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DEPARTMENT OF CLINICAL LABORATORY DIAGNOSTICS

A manual on

CLINICAL BIOCHEMISTRY

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The manual gives the fundamentals of clinical biochemistry that is necessary for understanding the mechanisms of formation of pathological processes in the human body, for the interpretation of methods of laboratory diagnostics, prevention and treatment.

The manual is compiled in accordance to the program of clinical biochemistry for students of faculty of general medicine and focused on training of physicians according to the qualified characteristics.

1. Topic: The basis for the organization of laboratory services. Clinical biochemistry in the structure of the clinical-diagnostic researches. The main tasks and methods of laboratory examination, clinical characteristics of laboratory tests. Taking and preparation of the blood and urine for laboratory research.

Clinical laboratory diagnostics (CLD) as a medical diagnostic specialty consists of a combination of in vitro studies of biological material of the human body based on the use of **hematology, general clinical, parasitic, biochemical, immunological, serological, molecular biological, bacteriological, genetic, cytological, toxicological, virological methods;** its objective is to compare the results of these methods to clinical laboratory data and to formulate conclusions.

Methods of CLD are used in clinical practice to:

- confirm the clinical diagnosis or clarify,
- determine the cause of the disease (genetic or infectious diseases, poisoning),
- characterize the form, severity and prognosis of the disease,
- choose of etiologic and pathogenetic therapy
- monitor the outcomes of treatment,
- detect of pathology in screening studies.

Fundamentals of the Theory of Clinical Laboratory Diagnostics:

1) development of laboratory methods;

2) development of requirements for quality of implementation of analytical methods and means of providing these requirements ;

3) the establishment of normal individual fluctuations of each studied parameter composition and properties of biological fluids and tissues ;

4) the study of natural connections laboratory abnormalities identified with the essence of the pathological process in specific diseases;

5) establishment of diagnostic , differential diagnostic and prognostic value of individual laboratory tests and their combinations;

6) establishment of diagnostic laboratory programs to optimize diagnosis.

In accordance with the objectives and methods of researches in CLD, the following sub-disciplines are distinguished:

- Clinical Chemistry
- Hematology
- Cytology
- Genetics Lab
- General clinical research
- Immunology
- Isoserology
- Molecular Biology
- Bacteriology
- Parasitology
- Virology
- Toxicology
- Coagulation

Session Purpose: Introduction to the basic principles of laboratory diagnostics. Knowledge of the methods of obtaining and preparing biomaterial for laboratory researches.

The student should know about:

- the basis of the organization of laboratory service;
- purposes, tasks, and the role of laboratory diagnostics in the practical medicine;
- the concept of diagnostic sensitivity and specificity of the test;
- the basic principles of obtaining biological material for biochemical studies.

The student should be able to:

- to evaluate the accuracy of obtaining biological material for biochemical studies;
- calculate the diagnostic sensitivity and specificity of the test.

For the correct choice of method and interpretation of the CLD indexes it is necessary to know the possibilities of each method, dependence of the results of tests on the conditions of taking material, its transportation, as well as the implementation of compliance tests.

The reliability of the results depends on the quality of laboratory methods, equipment, reagents, calibration materials, the carefulness of the personnel. If a deviation in test results is caused by a pathological condition, a repeat test in most cases shows the nature and recurrence of these deviations. In certain diseases specific changes in laboratory parameters are typical, like the number of white blood cell count, erythrocyte sedimentation rate, the content of some enzymes can be changed at the same time in acute inflammation, etc.

Some laboratory tests are specific for certain disorders or for a specific type of disease (e.g., organ-specific isoenzymes, paraproteins in multiple myeloma), but most tests give results with more or less probability. Thus, an increase in erythrocyte sedimentation rate is observed in bacterial inflammation, in an autoimmune process, and in case of tumors. The criteria of suitability of a laboratory test for the diagnosis of certain forms of disease are specificity, sensitivity, and efficiency of the laboratory test and the method of research. At the same time we should take into account the rate of both positive and false-positive and false-negative results.

• The diagnostic specificity of the test for a specific disease is the percentage of the rate of true negative test results in people not suffering from this disease. For example, consider a method of research with 90% specificity. If we examine 100 people not suffering from the disease, only 90 of these 100 healthy people will get "normal" results (indicating the absence of disease). The remaining 10 people are not affected by the disease as well, but the results will show the presence of this disease. For those 10% this "deviation from normal results" is actually a false-positive result. Specificity is especially important for studies that are used for confirmation of diagnosis of severe diseases**.** The more **specific a method of investigation, the less "false positive" results it produces. False-positive results may lead to misdiagnosis and unnecessary administrations of diagnostic and therapeutic procedures that may worsen the patient's quality of life.**

•Diagnostic sensitivity of a test for a specific disease is only a percentage of the rate of true positive test results in patients with this disease. For example, consider a method of research, which has 90% sensitivity. If 100 people suffering from this disease, are screened using this method, the disease will be detected in 90 out of 100 patients. The remaining 10 examinees also suffer from this disease, but this research method cannot detect it. For these 10% the obtained "normal" results of investigation will be false-negative. Sensitivity of a method is particularly important in cases where you want to exclude the presence of dangerous infectious diseases. **The**

more sensitive is a method of investigation, the less "false negative" results it produces. False-negative results are those, which do not allow an identification of the patient's illness.

Diagnostic significance of positive results is expressed by the ratio of true positives to the total number of positive results, which also includes false positives. The diagnostic value of negative results is the ratio of true negative results to the total number of negative results. The diagnostic efficiency of a test is the ratio of true (both positive and negative) test results to the total number of results. When making out these characteristics of laboratory tests, a correction for the incidence of this disease among the total number of patients is taken into account.

