rising titer results will give the support to the diagnosis of Chlamydia infection.

In case of Mycoplasma homionis and Ureaplasma urealyticum, half of the genital specimen will contain, these ubiquitous organism, with the interpretation of positive findings, being difficult to assess in the individual patient, routine isolation is difficult to justify (Taylor -Robinson and McCormack1980).

B) Post Gonococcal urethritis (PGU): 50% of men often the treatment, with the penicillin or amino glycosides developed post-gonococcal urethritis (PGU). Vaughan-Jacken et al (1977) studied that in urine, even presence of polymorphphonuclear leucocytes, gonococci can not be culture Chlamydia trichomatis has been implicated in the etiology of PGU in approximately, 50% of men who developed urethritis after the treatment of gonorrhea.

In one study, cell wall form of gonococci (L form) may be cause of PGU,
(Witkins and Geary, 1977) L form of gonococci are produced in vitro by treatment with penicillin (Holmes et al 1967a) L form are able to multiply in osmotically, favorable surrounding and because of their defective cell wall, are resistant to antibiotic that affect cell wall synthesis;

C) Non specific Genital infection (NSGI) in women:

Non specific genital infection (NSGI) is term that extends to the theoretically expected involvement of the female although it is less precise clinical entity than NGU in male.

Diagnosis may be unsatisfactorily based on exclusion of non gonococcal urethritis in the partner, symptoms of vaginal discharge or dysuria, inflammation of the cervix, vagina, urethra or greater vestibular glands (Bartholinitis).

Pelvic inflammatory disease (PID), involved fallopian tubes, can be found in some patient, considered to have NSGI and infertility may result from tubal involvement. Salpingitis especially, in young women etiological agent includes C.trachomatis, N.gonorrhoea and M. hominis.

**Aetiology:** C.trachomatis is responsible for as proportion of cases of NSGI in women C.trachomatis can be isolated from cervix have gonorrhoea due to contact of men having NGU. In one study, C trichomatis have been recovered in 57.5% from cervix.(Oriel and Ridgway, 1982).

Clinically no reliable criteria, for diagnosis do suggest, that edema, congestion,
mucopurulent discharge, are associated with Chlamydia infection of cervix (Rees et al 1977).

Although, some degree of inflammation reaction, is induces by Chlamydia trichomatis, it is unwise, however to imply that Chlamydia trichomatis can diagnosed from these appearance (Hare and Thin 1983).

In presence of coexisting infection with N gonorrhoea, the organism may be more readily detected by culture (Oriel and Ridway 1982)

Some evidence suggest, that Ureaplasma urealyticum and Mycoplasma hominis, may be implicated in a few case of pelvic inflammatory disease, although the precise extents of their involvement is not clear (McCormack et al 1973).

**Diagnosis of Non specific gonococcal infection:**

Diagnosis of NSGI is implicated that Neisseria gonorrhoea has been excluded as cause. Attempts should make to eradicate C.trichomatis.

Other organism, are like Bacteriodies fragilis may be pathogenic on occasion such as after surgical procedure and treatment of anaerobic organism with metronidiazole. Staphylococcus, streptococcus, Gram negative bacilli, anaerobes or facultative anaerobes, can become pathogenic may require, antibiotic treatment depending up on sensitivity.

In case of pelvic inflammatory disease (PID), a common aetiology practices to define the aetiology by isolating potential pathogens from the endocervix. The cervix and vagina of normal women may contain all the specimen of anaerobic
bacteria capable of causing pelvic disease.

Clinically in salpingitis, N.ganorrhoea was considered to be only pathogens, for which recovery from cervix is correlated with recovery from the fallopian tube, but Chlamydia trichomatis has been isolated from both sites (Cervix and fallopian tube) (Mardh et al 1977).

D) Urogenital Chlamydia infection in females:

The majority of the patient (60% or more) is asymptomatic. Symptoms are not diagnostic but are more or less to, that of gonococcal infection includes; vaginal discharge, dysuria and frequency (Urethral syndrome).

According to O.P.Arya and C.A.Hart 1998, 30% of C.trachomatis patents, do not have a concurrent infection.

On clinical examination are normal. Macrosopically 1/3 of patient shows cervicities i.e. mucopurulent discharge, oedema, and congestion of cervix or febrile cervix with contact bleeding. Cervical ectopy may become edematous and congested.

Mucopurulent discharge of endocervix is not always easy to recognized. Small portion of urethritis shows, urethral discharge and infection of Bartholin’s glands and their ducts passes mucous beds

Differential diagnosis:
Valuable, when patient has abnormal vaginal discharge, urethritis infection of
Bartholin glands or mucopurulent cervicitis.

Common differential conditions are gonococcal infection, bacterial vaginosis and yeast infection. A small portion of patient has cervicitis without any known cause, (Non-specific cervicitis).

**Diagnosis:**

In case of men, 30 or more number of polymorph nuclear, in gram stain smear, is diagnostic but not in similar in females, only in minority of patient detect. Swabs are taken to conform or exclude Chlamydia infection, preferable site of collection is cervix.

Other methods, ELISA, Nucleic acid amplification assay (LCR and PCR) and serological method are routinely use to diagnose Chlamydia infection.

**Choice of test:**

LCR and PCR are most sensitive and specific tests for Chlamydia trichomatis but expensive.

Culture is 100% specific but sensitive is no more the 75-80%, also laborious and expensive.

The most convent method is a Enzyme immuno assay (EIA) antigen detection, wildly available but less sensitive then 80%. Because of false positive results, conformation with other immunoflorescence tests requires. For Pharyngeal and rectal samples, EIA methods is unsuitable.
Recently researches have evaluated, first voided urine (FVU) and vulval and vaginal introits swab for Chlamydia antigen detection.

Nucleic acid amplification (LCR and PCR) have high sensitivity (90%) of these specimen. Multiplex PCR, aided at several infection agents, but may be more cost effective.

Time of Sampling and menstruation: The finding with regard between phase of the menstrual cycle and Chlamydia detection from cervix have been contradictory.

The mode of transmission is similar to that of N.ganorrhea. Cervical ectopy (often associated with use of oral contraceptive) facilities Chlamydia infection of the cervix.

E) Extra genital Chlamydia infection includes:

a) Chlamydia infection of eye: Occasionally as well as result of accidental infection (hand to eye) or infection directly during sexual activity may develop conjunctivitis (follicular conjunctivitis). Patient may asymptomatic. Conjunctivitis are self limiting but chronic recurrent infection result in conjunctivitis scarring and keratitis. On examination male reveals urithritis and female cervicitis, found or in both partner.

b) Chlamydia infection of Pharynx: It is uncommon in adult. No specific symptoms found. Pharyngeal swab detect C.trachomatis on selective basis. Infection is due to cross infection from infected mother during delivery though secretion.
c) Chlamydia Infection of the lungs: In, Immuno-compressed, pneumonia due to C.trachomatis is rare.

d) Chlamydia infection of the Rectum: Spread of infection is similar gonorrhea, in both sexes. In homosexual, proctitis occurs with C.trachomatis

Differential diagnosis: N.ganorrhoeae, Herpes simplex, Human papilloma virus, Treponema pallidum, Non syphilitic, Entamobe a histolytica, Campylobacter and Shigella spp; Trauma, ulcerative colitis.

Diagnosis: Culture is require. A gram stain show large number of polymorphonuclear leucocytes (PMN), search for sexually transmission before making, a diagnosis 'Non specific' prostatitis.

f) HIV and Chlamydia infection: There is no evidence that HIV infection alters the natural history of Chlamydia infection.

g) Chlamydia infection in infants and children: The incidence of Chlamydia conjunctivitis in the new born in the tropic is unknown. It may occur by direct infection though secretion of mother with Chlamydia infected during delivery. The risk to infant from mother is about 50%.

Incubation period ranges from 5-14 days may be unilateral or bilateral.
Infant develop sticky eyes conjunctiva are inflamed and lid so edematous, discharge may be thin watery or fucoids sometime mucopurulent.
The infection, may extent, to nasopharynx and finally, result in pneumonia
Differential diagnosis: The gonococcal infection is most important is ophthalmimia. Other bacteria include staphylococcus aureus, streptococcus pneumoniae, Escherichia coli, Hemophilus influenzae, H. manigitis, Klebsiella spp; Proteus and Pseudomonas.

Diagnosis: Gram stained smear of conjunctiva exudates show polymorphonuclear leucocytes (PMN), no gram negative bacilli. Some other bacteria may show it is non gonococcal and non Chlamydia. But if smear show PMN but no other bacteria, the cause may possibly be Chlamydia trachomatis.

With Geimsa stain, demonstration of inclusion of Chlamydia trachomatis, is possible but, can be conformed, by culture or antigen detection.

Vulvovaginitis and infection of other site: The immature vaginal epithelium, in Prepubescent girls are susceptible to infection. Vulva is often. Urethra and rectum occasional infected with Chlamydia infection.

Clinical feature are similar to gonococcal infection.

Differential diagnosis: Infection excluded is staphylococcus spp, streptococcus spp, H influenzae, Candida albicans, Thread worm (enterobius varmicularis)

Diagnosis: Chlamydia trachomatis is detected in cell culture (in case of medico-legal) from appropriate sample of vagina, urethra, rectum, nasopharynx and conjunctiva.
Isolation of Chlamydia trichomatis: In female specimen obtained, for testing after exposing the cervix with, bivalve speculum. The cervix should be cleaned with sterile gauze and sample obtained by rotating a swab in cervical canal at squamoculum junction, any inflamed area or where follicle is seen.

Tetracycline and erythromycin will reduced, the likehood of isolation the organism. With treatment of Cephalosporin or Penicillin negative, result in primary isolation.

LABORATORY DIAGNOSIS OF CHLAMYDIA:

Specimen should be sent to the laboratory, with in 2 hr, when possible, otherwise they should be taken, in Cryoprotectant transport medium (e.g. 2 SP or Sarbitol 10%) and store -70- *c before inoculating monolayer

A) Microscopy:

- Giemsa, Castanedas, Machiavello or Giemnez may be used to stain Chlamydia elementary bodies and inclusions.
- The DFA (Direct fluorescent antibody assay) uses fluoresceinisothiocynate-conjugated monoclonal antibodies to the outer membrane proteins or lipopolysaccharides of C. trachomatis. This is used to detect elementary bodies and also visualize Chlamydia inclusion. It also helps make an assessment of specimen adequacy.
- It is important that samples contain urethral or endocervical columnar cells and not exudates for detection of Chlamydia.
• Presence of 10 or more elementary bodies is considered as positive. DFA is a useful confirmatory test for samples found positive by antigen and DNA detection assays.

Leukocyte esterase assays: It detects the presence of an enzyme leukocyte esterase (LE) found in urine when leukocytes are present. The test can diagnose urethritis but cannot detect the causative organism present.

B) Culture: Cell culture lines like McCoy and Hela, treated with DEAE Dextran are used. The sample inoculums should be driven into cells by centrifugation up to 15,000g to get a good growth.

C) Detection of C. trachomatis antigen:
  • Enzyme immunoassay (EIA) can be used to detect Chlamydia genus specific lipo-polysaccharide (LPS) antigen in cervical and urethral swab specimens and urine samples from men.
  • Species-specific major outer membrane protein (MOMP) can also be used to detect Chlamydia and this test is more sensitive than the LPS antigen test.
  • Radio immunoblot or latex agglutination assays are simple test in which the swab sample is mixed with Chlamydia LPS antibodies coated on a membrane or card. The antigen-antibody complexes are observed visually as agglutination.

D) Detection of C. trachomatis RNA:
  • It is detected by hybridization with a chemiluminiscent DNA probe endocervical swab, urethral swab and urine samples from men can be detected.
• Specimen collection kits containing swabs and transport medium that lyse the organism and release RNA are provided with the kit.

E) Amplification and detection of C. trachomatis DNA or RNA:
• PCR and LCR are excellent tests for detection of C. trachomatis in endocervical, urethral and urine samples from both men and women.
• Transcription Mediated Amplification (TMA) of 16s ribosomal RNA is also used.

Mycoplasma & Ureaplasma:
The Mycoplasma and Ureaplasma are characterized, by totally devoid of cell wall and being bounded by plasma membrane only (Razin and Freundt 1984). Both are gram negative, stain poorly with aniline dye, best observed in Giemsa stained smear.

Grow well in cell free medium; require cholesterol and related sterol for growth. Basic medium required, peptone water, enriched beef heart infusion, broth containing horse serum and yeast extract with penicillin to inhibited bacterial growth. Grow under atmospheric condition, but in solid medium under CO2 enrich atmosphere.

Mycoplasma:
Mycoplasma associated in human infection, mostly are Mycoplasma hominis and mycoplasma genitalium.

In case of non gonococcal urethritis, in aetiology other then, Ureaplasma urealyticum is Mycoplasma. It is of low invasiveness causing recognizable infection in small proportion of colonized men (Taylor Robinson and
Mycoplasma metabolized arginine to form ammonia and ureaplasma produced urease, brake down urea to form ammonia.

Ureaplasma urealyticum are distinguished by their ability to hydrolyzed urea. In non gonococcal urethritis attention is centered on Ureaplasma urealyticum. Where evidence suggesting that these species causing recognizable infection.

Serologically, distinct serovas include, in Group A serovars are 2, 4, 5, 7, 8, 9, K2 and U24. In Group B serovas are 1, 3, and 6. Subdivision based on DNA hybridization.

Ureaplasma occur infection predominantly in mouth, respiratory tract and urogenital tract infection.

Taylor, Robinson and McCormack 1980, noted in half of the sexual transmitted disease Ureaplasma and Mycoplasma encountered on genital mucosa.

Mycoplasma and Ureaplasma are found in 2% in genitourinary tract of prepubertal boys and more often in prepubertal in girls 11% (Lee et al 1974).

Colonization is increase as result of sexual contacts i.e. increased with number of partner.

Taylor, Robinson and McCormack 1980 also, suggest prevalence of both organism diminish, after menopause.
REITER'S DISEASES: Although Hans described the diseases which now bears his named, in 1961. This disorder had been reported more than century earlier by stoll in 1776.

Reiter described the occurrence of urathritis, conductivities and arthritis in young man.

Following the first acute attack, the patient may appear to recover completely and have no further episodes. With each recurrence fever, features accompany the arthritis which may be the sole indication of the Reiter disease in the later stage. Reiter disease may lead to considerable pain and disability and anxiety.

Reiter's disease is one of the groups of the disorders known as the seronegative spondarthritis. Clinical and familiar inter-relationships can be demonstrated between numbers of this group, which must satisfy certain criteria. (Moll et al 1974)

1) Negative test for rheumatoid factor.
2) Absence of subcutaneous rheumatoid nodules.
3) Inflammatory peripheral artharitis
4) Radiological serco-illitis with or without classical ankylosing spondylitis
5) Evidence of clinical overlap between the members of the group.

Seven diseases fulfill these criteria and are called seronegative spondartharites are:

1) Idiopathic ankylosing spondylitis 2)Psoriatic arthariti 3)Reiter's diseases
PATHOGENESIS: Numerous diseases have been shown to be associated with one or other at the various loci within the major histocompatibility complex (MHC). This terms is described the Super gene or Cluster genes concerned with the expression of major histocompatibility antigen in the man. Ten loci have been identified namely HLA-A, HLA-C, HLA-B, C2, -C4A, -C4B, -BF, -DR and perhaps other 210H, and other related D, DR, MT etc. (Dawkins et al 1983)

It has been observed certain environmental agents; probably certain enteric bacteria are involved in pathogenesis, Klebsiella in pathogenesis of ankylosing spondylitis. Reiter disease is also followed by desentery caused by Shigella flexineri, rarely by Salmonella and the Camplylobacter.

The possible role of Chlamydia, in the aetiology of non gonococal urethritis about 30% of cases in men with Reiter’s disease has associated. Chlamydia infection is as detected by culture or serological findings. Titer of anti Chlamydia IgG is higher, in a patient with Reiter’s disease in men, with uncomplicated NGU (Kousa et al 1978, Keat et al 1980).

The infection with Yarsinia entrocolitia usually produces, a mild gestoindustrial illness complicated uncommonly by arthritis including the Sarco-illitis. About 90% patients had arthritis were found to have HLA B27 antigen. 15% developed diarrhoeeria but not arthritis (Aho et al 1974).

CLINICAL FEATURES: Reiter’s disease is a disorder of young adult at the age of onset usually between 18 to 50 years. Very rarely children under the age of 12 are involved. Males 50 times more frequently affected than females.
Incubation period is variable, but the disease usually manifested itself, 10 to 30 days after sexual intercourse or after the attack of dysentery.

The mode of onset is variable but the commonly urethritis, precedes the appearance of conjunctivitis, which is followed by arthritis. Any three features however, may appear initially.

The duration of the first attack of the Reiter's disease varies from between two weeks to several years. In general the (more than 70%) first episodes resolve within 12 weeks.

Half of the patient develops recurrence, the interval between the initial episode and the recurrence varying between 3 months and up to 30 years. Recurrence may be precipitate by urethritis, dysentery and surgical operations on the urinary track.

The inflammation of the genitourinary tract in male includes non gonococal urathritis (NGU) is a most commonly found about 70% cases is also with, the post dysentery form of disease, (Paroneu 1948, Csouka 1960).

In patient mixed gonococcal and non gonococcal urathritis a urethral discharge usually persists the following treatment of the gonorrhea with penicillin. In general inadequate treatment developed Reiter’s Syndrome.

The clinical features of the associated non-gonoccocal urethritis are identical to those of uncomplicated urethritis, in both conditions the severity various
considerably. If untreated, urethritis usually subsides after some two to four weeks but occasionally may persist for several months.

**Cystitis:** Cystitis often found mild and causing a little inconvenience may be associated with Reiter disease in 20% cases according to Csonka (1965). More severe form of cystitis is that of acute hemorrhagic cystitis. Acute prostatitis which followed by formation of prostatic abscess has been demonstrated in the Retier disease but is rare. Epidiymo-orchitis secondary to concomitant non gonococal occurs urethritis uncommonly. Renal parenchmal involvement in Reiter disease is a rarity.

In females, evidence of inflammation of the urinary tract and / or reproductive system is less obvious and less easily defined. As urethra of females is shorter relatively male uretharitis is rare. Non-specific inflammation is a sign may show cystitis, on occasional vaginitis or cervicitis (Oates and Young 1959)

The most common ocular manifestation of Reiter’s disease is conjunctivitis may be unilateral or more frequently bilateral. Severity of the conjunctivitis varies mild irritation to severe inflammation with sub conjunctival hemorrhage.

During the recurrent episode of the disease, a late course of initial acute episode, the complication is onset of pain and blurring vision of eye. On inspection eye is red because of there congestion of anterior ciliary blood vessels. In some case, oedema, pupil become small and reacting poor to light also observed.
**Arthritis:** During the acute initial episode of Reiter disease, some 95% of the patient develops the symptoms of joint involvement. In patient because of inflammations, joint swelling and majority develops arthritis.

**Mucous membrane:** Lesion of the mucous membrane of the buccal cavity is occurring in about 10% of the Reiters disease. On palate the lesions are whitish slightly elevated and surrounded by narrowed erythematous xone such lesion s are appear sharply from surrounding normal epithelium on tongue.

**Visceral lesions of Reiter disease:**
Clinical evidence of involve of organ of body.
Cardiovascular: Chest pain with pericardiatis, myocardiatis (less than 5%), aortitis.

Respiratory system: Pleurisy has been found 8%. Lymph nodes have been observed uncommonly in Reiter disease less than 1% of the cases.

Central nervous system (CNS): Various neurological abnormalities, meningitis, multiple peripheral neuropathies.

**LABORATORY TESTS IN RITER’S DISEASES:**
Heamatological investigation: In severe case, the result of these indicate mild normocytic, normochromic, anaemia,

In acute episode of the Reiter’s disease, there is polymorphnunuclear leukocyte in 20 to 30% cases.
Erythrocyte Sedimentation Rate (ESR):
ESR is elevated in more than 90% of the cases of acute Reiter's syndrome. Fall slowly, after first month and may remain elevated, in some patient up to the six to ten weeks of onset.

Rheumatoid Factor:
Incidance of Rheumatoid factor positive in about 4% Rheumatoid arthritis by latex agglutination test, usually negative (96%) in Reiter disease.

IgM agglutination may be negative, whereas IgG antibody may demonstrate in patient serum.

Anti Streptolysin "O" titer:
Result of these tests is within normal range.

Uric Acid:
Uric acid may be positive, in 2% of the cases of Reiter's disease, difficult to differentiate gout.
SYMPHILIS:
Syphilis one of the treponeamatoses is caused Treponema pallidum and a multisystem chronic infectious disease. It can be passed from pregnant women to her unborn child.

EPIDEMIOLOGY:
On global scale, infections with T.pallidum remain a major health problem especially in view of the facilitating role of ulcerative STI in HIV transmission. However as a cause of genital ulceration, syphilis in most developing countries is secondary to infection with H ducreyi (Goeman et al 1991)

Nearly 12 million new cases were occurred worldwide in 1995, among half of them being them south and south East Asia (WHO 1995). In Chandighad 1987 infection rate of syphilis 10.5 % in Orissa it was 16.27%

Causative organism: Treponemà pallidum the causative agent is thin spirochete with tapering ends, 6-15 μ in length and about 0.2 μ in width, which is below the resolution of ordinary microscope.

