**LESSON 21.
Microbiological diagnosis of infections of the central nervous system and urogenital system**

**LESSON PLAN:**

1. Central nervous system, brief anatomical and physiological information

2. Inflammatory diseases of the brain and meninges, meningitis, encephalitis, causative microorganisms.

3. Microbiological examinations conducted during inflammatory diseases of the brain and meninges.

4. Liquor collection, shipping and examination methods.

5. Organs of the urinary system, brief anatomical and physiological information.

6. Normal microflora of the urinary tract, inflammatory diseases and their causative agents.

7. Microbiological examination of urine. Bacteruria, its determination and evaluation. Diagnostic value of microscopic examination of urine

8. Genital organs, brief anatomical and physiological information.

9. Sexually transmitted diseases.

10. Inflammatory diseases of female genital organs, material collection and examination rules.

11. Understanding of TORCH infections. Transplacental diseases and their diagnosis.

12. Inflammatory diseases of male genital organs, material collection and examination rules

**Pathogenesis and clinical forms of meningitis**

• Meningitis is an inflammation of the meninges of the head and (or) spinal cord. Depending on which membrane of the brain is involved in the process, such as leptomeningitis (inflammation of the soft and arachnoid membranes), arachnoiditis (isolated inflammation of the arachnoid membrane), pachymeningitis (inflammation of the hard membrane) Forms are distinguished.

• During meningitis, inflammatory changes occur not only in the membranes of the brain and spinal cord, but also in the ependyma of the ventricles and vascular sheaths, which is accompanied by hyperproduction of cerebrospinal fluid and an increase in pressure.

• During meningitis, there are changes in the composition of the cerebrospinal fluid: a change in cell composition - an increase in the number of leukocytes with polymorphous nuclei, a decrease in the amount of glucose and an increase in the amount of protein are observed.

• Meningitis, which can be caused by practically most microorganisms, is a polyetiological disease.

• Meningitis is often caused by bacteria. Neisseria meningitidis, Streptococcus pneumoniae, Haemophilus influenzae are the main causes of bacterial meningitis in children.

• *Sometimes Staphylococcus aureus, S.epidermidis, Streptococcus pyogenes, E.coli, Klebsiella, Proteus, Pseudomonas, Listeria monocytogenes, Brucella, as well as Candida* and other fungal etiological meningitis. comes st.

• primary and secondary meningitis

• acute and chronic meningitis. Chronic meningitis is mainly caused by M.tuberculosis and fungi.

• Since most bacterial meningitis has a purulent character, they are sometimes called purulent meningitis.

• In some cases of meningitis, it is difficult to detect microorganisms in the cerebrospinal fluid, or they are not detected at all. Such cases are characterized as aseptic meningitis. Aseptic meningitis is mainly caused by viruses, but meningitis caused by *Mycobacterium tuberculosis, Leptospira, Cryptococcus, and Tochoplasma gondii* are also referred to as aseptic meningitis.

• When the submeningal structures of the brain - the brain stem are also involved in the inflammatory process, the process acquires the character of meningoencephalitis. Inflammation of the brain stem is called encephalitis, and inflammation of the spinal cord is called myelitis. Encephalitis, myelitis, as well as encephalomyelitis are mainly caused by viruses.

• Brain abscesses are caused by anaerobic streptococci, Bacteroidetes, and post-traumatic abscesses are more often caused by *staphylococci and streptococci. In less cases, abscesses caused by H. influenzae, A. israelii, N. asteroides and amoeba* are also observed.

• The clinical picture of all forms of bacterial (purulent) meningitis is primarily characterized by high fever and meningeal syndrome.

• N.meningitidis, S.pneumoniae and H.influenzae are the main causative agents of meningitis.

• E.coli is the main etiological agent of meningitis among bacteria from the Enterobacteriaceae family.

• *Staphylococcus aureus, S.epidermidis, Streptococcus pyogenes, Klebsiella, Proteus, Pseudomonas, Listeria monocytogenes, Brucella,* etc. Etiological meningitis is also found.

• Although viral meningitis and encephalitis manifest with symptoms similar to purulent meningitis, they usually present with milder clinical symptoms. Changes in the cerebrospinal fluid are weak compared to purulent meningitis, bacteriological examinations usually give negative results (aseptic meningitis). In viral meningitis, the disease manifests itself as meningoencephalitis, as the brain tissue is often involved in the process.