Conditions of taking biomaterial for clinical laboratory researches

The most common materials for laboratory tests are blood, urine and other body fluids.

It is necessary to take the material for laboratory tests before eating (fasting). The last meal should be for 8-12 hours (12 hours for studies of lipid profile) before taking material.

The time of taking biomaterial is from 7 to 9 am during the planned studies and at any time for emergency conditions. It is not allowed to take blood sample for routine biochemical analysis the night before.

Significant physical and muscular stress should be avoided for at least 3 days before taking the biomaterial, as they influence on the results.

Obtaining blood for clinical laboratory researches

1. Native venous blood from large veins (usually from the elbow) without anticoagulants.
2. Venous blood with the addition of anticoagulants.
3. Capillary blood from the finger to determine glucose, clinical analysis of blood and other components;
4. Arterial blood taken from the large arteries (usually the femoral or subclavian) - to determine blood gases.

Venous blood

The use of venous blood for biochemical studies is the most preferable.

Nowadays taking venous blood is done with a thick needle into a glass or plastic tube or industrial vacuum systems, such as Vacutainer. Depending on the material you need to get (serum or plasma), blood is collected in a clean, dry centrifuge tubes without additives (for serum), with the addition of anticoagulants (for plasma).

Capillary blood

Capillary blood is most often used to determine the glucose or blood count. For capillary blood sampling is used disposable sterile lancet or laser drills. Between the amount of received blood and the depth of the puncture is direct relationship. Therefore scarifier should be selected according to the puncture site and the amount of blood required to perform various studies with blades of different sizes.

Clotted and hemolyzed samples are not investigated. Amount of collected blood depends on the number of lab tests and their required amounts of biological material. For biochemical researches at least 6 ml, for reaerches of system of coagulation - 4.5 ml.

The main chemical additives used for blood analysis:

Ethylenediaminetetraacetate (EDTA) - an anticoagulant that prevents blood from clotting by binding and effectively removing the calcium ions present in the plasma (calcium is necessary for blood clotting). EDTA also protects red blood cells from destruction. It is added to the blood for the complete blood count and performance of some other hematological tests

Heparin - an anticoagulant that prevents blood from clotting by inhibiting the conversion of prothrombin to thrombin.It is added to the blood to conduct biochemical studies that require plasma. Anticoagulant properties of heparin are used in therapy

Citrate (as sodium salt, ie, sodium citrate) - an anticoagulant that prevents blood from clotting by binding calcium ions (like EDTA). It is added to the blood to examine the processes of coagulation

Oxalate (as sodium or ammonium salts, ie, sodium or ammonium oxalate) - an anticoagulant that prevents blood from clotting by binding calcium ions (like EDTA). Used in conjunction with sodium fluoride for the determination of glucose in the blood

Sodium Fluoride - is an enzyme poison which stops metabolizing glucose in the blood after it is collected, ie, retains its concentration. It is used in conjunction with ammonium oxalate specifically for the determination of glucose in the blood

Serum

Serum is obtained from spontaneously coagulated whole blood by centrifugation (1000-1200 about 10-15 minutes). It does not contain clotting factors.

Plasma

Plasma is obtained from the blood by removing blood cells. In comparison to serum it contains clotting factors, that is a cell-free supernatant obtained by centrifugation of blood clotting of which is inhibited by addition of anticoagulants. The resulting plasma (upper phase) to select individual filter tips (aerosol barrier) in an amount not less than 1 ml dry sterile plastic tube type Eppendorf.

Obtaining urine for clinical laboratory

Urine collection is carried out after a careful toilet of external genitals to avoid penetration of various substances in the urine.

Urine collected for analysis, can be stored for more than 1.5 - 2 hours (required in the cold). Prolonged standing leads to a change in the physical properties, the growth of bacteria and destruction of elements of urine sediment. The pH of the urine will be shifted to higher values because of the ammonia in the urine excreted by bacteria. Microorganisms consume glucose, so if patient has glycosuria you can get negative or low results of glucose. Bile pigments are destroyed by sunlight. The most appropriate way to preserve urine - cooling (can be stored in the refrigerator, but do not bring to freezing). While cooling corpuscles are not destroyed, but the relative density may influence on the results.

Further collection of urine, depending on the type of study has its own characteristics:

1. For carrying out the general analysis of urine collect only morning urine taken in the middle of urination as it is more concentrated and the pathological elements which have accumulated in kidneys and in urinary ways at night are washed away.

2. For carrying out assay on Zimnitsky (an assessment of concentration ability of kidneys) 8 portions of urine are collected per day

3. To determine the number of formed elements in 1 ml of urine by the method of Nechiporenko (revealing hidden inflammation) middle portion of the first morning urine is collected, no more than 15 - 20 ml.

4. Two glasses test is more often used in urology for women. Urine during urination is divided into two parts. It is important the first portion in this case to be small on volume. Glasses are also prepared in advance and the number of portion is indicated on each container.

5. Collecting of daily urine. The patient collects urine within 24 hours, keeping a usual drinking regimen (1,5-2/ l per day).

DISCUSSION

1. The main tasks of laboratory testing.
2. Basic laboratory methods.
3. Structure and equipment of modern laboratories.
4. The diagnostic specificity of the test.
5. The diagnostic sensitivity of the test.
6. Basic principles and methods of taking blood for biochemical studies.
7. Methods for the production of plasma and serum, types of anticoagulants.
8. Basic principles of taking the urine for laboratory tests: urinalysis, Zimnitsky test, Nechiporenko, collection of daily urine, two glasses test.

INDEPENDENT WORK OF STUDENTS

1.Write down practice session protocol indicating the purposes and tasks, methods of sampling and preparation of biological material for research.

2.Write methods of sampling and preparation of urine for general clinical and biochemical studies.

3.Write down the basic principles of sampling and preparation of blood for biochemical studies.