It has 8-20 rigid coils with distance of 1 μ between each coil. It recognized under dark ground illumination by its shape, corkscrew movement (forward and backward movement), rotating on its long axis and compression, explanation bending (flexuous) movements.

Electron microscope shows its structure comprises amorphous outer layer, outer membrane containing different proteins peptioglycan layer and axial filaments. In stain poorly with usual dyes, but in tissue section the organism can be
detected by silver impregnation or immunofluorescent techniques.

MODE OF TRANSMISSION:
Direct contact by sexual intercourse in adult is main mode of transmission. Needle stick or blood transfusion, kissing are other direct method without the appearance of primary lesion. Indirect method include drinking water from infected communal utensils etc, is rare.

PATHOLOGY AND IMMUNITY:
Polymorphonuclear leucocytes (PMN) are appear first, replace by T lymphocytes plasma cells and macrophage with 48 hr.

Basic pathology, perivasculcar in filtration with lymphocytes and plasma cells with endothelial proliferation is same in all syphilis stages. In primary and secondary syphilis, endothelial proliferation leads occlusion of small blood vessels result in impairment of blood supply and finally in necrosis and erosions.

Cellular and vacular response determines the indurations and skin rash may be macular, maculopapular, papular, papulosquamous.

Both humoral and cell mediated immunity in syphilis involve in various stages.

The patient has both anticardioliopin and anti-treponemal IgG and IgM antibodies in early syphilis, in later IgM antibody production may stop.

Healing of primary lesion, appear by fibroblast involves distraction treponomes
by T cell activated macrophages

Incubation period: It depends on 1) size of inoculation 2) the virulence of organism 3) response of the host and 4) recent medication. Usually it is 2-4 weeks range 10-90 days.

STAGES OF SYPHILIS:
There is a two broad stages early syphilis and the late syphilis. Early syphilis includes the primary, secondary and the early late syphilis, within two years of infection. The late syphilis includes the late latent syphilis, benign late syphilis, cardiovascular syphilis and the neurosyphilis.

Primary syphilis:
Almost 90% of the primary syphilis lesion occurs on genitalia and the rest are extra-genital. The primary lesion called chancre begins as a macule which progresses to be red papule. Papule becomes eroded resulting in shallow ulcer. The appearance of lesion is determined by the site of affected any treatment and the secondary infection.

A typical lesion is single, well defined, round or oval with red smooth surface, exuding clear serum and painless. The lesion is also indurate because of cellular infiltration of the base and surrounding tissue. The primary lesions is atypical may be painful because of secondary infection and/or non-indurate.

The genital lesions in females are often invisible if painless, patient may remain unaware. Common sites are labia (indurate scabbed lesion on the outer aspect, kissing chancer on the inner aspects) clitoris, urethral orifice, the wall of vagina
and the cervix. The infection of the cervix may manifest as local congestion indurations, ulceration or invisible endocervical lesion.

A cervical lesion remains unaware, hence a female patient speculum examination should be carried out and the secretion should be subjected for the dark field examination, if the clinical examination is clear.

Inguinal lymph nodes will be enlarged when lesion is on the external genital or lower 1/3 of vagina.

Up to about 10% of the primary lesions may occur on extra-genital sites, such as the lips, tongue, tonsils, nipples, fingers, anus, and rectum especially, in homosexual. Regional lymph nodes drain and enlarged.

**Differential diagnosis of primary syphilis includes:**

1) Chancroid is most common condition, in differential diagnosis but incubation period is much shorter. The lesions are multiple, painful, tender, the edges are undermined, surface grey and the lesions bleed easily, a bubo may form.

2) In case of ano-genital herpes, the patient shows vesicle, history of recurrent attack with or without prodromal symptoms.

3) Lesion of lymphogranuloma venereum are transient, disappear by time. Patient comes with inguinal lymph node enlargement and painful tenderness.

4) Granuloma inguinal, ulcer are well defined with raised edge. The pseudo bubo may be mistaken for lymph node enlargement.

Some time genital trauma, during the sexual intercourse involve fraenum and prepuce secondary infection, results in ulcer difficult to differentiate in ulcerated chancre
Differential diagnosis of extra genital primary lesions includes Bowen’s disease, fissure, hemorrhoids, herpes and Crohn’s disease, in case of anal lesions.

Bechcet’s syndrome, carcinoma, chellosis due to vitamin deficiency, herpes and tuberculosis are in case of lips and tongue.

Agranulocytosis diphtheria, infectious mononucleosis, Streptococal infection, vincent’s angina, in case of tonsils.

Carcinoma and paget’s disease in case of nipple. Filariases, Hodgkin’s disease, infectious mononucleosis, lymphatic leukaemia, lymphosarcoma, septic lesion, are in case of lymph nodes enlargement.

**Diagnosis:**

**Dark Field examination:**

In primary syphilis material from the lesion shows Treponema pallidum.

Ulcer is cleaned gently with gauze moistened with normal saline or water, after wearing rubber gloves. Clean serum from the ulcer is than allowed to ooze and any blood should be removed, with a swabs. Serum is collected on clean clover slip, which turns to place over the glass slide. Slide examined under dark field microscope immediately.

If the laboratory is at distant place from the clinic, then a clear serum is collected, in capillaries and the end of which are seal with wax.
A proper experience required to differentiate T. pallidum from the saprophytes of genital and oral spirochetes. Genital spirochetes are the T. rejignes, T. balanitidis, and T. gracilis, all are vary in size are thicker than, T. pallidum, have fewer coils and coarse movements.

The T. balanitidis has only 3 or 4 coils and has lashing eel like movement. The T. gracilis resembles to T. pallidum, but is thicker with more open coils and no characteristics movement. In case of oral lesions T. microdenticum and T. macrodentium have less regular coils, no rotation or bending movement.

Direct fluorescent microscope is better, than direct dark field microscopy. It is not yet possible to differentiate T. pertaue, T. endemium and T. carateum.

A negative dark field examination does not exclude syphilis; examination should be repeated for 2 to 3 days daily. A patient should not to use the shop only saline is use. To clear the secondary infection non-treonemicidal drug such as sulphonamides use. If in a clear serum T. pallidum is not observed than aspirated material from lymph nodes and serological test are used to diagnosis syphilis.

Lymph node puncture is generally carried out in a patient has a healed ulcer. Skin of the enlarge lymph node sterilized with an anti-septic and infiltrated with 1% lignocaine hydrochloride for local anesthesia. First of all with 2 ml. syringe 0.2 ml of saline is injected into the lymph node. Node is than massage gently and fluid is aspirated, expressed on the glass slide, examine under the dark field microscope for T. pallidum.
**Serological Test:** A positive serological test, in patient with genital ulcer, does not mean that ulcer is due to syphilis. Usually positive serological test requires time of about 3 to 5 weeks, after infection or 1 to 2 weeks following appearance of chancre so seronegative or seropositive may found. If baseline tests, first are negative, than it is necessary to repeat, monthly for 3 months before exclude the syphilis.

In fluorescent antibody absorption (FTA-ABS), usually the first become positive followed by RPR (or VDRL) and then the TPHA. Generally the facilities of FTA-ABS are found in larger city, in tropical countries, reliance will have to be placed on RPR (or VDRL) test will need to repeat to allow comparison with repetition of first, with baseline result or titer.

The chancre heals in 2 to 6 weeks with or without a thin atrophic scar. The lymphadenopathy takes longer to resolve. Many patients developed secondary syphilis within 8 weeks of the appearance of the primary lesion and about quarter enter the secondary stage while chancre is still present.

**Secondary syphilis:**
Secondary syphilis is characterized by wide spread manifestation of skin and mucus membrane, being the most obvious. However any organ of the body may involve.

The constitutional symptoms, like fever, malaise, headache, aches and pains, anorexia and the loss of weight, express in the individuals. 75% patient with secondary syphilis shows skin rashes known as syphilides may be generalize in early stage.
The initial rash is usually macular, on trunk and limb, in pink colour in patient with fair complexions. Papular syphilis develops after, macules in whole body. The rash is maculopapular and polymorphic with the papules at various stages. Rash usually, on the flanks, shoulder, back, arms, palms and soles.

Later on papulosquamous or psoriasiform syphilide developed, from the papule, more common on the face in dark skinned people. With increase in vascular damage, the centre of the papule becomes necrotic, resulting acneiform pustule, when dry form layer of thick crust on surface known as rupial syphilide.

A mucocuteneous junction, as well as where there is a warmth, moisture and friction lesion become confluent hypertrophic and flattened called condylomata which occur in parianal region, vulva and scrotum. Less frequent sites are angles of mouth, behind ears, axillae, and pendulous breasts, between toes and in the umbilicus.

Skin rash of the secondary syphilis is not itchy. Condylomata lata and psoriasiform or follicular suphilide may irritative. Skin rash of the secondary syphilis shows to evolve, may last for 4 to 12 weeks. Healing is take place without any scarring. Patchy loss of pigmentation at back of the neck may occur.

Lesion of hair follicles may results hair loss especially, side and back of the head. The bread and eyebrow may also be affected. The nails may affect as a result of onychia or paronychia.
Mucous membrane becomes eroded to form oval or circular shallow erosions, covered with grayish-white membrane and surrounded by red areola. Lips, cheeks, tongue, tonsils, pharynx, larynx, glands of penis, prepuce, vagina, cervix and anal canal are other mucous membrane sites affected.

Generalized enlargement of lymph nodes occurs in over half of the patient with secondary syphilis. The inguinal axillary, cervical and occipital nodes are commonly involved persists several weeks. They are painless, discrete, firm and freely mobile.

Bone pains, aching pains, worse at night, caused by perivascular infiltration of parietovertes. There is tender elevation of anterior surface of tibia. Radiological examination is usually normal.

**Neurological Manifestation:** 30% asymptomatic neurosyphilis with early syphilis. The cerebrospinal fluid may show, increases cells and proteins, but without neurological symptoms or signs.

Symptomatic neurosyphilis is very infrequent, in first two years of the infection. Meningitis is common may be focal or generalized.

Symptoms are persistent headache, neck stiffness, papilloedema, nausea, vomiting, clouding of consciousness. Basal meningitis may result in cranial-nerve palsies when 3rd or 6th nerve affected most frequently. 8th nerve involvement, result in vertigo tinnitus and deafness.
Differential diagnosis of secondary syphilis:

History of primary syphilis, presence of skin rushes and/or mucous membrane and generalized lymphadenopathy or evidence of primary chancre helps to exclude other conditions.

Skin lesion includes, macular lesions need to differentiate from illness of HIV infection, measles, rubella, drug rashes, infectious mononucleosis, leprosy, rose spots of typhoid are interfere.

Papular lesion will include consideration with HIV infection, drug rashes, chickenpox, scabies, lichen planus and leprosy. The papulosquamous lesion should be differentiating from psoriasis and seborrheic dermatitis. Pustular lesions may minic acne vulgaris, scabies, pyoderma, molluscum contagiosum and impetigo.

Anal condylomata lata may be mistaken for anal tags, anal wart and hemorrhoids.

Mucous membrane in mouth and throat may be differentiating from the aphthous ulcer, herpes labialis, tonsillitis, diphtheria, vincent's angina, thrush and Reiter's syndrome. The lesions of genital mucosa are indistinguish from genital herpes lichen planus, psoriasis, erythroplasia of Queyrat in females.

Diagnosis of secondary syphilis:

Demonstration of T.pallidum from moist lesion is such as, those on the genitalia and Condylomata lata. Dry papules will need abrading to obtain serum.

The demonstration of T.pallidum is, also from oral lesion.
Serological test for syphilis almost positive and the titers are high. Examination of CSF is mandatory, if there is indication of neurological involvement. CSF shows increase in cell (1000 leukocyte mm$^{-3}$) increase protein, elevated globulin level, VDRL and treponemal test positive from the blood and CSF.

A relapsing early syphilis is due to inadequate or no treatment may be different to reinfection. A primary chancre may reappear at original site. The mucocutaneous lesions are commonest.

**Latent syphilis:** Latent syphilis is diagnosed, when the blood test (specific syphilis test on repeated specimen) is positive, but patient has no symptoms and no abnormal physical sign, on through physical examination, radiological examination of the heart and aorta and examination of CSF are normal.

Latent syphilis found above age of 50 years. Usually such type of syphilis detected, during the routine screening e.g. STI clinics, antenatal screening and blood donors.

Latent syphilis divided in two (a) early latent stage to first 2 year of infection (b) late latent syphilis, is non-infectious sexually, but infection can transmitted by a pregnant women to her fetus, rarely by blood transfusion.

The differentiation from yaws is often impossible, a history or evidence of genital ulcer, positive treponemal serology in the partner, history of miscarriage in the female patient and a course of penicillin injection in the past, may suggest venereal syphilis.
**Benign late syphilis:**

The benign late syphilis becomes manifestation about 3-10 years, after the initial infection.

**Clinical Manifestation:** The majority the lesion are from the skin, subcutaneous tissue, mucus membrane, sub mucous tissue and bones.

In skin lesion, the cutaneous gummas may be occur in any part of the body, usually localized opposite to generalized rush of secondary syphilis. They may be modular or ulcerative, usually painless. They heal from the center and spread peripherally. The scars are like tissue paper, retain configuration of the original lesion.

Mucosal Lesions: Common sites are the mouth, palate, pharynx, nasal septum. The lesion originates in submucosa as painless swellings which gradually enlarge in size and encroach the mucous membrane and deeper structure including the bone. They subsequently may be eroding to form ulcer with wash leather slough in the bases. Depending upon, the site and the extent deformities include perforation of the hard palate, destruction of uvula, laryngeal stenosis nasal deformity.

The lesion of the tongue starts painless, single, circumscribed gumma or multiple gummata. The pain is due to with hot drink and vinegar. Impaired the blood supply, results in the white area of the necrosis (leucoplakia) and tongue become smooth and glazed because of the loss of papillae this condition is prior to malignant changes. Long term follow up necessary.
Bones, commonly involve are the clavicles sternum, cranial and facial bones and the tibia. Trauma may be predisposing factor.

Gumma of nasal bone, there is a pain, nasal discharge and perforation of the nasal septum. Also perforation of hard palate is result, in nasal voice. X-ray of the skull show a worm eaten appearance.

The lesion of the supporting structure muscle joints, tendon sheath and the bursae are rare.

Eye, iritis and choroidoretinitis may occur, result in the pain in eye, photophobia, and lachrymation and impaired of vision.

Gummatous involvement of the internal viscera is rare, but liver is the most common organ affected.

Testes gumma may be localized extended to subcutenous tissue and the scrotal skin causing ulceration Brain and spinal cord gumma results in neurosyphilis.

**Differential diagnosis**: Mucucutenous Lesion: Some common conditions include tuberculosis, leprosy, chronic eczema, psoriasis, dermetophytos, kaposis sarcoma varicose and tropical ulcers on the legs, rodent ulcer on face, lichen planus and thrush of the mouth.

Bone Gumma lesions may be needed to be differentiated from the pyogenic osteomyelitis, endemic syphilis, yaws, tuberculosis, leprosy, malignancy, myeloma and Peget’s disease.
Differential diagnosis of iritis and chroidoretinitis includes tuberculosis, leprosy, sarcoidosis, toxoplasmosis, diabetes and Reiter’s diseases.

Syphilis of liver may be differentiating from the alcoholic cirrhosis, hepatoma secondary carcinoma, amoebic abscess, hydatid cyst, carcinoma of the gall bladder.

**Diagnosis:** Diagnosis generally based on the clinical features. History of primary, secondary and / or syphilis elsewhere in the body may helpful. Non-treponemal serological test are positive. Dark field examination of *T.pallidium* is futile in late syphilis. A radiological examination of hart and aorta examination of CSF excluded cardiovascular syphilis and the neurosyphilis.

**Cardiovascular syphilis:** Cardiovascular syphilis involving thoracic aorta, aortic valves and coronary ostia, about 10-15 % of syphilis patient. Aorta involves in the early stage of infection, but the clinical condition is manifested, until 10-30 years later.

The clinical features: Uncomplicated aortitis is usually asymptomatic. Some may be complain of substernal ache. Radiological examination may show irregular outline and the calcification of the ascending aorta.

**Signs:** Impulse at the apex displaced downward and outwards, hammers pulse as a result of pulse increase, pressure diastolic murmur often louder at the left sterna edge and finally accompanied by systematic murmur at the base of heart.
Differential diagnosis: Aortic incompetence includes rheumatic heart disease, bacterial endocarditis, atherosclerosis of the aorta, hypertension, congenital bicuspid valve.

Diagnosis: Diagnosis of cardiovascular syphilis is made after all the information is available compromising history, clinical features including evidence of syphilis elsewhere in the body. Specific serological test for syphilis will be positive and non treponemal test, in most case.

Prognosis: Prognosis of cardiovascular syphilis is variable but is probably best in uncomplicated aortitis. Angina pectoris include, poor prognosis in some cases appropriate surgical intervention may be improve survival.

Neurosyphilis:
Neurosyphilis may be divided into four categories:
(1) Asymptomatic neurosyphilis (2) Meingovascular syphilis,
(3) Parenchymatous neurosyphilis (4) Gumm atous neurosyphilis.
The above four categories are not mutually exclusively and may the overlap they reflect predominant clinical and pathological involvement.

1) Asymptomatic syphilis: Most common form of neurosyphilis defines in section on secondary syphilis. 30% of the patient shows evidence of asymptomatic neurosyphilis in early stage will be continued in later stage. The prognosis of asymptomatic neurosyphilis with the adequate treatment is excellent.
2) **Meingovascular syphilis:** Both the meninges and blood vessels are involved. The clinical features may be predominantly meningeal or vascular blood vessels of the meninges, brain and spinal cord basically in all types of neurosyphilis.

Clinical manifestation occur early or late stages of the diseases. In brain, avascular occlusion may involve any arteries of the brain, middle cerebral and posterior cerebral and their branches. Symptoms are headache, dizziness, and transient paralysis of limb, or sudden onset of monoplagia, hemiplegia and other manifestation depends upon the site of the vascular accident.

Spinal Code: Maningomyelitis has a gradual onset and depending on the site affected may manifest, as a spastic weakness of the legs, impairment of bladder sphincter control, sensory changes.

**Differential diagnosis:** Cerebrovascular syphilis will need differentiated from cerebrovascular accident associated with hypertension or cerebral emboli. Spinal meningeal syphilis should be distinguished from the tuberculosis, disseminated sclerosis, motor neuronel disease, neoplasm and spinal vascular lesion.

**Diagnosis:** Specific blood test for syphilis (TPHA, FTA-Abs) are always and non treponemal test (RPR VDRL) almost always positive. CSF change (increase in lymphocyte and the protein, positive VDRL) are more predominant in cases of meningitis, than patient with vascular lesions in whom the CSF may even be normal in some cases.
Prognosis: With early adequate treatment, prognosis of maningo syphilis is good.

3) Parenchymatous neurosyphilis: Parenchymatous neurosyphilis include general paralysis (GPI) of the insne and tabes dorsalis. Both may occurs together (taboparesis)

Clinical features of GPI: More common in men becomes manifested about 10-20 years after infection.

Symptoms: Earlier symptoms include lack of concentration and intellect irritability, head ache, and impaired memory. After such type symptoms further there is a defective judgment, lack of insight sexual aberration, manic symptoms with paranoid features confusion, euphoria.

Sign: Flat expression, disorientation, small and irregular pupils with sluggish reaction to light, tremers of the lips, tongue, hands and respective speech.

Differential diagnosis of GPI: GPI will be differentiated from HIV infection, various psychiatric illnesses, chronic alcoholism, hepatic failure, drug addiction, cerebral atheroma, miliary tuberculosis, trepanosomiasis, and lead encephalopathy Parkinson’s diseases.

Diagnosis: Careful history and clinical examination may suggest the diagnosis. CSF of untreated patient will show increase in lymphocyte up to 100 mm⁻³, total protein above 50 mg100 ml⁻³ with excess of globulin and positive VDRL. Blood test for the syphilis will positive in all cases. X-ray chest show evidence of aortitis.
Prognosis: Prognosis patient may die within 5 years. Early adequate treatment may give good result. In late cases neurological change is permanent.

4) TABS DORZALIS:
The main features atrophy of posterior nerve roots in the lower thoracic, lumbosacral region and ascending degeneration of the posterior columns. Maningovascular changes involved in mid brain and ciliary ganglion the optic nerve sensory fiber of the trigeminal, other cranial nerves, 3rd, 4th, 6th and 8th.

Symptoms: The disease is most common in men, appears 10-30 years after infection. The onset is usually insidious. Stabbing pain of varying severity usually in the legs, occasionally, in arms lasting, for up too few minutes, and may repeat over the several days. The pain may free intervals of the weeks or months. Numbness of the feet, feeling of walking on a cotton wool, and a girdle sensation tight constricting bands around the trunk is other symptoms.

Sudden onset of the epigastric pain, anorexia, vomiting, cough, dyspnoea, bladder disturbances, difficulty in starting maturation, fecal, incontinence loss of testicular sensation, failing vision, loss of smell, loss of taste, deafness with / without vestibular.

Diagnosis: Detail history and through physical examination The CSF may be normal in an old bunt-out cases either cells or proteins or both may elevated in some cases Treaponemal test are always positive in blood and in the CSF.
Congenital syphilis:

**Epidemiology:** Although pregnancy does not have any effect on symptoms and sign of the syphilis. Syphilis remains an important cause of fetal and infant loss in same tropical countries, probably in sub Africa.