• Viral meningitis and encephalitis are mostly caused by enteroviruses (poliovirus, coxsackievirus and ECHO virus) and epidemic parotitis virus. Sometimes it can be caused by herpesviruses, including cytomegalovirus, measles, rabies, and arboviruses.

• Systemic endemic mycoses, as well as opportunistic mycoses, can be accompanied by damage to the CNS.

• Recently, the increasing role of Candida fungi, especially C. albicans, in the etiology of purulent meningitis has been observed.

• Cryptococcal meningitis is observed in approximately 5-8% of AIDS patients. In rhinocerebral mucoromycosis caused by fungi from the Mucorales family of the Zygomycota type (genera Mucor, Rhizorus, Absidia, Rhizomucor, etc.), sporangiospores enter through the nasal cavity and invade the blood vessels. transformation into hyphae causes thrombosis, infarction and necrosis.

• Liquor material is the main examination material in CNS infections.

• Liquor for the examination is obtained by a specialist doctor, strictly following the rules of aseptic.

• Any microorganism detected as a result of microbiological examination (contamination must be excluded!) confirms the etiological diagnosis, since the liquor is normally sterile.

• Analysis of the cellular composition of the cerebrospinal fluid may be important to indirectly determine the etiology of CNS infections.

• In the microbiological examination of the liquor, smears are prepared for microscopic examination from its sediment obtained by passing it through a centrifuge, after staining with methylene blue and Gram method, microscopy is performed.

• If the liquor is very cloudy, it can be examined without centrifugation.

• The rest of the liquor is used for bacteriological examination. Bacteriological examination is carried out by inoculating liquor sediment to various nutrient media - EPA, glucose EPA, serum EPA, blood agar, chocolate agar, Saburo medium.

• Obtained cultures are mainly identified by morphological, cultural, biochemical and antigenic characteristics.

• In most cases, the recovery of microorganisms from cerebrospinal fluid indicates their etiological role.

• Virological tests are applied during aseptic meningitis and are carried out by inoculation of cerebrospinal fluid into cell cultures, sometimes into laboratory animals.

• Serological examinations. In acute and convalescent periods of viral diseases of the CNS, a four-fold or more increase in the titer of anti-virus antibodies in the blood by means of IFA confirms the diagnosis.

**Urinary tract: normal microflora**

• The organs of the urinary system include the kidneys, renal pelvis, ureters, bladder, and urethra.

• Normally, the kidneys, renal pelvis, ureters, and bladder are sterile and microorganisms are not found here.

• However, in the distal part of the urethra - in its lower third, coagulase-negative staphylococci, greening and non-hemolytic streptococci, lactobacilli, Corynebacterium and Neisseria. pathogenic bacteria, gram-negative aerobic bacteria, as well as some representatives of the family Enterobacteriaceae, anaerobic cocci , Propionobacterium genus, gram-negative anaerobic cocci and bacilli, commensal species of Mycobacterium and Mycoplasma genera, Candida and other genera yeast-like fungi can be found.

• The clinical manifestation of pathological processes in the urinary tract depends on the localization of the process.

• Fever, hematuria, leukocyturia, sometimes proteinuria, dysuric symptoms are observed during pyelonephritis.

• During cystitis, pain in the inguinal region, often painful, burning urination and transient hematuria, changes in the color, transparency and smell of urine, etc. is observed.

• Dysuria in urethritis, swelling in the urethra, pain, dysuric symptoms, etc. is considered the main sign. Sometimes, symptoms of urethritis can be observed in the absence of clinically significant bacteriuria. This condition, which is observed in more women, especially in sexually active women, is called acute urethral syndrome.

• The number of microorganisms in the urine of practically healthy people usually does not exceed 104 per ml.

• The number of microorganisms in 1 ml of urine exceeding 105 is considered as an indicator of urinary tract infection - clinically significant bacteriuria.

• When this condition is not accompanied by clinical signs, it is called asymptomatic bacteriuria.

• Sometimes many diseases and pathological processes unrelated to the urinary tract, as well as manipulations, can be accompanied by transient bacteriuria. In the absence of pathological processes in the urinary tract, bacteriuria is usually secondary and is not detected in subsequent examinations.

• Microbiological examination of urine is one of the main diagnostic methods for urinary tract infections.

• For examination, the middle part of the morning urine is taken in a sterile glass container. If it is not possible to conduct the examination on time, the urine can be kept in the refrigerator at +40C for 24 hours.

• If the patient is unable to urinate freely, then urine is taken for examination by means of a catheter or by puncturing the bladder from the inguinal region.