4.Determine the diagnostic sensitivity and specificity of the test in clinical tasks.

2. Topic: Principles of clinical biochemistry and clinical laboratory diagnostics. Organization of quality control of laboratory researches. Types of laboratory researches. The unification of biochemical methods. The standardization of researches.

Correct diagnostic information through laboratory studies can be obtained by analyzing the normal values of the laboratory test, the limits of intra-and inter-individual fluctuations and the effects of various factors.

Sources of variability of indicators of clinical laboratory diagnostics (CLD) are such biological factors as age, sex, weight, and body surface area (especially important when examining children), monthly and seasonal circadian rhythms, ethnic origin, the conditions in which the material is extracted for analysis (posture, physical stress , fluid intake, smoking, drugs, stress, etc.), as well as climatic conditions and ecological environment in the community patient.

Truly normal (reference) consider the value of laboratory parameters, set in a group of carefully studied healthy subjects aged 20-30 years, and the normal to the contingent, which differs on any grounds (gender, age, profession, place of living, etc.) - the value of these parameters in healthy individuals of this contingent.

Session Purpose: Introduction to the principles of clinical biochemistry, organization of quality control of laboratory researches. Know the concept of screening, preventive and differential diagnostic studies, rapid diagnosis, the unification of biochemical methods.

The student should know about:

- the organization of the quality control of laboratory researches;
- the concept of screening, prevention and investigation of differential diagnosis, rapid diagnosis;
- the concept of standardization of studies.

The student should be able to:

- evaluate the correctness of the quality control in the laboratory;
- select the appropriate tools and techniques of quality control.

Quality control assessments.

To identify and assess the systematic and random errors in the results of measurements made in the laboratory internal and inter-laboratory quality control is performed.

A number of quality criteria are used:

•**Accuracy -** how close the results approach the true value of the measured parameter.

•**Accuracy -** the deviation of the measurement result from the true value;

•**Systematic measurement error -** an error that remains constant or regularly varying upon repeated investigations;

•R**andom measurement error** - an error that varies randomly upon repeated investigations

•**Correctness of measurements** - absence of systematic errors in the results (for the control of the correctness we only take the material with investigated components);

•**Precision of measurements** - lack of significant variation between the results of measurements performed under identical conditions (control of precision and reproducibility of research results may be achieved by using control material with unexamined contents);

•R**eproducibility of measurement results** - no significant differences between the results of measurements performed in different conditions (at different times in different places) Reproducibility of results is characterized by their degree of coincidence in multiple studies of

the same sample of biological material. Reproducibility is expressed by an inverse value of the coefficient of variation. The lower the coefficient of variation, the higher reproducibility;

Laboratory quality control is carried out by comparing the results of measurements made in the laboratory, with the control and determination of the deviation.

For control measurements control materials are used: aqueous solutions of standards, drained blood serum prepared in the laboratory, biological material, made by both with explored and unexplored content of components (serum, plasma, blood cells, urine, cerebrospinal fluid, and t . etc.), synthetic materials.

The main requirements for control materials are identical in physical and chemical properties of the analyzed samples, the stability during storage, minimum variability in the composition and properties of the series, to determine the suitability of systematic and random errors. Precision and reproducibility of research is carried out by control material with unknown contents, to check the correctness it is used the material only with examined contents of the components.

Intralaboratory control includes precision of measurements, reproducibility and accuracy of measurements.

Reproducibility is considered adequate if the value of the coefficient of variation of results in studies of substrates does not exceed 5%, when determining enzyme activity - 10%, which corresponds to the percentage ratio of about 1/8 of the range of normal variations of the studied parameters to the average norm. To evaluate the reproducibility of the results it is convenient to use the following checklists, where we mark the daily results of controlled trials.

Рисунок 1

Рисунок 2

Рисунок 3

If the coefficient of variation is larger than allowed, check the entire course of the analysis, eliminate the causes of unsatisfactory reproducibility and repeat the preliminary stage. Checklist constructed for each monitored parameter and only for this series of the control material. The results of the daily study of the control samples of the same series in the next few days put on the map in the form of points and is used to assess the reproducibility of laboratory testing.

Control of correctness of the results of measurements is carried out if it is good precision. Control methods can be compared with the results of our definitions of the nominal value of the test materials, comparing the results with the results of the reference method, participation in inter-laboratory quality control experiment, additional study samples of the material to which the

previously added the exact amount of pure substance; study samples with different concentrations. The results obtained were processed statistically.

Interlaboratory control - a comparative quality control of results obtained in a number of laboratories using a single control material. It includes control of reproducibility and accuracy, is carried out at least once a quarter under the methodological guidance of the control centers of the national, provincial and regional levels. Control centers define the goals, objectives and procedures for the control experiment, collect and study the results of the control definitions and make recommendations to improve the quality of the laboratory.

An important prerequisite for eligibility of patients in various hospitals (in the clinic, hospital, in different cities, and so on) is to standardize laboratory diagnostic techniques. diagnostics at the country level. Implementation in clinical and laboratory practice of the international system of units has helped to unify the results of laboratory methods. diagnosis in different countries.

Types of laboratory researches

Depending on the clinical tasks laboratory researches can be performed once and repeatedly (over time) as well as in the process of functional or pharmacological tests with the stimulation or inhibition of the test phases of the form of metabolism, cellular or humoral responses, or other functions, expressions, or the quality of which is reflected in the parameters determined in laboratory parameters.

In mass examinations of the population, as well as at the first contact with the patient in the clinic or hospital use multilateral laboratory examination, that does not preclude targeted research conducted as necessary to clarify the diagnosis.