**Mode of transmission:** Syphilis is not an infectious sexually after the first two years of infection. An infected mother can transmit the infection to the fetus for five years possibly longer. The organisms may cross the placenta but pathological changes in infants do not take place until after four months of gestation, when the fetus become immunocompetent.

**The outcome of the pregnancy in untreated syphilis:** The stage of infection of syphilis in mother also important. Congenital is more likely to be more severe in early stage of the active syphilitic infection as larger number of treponemes circulates and cross the placenta, result in mid-trimester abortion or premature or form stillbirth, delivery of the syphilitic infant. Health baby may develop the symptom and sings of congenital syphilis after a week or a month. Health child remain normal without positive serological test.

**Pathology:** Treponeme have disseminated throughout the body. Parivascular infiltration, with lymphocyte and plasma cells, and obliterate endarteritis result in necrosis, fibrosis and the formation of gummas.

The placenta: There is infiltration of lymphocytes and plasma cell and endarteritis of the chronic villous vessels ultimately fibrosis result in bulky, firm and pale placenta. Nevertheless placenta may look completely normal.
**Syphilis of the fetus:** In 1/3 of the fetus may die in the utero, if mother with early syphilis not treated. Fetus show characteristic changes bellous, eruptions containing hemorrhagic fluid, a collapsed Skull, hepatosplenomegaly, diffused interstitial inflammation of the lung changes in the kidneys and the pancreas and abundance of treponemes in the skin lesions liver, spleen and lungs.

**Clinical Features:**
Clinically divide into the two infectious stages. (1) First 2 years life and (2) late infections stage including, the stigmata.

Early congestion syphilis is usually up to the 2-8 week after birth. The symptoms and sing do not usually appear until about 2-8 weeks after birth. Infant born with physical signs carry a poor prognosis.

General symptoms and the sign failure to their and gain height may be first sign to notice. Anemia and thrombocytopaenia associated with bleeding disorders. Pneumonia, gastroenteritis and possibly also pancreatitis may be observed.

**Skin lesions:** Bullous eruptions on the palms and the soles contain haemorrhagic fluid with many treponemes. Generalized rush: Macular, papular and the papulosqamous on the palms and sloes. Linear scar is on the angles of the mouth, around the nose and anus.

**Mucous membrane lesion:** In mouth, nose pharynx and the larynx mucous patches may found. Rhinitis may occur early, nasal discharge with treponosomes may be mucoid mucopurulent bloody. Later on septal perforation and saddle nose deformity.
**Lymph node:** Generalized lymphadenopathy, the lymph nodes being rubbery, discrete and not tender.

**Bone Lesion:** Osteochondritis and periostitis present at the birth but usually manifests after the some week. The lower bones chiefly affected are the distal ends of the radius and ulna and upper end of tibia. X-ray of long bones shows irregular thickening of the temporary zone of calcification in the distal metaphysic giving saw toothed appearance.

Other lesion includes hepatosplenomegaly jaundice (low serum protein and reverse albumin / globulin ratio), meningitis (convulsion / vomiting / neck rigidity / CSF shows increase cells) and positive VDRL. The nephrotic syndrome is due to the deposition of immuno complex in glomeruli (oedema ascetics).

Differential diagnosis of the early congenitalsyphilis: Hepatosplenomegaly lymphadenopathy, intrauterine growth retardation may be due to other intra utrine infection such as rubella, cytomegalovirus infection and toxoplasmosis.

**Diagnosis of early congenital syphilis:** In serum Treponema pallidium are observed, in early congenital syphilis and also from the skin and mucous membrane and nasal discharge. Radiological examination of the long bones may indicate osteochondritis and periostitis and repeated after two to three week for the progress for more characterize the picture.
A maternal syphilis, transfer of the infant even if clinically normal must be following up positive. The serological test may be positive due to passive maternal IgG antibody with in 4 fold titer should be fall within 2 months after or non-treponemal test (RPR, VDRL) should be negative within 3 to 6 months.

The treponemal test is specially TPHA remain weekly positive up to the 15 months. After 3 weeks 6, 9, 12 and 24 week serological should be carried out. The child develop and old man

Rising titer of VDRL and RPR in infant than in mother or the test become positive, after having been negative. The IgM antibody positive i.e. FTA-Abs (IgM) in the absence of placenta leakage may indicate the congenital syphilis

**Late congenital syphilis:** There is very similarity between the late congenital and late acquired syphilis. Late latent congenital syphilis over half of the patient diagnosed with positive serology as the only abnormal findings.

**Benign Late congenital syphilis:** Lesion involved the skin, mucous membrane, subcutaneous and submucous tissue, viscera, bones and joints are rare before the age of 5 years. The septal perforation result in a saddle nose. Perforation of the palate is resulting in nasal voice.

Clutton's joins, Painless bilateral hydrarthrosis of the knees appearing around at the age of 10 years.

**Interstitial karatitis:** It is the common manifestation at late congenital syphilis onset is between 4 to 40 years. The symptoms are sepraorbital pain and
photophobia lachrymation and blurred vision. The prognosis is good in well managed cases but the worse if the choroidoretinitis is also present.

**Cardiovascular syphilis:** Very rare in congenital syphilis.

**Neurosyphilis:** It may be asymptomatic or symptomatic. The symptomatic involve meningeal cranial nerve palsies vascular (hemiplegia monoplegia) parenchymatous. Urinary incontinences or visual impairment may be first symptoms of tabes dorzalis.

**8th nerve deafness:** The age of onset from 3 to 50 years, usually 6 to 15 years. The CSF may be normal. Prognosis is unpredictable even with treatment. Paroxysmal cold haemoglobinuria is also found in benign late syphilis.

**Differential diagnosis of late congenital syphilis:** Manifestation of late congenital syphilis is from among the various conditions. Deafness may be due to non syphilitic middle ear disease drug ototoxicity, tumor and Meniere's disease.

**Diagnosis of late congenital syphilis:** The diagnosis is usually base on history clinical futures including stigmata and blood tests CSF should be examine if neurological involvement is a possibility.

**LABORATORY DIAGNOSIS:**

**Microscopy:** Diagnosis by microscopy can be done in primary, seconda ry stages and in case of congenital syphilis with superficial lesions.
Collection of sample: The lesion is cleaned with gauze soaked in warm saline and margins gently scraped so that the superficial epithelium is abraded. Gentle pressure is applied to the base of the lesion and the serum that exudates is collected preventing admixture with blood.

Wet film is prepared with the exudates and after applying thin cover slips, examine under dark grand microscope. T. pallidum is identified by it's slender spiral structure and slow movement.

Direct fluorescent antibody test:
Smears of the exudates are fixed with acetone and the test is done using fluorescent tagged anti palladium antiserum.

Serological tests for syphilis are classified as follows:
(A) Non specific (Reagin antibody) tests using the cardiolipin antigen (standard tests for syphilis).
1) Wasserman complement fixation test.
2) Kahn flocculation test.
3) Venereal disease research laboratory (VDRL) test
4) Rapid plasma Reagin (RRR) test
5) Automated RPR test
6) VDRL-ELISA test
7) TRUST-Tellurite Red Unheated Serum Test

(B) Group specific test using cultivable treponemal (Reiter strain) antigen.
1) Reiter protein CF (RPCF) test
Specific tests using pathogenic treponemes (T. Pallidum)

1) Treponema pallidum immobilization test (TPI)
2) Fluorescent Treponemal antibody Absorption (FTA-ABS) test
3) Treponemal pallidum Hemagglutination Assay (TPHA)
4) Treponemal pallidum Enzyme Immunoassay (TP-EIA)
5) TPPA (T. pallidum particle agglutination assay)
6) IC (Immunochromatography) rapid test
7) Latex agglutination syphilis fast test.

Frequency of reactive serological tests in untreated syphilis (Percentage)

<table>
<thead>
<tr>
<th>Stage</th>
<th>VDRL/PPR</th>
<th>FTA-ABS</th>
<th>TPHA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary</td>
<td>70-80</td>
<td>85-100</td>
<td>65-85</td>
</tr>
<tr>
<td>Secondary</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Latent/late</td>
<td>60-70</td>
<td>95-100</td>
<td>95-100</td>
</tr>
</tbody>
</table>

- Quantitative tests are useful in monitoring the patient's response to treatment.
- Reagin tests are preferred because they usually become negative following treatment.
- Specific treponemal following test are not little value as indicators of clinical cure, as they remain positive in spite of treatment.
- TPHA and FTA-ABS are helpful in excluding or confirming the diagnosis of syphilis, and for identifying BFP reactions.
- A negative TPHA excludes the diagnosis of syphilis, part or present, except in very early stages.
• Detection of specific IgM antibody may be helpful in some situations. Begin the initial type of antibody to appear, IgM is detectable by the second week of infection. IgM antibody production ceases soon after the elimination of infection by treatment. Persistence of IgM antibody indicates continuing active disease and need for treatment.

**VDRL:**
In the VDRL test, heat inactivated serum (to destroy complement) is reacted with freshly prepared cardiolipin-cholesterol-lecithin antigen and the resulting flocculation. (Suspended antigen-antibody complex) is read microscopically using a 10X objective and 10X eyepiece. Reactive tests are quantitated to obtain the antibody titre.

**RPR:**
In the RPR test, the cardiolipin-cholesterol lecithin antigen has choline chloride added to it which removes the need for heat-inactivation of samples and enables plasma as well as serum to be used in the test. It also enhances the reactivity of the antigen. The antigen is supplied in a ready to use stabilized form which can be kept for up 6 months when stored at 4-10°C. Carbon is also added to the antigen, enabling test reactivities to be read macroscopically. In the RPR, the patient's serum or plasma is spread within a marked circular area on a plastic coated card, antigen is added, and the mixture rotated at 10 rpm for 8 minutes using a mechanical rotor. Reactive tests are quantitated to obtain the antibody titre.

**Trust:** This test is performed and read in a similar way to the RPR test except red particles instead of black carbon particles are used to visualize reactivities. The
TRUST antigen can be stored at room temperature for approximately 6 months. False reactions and difficulties in the interpretation of cardiolipin tests for syphilis are due to:

1) Biological false positive, reducing the specificity of tests.
2) Prozone reactions.
3) Tests being performed incorrectly
4) Lack of and unexpected serological responses in those infected with HIV.

Specific Treponemal tests: A specific treponemal test is required to confirm treponemal infection in a person with a reactive non-specific cardiolipin test or in late stage syphilis when a cardiolipin test may be non-reactive. Unlike cardiolipin antibody tests, specific treponemal tests do not produce BFP. Because T. pallidium IgG antibody can persist in the serum for long periods, specific tests can remain positive for many years, even after successful treatment.

TPHA:
The IgM binding capacity of TPHA reagents varies and reactions can be detected around the fourth week but may take longer. Titres tend to be low in primary syphilis (80-320) but rise sharply in the secondary stage (5120 or greater). Although a drop in antibody occurs in latent and late syphilis, a positive TPHA test may still be found 20-30 years after treatment. A positive TPHA indicates either present or past infection.

In the TPHA, patient's diluted serum samples are mixed in the wells of a microtitration plate with sheep or avian read cells coated (Sensitized) with
T. pallidum antigen (Nichol’s strain). If antibody is present the sensitized cells are agglutinated and they settle in a characteristic mat pattern in the bottom of the well. Unagglutinated cells in a negative test form a bottom or smooth ring at the bottom of the well.

Non specific cross-reacting antibodies which may be in the patient’s serum due to the presence of commensal treponemes, are removed by an extract (from the non-pathogenic Retier’s treponema) contained in the diluents used in the test. False positive reactions can occur in connective tissue disorders and lepromatous leprosy.

**Interpretation and the use of serological test for syphilis:** A positive serological test for syphilis may be due to clerical or technical error a biological false positive reaction or post or present infection. In absence of any evidence of syphilis, a positive test must always be repeated.

**Non-treponemal test:** Non treponemal test (RPR or VDRL) are usually used as a screening test in case of reactive should be repeated and confirmed by treponemal test if later is negative then non-treponemal test is to be biological false positive. A positive treponemal test confirmed treponemal infection which may be treated or untreated syphilis or non venereal treponemotosis such as yaws.

A non-treponemal test becomes positive, about 3-5 weeks after the infection. The titer peaks when secondary syphilis makes its appearance and persists at the level for about 2 years and gradually declined even without treatment.
The non-treponemal test in large majority of the patients become negative with 6 months after the adequate therapy of primary syphilis and in 12-18 months the treatment of secondary syphilis.

In some instant non-treponemal test in low titer (1:8 or below) are found. In such cases careful history and the thorough clinical examination and also repetition of test after few weeks are essential. If the repeat test is negative or shows a falling titer syphilis is unlikely and BFP reaction should be suspected. If there is no change in titer, yaws in childhood or chronic BFP reaction.

**Biological False Positive (BFP) reaction:** A reactive non-treponemal test in the serum of the patient who has never had a treponemal infection and has a negative treponemal test is termed a BFP reaction. The titer of BFP reaction is usually not higher than 1:8. BFP reaction may be acute or chronic.

Acute BFP reaction includes infective hepatitis infectious mononucleosis, viral pneumonia, chicken pox, measles, immunization, lymphogranuloma veneram, malaria, tryponzomasis, pregnancy etc.

The chronic BFP reaction may be encountered in autoimmune diseases as systemic lupus erythromiaosis, thyroiditis, and rheumatoid arthritis, chronic infection such as tuberculosis and lepromatus leprosy.

When BFP continues the patient should be fully assessed and advice for the further investigation and follow-up.
Treponemal Test: These tests (FTA-Abs and TPHA) are used mainly as a confirmatory test or to distinguish true non-treponemal reactivity from BFP and also to obtain serological evidence in case of late stage syphilis, when non-treponemal test may be negative.

The fluorescent antibody absorption tests an indirect immunoflorscent antibody. In genital ulcer patient is being first test to become positive in primary syphilis. Also FTA-Abs Dr test is a double staining procedure for the FTA-Abs test designated as used.

TPHA, Treponema pallidum haemaglutination test: The sensitivity of test is superior the VDRL and FTA Abs except in primary syphilis. It is more specific and sensitive than the VDRL.

The TPHA usually fall significantly and fairly rapidly after treatment of secondary syphilis. A significant rise in TPHA titer may be indicating re-infection and in relapsing a rise in TPHA titer may precede that of VDRL.

SUMMARY:
As far as in diagnosis of syphilis dark field microscopy is a most significant as spirochates are demonstrated from the lesions. Florescent microscopy gives better results. Lymph nodes aspiration also helps in diagnosis of primary syphilis.

In secondary syphilis from skin lesions T.pallidum can be demonstrated. In serological test, indirect diagnosis includes, TPHA, VDRL, RPR test are carried out. RPR is most universally accepted as it is easy to perform, require less
equipment and because of charcol added to antigen, enabling test reactivities to read microscopically and also CSF examination is significant test.

In benign late Syphilis, combination of clinical examination, demonstration of Treponema pallidum, serological test i.e. non-treponemal test, CSF examination and other supportive test e.g. Radiological investigation are important for the diagnosis.

In cardiovascular as well as neurosyphilis careful clinical examination history non-treponemical serological test and CSF examination are significant.

TREATMENT: Benzathine pencillin G 2-4 million units, IM single dose in primary, secondary or early latest syphilis. In case of latent syphilis, 3 doses of 2-4 million units IM each at one week intervals.
BACTERIAL VAGINOSIS:

Bacterial vaginosis is very common cause of vaginal discharge the condition encounter frequently in STI clinics and commonly it concurrent with Chlamydia, gonococcal and trichomonal infection. Although sexually active women are predominantly affected but there is no evidence that Bacterial vaginosis is sexually acquire. Bacterial vaginosis is formerly known as, non specific vaginitis.

Gardner described in 1955, that Bacterial vaginosis is polymicrobial infection in which decrease in vaginal acidity and lactobacilli is accompanied by increase of 100 fold or more in the concentration of other micro organism Gardenerall vaginalis, Bacteriods bovins and other bacteroid spp.

Gardner and Dukes had said in 1957 that 90% cases of bacretial vaginosis are caused by gram negative pleomorphic organism, Gardenerella vaginalis.

By Catlin in 1992 the characteristic of G.vaginalis and its role in bacterial vaginosis have been described fully.

G. vaginalis is associated with bacterial vaginosis but can also isolate from women without any signs or symptoms of infection. Because of this routine culture of vaginal specimens for G. vaginalis should be discouraged.

ETIOLOGY:

The mixture of organism associated with Bacterial vaginosis includes facultative anaerobic bacteria such as, Bacteriodes, Prevotella, Porplyromonas, Peptostreptococcus and Mobilincus spp, Gardenerella vaginalis and Mycoplasma hominis. Many of these bacteria are found in the normal vagina of asymptomatic
women but in Bacterial vaginosis their number increase by 1000fold.

There is marked reduction in the number of the normally predominant hydrogen peroxide producing lactobacilli and production of amines and ammonia by other organism resulting in higher pH of the vagina (Normal 3.5-4.5). This allows the anaerobes and other organism to increase 1000 fold.

The number of organisms is decrease or increase intermittently in some individual, perhaps due to hormonal changes. Some organism may produce cytotoxins which may damage vaginal epithelium resulting in vaginal discharge. The precise mechanism of the formation of clue cells (which provide a clue to the diagnosis) is not known. The exact role of M.hominis apart from that of colonizer in a favorable environment is also unclear.

The vaginal infections are limited to the production of a discharge with an offensive odor, presumably resulting from the breakdown of proteinous products of parasitized degenerating squamous epithelial cells that are sloughed in to the vaginal secretion. Segmented neutrophilis are not a prominent component of the vaginal secretion suggesting that organisms do not invade the subepithelial tissue. For this reason the condition is called "Vaginosis" rather than "vaginitis."

CLINICAL SYMPTOMS:
Foul smelling vaginal discharge:
Vaginal exudates characteristic of BV is gray or grayish white in colour homogenous and thin with the consistency like heavy cream or a thin flour paste. It has tendency to adhere to the vaginal wall like a thin film.
**Complication:** Bacterial vaginosis has been associated with serious complications such as, risk of chorioamnionitis, premature rupture of membrane, pre-term labor, late miscarriage, postpartum endometriosis, pelvic infection and infective complication gynecological surgery.

Controlled trials are needed to prove that treatment of bacterial vaginosis during pregnancy will prevent the related complication. A definitive outcome will also resolve the uncertainty about the treatment of asymptomatic patients. Majority of the patient will deliver at term even without treatment and there are several other causes of preterm birth which will need to be sorted out.

In fact, that in mostly treated patients the condition women, with history of second trimester fetal loss, if found to have Bacterial vaginosis should be treated with oral matronidiazole, early in the second trimester. It is certainly prudent to screen the BV and treat those testing positive prior to termination.

Some males may develop balanitis. Their treatment has not proved to be of any benefit with regard to recurrence in the female partner.

The condition usually resolves within one week, after treatment. However recurrence is common almost, 50% relapse within 6 months. The reason may serve as reservoir of infection.

**Clinical Diagnosis: Diagnosis by Amsel's Criteria**

1. A homologous vaginal discharge which usually adheres to the vaginal wall and frequently is noted at the introitus.
2. Elevated vaginal PH (above 4.5-4.7).

3. Characteristic odor after addition of 10% KOH Solution to vaginal secretion(The Whiff Test)

4. Presence of clue cells (Epithelial cell with adherence of bacteria mostly Gardenerella vaginosis to the cell surface and its margins. The presence of three out of four clinical observation is sufficient to establish the diagnosis

Recent studies on the morphology of gram stained film from vaginal fluid showed that there is an inverse relationship between quantity of large gram positive rods (Lactobacilli morphotypes) and the small gram negative rods (Gardenerella morphotypes), when the lactobacillus morphotypes is absent or present in low number, the Gardenerella morphotypes may be number, but is not used as a basis for diagnosis.

When other forms of gram rods (possibly Bacteriod spp.) gram negative or gram variable curved rods (possibly the motile Mobiluncus) are present then the diagnosis is consistent with bacterial vaginosis (Spiegel et al 1983 a, b)

An aerobic bacteria are called anaerobic vibrios as high concentration of anaerobic bacteria in bacterial vaginosis,(Blckwell et al 1983 spiegel et al 1983) now designated mobiles rod shaped bacteria more resist the alkaline solution.

Other anaerobic nonhemolytic peptostreptococci and Bacterioids spp other than B fragilis e. g B bivius have also been found in high concentration. Other aerobes, such as alpha, beta, hemolytic streptococci (group B and occasionally coliform), Mycoplasma and Ureaplasma urealyticum are also often present.
Diagnosis by Nugent’s Criteria

1 Roll the swabs of vaginal discharge over the surface of a slide.
2 Allow the smear to air dry, fix with methanol and gram stain.
3 Assign scores,

Scoring vaginal gram stains for bacterial vaginosis.

Table. Scoring vaginal gram stains for bacterial vaginosis

<table>
<thead>
<tr>
<th>Organism Morphotype</th>
<th>Number / Oil Immersion field</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactobacillus like (parallel-sided, gram positive rods)</td>
<td>&gt; 30</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>5-30</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1-4</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>&lt; 1</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Mobiluncus like (curved, gram negative rods)</td>
<td>&gt; 5</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>&lt; 1-4</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Gardnerella Bacteroids like (tiny, gram variable, coccobacilli and rounded, pleomorphic, gram negative rods with vacuole)</td>
<td>&gt; 30</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>5-30</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>1-4</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>&lt; 1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Total Score Interpretation

0-3 Normal
4-6 Intermediate, repeat test later
7-10 Bacterial vaginosis
Gardenerella vaginalis:
Gram negative to gram variable, vaginitis leads to complication of pregnancy, preterm toxin produce endomateriosis.