• **Microscopic examination.** Microscopy of the urine sediment obtained after centrifugation shows the presence of microorganisms, signs of inflammation, salts, etc. allows to draw conclusions about its existence.

• A smear is prepared from the obtained sediment and usually subjected to microscopy after Gram and Giemsa staining. However, microscopy of urine sediment does not allow to determine the degree of bacteriuria.

• To quickly calculate the quantity of bacteria in a urine sample, a smear is prepared from uncentrifuged urine and subjected to microscopy. Detection of one bacterial cell or one leukocyte in each visual field is equivalent to clinically significant bacteriuria.

• The most size (caliber) loop method is used to evaluate bacteriuria.

• After the examined urine is carefully mixed, it is inoculated into a solid nutrient medium with a loop of known capacity. For this, the loop is removed by inserting it into the examination material in a vertical direction. The material on the hook is initially inoculated on the surface of the nutrient medium in the petri dish in a straight line along the diameter of the dish, and then spread by means of lines perpendicular to this line.

• After incubation, the developed colonies are counted and the degree of bacteriuria is determined by taking into account the capacity of the loop. For example, if the capacity of the loop is 0.001 ml, then the number of bacteria in 1 ml of urine is calculated by multiplying the number of colonies by 1000.

• Received pure culture is identified and sensitivity to antibacterial drugs is determined.

• The detection of more than two types of microorganisms in the examined urine indicates that the sample was taken incorrectly, in such cases, the examination is repeated.

• Most agents of sexually transmitted diseases in men enter the body through the mucous membrane of the urethra. In such cases, urethritis develops. Neisseria gonorrhoeae, Trichomonas vaginalis, Chlamydia trachomatis, Mycoplasma hominis, Ureoplasma urealyticum are the main causative agents of urethritis.

• Urethritis is named after the causative agent. For example, gonococcal urethritis, chlamydial urethritis, etc. The term non-gonococcal urethritis is also used to distinguish other urethritis not related to gonococci.

• Prostatitis - inflammation of the prostate gland can be of non-infectious or infectious origin. Infectious agents enter the prostate gland usually through the urethra.

• In some cases, the causative agents of prostatitis are the causative agents of urinary tract infections, especially enterobacteria (E. coli, Klebsiella, Proteus genera, etc.), as well as P. aeruginosa, enterococci, S. epidermidis, Candida fungi. it can happen.

• During sexually transmitted diseases, the causative agents enter the prostate gland usually through the thyroid gland. During urethritis, causative agents can enter the posterior urethra, including the prostate gland, and damage it.

Thus, prostatitis caused by sexually transmitted microorganisms such as gonococcus, trichomonad, chlamydia, etc. may be of origin.

• Infections of the female genital organs can be conventionally divided into two groups: non-sexually transmitted diseases and mainly sexually transmitted diseases. Sometimes it is not possible to detect such big differences between these infections.

• Vulvovaginitis is an inflammatory disease of the vulva and the genital tract, often occurring together. Sometimes it is caused by facultative microflora in the vulva and genital tract.

• Although the main causative agent of bacterial vaginosis is Gardnerella vaginalis, a number of anaerobic bacteria, Mobilincus genus bacteria have a synergistic role in the development of pathology.

• The causative agents of vulvovaginitis caused by sexually transmitted infections can be gonococci, T. vaginalis, C. trachomatis, M. hominis, U. urealyticum. In all cases, the infection spreads through the thyroid gland, inflammation of the cervix - cervicitis, inflammation of the cervical canal - endocervicitis, inflammation of the mucous membrane - endometritis, inflammation of the fallopian tubes - salpingitis, inflammation of the ovaries - can cause oophoritis.

• The causative agents of some sexually transmitted diseases can penetrate directly through the skin and form vesicular lesions and superficial wounds.

• Vesiculosis lesions are mostly caused by viruses, mainly herpes simplex virus. SHV type II infections are also known as genital herpes because they are sexually transmitted.

• Infectious damage to the skin of the genitals can sometimes manifest itself in the form of superficial wounds and erosions. During syphilis, a hard chancre is formed on the surface of the skin where Treponema pallidum enters. Damage caused by Haemophilus ducrei on the skin surface is in the form of soft chancre - chancroid. During a rare disease called Donovanosis (caused by Klebsiella granulomatis), red pus-free wounds with white edges are observed.

• Growth of inguinal lymph nodes (buboes) is sometimes observed in genital infections. This is characteristic of primary syphilis, genital herpes, lymphogranuloma venereum, and mild chancre. Generalized lymphadenopathy is also characteristic of AIDS.