There are also one-time mass preventive screening tests for one indicator. This preventive examinations of large segments of the population with a method having a high capacity to detect a relatively small risk groups in need of further examination. Further examination of high risk is done by the methods, resolution of which allows a final diagnosis of cancer in the early stages of development. Typical examples of screening methods are questioning, fluorography, finger study rectal palpation, study of mammary glands, fecal occult blood, determination of prostate-specific antigen in the blood, CA15-3 marker, identify genetic diseases in children (phenylketonuria and other congenital enzimopathy.) Basic requirements for screening methods: the ideal screening test should not only have a high sensitivity and specificity, but to be safe, widely available and affordable.

Rapid laboratory diagnostics - quick laboratory testing methods that provide a study in 10-15 minutes after receiving the material. Rapid methods are based on the same or similar chemical reactions as the classical methods of analysis. The extensive development of rapid tests was made possible by the achievements of clinical biochemistry and industrial sets of dry reagents (rapid tests) to determine various components of blood, urine and other biological fluids.

There are monotests, ie dry reagents for the determination of any one substance in bioliquid and politests - combined reactive strips, which has several indicator zones, designed to study 5 or more biochemical parameters simultaneously. The analysis can only be qualitative or allow us to determine the concentration of the substance in the test liquid, ie are quantitative. The most common are methods of rapid diagnostics of urine and blood. The use of such tests is particularly valuable in the intensive care unit, where in the clock surveillance of patient needs frequent monitoring of the dynamics of a number of laboratory tests that reflect the patient's condition and treatment efficacy. For rapid testing usually is not required sophisticated

equipment, so they should be used in the emergency department of hospitals, outpatients, visiting patients at home doctor or nurses.

The main sections of laboratory research

Clinical biochemistry - one of the most extensive sections of laboratory medicine, including studies of organic and inorganic substances that are formed during the biochemical reactions and enzyme activity in serum, plasma, blood, urine, cerebrospinal fluid and other biological fluids. Modern instruments for biochemical studies automatically determine simultaneously up to 20-30 indicators, using several microliters of blood. The widespread introduction of methods of "dry chemistry" allows you to move a number of biochemical analyzes of the tube to the special test strips and devices without many indicators to determine almost instantly.

Biochemical analysis of blood and other body fluids is about 40% of all laboratory analyzes. They can be characterized as a state of the whole organism, for example, indicators of acid-base balance, and individual organs, such as organ-specific enzymes. Since the exchange of substances between the organs and tissues is mediated by blood flow in the blood plasma containing different concentrations of all substances entering the body and synthesized it.

Clinical and laboratory immunology - section of laboratory medicine, which provides the definition of the degree of anti-infective and anti-tumor protect of the body using a set of indicators, and laboratory diagnosis and monitoring the effectiveness of treatment of allergic diseases. Man is constantly surrounded by a vast variety of pathogenic bacteria and viruses that are in the air, water, soil, and on surrounding objects, food, and the body of the man itself. They can cause many diseases, but it happens in life is relatively rare, because the body has a complex system of defense against foreign agents - the immune system. The human body can be compared to the state, has a large well-equipped army - immunity. A huge number of "soldiers" - immune cells - is circulating in the blood, "patrolling" all organs and tissues and eliminating not only infectious agents (bacteria, their toxins, viruses, etc.), but also cleansing the body of abnormal, malignant, dying and the transplanted cells (organs). Thus, the main function of the immune system is to recognize and destroy the alien substances. The definition of human immune status includes a set of analyzes that provide a qualitative and quantitative description of immunity and becomes a necessary condition for the successful treatment of many diseases.

Clinical microbiology (bacteriology, mycology, virology)

Microbiological laboratory studies are conducted to identify the causative agents of infectious and inflammatory processes, determine their sensitivity to drugs and monitor the effectiveness of treatment. In recent decades, this area made great progress thanks to the wide introduction of immunological and molecular genetic techniques to accurately determine the specific surface antigens and DNA fragments of viruses, bacteria, fungi, protozoa by immunofluorescence (IFA), enzyme-linked immunosorbent assay (ELISA) , polymerase chain reaction (PCR), DNA probes. This makes it possible to pinpoint agents who are using culture and serological methods can not be identified.

Cytology (exfoliative and puncture)

Cytological diagnosis is to examine the structure and identify abnormalities in the structure of cells derived from exudates, synovial and cerebrospinal fluid from the surface of the mucous membranes, as well as tissues and organs in their biopsy. Puncture cytology is the primary method of preoperative diagnosis and operating benign and malignant neoplasms. Modern methods of automated cytometry, histochemistry, radioisotope studies make cytological analysis quickly and accurately.

Clinical Molecular Biology and Genetics Diagnostic

Genetic material is explored - chromosomes, genes, nucleic acid for detection of different types of mutations underlying hereditary diseases and malformations. Modern methods of DNA diagnostics - hybridization analysis, the amplification of genome, polymerase chain reaction, DNA probes, and other irreplaceable in prenatal diagnosis, and is widely used to identify viruses and bacteria.

Clinical Toxicology

Provides laboratory diagnosis of acute and chronic poisoning caused by organic and inorganic substances, drugs, etc. The high degree of pollution, production of harmful conditions, technological accidents, and many other factors determine the contemporary relevance of this field of medicine.

Clinical and laboratory parasitology

Detects and identifies the agents of parasitic diseases - insects, worms, protozoa. These diseases have a certain geographical and social characteristics of distribution, but due to the high population of migratory activity in humans appear parasitic diseases are not typical for permanent residence, so the parasitology laboratory currently holds the high relevance and importance.

Laboratory control (monitoring) of drug therapy

Using a set of biochemical, physical, chemical, cytological and other techniques, the control of the ratio of the dose and the effect of drugs, their individual pharmacokinetics. This laboratory monitoring is not yet widely distributed, although necessary and effective in drug therapy of tumors, emergency conditions, long-term chronic diseases, etc. Modern automated registration systems provide high speed and accuracy of the analysis.