Initially Gardenerella are called as Heamophilus aginalis, but not fit in this, then called as, Chlamydia vaginalis, but due to corynform appearance, it was also unsuccessful, put in genus Gardenerella is named.

It’s features, are 1) Gram negative to variable rod shaped bacilli 2) Laminated cell wall in vaginal epithelium 3) Non capsulated and non motile 4) Facultative anaerobes 5) Oxidase negative 6) Acetic acid produce, by fermentation 7) Ferment fructose galactose, glucose, manol, ribose, sucrose, starch, but variable in lactose, sucrose, and xylose

Isolation of G. vaginalis:
The growth requirements of G vaginalis include fine B vitamins (riboflavin, thiamine, niacin, folic acid and biotin) various purines, pyrimidines inorganic salts, trace metals, some amino acid and fermentable carbohydrate. Growth of G vaginalis enhanced in an atmosphere with increased carbon dioxide and its grow well under anaerobic condition.

The most successful media are those using bloods as an enrichment based on the original medium described by Casman. The use of human blood agar gives good growth of G. vaginalis and a characteristic beta heamolysis.

Two layer plates give the best results, with columbia agar alone as a base and a top layer of Columbia agar containing 5% human blood.
The beta hemolysis is less clear when single layer plates of 5% human blood are used. Tween 80 and 1% proteose peptone are sometimes added to enhance the hemolysis but this shortens the life of the medium as the tween 80 causes lysis of blood on storage.

Rabbit blood can be used as an alternative. The use of sheep or horse blood results in poor growth and a loss of characteristic hemolysis.

Peptone starch dextrose (PDS) agar is another medium. Neither blood nor serum is needed, for PSD agar but as it is time consuming to prepare and interpretation of colonial morphology needs experience, it is generally unsuitable for most clinical laboratory.

Selective agents can be used like colistin 10mg/lit, nalidixic acid 15mg/lit and amphotericin 2mg/lit or alternatively gentamycin 4mg/lit. Nalidixic acid 30 mg/lit and amphotericin 2mg/lit are combination that have been used successfully with human blood agar.

G. vaginalis is associated with bacterial vaginosis but can also be isolated from women without any signs or symptoms of infection. Because of this routine culture of vaginal specimen for G. vaginalis should be discouraged.

**TREATMENT:** Clinical evidence shows, that metronidazole is effective in short term treatment of bacterial vaginosis. It is relatively effective an obligatory anaerobes other than on facultative organism G. vaginalis The treatment regimen based on 500 mg metronidazole twice daily for 7 days.
Treatment in pregnancy: Recently a data analysis confirmed the safety of metronidazole in pregnancy including in the first trimester. However manufactures advise avoidance of high dose regimens in pregnancy and some specialists avoid the oral regimens in the first trimester. A low dose regimen is metronidazole 200 mg orally three times daily for 7 days.

Clinamycin is not known to be harmful in pregnancy 300 mg orally twice daily for 7 days.
CHANCROID:
Chancroid is an STI characterized by painful genital ulceration and suppurative inguinal buboes. It is prominent cause of genital ulceration in many tropical areas. The facilitating effect of genital ulcer in the transmission of HIV infection has added urgency to the control of Chancroid.

EPIDEMIOLOGY:
Chancroid is a common in Africa, Asia, Central and South America Promiscuity prostitution and the poor hygiene contribut to its spread.

ETIOLOGY:
The causativ organism haemophilus ducrey is a short non-motile, non-sporing, gram negative, facultative anaerobic bacterium requiring haemin for growth.

TRANSMISSION:
Transmission is by sexual contact. The pathogenesis is not a clear. After penetrating the skin through a minor abration, organism invades the epithelial cells before causing cytopathic effect.

CLINICAL FEATURES:
The incubation period is short usually not larger than 10 days. The lesion begun as a tender papule develops into pustute breakdown to for and ulcer. The ulcers are non-indurated shallow with undermine ragged edges. Ulcer is very painfull, tender and the bleed easily. However, the pain may be absent in some, especially in females, who may be unaware of the presence of ulcers. The labia are the usual sites, then other area in case of female. Half of the patient is the inguinal lymph node became enlarge on one or both sides within 1 week. Extra genital lesions as
a result of direct inoculation systemic spread do not occur. HIV-infected patient with Chancroid may develop extensive genital ulceration.

DIFFERENTIAL DIAGNOSIS:
Chancroid lesion have to differentiated from the other cause of genital ulceration i.e. syphilis, genital herpis and septic adenitis. Careful history and physical examination may help to make a fairly accurate diagnosis. In some cases the sensitivity and specificity of the clinical diagnosis of genital ulcer disease are rather poor.

DIAGNOSIS:
Direct smear: By direct smear demonstration of H.ducreyi from the ulcerative material is unreliable. Both sensitivity and the specificity of the gram stained smear are less than 50%.

Culture: Culture (of the material from the ulcer or bubo aspirated) is essential for a definative diagnosis. Culture media are expenses and have limited shelf life and therefore may not be available at the site. Amie's transport medium may maintain the viability of H ducreyi for upto 3 days at 4°C.

PCR: DNA amplification using a PCR is expected to be highly sensitive and specific test. A multiplex PCR assay is shown to be more sensitive than the standard diagnostic test for the detection of this organism.

Serological Test: There is no widely available serological test for the diagnosis of Chancroid. Enzyme Immunoassay for the detection of circulating anti H ducreyi
serum antibody has been used for epidermiological studies. Histology of the ulcer biopsy material may be used as aid of the diagnosis.

**PROGNOSIS:**
The prognosis of the Chancroid is excellent with appropriate treatment. However repeated infections may occur in subsequent exposure to the organism.

**TREATMENT:**
Considerable geographical variation in the sensitivity of H. ducreyi to antimicrobials is exist. Many strains are β-lactamase producer. Plasmid mediated resistant to sulphamethizide, ampicillin, streptomycin, chloramphenicol and tetracyclin has been observed. Azythromycin, ciprofloxacin, ofloxacin, ceftriaxome and septinomycin are effective antibiotic treatment.
HUMAN IMMUNO DEFICIENCY VIRUS AND ACQUIRED IMMUNODEFICIENCY SYNDROME (AIDS):

Human Immunodeficiency Virus (HIV) infection and acquired immunodeficiency Syndrome (AIDS) follow the path of other infectious disease, by being more prevalent in the tropics, then in other part of the world. The reason includes, low income, low education, poor hygiene and lack of resources. These factors can potentially explain the rapidly spreading epidemic of HIV and AIDS in sub Saharan Africa, and south East Asia.

Clinical manifestation of HIV infection, in the tropics may differ, from other part of the world, due to difference, in endemic or environmental pathogenic, genetic susceptibility and nutritional and immunological status.

EPIDEMIOLOGY:

With HIV, endemic in the developed world, where HIV infection is, predominantly a disease, of homosexual and bisexual men, and intravenous drug users, mainly the infection in tropics occurs, among heterosexuals. Intravenous drug users are also risk group, in many countries, in the tropics such as, Thailand, India and Myanmar.

<table>
<thead>
<tr>
<th>Wave</th>
<th>Starting Year</th>
<th>Population group</th>
<th>Size of the population</th>
<th>Estimated number of HIV infection 1997</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1984</td>
<td>Male homo/bisexuals</td>
<td>100,000</td>
<td>10,000 (10%)</td>
</tr>
<tr>
<td>2</td>
<td>1988</td>
<td>IVDU</td>
<td>100,000</td>
<td>40,000 (40%)</td>
</tr>
<tr>
<td>3</td>
<td>1989</td>
<td>Female sex workers</td>
<td>200,000</td>
<td>56,000 (28%)</td>
</tr>
</tbody>
</table>
Table: Five waves of HIV epidemic in Thailand and estimated numbers with HIV infection individual risk groups.

Because of high risk sexual behavior, heterosexual is largest risk group.

In Thailand, the HIV endemic began in 1984, in foreign and Thai homo/bi sexual. The second, wave were starts, among IUCDs in 1988.

This was followed, by the third wave, in 1889 among female's sex workers.

Client of sex worker is, in 1990 as, the fourth wave.

In 1991, fifth wave occurred, in house wife and new born.

Promiscuous hetero sexual behavior, poses greatest threat of large epidemics simply because of heterosexual, are potentially the largest group, in the population.

The spread of human deficiency virus (HIV) infection has posed a serious challenge worldwide. In India when the first case of AIDS was reported in 1986, there were around 20,000 reported case of worldwide. At that time HIV/AIDS
was gradually being recognized as an emerging public health problem. Since then in last two decade, the epidemic has grown into a public health problem and unprecedented magnitude and has become a threat to human survival and development.

Globally the estimated number of people living with HIV/AIDS is 39.5 million (December, 2006) of which 17.7 million are women. Children under 15 years of age contributed 2.3 million case during 2006 4.3 million people were newly infected. There were 2.9 million AIDS deaths in 2006 and in 2007 around 3,70,000. Children’s under 14 years become victim of HIV infection.

Presently the main global trend is that new HIV infections are heavily concentrated among young adult (15-24 years) and infection in women rising substantially.

Sub-urban Africa continues to remain the worst affected region accounting for 63% of global HIV burden and 72% of total HIV death globally. Unlike women in most regions, in the world, African women are considerably more, likely 1.4 times to be infected with HIV, than men.

In India it was initially thought to be this problem of few large cities or urban agglomerate, such as Chennai and Mumbai. But there is increasing evidence that the epidemic is now growing in other area as well.

PATHOGENESIS:
Although, some still feel that, HIV may be a mere passenger in AIDS, most now accept that, HIV is the primary infection against, leading to AIDS.
The virus is abundant in blood, semen and the vaginal secretion, which makes the exchange of, or the contamination with, blood and genital fluid, are the most important routes of HIV infection.

Among infection of the cells, CD4 +T helper is most important, after entry. In CD4 T helper cell, they may remain, dormant, for a reliable time or also replicate constantly, in the body particularly, within the lymph node.

The immune system, also stimulate constantly, by the invader and produce, the humoral or cell-mediated immune response to the virus.

Finally, the progressive deterioration of the cell-mediated immune system, leading to, secondary infection with a verity, of opportunistic pathogen, and to the development of neoplastic growth, the hallmark of AIDS.

It is essential to dissect the pathogenesis of HIV infection, in to viral factor host factor and their interactions.

STRUCTURE:
HIV is a member of the retro virus, family in sub family lenivirinae. HIV is spherical enveloped virus, about 90-120 nm in size. The nucleocapsid has an outer icosohedral shell and an inner cone-shaped core, enclosing the ribonucleo proteins.

It contains two copies of single standard RNA genome. Viral RNA, after infect the cell first transcribe by reverse transcriptase enzyme, in to single stranded
DNA and the in to double stranded DNA (Provirus), which integrated in to the host cell chromosome. The provirus remain, latent for long period, then synthesis viral RNA and viral component.

During viral replication, virus buds out through the host cell surface membrane, it acquires a lipoprotein envelop, which is lipid derive, from host cell membrane and glycoprotein. Also, the virus coded envelop proteins are projecting knob like spikes on the surface, and the anchoring transmembrane pedicles. The spikes constitute the major surface component of virus, which binds to the CD4 receptors, on susceptible host cell.

RNA a genome of HIV, which encodes the three polycistronic structure of regulatory genes (gag, pol and env) and number of regulatory genes.

The Gag precursor protein, P^55, is cleaved by viral protease, to P^24 inner core protein or capsid antigen, P^17 center core protein or matrix antigen and P^15 which is further calved in two nucleic acid binding proteins (P^9 and P^7).

The Pol precursor antigen, gives rise reverse transcriptase (RT, P^51), the protease and integrate (P^31) Proteins.

The env precursor glycoprotein, gp160 gives rise to gp120 (surface glycoprotein) and gp 41(transmembrane glycoprotein), which form spikes, projecting out from lipid blazer or the viral envelop.

Among various regulatory proteins of HIV are a) Tat (transactivator of transcription) b) Rev (Regulator of expression of virion protein c) Nef (Negative
factor whose function is not clear). In production of virus, such as assembly and budding, Vif, VPu, VPr, VPx and VPt are involved.

Infection is transmitted, when the virus enter, the blood or tissue of person and comes in to contact, with suitable host principally, CD4 lymphocytes

![HIV-1: schematic structure](adapted from Shaw et al., 1988)

The CD4 antigen, on the host cell surface is, serve as, the primary receptor for HIV. It binds, via the CD4 binding site on gp 120. This results, in displacement of gp 120 and proteolytic cleavage of the third variable loop (V3), which allows, interaction between fusion receptor, on the cell surface, following membrane fusion, viral RNA enter in to the cell.

Some other immune cells, CD4 antigen on surface, susceptible to infection, about 5-10% of B lymphocytes and 10 to 20 % of monocytes and macrophage, such as alveolar macrophage in the lung, a Lyangerhans cell, in dermis.
Binding to CD\textsubscript{4} receptor, is not by itself enough, for cell fusion and virus entry. This require, participation of co-receptor molecule, which has been identified, as CxCR4 for T cell tropic, HIV strain and CCR5 for macrophage tropic strain.

Following membrane fusion, viral RNA enter, in to the cell and is reverse transcribed, in to DNA by reverse transcriptase.

The transcribed viral DNA provirus is transported in to nucleus and integrated in to chromosomal DNA. The proviral DNA may remain latent, in the cell for variable time, before starting to produce, viral genomic RNA and viral m RNA. The viral mRNA is used, to synthesize viral poly peptides.

The viral genomic RNA, packed in to viral capsid and buds, through cell membrane, while in cooperating, the viral envelope glycoprotein, present on the cell membrane.

HIV variants mainly are of two, designated HIV I & HIV II. HIV I is the
predominant, variant in most part of the world. HIV II is endemic mainly, in West Africa.

HIV II is 55% genetically, different from HIV I and is more closely related, to simian immuno deficiency virus, then to HIV I.

HIV II is believed to be less transmissible and less pathogenic then HIV I. With in HIV I and HIV II, biological and serological heterogenicity exist.

HIV I have been classified, in two major groups, Group M, common and contain at least eight genetic sub types, while group O, contain several heterogeneous viruses.

Intra sub type variation does occur termed isolated. The genetic difference within subtypes will usually not exceed 15%, where as inter-subtype, variation will beyond the 25% range.

HOST FACTOR (THE IMMUNE RESPONSE TO HIV INFECTION):
Like any antigen, HIV induces a spectrum of antibodies, following infection. Antibodies are directed against, the various components of the viruses, as evident in western blot assay.

Mainly IgG antibodies against HIV antigen, is present in patient also IgM, IgA and IgE described, but the functional role of these antibody, is incompletely understood. There may neutralizing cytotoxic (ADCC=Antibody dependent cell mediated cytotoxicity) or enhancing antibodies. Antibody enhancing can be visualized, in vitro but its vivo role is unclear. Allergic or autoimmune process of
antibody, responses to HIV may found, in same patients.

In cell mediated immune response, like B cell-T cell also, respond to HIV infection, both CD$_4$ and CD$_8$ T cells are involved.

CD$_4$ T cells can either be, T$_{H1}$ 1 cells or T$_{H2}$ 2 Cells. TH1 Cell produce, interleukin-2 (IL-2) and y interferon (Y-IFN), which results in cellular immunity against the virus particle activate through the CD$_8$ Cytotoxic T Lymphocytes (CTL) and partly through the CD$_4$ + CTL(Cytotoxic T lymphocytes)

Interlukin 2 (IL2), IL-6 and IL10 produced by TH-2 cell, which enhance antibody production may responsible, for hypergamma-globulinemia found, in HIV infection.

Usually CD$_4$ T cell responses, is brisk and strong, in early HIV infection, but CD$_4$ depletion is a common event, in the late stage of HIV infection.

CD$_4$+ cell depletion, may be a result of

1) Killing by cytopathic virus, through altered cell membrane integrate, during virus budding through, intracellular accumulation of toxic unintegrated provirus DNA.

2) Or as result of syncytium formation, between infected and uninfected cells.

3) Also by antibody and CTL directed against, virus envelope protein (gp120) expressed, on the infection cells or an uninfected cells, via budding of soluble gp120 on to the surface CD$_4$.

4) CD$_4$+ T cells may be depleted or rendered anergic by exhaustive stimulation of receptors, by the HIV super antigen or by persistent stimulation of CD$_4$, by gp120 antibody complexes.
Finally, programmed cell death of HIV infected T cells, may also contribute to the overall CD4+ depletion, in HIV infection.

Clinically depletion of CD4 results in the onset of various opportunistic infections and neoplasm, characteristic of HIV disease.

The numbers of CD8 cells are also greatly increase in early HIV infection and then decreased at later stage probably through mechanism similar to CD4 depletion such as apoptosis and super antigen stimulation.

Functionally CD8 T cell are the main and the most effective CTL, against HIV. Constantly exposed but uninfected individual or patient, who are long-term survivors are found to have strong CTL responses. In addition CD8 can secrete an antiviral factor which can suppress viral replication non-specifically.

The clinical outcome of HIV clinically depends up on a) the viral properties and b) protective immune response. The virulence and the infecting dose of HIV, play a vital role. Virulence related to subtypes and biological characteristic of HIV, such as cell tropism rate of replication cytopathicity or syncytium-inducing properties and pattern of antiviral resistance.

The viral expression also depends upon expression of various cofactors such as, co-infection or use of illicit drug.

In clinical latency, the virus multiplies rapidly in the lymph node. During early phase of HIV infection very few viron are found in the peripheral blood. The
discrepancy in viral load between peripheral blood and lymph node tissue is result of an intact immune system. Within lymph node many viron are trapped by follicular dendritic cells of T and B cells.

As the humoral and cell mediated immune responses stimulated, will keep the virus load in check

Because of result of dysfunction of the dendritic cells, including decreased ability to present the antigen to immune system and of HIV related depletion of CD4 and CD8 will pass in to blood signaling the onset of HIV diseases.

CLINICAL MANIFESTATION:
The Center for disease control USA, has classified the clinical course of HIV infection under various group 1 to 4.

A) Acute primary HIV Infection (serocversion): The mononucleus like syndrome occur in 60% of individual, with documented time of HIV infection. It develops within 3-6 week after infection last for 1 to 2 week resolving spontaneously.

The symptom and sings includes, fever, headache, sore throat, oral ulcer, diarrhoea, generalized mucopapular, erythematous skin rash, lymphadenopathy, myalgia and menigoencephalitis.

The clinical illness is usually accompanied by transient but marked decline in CD4 cell count. Positive anti-HIV serology will observe after resolution of acute illness.
AIDS is only the last stage in the wide spectrum of clinical features in the HIV infection.

The test for HIV antibodies are usually negative at the onset of the illness but become positive during its course. Hence this syndrome has been called seroconversion illness through in many of these infected 'acute retroviral syndrom' or serocoversion occur without any apparent illness. HIV antigemia (p24 antigen) can be demonstrated at the beginning of this phase. The pathogenesis of seroconversion illness is believed to be due to immune complexes as well as to direct effects of viral multiplication.

B) Early Asymptomatic disease:
Asymptomatic HIV infection found in known area of HIV infected zone and also relatively recent zone of infection

All people infected with HIV whether or not they experience seroconversion illness pass through a phase of symptomless infection (clinical latency) which may last up to several years.

Patient shows positive HIV antibodies during this phase and are infectious. The infection progresses in course of time through various stage CD4 lymphadenopathy, minor opportunistic infection, persistent generalized lympho adenopathy, AIDS related complex (ARC) ultimately terminating in fall down AIDS with characteristic infection and malignancy.

This is longest period of HIV infection, in which the patient is asymptomatic and
remains apparently healthy. It is 8 to 10 years in the western countries but in India, it has been found to be 5-7 years.

HIV cases are detected through pre-employing screening, preoperative center; at antenatal care clinics, during blood donation and screening in various risk groups. Most of such screening is performed without proper counseling.

Asymptomatic HIV infected patient have CD$_4^+$ cell count decrease steadily from 1000/microliter to about 500 (by stage of acute infection, when count fall to 200 or less, clinical AIDS usually set in.)

C) Intermediate HIV Infection or Early symptomatic HIV infection (CD$_4$ counts 200-500mm$^3$)

Early symptom of HIV infection covers a) Persistent generalized lymphadenopathy define as the presence of enlarged lymph node, at least 1 cm in diameter, in two to three extra-inguinal sites, persist for at least three to four months without any current illness or medication that cause lymphadenopathy.

b) AIDS related complex (ARC), this includes patients with considerable immunodeficiency with various constitutional symptoms or minor infection. The typical symptoms and sing can be regarded as reliable clues to HIV infection. These include herpes zoster, oral candidiasis, oral hairy leucoplakia, prolonged fever, chronic diarrhea, progressive weight loss and pruritic, popular, eruption (PPE). PPE is descriptive term, for a form of generalized pruritic eruption commonly, found in patients with HIV infection, in tropical countries.

Some clinical manifestations are found more frequently or more sever in HIV...
infected individuals. These includes psoriasis, psoriatic, arthritis, seborrhoeic dermatitis, muscle-wasting in the absence of significant weight loss, HSV1 and 2 infection, chicken pox, warts, mucocutaneous fungal and bacterial infection, skin hyper-pigmentation and pre-mature grey hair.