• Examination materials and their collection rules. During urethritis, material is taken from the urethra for examination. The material from the urethra should be taken in the morning, before urination. Depending on the amount of urethral discharge, its removal is carried out by different methods.

• If the discharge is abundant, the material can be removed with a bacteriological loop or a special cotton swab.

• If there is little discharge, it is necessary to take scraping material from the mucous membrane of the urethra, not the urethral discharge itself, especially when examining for chlamydia and other intracellular microorganisms. The material taken in this way contains a large number of epithelial cells, which is necessary for microscopic examination in the indicated cases. For this purpose, it is possible to use other cytological brushes (cytobrush) than the urethral tampon.

• Examination materials and their collection rules. The material from the vagina and vulva is removed with a sterile cotton swab.

• Gynecological mirrors are used to increase the visualization effect during the removal of material from the birth canal, and fluid accumulated in the rear arch of the birth canal is obtained.

• In order to detect intracellular microorganisms, the material is removed from the mucous membrane by scraping with a cotton swab.

• Mucus from the cervical canal - endocervical canal is first removed with a sterile tampon, then a special (urethral) tampon is inserted into the cervical canal, rotated and removed while touching its walls.

Rules for conducting microbiological examinations.

• A smear is prepared from the secretion taken from the urethra, and after staining with Gram's method and methylene blue, it is subjected to microscopy.

• With this method, the diagnosis of gonococcal and trichomonad urethritis is often determined.

• It is possible to determine the diagnosis of bacterial vaginosis by determining "key cells" by means of microscopy of smears prepared from the material taken from the genital tract and stained by the Gram method.

• Ch.trachomatis, M.hominis/genitalium and U.urealyticum/parvum antigens can be detected by IFR in smears.

• When conducting an examination by means of IFR, the material (rich in epithelial cells) taken with a swab is spread on a glass slide and fixed with acetone.

• After working with a specific antibody conjugated with fluorochrome, the preparation is carefully washed with a buffer solution, dried and examined under a luminescent microscope.

• All the means and reagents required for the indicated procedure are included in special test systems for determining chlamydial antigens and are commercially available.

• Recently, PCR, cultivation and microtest systems are increasingly used to detect the indicated microorganisms.

• Microbiological examination of prostate gland secretion, ejaculate, as well as urine is performed during prostatitis, prostatitis and vesiculitis.

• Prostate secretion is usually achieved by massage after urination. Smears of the secretion can be prepared and subjected to microscopy. A native crushed drop preparation can be prepared.

• Ejaculate is obtained artificially for examination. In microbiological tests, a crushed drop preparation is prepared and inoculated into nutrient media. Examination of ejaculate is also recommended for epididymitis and orchitis.

• In order to clarify whether the pathological material is really in the prostate gland, it is recommended to conduct an examination of the urine taken before and after the massage of the prostate gland.

• Cultural examinations. From the urethra, the urethra, the neck, etc. obtained excreta, aspirate materials, ejaculate, prostate gland secretion, urine, if necessary, cultural examinations are carried out by inoculation into appropriate nutrient media.

• Serological examinations. In the diagnosis of sexually transmitted diseases, reactions based on the determination of specific antibodies in blood serum are also used.

• In syphilis, VDRL and RPR-tests allow to detect non-specific - non-treponemal antibodies. Only specific antitreponemal antibodies can be detected by means of TPHA and IFR.

• ELISA is used in the diagnosis of herpes and CMV infections. Separate determination of IgM and IgG antibodies allows to distinguish past and current infections.

• IFR is important in the detection of causative agents and their antigens in pathological materials in the diagnosis of sexually transmitted infections.

• In some cases, in microbiological practice, it is necessary to determine the diagnosis of transplacental infections that damage the fetus, cause its death or abortions.

• Some of these diseases are sexually transmitted, and in their diagnosis, the examination of the stillbirth, placenta, and fetal fluid is of certain importance. Transplacental diseases include listeriosis, toxoplasmosis, rubella, cytomegalovirus, genital herpes, parvovirus infections, syphilis, AIDS, etc. belongs to

• Reagents for specific IgM-antibody tests are now commercially available to detect TORCH infections (toxoplasmosis, rubella, cytomegalovirus, herpes). The detection of IgM in the blood serum of newborns in the indicated infections indicates the infection of the newborn. Thus, IgM antibodies are not transplacentally transmitted to the fetus.