Clinical blood

When people talk about a blood test, you should always keep in mind that the actual blood is only part of the system, which includes hematopoietic organs (bone marrow, spleen, lymph nodes, liver) and organs, where is disintegration of blood (spleen tissue). All parts of this system are interrelated and interdependent.

Bone marrow is the body in which are born and mature blood cells. After some time the cells enter the blood stream, in which the red blood cells live about 120 days, platelets - 10 and neutrophils only about 10 hours. Moreover, if the red blood cells and platelets in the blood stream function, the granulocytes (neutrophils, eosinophils, basophils) and macrophages - and even in tissues.

Counting the number of cellular elements, which can be made, as in the manual, with a microscope, and automatically determines the functional state of the bone marrow, to diagnose a range of diseases related to the violation of its activities.

In addition, a number of red blood cells, white blood cells, platelets and other elements, the concentration of hemoglobin and erythrocyte sedimentation rate (ESR), can detect the presence of inflammatory disease (pneumonia, rheumatism, arthritis, tuberculosis, etc.).

Studies of blood coagulation

Blood - a unique liquid tissues, has not only flow, but also the ability to clot (coagulate), that is to thicken and form dense clumps (thrombi). Flow properties prevents cells from sticking together, and they are easy to move around to all vessels, including the most subtle - the capillaries. Thanks to clotting ability damaged small and medium-sized blood vessels while bleeding after some time alone stop, because the gap is closed in the vessel thrombus. As flow and blood clotting provided many substances and cells, which interact with each other, form a system of hemostasis.

Disorders of hemostasis can be the cause of a separate disease, but more often they play a very important role in the course of, and sometimes in the outcome of other diseases in the first

place, injuries, surgeries, heart disease, extensive inflammation, birth. Therefore the determination of blood coagulation system (homeostasis) is a very useful tool to assess the status, prognosis, and effective treatment of many acute and chronic diseases.

Study of the endocrine system

Endocrine glands and endocrine glands - pituitary, pineal, thyroid and parathyroid glands, adrenal glands, pancreas, male and female gonads - got its name due to the fact that the release of synthesized substances - hormones - directly into the blood. This provides with a very mature vasculature glands.

Production of hormones is controlled by the nervous system, which through the hypothalamus regulate the synthesis of hormones in the pituitary gland. Hormones peripheral glands, particularly the adrenal medulla, in turn, control the secretion of hypothalamic hormones. Due to this close mutual influence and control of the endocrine glands form a single endocrine system. Therefore, an increase or decrease of the hormone in the body can result not only from changes in the gland itself (tumor, atrophy, sclerosis, etc.), but also as a result of dysregulation of the other systems.

Get information about the activity of the endocrine glands can be achieved by direct determination of the appropriate level of hormone, intermediates of its synthesis or transformation, but also by determining the biochemical, physiological, and other process parameters that are affected by a particular hormone.

Studies of renal function

The kidneys are involved in the removal of the end products of metabolism, heterogenous and toxic substances entering the body from the external environment, maintain constant levels of osmotically active substances, acid-base balance, are involved in the regulation of water balance, produce substances that regulate blood pressure, erythropoiesisFinally, the main function of the kidneys - urine formation.

Samples used for the study of renal function, in some cases, allow the assessment of their ability to concentrate urine and remove the water in the other - to characterize the individual processes involved in urine formation (glomerular function, convoluted tubules, to investigate renal blood flow, etc.)

Liver function tests

The liver is central to the metabolism of human body. Large amount of blood passing through the liver, the body allows it to allocate the bloodstream and extract from it many biological agents. Bile - just one of the functions of the liver.

The liver is involved in the synthesis of proteins, carbohydrates, fats, pigment metabolism, urea, creatine, and a number of other compounds. The role of the liver in the removal of various toxic substances by forming harmless complexes that are removed from the body through the kidneys.

Tumor markers

Tumor markers - proteins with carbohydrate or lipid components, which can be detected in the tumor cells or serum, as an indicator of malignancy in the body. These proteins have the same degree of specificity - some may appear in several types of tumors in different locations, while others - only at any one specific malignancies. Different frequencies of detection and diagnostic value, as in 10-35% of cases (for different tumors, these values are different) protein marker could not be detected in the presence of a tumor.

Tumor markers are used to monitor the course of disease and the effectiveness of chemotherapy, surgery and biological treatment. Dynamic monitoring of serum tumor marker allows

to draw conclusions about a full stop or further progression, development of metastases. Often the increase in the concentration of tumor marker notes much earlier any clinical signs of disease.

In accordance with state standards, in all fields of science and technology, including in medicine, it is obligatory to use units of the International System of Units (SI). Unit volume in the SI is the cubic meter (m3). For convenience, the medicine may be used per volume liter (l, 1 l = 0.001 m3).

The unit of the amount of substance that contains as many elementary entities as there are atoms in the carbon nuclide 12C weighing 0.012 kg, is a mole, mole that is - is the amount of substance in grams, equal in number to the molecular weight of the substance.

Number of moles of the mass of a substance in grams divided by the relative molecular mass of the substance. 1 mol = 103 mmol mkmol = 106 = 109 = 1012 nmol pmol. The content of most of the substances in the blood is expressed in millimoles per liter (mmol / L).

For indicators, the molecular weight of which is unknown or can not be measured, as devoid of physical sense (total protein, total lipids, etc.), the unit of measure is the mass concentration - grams per liter (g / l).

The enzyme activity is expressed in SI units, the number of moles of the product (substrate) emissions (turns) to 1 in 1 liter of solution - mol / (s-l), mol / (s-l) nmol / (s-l).