D) Stage IV/Late stage of HIV disease (CD4Counts 50-200mm$^3$)/AIDS:
This is the end stage disease, representing the irreversible break down of immune defense mechanism and some advance clinical illness, including opportunistic infection and malignancy.

In early AIDS, many patients are ill only during episodes of infection, which may respond to treatment. Between episodes they may relatively well and able to resume normal life.

In advance clinical illnesses including infection malignancies and other are found of list Table

<table>
<thead>
<tr>
<th>Box: CDC 1993 revised AIDS-defining illnesses.</th>
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<tr>
<td>Infections</td>
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<tr>
<td>Candidiasis of bronchi, tranchea, lungs or oesophagus</td>
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<td>Coccidioidomycosis, disseminated or extrapulmonary</td>
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<tr>
<td>Cryptococcosis, extrapulmonary</td>
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<tr>
<td>Cryptosporidiosis, chronic intestinal (&gt;1 month duration)</td>
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<td>Cytomegalovirus infection including retinitis (excluding liver, spleen or nodes)</td>
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<td>Condition</td>
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<tr>
<td>Herpes simplex bronchitis, pneumonitis, oesophagitis or chronic ulcers (&gt;1 month)</td>
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<tr>
<td>Histoplasmosis, disseminated or extrapulmonary</td>
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<td>Isosporiasis, chronic intestinal (&gt;1 month)</td>
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<tr>
<td>Mycobacterium avium complex or other mycobacterium, disseminated or extrapulmonary</td>
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<td>Mycobacterium tuberculosis, any site</td>
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<td>Pneumocystis carini pneumonia</td>
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<td>Pneumonia, recurrent</td>
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<td>Salmonella septicaemia, recurrent</td>
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<tr>
<td>Toxoplasmosis of brain</td>
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<tr>
<td>Malignancies</td>
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<tr>
<td>Cervical cancer, invasive</td>
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<tr>
<td>Kaposi’s sarcoma</td>
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<tr>
<td>Lymphoma (Burkitt’s, immunoblastic or primary brain)</td>
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<tr>
<td>Others</td>
</tr>
<tr>
<td>Encephalopathy, HIV-related</td>
</tr>
<tr>
<td>Progressive multifocal leukoencephalopathy</td>
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<tr>
<td>Wasting syndrome due to HIV</td>
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<td>&lt;200 CD4+ T cells µl⁻¹</td>
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Mycobacterium tuberculosis is the most common AIDS define opportunistic infection, followed by Pneumocystic carini pneumonia (PCP) and cryptococcal meningitis. There are geographic differences, in prevalence of various opportunistic infections. PCP is constantly infrequent while disseminated penicilliosis is consistently more prevalent in northern Thailand.
Gastrointestinal system, also involve the mouth with thrush herpetic stomatitis, gingivitis, hairy leukoplakeia or kaposis sarcoma are found. Dysphagia may be due to esophageal candidiasis. A characteristic intestinal pathogen in AIDS is cryptosporidium, Salmonella, Mycobacteria, Isospora, CMV or adenoviruses, also frequently cause intestinal infection.

Central nervous system involves, with toxoplasmoasis, CMV, herpes simplex, papova viruses Mycobacteria, Aspergillous and Candida. Lymphoma, of the central nervous system, is common.

Other then kaposis sarcoma tumors commonly seen are lymphomas both the Hodgkin and non Hodgkin types. Demanetia is because of damage in the Central Nervous system.

Cross cultural and cross geographical differences is also due to the incidence of endemicity of the pathogens in environment. In proportion various risk behaviors among AIDS patients, the AIDSs defining illness is multifactorial. Kaposis sarcoma is common in homosexual.

**Tuberculosis:** Mycobacterium tuberculosis is the most common infection, encountered in HIV infected patient in developing countries.

In one survey out of 200 HIV infected patient, 101 case were found to have tuberculosis of various organ, 53(50.5%) pulmonary tuberculosis, 15(30%) had tuberculosis of lymph node, 12(24%) had disseminated tuberculosis, pericardium six, pleura five, meanings and peritoneum two each, esophagus ileum and joints
Cervical lymph nodes are most common site of tuberculosis lymphadenitis. It is characteristically unilateral and one to three glands may involve which may coalesce.

Compared with reactive lymphoid hyperplasia as seen early HIV infection tuberculosis, lymph node are several times larger and show sing of inflammation. Patient may have constitutional symptoms such as after noon fever, night sweats weights loss. Generally CD\textsubscript{4} count may found, higher over 200 cell mlu and tuberculin test frequently shows strongly positive, through there is T cell deficiency.

Patients are severely immunosupressed and cachectic with high grade fever, hepatomegaly, with or without splenomegaly, is most frequent. The liver is firm and non tender, mild to moderate elevation of serum transaminses, marked elevation of alkaline phosphates. Blood culture of Mycobacterium tuberculosis is usually positive.

Nosocomial spread of mycobacterium tuberculosis, especially multi resistant bacteria is higher among hospital staff, doctor, and nurse, other directly involved with AIDS patient care.

Cryptococcal meningitis:
The most common cause of meningitis, in AIDS patient is Cryptococcus neoformans. Rate of meningitis is more than 20% in Thailand and progressive higher with decrease in CD\textsubscript{4} count. Headache, nausea, vomiting, fever, blurred
vision, mental changes, radial pain to leg and buttock are common symptoms.

Diagnosis is usually made by examination of CSF with positive India ink and positive culture for Cryptococcus neoformans. CSF shows little changes in protein, sugar and cells. Cryptococcal antigen levels are used to confirm diagnosis in CSF.

**Penicilliosis:**

In Thailand, systemic infection with P. marneffei is considered as AIDS defining illness. In Northern Thailand, south China, Vietnam and Hong Kong, organism is found to be endemic.

Bamboo rats are one of the natural reservoirs. Route of transmission to man is yet uncertain. Fever, anemia, weight loss and skin eruptions, are common symptoms.

Presumption diagnosis is made by microscopic examination of wright-stained of bone marrow. Also touch smear of skin/lymph node biopsies show intracellular and extracellular oval and elliptical yeast cells with clear septation.

**MANAGEMENT OF HIV/AIDS PATIENT:**

The management includes diagnosis, treatment, prevention and community care aspect. It requires multidisciplinary approach, including specialties, in sexually transmitted disease and other infection disease, e.g. chest disease, dermatology gastroenterology, ophthalmology, neurology, etc.
DIAGNOSIS OF HIV INFECTION:
A compressive base line assessment of Patient with HIV infection, will include a detailed history, clinical examination, STI screen including, syphilis serology, hepatitis B screen, cervical cytology in women, cell blood count, chemistry, HIV load, toxoplasma antibody test, tuberculin skin test, and chest X ray.

Counseling education, partner notification, nutrition, vaccination against hepatitis B and influenza, antiHIV therapy and follow up, are other consideration.

LABORATORY DIAGNOSIS:
For laboratory diagnosis of HIV Infection immunodeficiency as well as specific test is commonly employed.

A) Immunological Test: Following test help to establish immuno deficiency in HIV Infection:

1) Total leucocytes and lymphocytes count to demonstrate leucopenia and lymphocytes count usually below 2000/mm³.
2) T cell subset assay: Absolute CD4 cell count will be usually, below 200/mm³; T4: T8 cell ratio reversed.
3) Platelet count shows thrombocytopenia.
4) Raised IgG and IgA levels.
5) Diminish CMI as indicated by skin test.
6) Lymph node biopsy shows profound abnormalities.

B) Specific tests for HIV infection: These includes, demonstration of HIV 1) it’s antigens or other component 2) antibody and 3) Isolation of the Virus
Antigen detection: After single massive infection, as by blood transfusion, the virus antigen may be detected in blood after about two weeks. The major core antigen P²⁴ is earliest viral marker to appear in blood. One can test for IgM will appear in about 4-6 weeks, to be followed by IgG antibodies.

The appearance of p²⁴ antigenemia and viremia followed by IgM antibody response coincides with acute or seroconversion illness. Free P²⁴ antigen is disappears from circulation and remain absent during the long asymptomatic phase to reappear only when sever clinical disease sets in.

Antibody bound P²⁴ antigen, continues to be demonstrable, after dissociation ELISA, the p²⁴ antibody capture assay, uses ant p²⁴ antibody, as solid phase can be used. About 30 % of HIV infected person, show positive results with dissociation antigen antibody complex, the positive rate increased, to about 50%.

The test is positive, in first few weeks, after in first few weeks, after infection and terminal phase. The test is use for recently exposed individual, to HIV. In USA, current test is use, for screening blood donor, along with HIV ELISA.

Virus Isolation: Once individual infected, with HIV Infection remained infected for life. The virus is present, in circulation and body fluids, within lymphocytes or cell free.

The graph of viral titer, is similar to P²⁴ titer, high in initial phase, low and antibody bound during a symptomatic phase and again high toward, the end.
Infectivity is higher, in early phase and again high, at terminal stage of illness. The virus present, in many parts of body and isolated from the peripheral lymphocytes. The technique of isolation is by co-cultivation of patient lymphocytes with uninfected lymphocytes, in the presence of interlink-2.

Viral replication can be detected, by the demonstration of reverse transcriptase activity, as well as antigen, in the system.

Viral isolation is not suitable, as routine diagnostic test, because of risk involve. The test will be positive, only in proportion of person infected with HIV. Isolation of virus carried out only in laboratories with adequate contaminant facilities.

Polymerase Chain Reaction is most sensitive and specific test. PCR has become the gold standard for diagnosis, in all stages of HIV infection. Two form of PCR have been used 1) DNA PCR and 2) RNA PCR

In DNA PCR peripheral lymphocytes from subject, are lyses and proviral DNA amputed, using primer, prior from relatively constant region, from gag and L.tr.

The amplified DNA is characterized by nucleic acid hybridization. The test is highly sensitive and specific when done with control and can detect, HIV DNA one copy/1000 cells. HIV RNA PCR can be used for diagnosis as well as monitoring level of viremia.

The PCR test is complex and costly, indicated when other test cannot give definitive result.
Antibody Detection: Demonstration of antibody is simple and most widely employed technique, for the diagnosis of HIV infection.

However it needs to be emphasized that it may take 2-8 weeks to months for antibody appears after infection and during part of this period for individual may be highly infectious. This seronegative infective stage is known as the window period. For this reason antibody screening is not totally dependable for spotting diagnosis of infection, for example for blood donation. Infection can be detected, during the window period by P24 assay.

In many instant HIV transmission by blood transfusion seronegative donor, have been documented following sexual exposure, HIV antibody may appear, after 2 month duration, test is perform after 2-6 months to ascertain, whether infection, has occur or not.

Once antibodies appear they increase in titer for the next several months. IgM disappear in 8 to 10 weeks, while IgG antibodies remains though out life. When immunological deficiency become, severe following clinical AIDS, some component anti HIV antibody, like anti P24 antibody disappear.

Serological test for anti HIV antibodies are of two types, screening and confirmatory test. First screening tests possess high sensitivity, have broadly reactive spectrum can be automated for handing large no of sample, but not specific, may give false positive reaction.

All sera positive on screening test, are to be rechecked, before declared positive
The most widely screening test is ELSA.

a) ELISA TEST: Direct solid phase antiglobulin is the method mostly commonly used.

Antigen is prepared from the growth in continues T lymphocytes cell line or by recombinant technique and should represent all groups and sub types of HIV & HIV II. The antigen is coated on micro-titer wells or other suitable solid surface.

The test serum is added, if antibody is present it binds to the antigen. After washing away, unbound serum antihuman immunoglulin, linked to a suitable enzyme, is added followed by colour-forming substrate.

If the patient serum contain anti HIV antibody a photometrical detectable color is formed which can be read by special ELISA readers.

ELISA is simple and relatively inexpensive but false positive reactions are not uncommon, particularly with sera containing rheumatoid factor, anti-lymphocytes and auto antibodies. False positive results also occur in hepatic disease.

Modifications of ELISA, in which the antibody in test serum either competes with enzyme conjugated anti HIV antibody or is captured by antihuman immunoglobulin on to solid phase, are more specific. Capture ELISA specific for IgM antibody is also available. Immunoassay is highly sensitive and specific.

A number of ‘rapid test’ have been introduced for this purpose such as
cylinder, or cassette ELISA immune chromatographic, coated partical agutination, immunoperidase or dip stick tests.

b) **Western blot test**: The confirmatory test commonly employed is the western blot test. In this test HIV protein is separated according their electrophoretic mobility (and molecular weight), by polyacrylamide gel electrophoresis are bottled on to strips of nitro cellulose paper.

These strips are reacted with test sera and then with enzyme conjugated antihuman globulin. Prominent colour band produces after suitable substrate is added where specific antibody reacts, with the separated viral protein. The position of bands on the strip indicate antigen with which the antibody has reacted.

The positive serum bands will be seen with multiple proteins, typically with P\(^{24}\) (gag, gene core protein), p\(^{31}\) (pol, gene reverse transcriptase) and gp\(^{41}\), gp\(^{120}\) or gp\(^{160}\) (env, gene surface antigen). A positive reaction with protein presenting three genes gag, pol, and env is conclusive evidence of HIV infection.

The test may be considered positive, even if it shows bands against, at least two of following gene products, P\(^{24}\), gp\(^{41}\), gp\(^{120}\), gp\(^{160}\). However interpretation become difficult, when bands that does not satisfy these criteria. This may happen in early infection or may also be nonspecific. Intermittent results are not uncommon. In such case western blot may be repeated. If no definitive result can be given even, and then it may necessary to have p\(^{24}\) assay done A positive result, in any one screening test may not be accepted without conformation.

Western blot test is use in practice for conformation and considered the good
As test is costly, in the practice now prefer two different types of ELISA or ELISA with any of the rapid test. A serum positive, in both test is considered positive. When in doubt, retesting after 1 or 2 months may be useful.

Apart from diagnosing HIV infection, laboratory would be called up on to identify the opportunistic infections that are feature of AIDS.

Application of serological test for HIV infection are employed in following situation: a) In screening is defined as, the systemic application of HIV testing whether voluntary or mandatory to entire population or selected target groups.

Screening of entire population is neither feasible nor practicable. However screening of target population is valuable, as iatrogenic transfer of HIV, is an important mode of spread of the infection to unsuspecting recipients. It should be mandatory that all donors of blood, blood products, semen cells, tissue and organ be screened.

As antibody tests are negative during the early stage of HIV infection, when the individual is infective, screening may not detect all dangerous donors but can still eliminate large majority of them.

P24 antigen can detect those in the window period. a) In Blood donation, b) also the infection, can be transmitted from mother to baby or after birth, antenatal screening is useful.

Antibody surveys are most useful in identifying the geographical extent of HIV infection.
In diagnosis; Serological investigation are always positive in person with clinical features of AIDS, but may be negative in early stage of illness or sometime in very late cases where immune system is non-reactive.

Antibody testing may also help to check whether infection following on exposure such as sexual contact, blood transfusion or needle stick injury. Two serological methods are if negative after six months would be sufficient.

The CD4 cell count is the most frequently used as marker for immunological evolution of HIV patient, as well as for, prognosis and assessment of therapeutic response P24 antigen, B2 microglobulin and neoptein, can be of prognostic value. They are used infrequently in clinical practice. Viral load measurements have been started in some research laboratories and may prove practical for clinical use if the coast can be decreased.

**DIAGNOSIS, TREATMENT AND PROPHYLAXIS OF OPPORTUNISTIC INFECTION:**
Consequently the diagnosis is usually and often correctly made on a presumptive and therapeutic base.

The treatment to the more common opportunistic infection includes cotrimoxazole, ketoconazole, isoniazid, rifampicin, ethambutol, pyrazinamide, amphotericin B and acyclovir. Itraconzole, flucanazole, ganciclovir, foscarnet and liposomal formation e.g. amphotericin and daunorubicin, some requiring prolonged administration are expensive to patient with moderate income, cannot afford these drugs.
Patient with tuberculosis respond satisfactory to standard antituberculosis treatment regimen. The regimen used most frequently is four drug combinations of isoniazid, rifampicin, ethambutol and pyrazinamide for the first 2 month followed by 7 month with isonizaid and rifampicin.

Drug resistance is found nearly in all drugs and also with isonizaid and rifampicin combination. In developing countries, it is not known whether primary tuberculosis prophylaxis is effective, which drug is not effective to whom prophylaxis should be given. There are uncertainties over secondary prophylaxis.

The optimum treatment for Cryptococcal meningitis is unclear. Amphotericin B is less expensive and somewhat more effective then fluconazole. Administration of Amphotericin B is requiring prolonged hospitalization. In certain countries fluconazole, used as an alternative either initially or after a short indurations course of Amphotericin B where there is shortage of hospital beds. In both primary as well as secondary prophylaxis of Cryptococcal meningitis, Itraconazole is less expensive then flucanozole.

The infection responds well to amphotercin B or itraconazxole but relapse is common.

TREATMENT:
Antiretrovirals: Currently the nucleoside analogues, i.e zidovudine (ZDV), didanosine(ddI), Zalcitabine (ddC), stavudine (d4T) and lamivridine(3TC) and protease inhibitors are major drugs, use in practice. These are expensive and the
government allocates a budget to provide antiretroviral free of charge to low income patients.

The use of antiretroviral in Thailand was changed along with global trades. In 1977, guideline have shifted toward, earlier treatment (i.e. CD₄ cell count 500 Cell/mm³) with double nucleotide therapy protease inhibitors are recommended only for those who can self support. The same is also true for the use of viral load assay in guiding the therapy. When more antiretroviral are available, price is lower better treatment regimens can be designated for developing countries, at least for those with moderate resource Thailand, Brazil, and South Africa.

Alternative treatment approaches include unconventional treatment methods such as herbal medicine and home and community care.

**Herbal medicine:** Because of unresponsive to conventional care HIV/AIDS has generated many. In terms of treatment, many unconfirmed claim for various traditional herbal and spiritual treatment are being made. Some patients do get better in terms of appetite weight gain or diarrhea.

**Home and community care:** Non institutional care has become an increasing need in many countries, in Thailand, India, and Africa.

Many patients discharge sooner from the hospital and transferred to day care center for continued but less intensive care. Patient relatives and community volunteers have to be trained in basic HIV medicine, in order to provide care, to patient at home. The western style of organizes home care is not possible in developing countries because of shortage of professional home care providers.
HIV PREVENTION: AIDS education is considered the most cost-effective means of prevention. Education should be targeted, to both the risk groups and the general public. Attention should be given to socially disadvantaged groups, such as street children, homeless people, and factory workers.

Modern effective approach to AIDS education is the type of knowledge and information dissemination that will attract an individual's concern for and commitment to behavior changes.

Different methods to develop, rational thinking and judgment, techniques of self improvement, in order to stand against risk behaviors.

a) Program of exchange of needle and syringe,
b) the 100% condom use, is also one of the important programs, when condoms are used in almost every commercial sex. This was made possible through involvement of different groups/parties such as, public health officers, law enforcement officers, owner of sex establishments, commercial sex workers and their clients. In Thailand this has resulted in dramatic decline in the incidences of sexually transmitted disease and also risk group in HIV epidemic, 1994.

Vaccination:
Vaccination represents another facet of HIV prevention WHO program on AIDS in 1992 selected Thailand, Uganda, Brazil and Rwanda as sites for international HIV vaccine efficacy trials. This not well planned announcement attracted considerable criticism of using volunteers from developing countries as human guinea pigs for vaccine made in the developed world.
Enthusiasm for the prophylactic HIV vaccine has declined since 1994 when small numbers of US vaccines were found to be unprotected and when the proposal for the efficacy trial in US volunteers was turned down.

Phase I and II HIV vaccine trial in developing countries are essential. Infrastructure of host institutes feasibility and public acceptance has been established.

Evolution can also be made on the safety and immunogenicity of vaccine in ethically different population especially when the local HIV subtypes are incorporated in the vaccine.

In 1994 the First HIV vaccine trial in Thailand was started with very good acceptance from the public. It confirmed that HIV vaccine trail could be carried out successfully in Thailand.

When efficiency trial is ready for the ethical issue will be even more important especially for developing countries. Volunteers have to be well informed about the risk and major efforts in behavior modification have to implement even though larger sample size or longer duration of study is necessary. One cannot risk the life of the volunteer seven if they are from developing countries for the sake of scientific merit.

**Live attenuated vaccine:**
No adverse effect have been described in HIV infected individuals given measles, mumps, rubella or oral polio vaccines.
This vaccine should be given symptomatically or asymptotically HIV infected individual who are at increased risk from these condition.

There may not be a detectable antibody response in some of the vaccines
HEPATITIS:

Hepatitis is not a necessarily infective, in the nature e.g. in the inflammation can be caused by the toxin (by the alphatoxin) chemically by the carbon tetrachloride), drugs (e.g. paracetamol) or by auto immunity. Also, the infection (HHV4, HHV5, yellow fever, laptospirosis) caused hepatitis. But the virus that caused the hepatitis is known as viral hepatitis.

The viral hepatitis caused, by the human hepatitis virus A (HHVA), B (HHVB), C (HHVC), D (HHVD), E (HHVE), F (HHVF), G (HHVG) virus.