DISCUSSION

1. Organization of quality control laboratory.
2. Reference values and the median.
3. Screening, preventive and differential diagnostic study. Rapid diagnostics.
4. The basic SI units in biochemistry.
5. Means of quality control. Methods of quality control (control of reproducibility, control of correctness). Interlaboratory quality control.
6. Basic statistical tests in the quality control of laboratory researches.
7. Unification of biochemical methods.
8. Criteria for standardization: analytical, feasibility, and diagnostic value. Standardization of researches.

INDEPENDENT WORK OF STUDENTS

1. Record practice session protocol indicating the purpose and objectives, methods and principles of quality control in the laboratory.
2. Write methods of sampling and preparation of urine for general clinical and biochemical studies. Draw a conclusion into the protocol.
3. Write down the basic principles of screening, prevention, and differential-diagnostic studies. Rapid diagnostics.
4. Record basic SI units in clinical biochemistry.

3. Topic: Biochemical studies of liver diseases. Liver functions. Laboratory tests of liver disease diagnostics. Clinical and biochemical syndromes. Hypoalbuminemia and hyperglobulinemia. Enzyme diagnostics of liver diseases.

The liver plays an important role in the metabolism of proteins, carbohydrates and lipids. Liver cells metabolize, excrete and detoxify exogenous and endogenous substances.

Important function of liver is synthesis of plasma proteins. Bile acids are synthesized in the liver, necessary for the digestion and absorption of fats. There are hepatocytes or parenchymal cells, which constitute about 60% of all cell mass, and Kupfers cells, included in the reticulate-endothelial system and constitute 30% of all the hepatic cells, in the liver.

Session Purpose: To learn the basic laboratory methods of diagnostics of liver diseases , to learn the basic functions of the liver, clinical and biochemical disorders of liver damage, diagnostic significance of determination of enzymes.

The student should know about:

-the main functions of the liver;

-clinical and biochemical syndromes of liver damages;

-laboratory diagnostics methods of liver diseases.

The student should be able to:

-estimate the correctness of the choice of laboratory methods to the study of the liver;

-determine the nature of the diseases of the liver, based on laboratory data.

Liver functions are manifold:

-metabolism (regulating the metabolism of proteins and amino acids, lipids, carbohydrates and biologically active substances (hormones, vitamins), minerals;

-**deposition** (accumulation of carbohydrates, proteins, fats, hormones, vitamins and minerals);

-secretion (production of bile, which is an important way of removing a number of substances that are converted in the liver from the plasma; the liver is also involved in digestion by emulsifying fats);

-detoxication (Kupffer cells, hepatic macrophages, serve as a biological filter: they form slightly toxic etherosulfuric acids that later go to the intestine);

-excretion (toxic compounds indole, skatole, tyramine bind to sulfuric and glucuronic acids in the liver);

-homeostasis (the liver is involved in metabolic regulation, antigenic homeostasis).

The variety of hepatic functions is reflected in the abundance of laboratory test elaborated for assessment of the functional state of the body. The liver has a strong regenerating ability which allows for a compensation for a long while when a disease has developed. Various enzymatic proteins are found in the liver. Along with enzymes found in other organs, the liver contains specifically hepatic enzymes.

The role of the liver in protein metabolism

The liver plays a key role in the metabolism of proteins:

- synthesis of specific plasma proteins: albumin, prothrombin, fibrinogen, kappa factor and proaccelerin;

- the formation of urea and uric acid;

- synthesis of choline and creatine;

- transamination and deamination of aminoacids.

It is known that a pathological process in hepatocytes reduces their synthetic abilities dramatically thus causing the immediate fall of the albumin content in the blood plasma, which could lead to a decrease in plasma oncotic pressure, the development of edema, and later, ascites. It is noted that in hepatic cirrhosis with signs of ascites, the content of albumin in the blood serum is 20% lower than in cirrhosis without ascites.

In hepatic lesion the process of deamination of amino acids is disturbed, which leads to an increase in their concentration in the blood and urine. Deamination of amino acids is accompanied by formation of ammonia, which is cellular poison. Ammonia is neutralized through synthesis of urea. This process takes place almost exclusively in the liver, urea formation is one of the most important of its functions.

In addition to deamination, amino acids undergo transamination in the liver. The process of transamination is not specific to the liver, it occurs in other organs, but the intensity of these enzymatic reactions in the liver is significant. The activity of transaminases (ALT - alanine aminotransferase, AST - aspartate aminotransferase) is elevated in various destructive changes, such as myocardial infarction and hepatitis. It is very important given the specificity of enzymatic diagnostics: with the help of these enzymatic reactions we can judge about a lesion of these organs. When there are necrotic changes in the cardiac muscle, the activity of AST increases in the blood dramatically, while in hepatitis an increase in ALT is noted. It is very important that detection of transaminase activity can reveal hepatic disease before jaundice develops.

It is equally valuable to the organ-enzyme diagnostics liver disease have studies isoenzyme spectrum of lactate dehydrogenase (LDH) were measured: for example, increased activity of the fifth fraction of lactate dehydrogenase (LDH-5) in the spectrum with certainty isoenzymic evidence of destructive processes in the liver tissue, and increased activity of LDH-1 - damage to the myocardium. But there are also differences in the subcellular distribution of LDH isoenzymes, which depends on the specificity of the functions of cell organelles. Thus, the mitochondria of hepatocytes, where, basically, there are energetic processes in the liver and high levels of oxygen, rich in LDH-1 and LDH-2, whereas LDH-4 LDH-5 is mainly concentrated in the cytoplasmic fraction of hepatocytes.

The role of the liver in lipid metabolism

Liver provides such processes as synthesis of fatty acids, triglycerides, phospholipids, cholesterol (related to the lipids) and its esters, as well as lipolysis of triglycerides, fatty acid oxidation, formation of acetone (ketone) bodies, and synthesis of plasma lipoproteins.

It is known that enzymatic reactions of triglyceride synthesis are similar in the liver and in fat tissue. Triglycerides synthesized in the liver either remain in the liver or are secreted into the blood as part of lipoproteins, mainly VLDL (VLDL) and are transported to the adipose tissue.

A portion of cholesterol (lipids related to sterol or sterols, i.e., steroid alcohols) comes into the body with food, but much more of it is synthesized in the liver from acetate. The biosynthesis of cholesterol in the liver is inhibited by exogenous cholesterol. The more cholesterol comes from food, the less than is synthesized by the liver.

A portion of cholesterol synthesized in the liver is excreted from the body together with bile; another portion is involved in the formation of biliary acids, and is also utilized by other organs for synthesis of steroid hormones and other compounds.

Diseases of the liver are accompanied by a number of laboratory syndromes. In analyzing the results of biochemical studies in patients with liver disease it is advisable to allocate four laboratory syndromes, each of which corresponds to a certain extent to certain morphological and functional changes in the body: cytolytic syndrome, mesenchymal-inflammatory syndrome,

cholestatic syndrome, minor hepatocellular failure, usually in each case there is a combination of several biochemical syndromes.

Syndrome of disrupted integrity of hepatocytes (or *cytolysis, cytolytic syndrome*).

In this we see an increase of tracer activity of enzymes in plasma - AST, ALT, LDH and its isoenzymes - LDG4 and LDG3; specific liver enzymes: fructose-1-phosphataldolase, sorbitdehydrogenase, as well as serum ferritin, serum iron, vitamin B12, and bilirubin – mostly due to an increase of the direct fraction.

In assessing the severity of a pathological process, fundamental importance is attached to the activity of ALT and AST. An increase in their content in the serum in less than five times compared with the upper limit of normal is seen as moderate, 5 to 10 times – as an average degree and more than 10 times - as high severity. Morphological basis of this syndrome is acidophilic and hydropic degeneration and necrosis of hepatocytes with lesion and enhanced permeability of cell membranes.

Cholestasis syndrome (excretory-biliary syndrome, cholestatic syndrome, a disturbance of the excretory function of the liver). It is accompanied by increased levels of serum alkaline phosphatase, LAP, GGTF, cholesterol, P-lipoprotein fraction of conjugated bilirubin, bile acids, phospholipids, excretion of bromsulfalein (vofaverdin) and radiopharmacologic drugs decreases. Morphological basis of intracellular cholestasis is ultrastructural changes in hepatocytes: hyperplasia of smooth cytoplasmic network, changes in the biliary pole of hepatocytes, accumulation of bile components in the hepatocyte, which are often accompanied by cytolysis of hepatocytes. In intrahepatic cholestasis we can reveal accumulation of bile in the bile ducts, and in extrahepatic - expansion of interlobular bile ducts.

Syndrome of hepatocellular insufficiency (synthetic failure).

This condition is manifested through a decrease in serum total protein and especially albumin, transferrin, cholesterol, clotting factors II, V, VII, cholinesterase, alpha-lipoproteins, while bilirubin content is elevated due to the unconjugated fraction. Morphological substrate of the syndrome is pronounced dystrophic changes of hepatocytes and / or a significant decrease in the functioning of liver parenchyma as a result of necrotic changes.

Impaired function of hepatocytes can lead to a lesion of albumin synthesis, which is observed in chronic liver diseases. The most pronounced hypoalbuminemia is detected in portal cirrhosis, fatty liver.

Mesenchymal-inflammatory syndrome

It is characterized by hypergammaglobulinemia, increased rates of protein-sediment samples, increased erythrocyte sedimentation rate, presence of products of connective tissue degradation (C-reactive protein, seromucoid, etc.) in the blood. In morphological studies of the liver we see characteristic activation and proliferation of lymphoid cells and reticulohistiocytic cells, increased fibrogenesis, formation of active septa with hepatocytic necrosis, intrahepatic migration of white blood cells, and vasculitis.

Syndrome of cytolysis (cytolytic syndrome or syndrome of disturbed integrity of hepatocytes)	↑ AST, ALT, LDH and its isoenzymes - LDG4 and LDG3, fructose-1-phosphataldolase, sorbitdehyidrogenase, and serum ferritin, serum iron, vitamin B12, and bilirubin due to increased direct fraction
Cholestasis syndrome (excretory-biliary syndrome, cholestatic syndrome	↑ ALP, LAP, GGTF, cholesterol, P-lipoprotein fraction of conjugated bilirubin, bile acids, phospholipids
Syndrome of hepatocellular insufficiency (synthetic failure syndrome)	↓ total protein (especially albumin), transferrin, cholesterol, clotting factors II, V, VII, cholinesterase, alpha-lipoprotein ↑ bilirubin due to unconjugated fractions

Mesenchymal-inflammatory syndrome	↑ ESR, presence of blood C-reactive protein, rheumatoid factor, antibodies to subcellular fractions of hepatocytes, antimitochondrial and antinuclear antibodies, changes in the quantity and functional activity of T- and B-lymphocytes, increased content of immunoglobulins.

Enzyme diagnostics of liver diseases

- Aspartate aminotransferase.
- Alanine aminotransferase.
- Alkaline phosphatase.
- Lactate dehydrogenase.
- Glutamyltransferase.
- Glutamate dehydrogenase.
- Sorbitol dehydrogenase.

DISCUSSION

1. Liver functions.
2. Laboratory tests of liver diseases diagnostics.
3. Clinical and biochemical syndromes of liver diseases.
4. Syndrome of cytolysis.
5. Syndrome of hepatocellular insufficiency.
6. Cholestasis syndrome.
7. Mesenchymal-inflammatory syndrome.
8. Enzyme diagnostics of liver diseases.
9. Diagnostic value of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, lactate dehydrogenase, glutamyltransferase, glutamate dehydrogenase, sorbitol dehydrogenase.
10. Hyper-and hypoenzynemia.