Hepatitis B is 42-50 nm, enveloped double stranded DNA virus with icosahedral The capsid carries the hepatitis B core antigen (HBcAg) outside the nucleocapsid is lipid envelop with glycoprotein spikes which form the hepatitis B surface Antigen (HbsAg). The complete viron (some time called the Dene particle) carries HBSAg, HBcAg and viral DNA and is infective. The other two are vesicular and tubular particle which are in essence the viral envelope. During acute HBV infection there is large number of Dene particles in the blood stream. Chronic carrier will have varying rations of Dene to tubular and vesicle particles, higher ratio is more infectious HBV is assigned to a separate, family Hepadnaviridae (hepatotropic DNA viruses)

HISTORY: In 1965, Blumbreg et al, in Philadelphia, found an antibody in two hemophiliac patients which reacted with an antigen in a single serum in their panel which came from an Australian Aborganine. Later the antigen was found in patients with viral hepatitis. Because of it's discovery in aboriginal serum, the antigen was called Australia antigen.
It is now termed as Hepatitis B surface antigen.

**EPIEMIOLOGY:** HBV is a human pathogen and it is maintained by the 200-300 million persistently infected patients (carriers), worldwide. The prevalence of infection and the chronic carriage are highest in China, South Asia and sub-Saharan Africa, where 80-90% of the population has serological evidence of past or current infection or 20-30% are persistently infected.

About 4% of the populations are estimated to be carriers of HBV, giving a total pool of approximately 36 million carriers in India. HBV is reported to be responsible for 70% cases of chronic hepatitis and 80% cases of cirrhosis of the liver. About 80% of Indian patient with hepatocellular carcinoma have hepatitis virus associated liver disease.

**MODE OF TRANSMISSION:** HBV infection is predominantly acquired at an early age in developing countries, which includes vertical transmission from mother to child, parental transmission, and horizontal transmission from child to child. HBV can also be transmitted sexually and sexual transmission -both hereto sexual and homosexual, accounts for a majority of transmission occurring in adult life.

**CLINICAL FEATURES:** In the incubation period of the HBV infection is 2 to 6 months (average 4 months) approx. 30% of adults remains asymptomatic. HBV is more serous infection from the HAV.

The onset is insidious and 15-20% develops erythematous macular maculopapular or urticarial skin rashes. In some patient anorexia, abdominal
discomfort, nausea, vomiting and may developed jaundice. The risk of developing persistent infection is greater for intravenous drug users and for the immune-compromised patient. Up 3 of the chronic carriers will developed active hepatitis which may progress to cirrhosis.

Hepatocellular carcinoma is another long term results of persistent HBV infection. In some patient immunocomplex disease may develops chronic gronulonephritis and cryglobulinemia. HBV has a potential to promote HIV infection, but there is a little clinical evidence to suggest that HBV spread to progression to AIDS.

LABORATORY DIAGNOSIS:
Specific diagnosis of hepatitis B rests on the serological demonstration of the viral marker. It is therefore necessary to understand the sequence of their appearance in blood.

HBsAg is the first marker to appear in blood after infection. It remains in circulation throughout the icteric or symptomatic course of the disease. In typical case it disappears within about 2 months of start of clinical disease, but may sometimes last for 6 months and even beyond. When it is no longer detectable, its antibody, anti-HBs appears and remains for very long periods. Anti HBS is the protective antibody.
HBcAg is not demonstrable in circulation, because it is enclosed within the HBsAg coat, but its antibody, anti Hbc appears in serum a week or two after the appearance of HBsAg. It is earliest antibody marker seen in blood. As anti Hbc remain life long, it serves useful indicator of prior infection with HBV.

HBeAg appears in blood concurrently with HBsAg, or soon afterwards. Circulating HBeAg is an indicator of active intrahepatic viral replication, and presence in blood of DNA polymerase, HBV DNA and virions, reflecting high infectivity. The disappearance of HBeAg coincides with the fall of transaminase level in blood. It is followed by the appearance of anti-HBe antibodies.

Like HBeAg, HBV DNA is also an indicator of viral replication and infectivity. Molecular methods, such as DNA: DNA hybridization and PCR, at present used for HBV DNA testing are highly sensitive and quantitative HBV DNA level in serum reflects the degree of viral replication in the liver and so helps to assess the progress of patients with chronic hepatitis under anti viral chemotherapy.
**Virus / Antibody Markers**

<table>
<thead>
<tr>
<th>HBsAg</th>
<th>HBeAg</th>
<th>Anti-HBc</th>
<th>Anti-HBs</th>
<th>Anti-HBe</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>IgM</td>
<td>-</td>
<td>-</td>
<td>Acute HBV infection, highly infectious</td>
</tr>
<tr>
<td>+</td>
<td>+</td>
<td>IgG</td>
<td>-</td>
<td>-</td>
<td>Late / chronic HBV infections or carrier state, highly infectious</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>IgG</td>
<td>-</td>
<td>+ / -</td>
<td>Late / chronic HBV infection or carrier state, low infectivity</td>
</tr>
<tr>
<td>-</td>
<td>+ / -</td>
<td>IgM</td>
<td>-</td>
<td>+ / -</td>
<td>Seen rarely acute HBV infection, infectious</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>IgM</td>
<td>+ / -</td>
<td>+ / -</td>
<td>Remote HBV infection, infectivity nil OR very low</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+ / -</td>
<td>-</td>
<td>HBV vaccination</td>
</tr>
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</table>

**TREATMENT:** Acute HBV infection in a majority of adults is a self limiting disease and hence doesn’t require any antiviral treatment. In patients with chronic HBV infection, antiviral therapy in the form of interferon-α or lamivudine or adifovir have been established for treatment of chronic HBV infection.

**PREVENTION:**

**General measures:** HBV infection can be prevented by screening blood for HBsAg, using disposable needle and syringes, using gloves and practicing safe sex.
**HBV vaccine:** Vaccination can be effectively used to reduce HBV infection rate. In healthy individuals, the recombinant vaccine is given in a dose of 10 μg. IM having schedule of 0, 1 and 6 months.

**Hepatitis B Immunoglobulin:** It is effective for passive immunization if given prophylactic. It is useful in post exposure prophylaxis (within 48 hours) and prevents maternofoetal transmission of HBV.
HUMAN PAPILLOMA VIRAL INFECTION AND GENITAL WARTS

Ano-genital Warts are the most common of viral infection and perhaps the most common condition seen at STI clinics in developing countries.

Not only are genital warts due to human papilloma virus unsightly, but their persistence and inconstant response to treatment gives rise to anxiety and introspection in the patient as well as the added burden of multiple attendances.

The high prevalence, high infectivity, long permanent period (average 3 month, range 2 week to 8 months) and often poor response to treatment, all make effective control by therapy and contact tracing possible, only to a limited extent.

Until, quite recently genital wart has been regarded virtually as benign growth. Which regress spontaneously but human papilloma viruses may not be solely causes of benign tumor of skin and mucosa, but in longer turn and possible in accumulation with, herpes virus type 2 and/or other cofactor such as smoking may play a part in etiology. Cancer particularly, of the cervix uteri, the case against Papilloma virus, in regard to cancer of cervix, is however not yet proven.

ETIOLOGY:

Human papilloma virus (HPV) or in colloquial term the wart virus consist of heterogeneous group of DNA viruses, which were placed until recently in family of papova viruses (pa=papilloma, po=polyma va=vaculating agent).

The viruses were originally, grouped together on the basis of similar structure of their icosahedra capsid (55 nm in size) and nucleic acid (Pfister 1984)
Examination of genital warts Condyloma acuminatum and common skin warts (verruca vulgaris) showed that both lesions contain morphologically identical virus, but with techniques using immuno electronmicroscopy, it was clear that the viruses were antigenically distinct.

Since virus cannot be propagated, in vitro in any cell culture system, progress in research, until recent years, been difficult and unrewarding. The quantities of wart material in the lesions were so low that, their characterization directly from such material was not possible.

The extraction of virus DNA from warts and its molecular cloning in bacterial plasmids or in bacteriophage lambda has now enabled. The preparation of sufficient quantity of virus is to develop methods, for classification of papilloma viruses from a variety of lesions, in variety of anatomical sites.

At the present time classification of HPV, in to separate types is based on nucleic acid hybridization studies; a virus is considered to be separate types if Such DNA Sstudies reveal less than 50% homology with known virus types.

Subtypes are those which show more than 50% cross hybridization, but differ in their restriction endonuclease cleavage pattern. The classification may be supported by serological data, but in most cases this will be difficult or impossible because of the very limited amounts of antigen (Pfiser 1984).

On basis of these nucleic acid studies more than 40 types have been reported of which 7 are specific for the uro-genital tract viz the common types are 6, 11, 16, and 18 (Singer et al 1984), the less common HPV are 31, 33, and 35 (Beudenon et
al 1986). Each type of virus usually produces lesions with distractive histological features, in particular an anatomical sites e.g. HPV 1 cause plantar warts HPV 6 and 11 cause Condyloma acuminatum of genital tract.

Skin warts appear to be acquired from environmental sources such as public bathing facilities or gymnasiums and occur primarily in children, while genital warts are acquire mainly by sexual intercourse.

The age incidence of genital warts shows similarity to that of gonorrhoea, to the commonest age of onset being 22 years in case of men and 19 years in case of female.

Vulvar and penile warts are more clearly associated with transmission by sexual intercourse, while anal warts are less certainly associated with anal intercourse, so their origin remains speculative.

It has been suggested that wart virus may be a normal inhabitant of ano-rectum in some patient and trauma may allow entry of the virus, into the epidermis but in fact are virus seldom seen in association with anal fissures (oriel 1971 b).

**BIOLOGY OF HPV INFECTION:**

Papilloma virus causes epithelial or fibroepithelial proliferation of the skin or mucosa. Only a few bovine papilloma viruses, affects the dermis. In wart lesion there is no direct evidence of papilloma virus DNA in basal cells of epidermis.

According to hybridization studies suggest that viral DNA, first occurs in stratum spinosum (Grussendorf and Zur Hausen 1079), where mature virus
parctical can be seen in association with nucleoli (Almeida et al 1962). In the stratum granulosum viron are spread though out the nuclei and appear, as paracrystalline arrays.

After breakdown of cell structure aggregation of virus seen in keratin of the stratum corneum. In beginning, epidermal cells are therefore nonpermissive, but latter on, become more differentiating. (Pfisfer 1984)

Hybridization studies shown that, HPV DNA is to be found apparently normal, skin at least 2 cm from the genital wart itself. ((Ferenczy et al 1985)

Available data on cervical carcinoma and derived cell line suggest that HPV 16 and 18 may be integrated, in cell genomes. While integration of HPV 33 DNA sequence, found in one case of valvar carcinoma in situ (Bowen's disease) Beaudenon et al (1986). It was concluded that integration of HPV 16 or 18 is risk factor for development of cervical cancer. Conclusion about the biological significance of integration can not be made out.

In this Study Mcanwell et al (1987) found that HPV 16 was found to be common, in those with cervical cancer and absent in those without, and a number of point were made, more on HPV 16 DNA.

1) Positive and Negative finding were obtained in adjacent area of the same cancer tumor.
2) HPV16 occurred as episomal molecules, in more then 2/3 of these 31 carcinoma cases.
3) HPV16 integration when found occurs, at mostly single host genome sites but
on one occasion of multiple sites.

4) The association of HPV 16 and cervical neoplasia is age related, the older patient the virus is more likely found.

Result of immune electron microscopy suggest that one way antigenic cross areas exists between the virus of common skin warts and the genital warts. Sera of patient of common genital wart reacts both the common skin and genital wart virus, while serum from patient with genital wart appear to react only with homologus genital wart virus (Almeida 1976). Genital wart produced, humoral antibody, IgM is commonest, but IgG also, found.

Studies on Cellular Mediated Immunity (CMI) have been hampared by the lack of a culture technique; to obtain sufficient antigen but modern technology should being important advances soon.

Spontaneous resolution of warts is often associated, with infiltration by macrophages and lymphocytes. In pregnancy, when CMI is depressed, genital wart enlarges and extended considerably, although they usually regress during the puerperium

Defective CMI is probably also the reason for, florid genital wart which is sometime seen in the patient with Hodgkins disease (Oriel 1976) and occasion on in renal transplantation.

Langerhans cells, HLA DR, positive dendric cells, are to be found in straitified mucosae, including the vagina and cervix, as well as thymus, spleen and lymphatic. These cells are to play a vital role in the progress of reorganization of
exogenous antigen. (Shelley and Juhlen 1976) The process of T lymphocytes activation is thought to be initiated by direct cell to cell contact between T lymphocytes and HLA DR positive cells which have been taken up antigens. T helper cell in the stroma may induce B lymphocytes maturation, into immunoglobulin producing plasma cell at site of circulation of antigen specific cells to regional lymphatic and lymphoid tissue would be important to the development of immunological memory (Morrix et al 1983)

The cervical tissue epithelial wart infected with virus consistently shows a partial depletion or absence of langerhans cells and T lymphocytes also. Few Langerhans cells which remain, show loss of their normal dendritic process and may be result in direct cytotoxic effect.

TRANSMISSION:
Genital papilloma virus infections are acquired through breaches in the skin and mucosa during sexual contacts and are considered to be sexually transmitted. Nearly 2/3 of sexual partner of person with genital wart will develop clinical wart with 2-3 months, incubation period varies from a few weeks to months or even years.

PATHOLOGY AND CYTOLOGY:
For centuries, the only recognized manifestation of genital papilloma virus was condylomatous wart. In the 1970, because of technique of colposcopy, histology and exofolitive cytology, specific morphological changes observed. Recently with advance molecular virological techniques e.g. hybridization and PCR it now evident that, genital papilloma virus can be present, as latent infection in epithelium classified by histological and cytological criteria as normal.
Virus initially enters through skin aberration to the basal germinal epithelium cell. Replication of virus occurs, exclusively in nucleus and result in keratinocyte. With growth pattern of host cell resulting in disordered differentiation and proliferation leading to wart formation.

Histopathological changes include parabasal hyperplasia, hyperkeratosis, parakeratosis, cytoplasmic vacuolation (koilocytosis) and range of other morphological changes. Cytological features are characterized by koilocytosis, dyskeratosis, and bi or multi nucleation. The nuclei show degenerative changes in form of pyknosis, borderline abnormalities or frank dyskaryosis.

Koilocytosis may be identified in both, histological as well as cytological and is the specific cytopathic effect of HPV infection. Koilocytes are of superficial or intermediate squamous cell types have a large perinuclear vacuole or halo from peripheral rim of condensed cytoplasm.

Viral particle and capsid protein absent in basal layer, but both early and late gene are expressed in superficial layer epithelium and there is large number of virus particles in the most superficial layers is source of transmission to an unprotected contact.

Acuminate wart is histological characterized papillomatosis structure consisting of branching fibrovascular cores covered by thicken squamous epithelium, elongation and thickening of the rete ridges and accentuation of intra cellular bridges. Clinical evident papular macular and flat lesion has been less pronounced branching. Warts may pigment in basal cells and in some
karatinocytes. Such wart may mistake for pigmented intraepithelial neoplasia, seborrhoeic keratosis.

The commonest type of subclinical lesions is invisible to the naked eye, but is evident on colposcopy described is flat wart non Condylomatous wart virus infection or sub clinical wart virons infection. Histological, such wart resembles to Condylomata acuminata without papillomatous configuration.

**CLINICAL MENIFESTATION:**

Clinically, most genital papilloma virus infections are asymptomatic. It may present due to discovering a growth or as contact of partner with warts. Its accidental finding, at clinical examination or on cervical cytology smears.

However some patient may have associated symptoms such as discomfort, pain and bleeding due to its location, size and extent trauma or rarely, malignant transformation.

Anogenital wart is polymorphic, variable in size and in growth, may present as flat and papular to markedly exophytic acuminate warts. Common warts occasionally appear in the genital area also, similar lesion found in non genital skin.

Condylomata acuminata warts are soft, fleshy and vascular, with pointed irregular, fissured surface. Highly vascular appear by necked eye on magnification show various capillary patterns. Colour may be pinkish red through reddish white to grayish white. Size of acuminate may be coalescing to form large cauliflower like growth often seen in pregnancy. They are
predominantly in moist and intertriginous area the site labile to trauma during sexual intercourse.

In women the accumulate warts are commonest clinical variety and usually appear first in vestibulum and on the posterior fourchette of vulva, extend to vaginal and also entire vagina may affected. The infection of cervix is usually subclinical but exophytic warts are not infrequent. Perianal and anal wart occur in both sexes. Anal warts in men traditionally have been associated, with practice of anal intercourse.

The method of transmission of anal wart, in heterosexual is probably though self inoculation from the penile lesion. In women, concurrent occurrence of peranal warts is common, posterior spread being attributed to close proximity to the vulva.

Wart tends to become more rounded or papular on fully keratinized drier genital area such as, lateral parts of the vulva, the perineum and perianal area.

Accumulate warts some time change in to more papular types irrespective of anatomical site. The colour of wart is varying from pinkish red to grayish white but often appear brownish. Surface is smooth and velvety unless covered by pronounced hyperkeratosis. They are often multiple, between 0.5 to 5 mm in diameter and tend to be dispersed than forming plaques.

Sub clinically, genital papilloma virus are at least ten times common as overt warts. These lesions frequently coexisting with and often indistinguishable from CIN were first described on cervix observed under magnification with a
colposcope on application of 3-5% acetic acid. In female, the vulva and the perineal area are often covered with isolated macular lesions which are seen to coalesce the medial aspect of the labia minora.

In both sexes, subclinical infection may be associated with superficial epithelial fissuring, especially in the posterior fourchette and perianal area, in the female and prepuce in male. This may occur with symptoms of severe dyspareunia and in male; itching, burring and discomfort in intercourse.

In prepubertal children, anogenital wart female: male, ratio is 2.6:1. HPV, 2, 3, 6, 11 and 16 are usually found. Anogenital warts, in infant several weeks after birth suggest transmission from mother during delivery, when occur in older children due to reactivation of latent infection acquire at birth or due to acquisition postnatal by nonsexual or sexual means.

Anogenital wart enlarge or appear for first time in pregnancy due to reactivation of latent or sub clinical genital papilloma virus probably due to natural immunosuppressant of pregnancy. Rarely cause obstruction to delivery, physical discomfort; bleeding and poor healing of the episiotomy site may result.

Human papilloma virus types 6 and 11 confirmed as aetiological agent of papilloma occurring in the respiratory tract. The papillary lesions develops on vocal cords may spread to other parts of larynx and to trachea.

Genital papilloma in chronic immune-suppressed or Immunodeficient patients associated with disease in herniated, acquire (e.g. AIDS) or due to immunosuppressive therapy. There is higher prevalence and more prone to
Giant condyloma (Buschke Loewenstein tumor) is a rare condition. Here, initial wart-like lesion undergoes relentless enlargement to produce a foul-smelling, exudating or hemorrhagic exuberant cauliflower-like non-metastasizing growth. The most lesions are single recorded cases where the penis has been affected. Deeper invasion, local lymph node involvement, and distant metastases are absent suggesting benign nature. Although, malignant transformation into metastasizing squamous carcinoma, has been reported.

The intraepithelial neoplastic changes (Primalignant and malignant) are now recognized, not only in the cervix (CIN) but also vulva (VIN), vagina (VaIN), penis (PIN), perianal area, and anal canal (AIN) according to severity as grades 1, 2, and 3.

Cervical cancer is worldwide, the second commonest cancer, in women with 46,000 cases reported per year, 77% occurring in non-industrialized countries. Good epidemiological evidence suggests sexually transmitted aetiology, notably HPV for CIN and Cervical cancer. The postulated role of HHV-2 has been undermined by failure consistently to detect HHV-2 specific DNA, in cervical cancer biopsy samples.

HPV type 6 and 11 are associated mainly with genital warts and low-grade CIN lesion with polyploid nuclear DNA content. HPV types 16 and 18 are associated with lesion and are found constantly in higher grade of CIN, vulval, invasive cervical, penile, and anal cancer.
Around 30-40% of untreated CIN3 lesions are progress to cancer. The majority of CIN 1 lesions have low malignant potential and progression to cause cervical cancer. It is believe that, the malignant transformation results from synergistic interaction with one or more external and internal cofactor such as concurrent infection with HSV, smoking or immunological status.

Natural history of valva (VIN), vagina (VaIN), anal (AIN) and penis (PIN) is limited and comparatively less malignant potential than CIN. Clinically presentation is variable, frequently being asymptomatic, puritus, burring and superficial dyspaeruina are commonest complains. The lesions may be solitary or multiple, partially pigmented, macular and popular in clinical appearance.

With Colposcopic evolution, with 3-5% acetic acid, identify subclinical lesion HPV DNA have been demonstrated in 40-70% of VIN3 and also invasive squamous vulval cancer 2/3 positive for HPV 16. The papilloma virus appears to be associated with neoplastic changes in young women. Malignant conversation of HPV associated VIN is low, varying from 0-0.6%.

The risk of anal cancer in homosexual men especially in those practicing anal intercourse has been recognized for some time. Many of the risk factors associated with cervical cancer. HPV 16 and 18 DNA have been identified, in AIN and anal malignancies and are implicated in the aetiology of anal squamous cancer.

**Differential Diagnosis:**

Exophytic ano-genital warts can differentiate from other papular lesions including normal anatomical variants, infective and neoplastic conditions. Of the
Infective lesions molluscum contagiosum diagnosed by its characteristics smooth umbilicated appearance. More important lesions are secondary syphilis (Condylomata lata), papular skin eruptions and granuloma inguinale (Donovanosis).

**DIAGNOSIS AND INVESTIGATION:**
Identification of virus in the lesion is as yet seldom undertaken for diagnosis owing to the ease of recognizing a wart clinically. With the new high technology it will be possible to study the epidemiology of heterogeneous wart viruses.

At present time diagnosis depends upon the use of biopsy and in the case of cervical infection the colposcope. Anoscopy is required when there are perianal warts or history of anal intercourses sexual partner should always to be examined carefully.

Very small lesions cause difficulty and to be certain that a wart has completely disappeared is often a problem as minor changes in the skin surface often persist as its site.

Skin lesion of secondary syphilis viz Condylomata lata and papular skin eruption require to differentiated by dark field microscopy or by test based, on monoclonal antibody for T.pallidum and serology test for syphilis.

Squamous cell carcinoma of the penis particularly if it is papillary in type may be difficult to differentiate from Condyloma acumulata.

Biopsy of vulva perianal and penile lesion is indicated when appearance is
typical or response to treatment is poor, to exclude neoplasia or other non-neoplastic condition. Colposcopic criteria distinguish CIN from HPV have been described. The Exophytic lesions of the cervix must be biopsies as they might conceal significant CIN or even frank carcinoma.

**TREATMENT:**

Although anogenital Condylomata accuminata tend to regress spontaneously, treatment is important as in the case of moist hyperplastic warts spread is sometimes rapid and sepsis can be troublesome.

The exclusion detection and if required treatment of other sexually transmitted infection in the affected individual and sexual contact(s) are essential first steps. The presence of genital warts often causes much anxiety in the patient and explanation and encouragement are important.

Before local treatment of genital warts is shorted it is important to attend to any other local infection whether sexually transmitted or not as warts tends to spread more readily in inflamed skin.

In women any cause of vaginitis or discharge must be discover and eradicated, particularly Candidiasis or Traichomiasis.

Treatment of genital wart is notoriously unsatisfactory and recurrence is common although when warts are discrete and few, responses to treatment may be rapid.
Podophyllin:
Podophyllin deserves full concentration because of its wide spread clinical use and its often spectacular initial effect when applied to hyperplastic condylomata accumulate of the genital skin.

Preparation and contain:
Podophyllin is an ethanol extract prepared from the dried rhizome and root of the plant Podophyllum. Greek word podos meaning foot and phyllon meaning is a leaf so named.

Preparation ethanol extract is poured into cold water which acidified with hydrochloric acid to form a precipitate which is dried and powdered. Light brown to greenish in colour. Extract also contain colorless ligans insoluble in water.

Painful local inflammatory reaction were increasingly recognized and various modifications such as washing off medication after application and protection of surrounding epithelial surface with inert pastes, cream or ointment were advocated.

Subsequently Sullivan and king (1947) described to apply 20% podophyllin in 95% alcohol and to allow drying to avoid the smearing and spreading effect of the preparation in liquid paraffin.

In studies it was observed that in 4 to 24 hrs. Condyloma decreased in size and in 48 hrs. There was a complete involution. Unwanted local effects like local itching, burning, tenderness and erythema to pain, swelling and the minor erosions
Electrocoutery treatment in case of genital wart is desecrating. After 1% solution of lignocaine is used as local anesthetic, wart is removed with cautery. The aim should be coagulate the wart down to the basement membrane and cause the minimal damage to surrounding skin.

Cryotherapy: The application of liquid nitrogen (boiling point -195.8° C) to desecrate wart is some time effective. The aim should to treat the wart until a hole of the frozen skin is just visible of the base. A cotton tips applicator can be immersed in vacuum flast of liquid nitrogen and then applied to wart. Within 30 min of the freezing cell begin to show the pyknotic nuclei edema and other cytoplasmic changes. Later changes are those seen in actuely ischemic area. The cellular infiltration is mainly of polymorphonuclear leucocytes. Resolution being within 3 days, healing usually occur without scarring.
MOLLUSCUM CONTAGIOUSUM: The Molluscum contagiosum is a benign skin condition, spread by contacts. It can be transmitted sexually but more often non-venereal.

EPIDEMIOLOGY: Most often this is a mild disease which may not be reported to physician. It occurs in all age groups, may spread sexually and non-sexually. It frequently occurs in those with multiple sexual partners or who buy the sex. It occurs tends to primarily on the lower abdomen, thighs, genitalia and buttocks.

In UK, in the decade from 1971, the number of cases attending STI clinics increase fourfold to represent 0.27% cases attending the clinics. Since the advent of HIV and AIDS, the number of cases has increased further.

The causative organism virus is large DNA virus of molluscipox family, it has poxviral brick shape, (300x220x100 nm) on electron microscopy. The virus has never been maintained successfully in artificial culture. Molluscum contagiosum are of two sub-types MCV-I and MCV-II on bases of using restriction endonuclease digestion of viral DNA obtained from lesions. MCV1 is most prevalent type; but neither type is associated exclusively with sexual and non-sexual transmission MCV-II has been found only rarely in children under the age of 15 years.

TRANSMISSION: It is occurs, directly person to person by close skin contacts, such as when sexually acquired. It can also occur indirectly via fomites such as swimming pools, towels, gymnasium equipments and benches.
PATHOGENESIS:
Molluscum contagiosum lesion, consist focal area of hyper plastic epidermis surrounding lobules filled with keratinized debris and degenerating molluscum bodies. Anti-MCV antibodies can be detected during and after infection but their role in immunity is unclear. In some cases inflammatory response in the lesions are also found. The infection is more common in children than adults suggest that immunity develop. In AIDS patient and other T-cell deficiencies tend to have more wide spread infection.

CLINICAL FEATURES: The incubation period ranges from 2 to 3 months. Most patients are asymptomatic, but in some cases lesion may be itch or be tender.

The lesions being, as small papule which grow to 3-10 mm in diameter, over 3 to 4 week. The mature lesions are small, firm, dome shaped, with central umbilication. The colour may be pearl grey, yellow or pink. In sexually acquired infection, common sites are genital region, lower abdomen and thighs.

Approximately 10% children will have a genital reason. More widespread infection occur eczematous skin, when the corticosteroids are used in the treatment. In the patient with AIDS, T cells deficiencies or immunosupression lesions are multiple widespread and disfiguring.

The average duration of the Molluscum contagiosum is two years but may persist two to six months, in some patients. In AIDS patients lesions may persist for longer continue to erupt and recur, despite treatment.
Differential diagnosis includes cutaneous and genital warts and cutaneous cryptococcosis, in AIDS.

**DIAGNOSIS:** The molluscum bodies can be shelled out or samples taken with a needle. Histopathological test will show the characteristics features of Molluscum contagiosum. Rapid diagnosis by the negative staining, under the electron microscopy will show the characteristics Molluscipox viral particlals. In most cases the diagnosis is made clinically except medico legal / AIDS.

**TREATMENT:** None of the suggested treatment has undergone controlled trials. In treatment includes application of phenol or tincture of iodine by the wooden sticks, to curettage, cautery and cryotherapy. In AIDS patients, no form of treatment (other than antiretroviral therapy) is highly effective. No vaccines are available.
HERPES GENITALIS: It is caused by alpha group of Herpes virus Viz. HSV-2 (more commonly) and HSV-1 very rarely.

History: Infection was first reported by Astruc and Morbis in 1736. Slavi and Gavett (1946) isolated herpes virus from herpetic vulvovaginitis in 1967. In 1968 Nahmias and colleagues noted the antigenic and biological differences between HSV type 1 and 2. They reported that HSV-2 caused genital infection and HSV-1 caused most labial infections. But frequency of HSV-1 causing genital infection is increased.

Primary herpes infection is usually followed by latency and variable periods of reactivation. Typical herpes lesions are thin walled umblicated vesicles that rapidly become pustules and Scabes and heal without scarring.

While most infections are asymptomatic or mild, some can be transmitted to neonates and are associated with other STIs and cervical neoplasia. Laboratory diagnosis is necessary to detect HSV in asymptomatically infected persons to prevent transmission to sexual partners and to children born to infected mothers.

PATHOGENESIS: Herpes genitalis is frequently associated with the presence of sexually transmitted diseases. Various STIs associated with genital herpes are gonorrhoea, syphilis, trichomoniasis, candidiasis, venereal wart and chlamydiosis.

Infection is transmitted by direct sexual contact, most commonly when an active virus-secreting lesion present. HSV has been recovered from asymptomatic male carrier and also from the cervix of asymptomatic women. In the presence of primary disease, HSV may occasionally continue to spill for prolonged periods
after resolution of primary infection. The virus is usually no longer recoverable from the cervix or vulva.

After the primary infection, HSV lies dormant in the sensory sacral ganglia that supply segments of the genitalia where the infection was clinically evident.

**Immunity**: Host response to infection with HSV influence the acquisition of disease, severity of infection, resistance of development of latency and frequency of recurrences.

Both antibody mediated reactions are clinically important. Immunocompromised patients with defects in cell mediated immunity experience more severe intensive HSV infection than with deficits in humoral immunity.

Recurrences may be triggered by specific physical or emotional factors and may be frequently associated with menses. Other factors are respiratory factors, fever, digestive disturbances, trauma, and exposure to UV light, sexual intercourse and emotional upset.

Incubation period: Ranges from 1-26 days (median 6-8 days)

**CLINICAL FEATURES:**
In primary infection of women, the cervix, vagina, vulva and perineum are affected when only cervix is involved, the infection may be asymptomatic.

The primary infection is usually more serious, accompanied by systemic features like fever and malaise.
Vesiculo ulcerative lesions are very painful.
Primary lesion persists for 2-6 weeks. There is no residual scarring after healing.

**Disseminated infections**: Usually seen in immunocompromised patients. It has been suggested that pregnant women in third trimester who develop primary HSV infection at increased risk of disseminated disease when present at time of labour approximately 50-60% of infants get infected with HSV by vaginal delivery.

**Recurrent infections**: Symptoms are milder than primary infection.

**LABORATORY DIAGNOSIS:**

A) Microscopy: The Tzanck smear is a rapid, fairly sensitive and inexpensive diagnostic method. Smears are prepared from the lesions, preferably from the base of vesicles and stained with 1% aqueous solution of toludine blue "O" for 15 seconds. Multinucleated giant cells with faceted nuclei and homogenously stained "ground glass" chromation (Tzank cells) constitute a positive smear. Intranuclear A inclusion bodies may be seen in Giemsa stained smear. The virus particles can also see under electron microscope.

B) Detection of Antigen: Direct Immunofluorescene assay.

C) Virus isolation: Virus can be isolated by using various cell lines like primary human embryonic kidney, human amnion, human diploid fibroblasts etc. Typical cytopathic changes may appear as early as in 24-48 hours but cultures should be observed for two weeks before being declared negative.

D) PCR based DNA detection.

E) Serology: Immunoglobulin M (IgM) antibodies to HSV-2 are increased to four times after the infection and the ELISA is specific, sensitive and
simple test which confirms the infection by HSV. Considering that herpes is a life long infection not cured by antimicrobial treatment, HSV-2 antibodies are much more reliable indicator of risky behaviour than T. Pallidum antibody.

TREATMENT: Acyclovir 400 mg TDS for 7-10 day.
CYTOMEGALOVIRUS

The name cytomegalovirus was chosen on account of swollen state of infected cells as seen in culture and in tissues. Nuclei of productive infected cells contain a large inclusion body, giving a typical "Owl's eye" appearance.

In human fibroblast cells are required for isolation of cytomegalovirus virus in vitro but in vivo replicates in epithelial cells salivary glands, renal and respiratory, epithelial particularly.

Replication: The temporal regulation of viral protein synthesis in a growth cycle is more obvious in laboratory culture of the slower growing CMV than with HSV appear in nuclei. Early Proteins appear within 16 hrs of the inoculation whilst late proteins are produced after DNA synthesis and the typical cytopathic effect is often not recognizable for 5-21 days. Foci of swollen cells slowly expand as infection passes from cell to cell passage and storage of best achieved by trypsinization and passage of infected cells.

PATHOGENESIS

CMV virus is persisting in the host for life. Reactivation is common and virus is shed in body secretion e.g. urine, saliva, semen, breast milk and cervical fluid.

Intrauterine infection: Maternal viraemia may result in fetal infection in approximately one out of three of cases primary CMV during pregnancy and may lead to disease in the fetus.

Perinatal infection: This predominantly acquired from the infected maternal genital tract secretion or from the breast feeding.
**Postnatal Infection:** Saliva containing CMV is profusely distributed amongst young children and shared by intimate kissing. Semen can high titer of virus and may be a source of sexual transmission.

CMV is transmitted by sexual intercourse, through transfused blood or marrow, and perinatally either transplacental or by virus through breast milk and, in childhood, transmission may occur through cross-contamination in nurses, as protected viral shedding from the urine and respiratory secretions of infected children occurs. Viral shedding occurs more readily in pregnancy and with advancing gestation. Advanced gestation along with primary maternal infection increases the risk of neonatal disease.

**CLINICAL FEATURES**

Congenital CMV infections are 95% asymptomatic, 15% of cases will go on to show sorineural internal deafness or intellectual impairment later on. The congenital infected infant excrete abundant virus in urine during first year.

Growth retardation, hepato-splenomegaly, jaundice and thrombocytopenia are common to various congenital infections. When central nervous system involves microcephaly, encephalitis and retinitis be may noted at birth. Congenital CMV infection may account for as much as 10% of mental retardation in children up to the age of 6.

**Mononucleosis:** Postnatal infection with CMV is seldom recognized clinically. A mononucleosis syndrome is seen occasionally when CMV is acquired from the blood transfusion. Hepatitis, fever and atypical lymphcytosis are noted but phyrangitis and lymph-adnopathy are unusual.
Primary infection in the adult may present with fever, malaise, atypical lymphocytosis, and lymphadenopathy and rarely may lead to pneumonitis, myocarditis, thrombocytopenia, meningoencephalitis and hepatitis. The virus can be cultured from urine and excretion of virus may be prolonged from weeks to months and occasionally even longer. Reactivation and re-infection are common sequelae.

Immuno-compression patient may develop symptoms as the results of primary or recurrent CMV infection. Complication of the disseminated CMV infection includes pneumonia, encephalitis, retinitis, colitis, hepatitis, pancreatitis or aderinalitis.

LABORATORY DIAGNOSIS

Detectons of CMV are from the samples, of urine, saliva, bronchoalveolar lavage fluid or biopsy tissue. Peripheral blood is collected into preservative free from the heparin. Rapid diagnostic methods will detect virus DNA in sample or CMV early antigen in cell culture. High titer of CMV will produce a cytopathic effect very quickly.

To demonstrate congenital infection, virus must be shown in a sample taken within the first 2 weeks of life.

Serological test for CMV: This is increasing important and sensitive screening tests are more widely available. Complement fixation test are adequate for showing secoconversion after primary infection in component hosts. To screen for 'siropositive' status, a more sensitive assay such as, enzyme immuno assay for the CMV IgG antibody or latex agglutination assay is appropriate.
CONTROL: Some protective action is under taken by way of screening organs of the donor.

TREATMENT: Some antiviral agent for CMV infections is available. Ganciclovir is the agent most often used for the serious CMV disease, given intravenously twice a day. Neutropenia because of narrow, toxicity and there is potential for long term loss of spermatogenesis.
TOXOPLASMOsis:
Toxoplasmosis is caused by the parasite Toxoplasma gondii that normally lives within the domestic cat.

Infection is often asymptomatic or may produce a lymphadenopathy or present with a glandular fever-like illness in the immunocompetent adult, but in immune-compromised individuals it may develop into a severe disseminated illness with chorioretinitis and encephalitis.

OBSTETRIC SIGNIFICANCE: In the fetus and the neonate, toxoplasmosis leads to a syndrome comprising chorioretinitis, microcephaly/hydrocephaly, intracerebral calcification, and mental retardation as the predominant features. The earlier the infection occurs in pregnancy, the more serious the neonatal consequences with spontaneous miscarriage being common in first trimester infections.

With primary maternal infection the risk of transmission rises from 17 to 25% and to 65% from the first through to the second and third trimester, with, overall, 70% of babies born without any damage and a further 10% with chorioretinitis only.

PREVENTION: Prevention of maternal diseases and therefore fetal infection is by avoidance of undercooked meat, unpasturized milk, and contact with cat litter, as well as through washing of garden produce and hands after gardening activities and handling raw meat or vegetables soiled by earth.
DIAGNOSIS: Routine serological testing, should be carried out in case of clinical suspicion and either a fourfold rise in IgG titers between acute and convalescent samples, concurrent high titers of IgG and IgM, or isolated very high IgM titers done by Enzyme-linked immuno sorbitent assay (ELISA) are indicative of acute infection or re-infection.

MANAGEMENT: The maternal infection is confirmed maternal treatment with antibiotic e.g. spiramycin 3 g/day reduced risk of transplantable infection.

Fetal infection in confirmed by demonstration of specific IgM at amniocepha and fetal blood sample.

Ultrasound scanning performed at around 22 weeks which may demonstrate ventricular dilatation

Postnataley it is important to carry out conformation test (placenta amniotic fluid cord blood and maternal blood serology) and a though assessment of neonate including ophthalmic review and cranial radiological assessment.
RUBELLA:
This is also known as German measles.

ETIOLOGY: It is caused by RNA togavirus and acquired by respiratory droplet exposure. The affected individual is infectious for the last week of incubation and the first week after the rash appears.

CLINICAL MANIFESTATION: After 2-3 week incubation, a rash with arthralgia, fever, sub occipital and postauricular lymphadenopathy occurs, but clinical symptoms are present only in 50-75% of those infected.

Obstetric Significance: Rubella infection, though mild or even subclinical, has marked embryopathic consequences when acquired by the fetus in utero.

Risk of transmission to the fetus with resultant congenital anomalies... may be over 80% in the first trimester dropping to approximately 50% by 13-14 weeks, and around 25% by late second trimester, compared with a less than 10% risk from rubella re-infection.

Rubella re-infection may occur in up to 50% of case of vaccine-induced immunity as opposed to 5% in cases of naturally acquired immunity.

PATHOLOGY: Rubella-associated defects, which are generally attributed to vascular damage or reduced mitotic activity, are present in almost all infants infected before 11 weeks gestation, in approximately 35% of those infected at 13-16 weeks (mainly deafness), with infection after 16 weeks rarely causing defects.
Congenital anomalies may be permanent such as congenital cataracts, glaucoma, heart disease, deafness, microcephaly, and mental retardation along with later development of diabetes, thyroid problem, precocious puberty, and progressive encephalitis. Other features may be transient findings such as purpura, splenomegaly, jaundice, meningo-encephalitis, and thrombocytopenia.

**DIAGNOSIS:** Rubella is extremely difficult to diagnose because of non-specific rash and commonly sub clinical infection, so serological assessment (latex agglutination, fluorescent immunoassay, or enzyme immunoassay) of paired acute and convalescent samples from women with suspect illness or exposure is the main method of diagnosis.

During pregnancy, in cases of suspected exposure in susceptible women, it is important to confirm serologically and diagnosis in the index case whenever possible.

Material infection is confirmed by appearance of IgM antibodies or a fourfold rise in IgG antibody titers. Assessment of fetal infection carried out by amniotic fluid culture and IgM in fetal blood.

Polymerase chain reaction (PCR) has low accuracy, so counseling is based on the gestation-age risk of congenital infection along with whether this was a primary maternal infection or a re-infection along with.

**PREVENTION:** If maternal seroconversion occurs in the first 12 weeks then a termination of pregnancy may be offered without invasive prenatal diagnosis. In cases of seroconversion between 12 to 18 weeks or re-infection in the first
trimester or where TOP is not an option, fetal blood sampling may be offered to confirm fetal infection, but after 12 weeks the main risk is one of hearing defects. Children with no clinical manifestations but persistent antibodies need to be closely followed up as sensoreneural deafness may be of late onset, bilateral, and progressive.

**IMMUNIZATION:** A pregnant women should be tested prior to or in early pregnancy to continue immunity. Prevention though child wood vaccination serological test at pre-pregnancy stage or while undergoing fertility investigation and treatment.

Te rubella vaccine is live virus preparation and therefore should not be used in pregnancy.
CANDIDIOSIS (of Genitalia):

(Candidiosis synonyms thrust) Convenient generic term for infection is caused by yeast acting as opportunistic pathogen.

The most important membrane of the group Candida albicans is capable of causing a very common superficial infection of mouth and vagina; Candida albicans are widespread or also cause more deep seated disease viz systemic candidiosis, an important hazard in modern medical procedures such as transplantation and immuno suppressive surgery. This affects young and middle aged females, particularly during their active reproducitive life. About 75% of all women suffer at least one episode of this condition during their life time and around 50% of then have recurrence.

HISTORY: As long ago as Hippocrates, fungi were known as agents of disease. J.S Wilkinson in 1849 first demonstrated their pathogenicity in the reproductive tract.

Plass et al and Hasseltine and his colleagues (1931-1934) rediscovered the relationship between yeast vaginitis in pregnancy and oral thrush of the newborn.

Within last 50 years or so we have reached our current understanding of this ubiquitous disease.

ETIOLOGY:

Various species causing Vulvovaginitis are

Candida albicans
Candida glabrata
Candida tropicalis

Vulvovaginitis Candidiosis characterized, by puritus with or without vaginal discharge, tends often to be regarded as minor condition and not seriously damaging. It is a frequent cause irrigative and enhance. In women, tendency to cause to recur, an important cause of morbidity.

Men tend to be less affected as the miliue of the gland and perputial sac is less favourable as a site for colonization by Candida,

Morphologically pathogenic yeast cells are typical of aerobic eukarucytes possessing intracellular organelles, including mitochondria, ribosome and double membrane nucleus containing chromosome.

All pathogenic Candida and Tolulopsis are multiply by production of buds (1.5-4.0 μ in diameter) from thin walled avoid blstospore (yeast cell). Buds enlarges, then mitosis stops septum takes places, between the parent cells and daughter cells

A hypha is long microscopic tube divided by septa. Hypha is arise as, branches of existing hyphae, or by germination of spores. A mycelium is an entire fungal cellular aggregate including hyphal with branches.

A pseudohypha arises by budding process. Of each generation of buds remains attached to its parent. First and subsequent generations are narrow elongated cells that do not resemble the parent blastophore end to end aggregation of
elongated blastospore or pseudohypha are distinguished from true hyphae by true junction at septal junction.

Chlamydospores are a yeast pathogen induced by low temperature incubation under poor nutritional conditions and have thick double layered cell walls.

**Germ tube:**
The formation of germ tube is accepted as reliable property for identifying Candida albicans and is used, in medical laboratories because of its rapidity. The germ tube is a thin filamentous outgrowth from the cell without constriction of its points of origin. Cells from 24 hr old culture (10^5 to 10^6 cells/ml) are suspended in serum and examined microscopically, for germ tube after 1-3 hr incubation at 37°C.

**Toxonomy:**
Other Candida like C pesudotropicalis and C kreusei have been established or conformed by DNA hybridization technique (Riggshy by1985) Base of composition guanine + cytosine (G+C) content in nuclear DNA have been recorded. G+C composition of Candida albicans is 34.4-35.6; C. tropicalis 35.9-36.1; C.parapsilosis 40.5; C. guillermondii 44.1-44.4; C. globrata 39.6-40.2.


In one data from human source in Europe and North America collected from literature by Odds(1979), seen that Candida albicans is the most commonly,
encountered of all species, in samples collected, in mouth. Also fecal sample Candida albicans are in high number%. In vaginal swabs collected in those patients of vaginitis. Candida albicans comprises a very high proportion of isolates (90%) in none of the study reported by Odds, was preparation less then 70%.

Kimura and Pearsal (1978), demonstrated the capacity of Candida albicans to adhere in vitro to human buccal epithelial cells and found that adhere use was significantly greater in human saliva than in phosphate buffered saline.

Similarly Sobel et al, 1981) showed that adherence of Candida albicans in yeast form to human buccal and vaginal epithelial cell occurred, also found that germ tube also increase adherence.

The adherence of Candia albicans to vaginal cells in vitro at ph 6 was considerably greater to than at ph 3-4 orange corresponding to that of normal vagina.

As Candidiosis is more common in mouth and vagina, in vitro more adhesions to exfoliated buccual and vaginal epithelial cells is measured. Adherence is increased by adding high concentration of Sugars (Galactose, maltose sucrose) in media.

Odds (1979) shows that the hyphal production in vivo is probably an indication of active growth but necessarily of tissue invasion and therefore cannot be regarded as absolute for diagnostic evidence for Candidiosis.
Factor Predisposing to Candidiosis:
Condition which favour transition from saprophyte to pathogen and are relevant to vaginal condiosis includes, pregnancy, diabetes mellitus, and use of immunosuppressive drugs, possibly oral contraceptive and antibiotic.

Pregnancy is undisputed factor in pathogenicity possibly by virtue of changes in cell mediated immunity, in glucose metabolism or by provision of a glycogen-rich vaginal epithelium (Winner and Hurley 1966, Crolla et al 1973). In pregnancy there is reduction of T cell activity which may help protect the fetus from rejection (Finn et al 1972) and there are changes in carbohydrate metabolism (Lind and Harris 1977) may favour the growth of Candida.

Yeast is highest in vaginal carriage, during third trimester and there is an abrupt reduction in the incidence of Candida take place in post partum period (Speellacy et al 1971, Odds 1979).

Oral contraceptive contains an oestrogen and a progestogen are the most effective preparations in general use and are called ‘combine’ type. The oestrogen enhances the susceptibility of the vagina to yeast over growth leading some time to vaginitis (Odds 1979).

Antibiotic given orally appear to enhance the carriage of yeast in vagina but it is more the matter of clinical consensus rather than, by scientifically controlled trails (Odds 1979).

Metronidiazole treatment, of Trichomonas vaginitis may predispose patients to
vaginal thrush (Moffett and McGill 1960) Corticosteroid depress the cell mediated immune system favour the growth of Candida.

In respect to infection with the Human deficiency Virus (HIV) and Acquired Immune Deficiency Syndrome (AIDS), there is profound damage to the cell mediated immunity and although it is oropharyngeal candidiosis that is often seen, disseminated candidiosis occur although rarely.

PATHOOGY:
Gardner and Kanfman (1969) noted that, there is mild to moderate inflammatory changes without invasion of vaginal tissue by yeast. It has been shown, however, that in mouth at least, in acute and chronic candidiasis there is invasion of epithelium by hyphae which grow downwards in more or less straight lines without respect for epithelial boundaries.

Certain changes in squamous cells such as radial clumping and emptiness of stained material in the cytoplasm as well as increased nuclear activity and perinuclear haloes have been described in cervical vaginal Papanicolaou smears (Heller & Hoyt 1971).

CLINICAL FEATURES:

Symptoms:
Discharge: thick, white cottage, cheese like form plaques, stick to walls and leave hemorrhagic spots when removed.
Intense purities: Purities is out of proportion to discharge.
Dyspreunia.
Signs:
Erythema and oedema of the libia and vulvar skin.
Odorless, Curdy white discharge per vaginum.
Tenderness observed on examination.

A character of curdy (White) material is found in 20% of non pregnant women and 70% in pregnant women. Plaque may be small (1mm) larger 1 cm) and thick accumulation of exudates.

LABORATORY DIAGNOSIS:

A) Direct microscopic examination:
Wet preparation: material collected from vagina or penis by the plastic loop or swab from posterior fornix or characteristic plaque. On clean glass slide, put a small drop of saline and covered with glass cover slip.

In 10% KOH /Dimethal suphoxide may observed, there by dissolving host cell protein an enhancing the visibility of fungal element, under microscope under 40 X magnification.

Gram staining: Shows presence of gram positive yeast and pseudohyphae of Candida spp.

B) Culture: Clinical specimen should be cultured on Saboraud's dextrose agar with antibiotics and incubated at 37*c.
Colony characteristic of Candida albicans:
Cream coloured, pasty and smooth.
Growth is rapid and mature in 3 days.

The majority of clinical isolates from the genitalia will be Candida albicans for which 2 rapid test are use to conform.

1) The germ tube test (Reynoldss-Braude phenomenon):
The culture of Candida albicans species is treated with normal human serum and incubate at 37\(^\circ\)c for 2-4 hr. A drop of suspension is examined on the slide under microscope.

The germ tubes are seen as long tube like projection extending from the yeast cells. There is no constriction at the point of attachment to the yeast cell as seen in case of Pseudohyphae.

The germ tubes are formed, within two hours of incubation, in Candida albicans and Candida dubliensis.

Occasional isolates of Candida stellotroidea also produced germ tube as its DNA is identical to that of Candida albicans.

2) Chlamydospore formation (test):
The suspected strain of the Candida isolates is grown on cornmeal agar (CMA) or rice starch agar (RSA). Incubate at 25\(^\circ\)c

It shows the formation of large, highly retractile thick-walled, terminal...
Candida albicans and Candida dubliensis forms chlamydosporas with two hours of incubation.

Biochemical Test:
Sugar fermentation and assimilation are of immense importance in identification of yeast isolates.

Accurate identification of the species Candida is important as prognostic and therapeutic implication new methods like:

a) Capacity of adherence epithelial and other surface

b) DNA: DNA hybridization provides more accurately Candida species and analysis of genetic relationship. (Riggsby 1985).

TREATMENT
In vaginal Candidosis treatment is based on the symptoms of puritus. On basis, of clinical appearance and detection of yeast on investigation, also a treatment is carried out.

In treatment of candidiosis, nystatin and amphotersin, imidazoles, clotrimazole and miconazole are routinely used.

In case of systemic candidiosis; imidazole, ketacanzole and 5-flourocytosine may use, may lead hepatotoxic as a side effects, so use with casino.
TRICHOMONIASIS:

Trichomoniasis is caused by sexually transmitted flagellated parasite, Trichomonas vaginalis. It is a common cause of vaginitis. Although asymptomatic in men, it is a most common curable STI worldwide. It is encountered in sexually active women of all age group, but less common after age of 40 years.

HISTORY: Donne reported his observation of "animalcules" in purulent discharges from the genital tracts of men and women. Ehrenberg and colleagues suggested the term Trichomonas vaginalis (T.V.) Hoehe correlated characteristic clinical vaginal disease with the presence of trichomonas. Isolation of Trichomonas vaginalis in pure culture d by Trussel and plass.

In 25% of women in the reproductive period, the parasites harbor in the vagina in asymptomatic state. The organism has affinity for squamous epithelium.

The vaginal infection (predisposing factor) includes when the PH of the vagina is raised to 5.5-6.5 example during and after menstruation. Suppressive of lactobacilli, increase anaerobic flora and Mycoplasma hominis found in many cases.

The inflammatory response includes infiltration with polymorphoneuclar leucocytes (PMN).
Also after sexual stimulation
The incubation period, as far as can be accessed is between 5-28 days.
MORPHOLOG OF PARASITE:

- Unicellular protozoan flagellate measures 7-23 μm x 5-12 μm.
- Exist in only trophozoite form. No cyst stage.
- They have four anterior flagella and one lateral flagellum which is attached to the surface of the parasite to form undulating membrane.
- The undulating membrane is supported at the base by a road-like structure known as costa.
- The axostyle runs down the middle of the body and ends in the pointed tail-like extremity.
- A round nucleus is located in the anterior portion.
- Actively motile
- Pathogenic organism such as Chlamydia and bacteria adhere to the surface of motile Trichomonas and are carried upwards in the genital tract to cause fatal disease and subsequent infertility.

Trichomonas vaginalis exist only single form that is trophozoite. It is pear shaped actively motile organism measures about 10 x 7 μm, replicates by binary fission. It inhabits the lower genital tract of females and the urethra and prostate of males. The parasite lives on the mucosa feeding on bacteria and leucocytes. Trichomonas vaginalis is an obligate parasite. The organism can survive for a few hours in most environments and it is sexually transmitted from person to person.

TRISMSION: The sexual contact is the usual mode of infection in adults. The accidental infection from the inadequately sterilized instruments, gloves, toilet sheets, most contaminated towels and the common bath is possible but difficult to prove.
CLINICAL FEATURES:

Symptoms:
1) There is sudden profuse and offensive vaginal discharge
2) Puritus of the vulva is the second most common complain
3) Dysuria and frequency of maturation

Signs:
1) There is thin, greenish yellow, frothy and offensive discharge per vaginum
2) Vulva is inflamed
3) Tenderness present. The vaginal walls become red and inflamed with multiple punctuate hemorrhagic spots. Similar spots are also found over the mucosa of the portio vaginalis part of the cervix giving “Strawberry” appearance.

Complication: Trichomoniasis does not usually produce any complication nevertheless the spread of organism to the fallopian tubes has been reported. There is no proof that trichomoniasis affected the outcome of the pregnancy. In males, the possibilities not proven, includes epidymitis and the chronic prostatitis. There is no proof of any interaction between trichomoniasis and HIV infection.

LABORATORY DIAGNOSIS:
A) Wet mount preparation: Demonstration of motile trophozoites of Trichomonas vaginalis in wet mount of high vaginal swab, after adding normal saline. It is simple and rapid method to detect the infection.
1) Fixed smears may be stained with any of three stains papanicolaou, Giemsa, Leishmen and periodic acid schiff stain.

2) Fluorescent stain: The parasite can be detected by fluorescent microscopy by staining with fluorescein labeled monoclonal antibody

3) Fluorescent staining by using acridine orange stain

B) Culture:
A high vaginal swab can be inoculated in various culture mediums like,

1) Trussell and Johnson’s medium-Consists of proteose peptone, sodium chloride, sodium thioglycollate and normal human serum.

2) Simplified trypticase serum medium

3) Diamonds’ medium

4) Plastic envelopes
- Optimum PH is 5.5 – 6.0
- After inoculation the medium should be incubated at 35-37°C and were examined microscopically on alternate days for up to 7 days for detection of presence of motile trichomonas. Culture techniques are most sensitive.

C) Other methods available:

1) PCR method

2) Nucleic acid hybridization

MANAGEMENT: The metronidazole with up to 95% cure rate is the drug of the choice. The recommended regimen is 200 mg. orally twice daily for 5 to 7 days or 2 gram orally single dosage. It should be taken with or following meal to reduce the possibility of the gastric side effects. Alcohol should be also avoided to prevent the disulfiram.. likely reaction.
All the sex partners including those who are asymptomatic should be examined and treated. The patient should be advised to abstain from the sexual intercourse until patient and the sex partner have completed treatment and symptomatic relieved.

The treatment failure in some case includes, the compliances, lack of absorption or inactivation of drug by the vaginal flora such as hemolytic streptococci and metronidazole resistance, double dosage 400 mg. orally, three times a day. A high vaginal swab taken for culture to identify other bacteria and should be treated appropriated before retreating metronidazole.
HISTORY TAKING, EXAMINATION OF PATIENT, COLLECTION OF SPECIMENS AND COMMON PRESENTING SYMPTOMS:

History Taking:
The attitude of the clinical staff must be friendly well coming and nonjudgmental as many patients who come to STI clinics are emotionally disturbed and embarrassed.

Accurate history and relevant physical including genital examination which are all essential components for the appropriate management of patient.

History includes method of contraception, menstrual history, pregnancies and their outcome, past history.

Patient should be given adequate explanation, to what the examination, entails and the test, which will be carried out. Patient should be examined in privacy. The patient should be told of normal finding and informed of any abnormal sings. The history of course, the least expensive way of working diagnosis, an accurate history, suggest the correct diagnosis, where as physical examination and subsequent investigation conform this impression.

Obtaining History:
It is useful, to make notes, whilst questioning the patient. At the end of, the history and examination, a detailed record is made. The record is must, be a sequential accurate account of the development and course of the illness or illnesses of the patient.
A sick patient some time emphasize irrelevant facts and target about very important symptoms for this vision a systematic approach to history taking and recording is crucial

Each of the presenting complains has to be talked about in detail with the patient. When writing down the history of presenting illness the event should be placed in chronological order or in chronological order for the genital (all) system and their

Current Symptoms: In general, a number of facts have to be uncovered about every symptom. Those include the duration, the mode of onset, the site, especially of pain, the character, the severity, aggravating or living factor and associated symptoms

**Duration:** when the symptom, first began and try to done, this as accurately as possible, for example ask the patient what was the first thing, she noticed that was unusual or wrong. Also she had a similar illness, in the past. It is often helpful, to ask patient when then last felt entirely well

**Mode of onset and pattern conformed** by whether, the symptom comes, on rapidly, gradually or instantly or date of onset. Also whether, the symptom has been present continuously or intermittently determine if the symptom, is getting worse or better and if so when the change occurred

In **case of site and Radiation**, where the symptom is and whether it is localized or diffuse, point to actual site on the body. Also determine if the symptom if localized radiates to a distant site. This mainly, applies if the symptom is pain
other symptoms, such as cough, dyspnoea, changes in weight or giddiness, weakness, diarrhea, vomiting.

**Character of symptoms is includes**, in case of pain, it is sharp or dull, stabbing burning or cramp like. Most such description of the character of pain is difficult to interpret

**Severity** is subjective; the best way to assess, severity is to ask the patient, if the symptom interferes with normal activities or sleep. Severity can be graded from mild to very sever.

A mild symptom, can be ignores while a moderate symptom cannot be ignored but dose no it interfere with daily activities. A sever symptom interfere with daily activities, while a very sever symptom markedly interferes, with most activity.

**Recurrent treatment:**
Asking the patient, if she is currently taking any tablets on medicines (the use of the word drug may cause alarm) often be described by colour or size rather than by name and dose.

Note the dose length of use and the indication for each drug. Specifically if a women is taking the contraindication pill because it is not consi dered a medicine or tablet by many who take it.

**Sexual history:**
The sexual history is important particularly if there is a history of urethral
discharge, dysuria, and vaginal discharge, a genital ulcer or rush, pain on intercourse or ano-rectal symptoms or if the acquired immune deficiency syndrome (AIDS) or hepatitis is suspected. It should be ask about, the last date of intercourse, number of contacts, homosexual or bisexual and contact with prostitute.

The type of sexual practice may also be important for examples, oro anal contact may predispose to colonic infection pre-rectal contact to hepatitis B and C or AIDS, while interesting object in to the rectum may cause trauma

Menstrual History:
In all cases menstrual history should be obtained it is particularly relevant for the patient with abdominal pain on endocrine diseases or genitor urinary symptoms write down the date of last menstruation period. The age at, which menstruation began. If period are regular or whether menopausal has occur.

Past history includes, she has had any serious illness or operation or admission to hospital in the past. It is important to obtain the particular of each past illness, including the symptoms experienced last performed and treatment prescribed. In case of medication or treatment, the patient may have had in past which remain relevant. These include corticosteroid, oral contraceptive anti hypertensive chemotherapy and radiotherapy. Note any adverse reaction which has occurred in the past. It is necessary to mention also, any allergy to drug and allergic reaction involved.

The social and personal history includes the whole economic, social, domestic and industrial about place of residence. The level of education obtained. Race is
important in some disease, such as thalasaemia and sickle cell anemia

EXAMINATION OF PATIENT:
All patient should be given adequate applicable as to what the examination entails and the test which will be carried out.

The patient should be examined in privacy. The patient should be told of normal finding and informed of any abnormal sings. The patient should be in a lithotomy position, for examination of female genitalia. An angle poise lamp is directed appropriately.

Examine first of all, skin of pubic, vulval, peri-anal area and thighs. The inguinal glands are palpated and pubic hair examined for lice and nits. The labia minora are then separated and any discharge from the introitus noted. The urethral meatus is wiped clean and inspected for redness and urethral discharge which may become obvious only after massaging the urethra with index finger in the vagina.

Beads of pus may be seen at para-urethral duct orifices if Skene's glands are infected. The gram stain and culture may be made from the pus. Specimen for Chlamydia and for smear and culture for gonorrhea should be obtained by insertion and gentle rotation of the swabs 1-2 cm into the terminal urethra.

The collection procedure is painful, should be performed with care after the patient has been so informed. Bartholin's glands are then palpated with finger in the introitus and thumb on the outer side. The normal glands are not palpable. If infected the glands may be tender and a beads of pus may be seen at duct
orifice(s) which may be inflamed. The duct may need to be massaged on each side and any discharge should be sampled for smear and culture. Even clear mucus may occasionally yield *N. gonorrhoeae* and/or *Chlamydia trachomatis*.

A bivalve vaginal speculum moistened with worm water is then passed, directed towards the posterior vaginal fornix. Vaginal wall inspected and the quality and quantity of any vaginal discharge noted.

Specimen are obtained to ascertain the vaginal pH and for the amine test, wet film for the detection of *T. vaginalis*, yeasts and clue cells, Gram stain for yeast clue cells and other morphotypes. Vaginal material for pH determination should be obtained with care, so to avoid contamination with cervical mucus, which has pH of about 7.0.

A narrow range pH paper is touched with vaginal swab or placed on the surface of speculum after removal from the vaginal or pH indicator held in the forceps may be applied to vaginal wall near the lateral fornix.

The external os of the cervix is then wiped with cotton wool held in a sponge holding forceps. The condition of cervix with regard to ectopy, congestion and character of cervical discharge are noted.

Nabothion cyst or follicles are sometime seen on the cervix when the duct of glands in cervix is blocked by the surface epithelial cells. The cervical specimens are then obtained by inserting cotton-tipped swabs about 1-2 cm into endocervix and firmly rotating them for about 10 sec to obtain adequate material.
For N.gonorrhoeae a smear is spread thinly on a microscopic slide for Garm staining and a swab for culture is taken in suitable transport medium.

Chlamydia are obligate intracellular organisms. The swabs are rubbed against the mucous membrane to obtained the infected cells for culture and antigen detection tests.

The swab for culture is then placed in the Chlamydia transport medium and stored in refrigerator at 4°C until sent to the laboratory. If inoculation is not possible within 24hr the specimen, the swabs are placed in appropriate transport medium and need not refrigerator.

Bimanual pelvic examination is then carried out to ascertain the condition of uterus and appendages. Movement of cervix and examination of fornices are not normally painful.

Rectal specimens are taken if indicated (with or without proctoscopy). Because of culture taboos rectal examination, may have to be restricted to proven gonorrhea contacts.

For female children the genital examination must be very gentle. A speculum should not be used, specimen obtained with a platinum or plastic loop or a cotton wool swab from the vagina, urethra and the rectum.

A blood specimen is taken for syphilis serology to indicate past or present infection and to serve as baseline for future tests. After, appropriate, information/counseling a hepatitis screen and HIV serology should be offered to
all patients.

Other investigation including darkfield examination for Treponema pallidum throat swab, urine culture, blood counts, blood chemistry, lumber puncture, radiography etc are carried out as indicated.

Records: The patient records should be legible and include the history, clinical finding, results of investigations, the treatment given and compliances, any side effects the dates of follow up, partner notification and outcome of treatment.

Common presenting symptoms: Some men and women with STI have no symptoms. They attended clinics contacts of patients known to have on STI after they have exposed themselves to risk of STI. They could be harboring one or more STI agents and hence should be examined and tested as thoroughly as those with symptoms.