INDEPENDENT WORK OF STUDENTS

1. Write down practice session protocol indicating the purpose and objectives, to list the main functions of the liver.

2. Write down types of clinical and biochemical syndromes in liver diseases. To characterize each of the syndromes, describing the basic biochemical parameters.

3. Write down the main enzymes that are used for diagnostics of liver diseases.

4. Topic: Biochemical studies of liver diseases. Types of jaundice. Hyperbilirubinemia and bilirubinuria. The formation of bilirubin and its fractions in the blood, liver, intestines and kidneys. Free (indirect) and conjugated (direct) bilirubin, urobilinogen and stercobilinogen, bile pigments. Bilirubin toxicity. Jaundice at newborns. Reference values , the differential diagnosis of liver diseases. Fraction of bilirubin in the blood, urine and feces.

Jaundice is innatural yellow coloration of the skin or sclera. This is due to the presence of bilirubin in plasma in concentrations greater than 40 mmol / l. The normal concentration of bilirubin in the plasma is under 22 mmol / l.

There are three main causes of elevated bilirubin content in the blood:

- The rate of synthesis of bilirubin is increased exceeding the excretory capacity of the liver (sythemolytic jaundice).
- Inhibition of conjugation and / or excretory mechanisms in the liver: the liver's ability to metabolize bilirubin synthesized in normal amounts is reduced (hepatic, or hepatocellular jaundice).
- Obstruction of the biliary system, which prevents the outflow of bile (cholestatic, obstructive, mechanical, obstructive jaundice).

Session Purpose: To learn the basic types of jaundice, the mechanism of bilirubin and its fractions synthesis, to study the differential diagnosis of liver diseases.

The student should know about:

- main types of jaundice;
- differential diagnosis of liver diseases;
- basic fractions of bilirubin;
- mechanism of the main bilirubin fractions synthesis.

The student should be able to:

- to determine the type of jaundice, based on laboratory data;
- to determine the nature of liver disease, based on laboratory data.

Types of jaundice

- **Hemolytic jaundice**

Most often hemolytic jaundice is caused by an increased destruction of red blood cells: both mature cells and their precursors. Destruction of mature cells may be the result of hemolysis or an effect of blood utilization after internal bleeding, like in soft tissue injuries. Ineffective hemopoiesis occurs in pernicious anemia (disturbed maturation of red blood cells) or thalassemia (anomalous structures and hemoglobin). Hyperbilirubinemia in hemolytic jaundice is caused by accumulation of unconjugated bilirubin that is not excreted by the kidneys. This increases the flow of bilirubin from the liver to the intestine. Urobilinogen is produced in large amounts, and its urine content is elevated.

- **Hepatocellular jaundice**

Congenital disturbances of bilirubin transport lead to jaundice due to imperfect absorption, decreased conjugation or impaired excretion of bilirubin. Generalized hepatocellular dysfunction may occur in hepatitis and decompensated liver cirrhosis. Pathogenesis of jaundice in these cases is complicated; the contributing factors are disturbance of the uptake, intracellular transport, and reduced conjugation of bilirubin. Drugs can cause hepatocellular lesion in

connection with its dose-related hepatotoxicity (e.g., acetaminophen) or idiosyncratic sensitivity (e.g., isoniazide). If hyperbilirubinemia is caused by disturbance of conjugation, bilirubin is not conjugated, and the flow of bilirubin in the liver is not increased. As a consequence, bilurubinuria is absent, and urobilinogen content in the urine is not elevated. If there is generalized dysfunction of the liver, the uptake of bilirubin is reduced, and, therefore, the kidneys excrete it in greater quantities. Serum bilirubin can be both conjugated and unconjugated, due to defective UDP-glucuronyl transferase and intracellular transport of bilirubin. If the rate of conjugation exceeds the excretory capacity, blood levels of conjugated bilirubin go up, and it can be excreted in the urine. This sometimes happens during convalescence after viral hepatitis.

- **Cholestatic jaundice**

Cholestatic jaundice may be the effect of obstruction of an outflow of bile from the hepatocytes in the duodenum. It can be caused by lesions in the liver (intrahepatic cholestasis) or in the bile ducts and pancreas head (extrahepatic cholestasis).

Intra- and extrahepatic cholestasis can be differentiated by ultrasound or biopsy of the liver; evaluative tests of liver function do not help.

Intrahepatic cholestasis is often the result of generalized hepatocellular dysfunction developing, for instance, in hepatitis or decompensated cirrhosis. This condition is also a sign of primary biliary cirrhosis. The branches of biliary tree can be blocked by malignancies. Some medications such as anabolic steroids, phenothiazines, and sulfonylurea may lead to intrahepatic cholestasis.

Extrahepatic obstruction is often the result of large biliary tract tumors, tumors of the head of the pancreas and lymph nodes at the porta of hepar. Obstruction of bile ducts can be also caused by gallstones or sclerosing cholangitis.

Jaundice is caused by a disturbance of excretion of conjugated bilirubin and its accumulation, which is filtered by the glomerules and appears in the urine. However, bilirubin may not be detected in the urine, possibly because changes in the processes of conjugation lead to formation of less soluble bilirubin bound to albumin. In complete obstruction, bilirubin cannot reach the intestine, urobilinogen is not formed or detected in the urine, and feces may have whitish coloration.

Hyperbilirubinemia and bilirubinuria. Bilirubin and its fractions in the blood, liver, intestines and kidneys. Free (indirect) and conjugated (direct) bilirubin, urobilinogen and stercobilinogen, bile pigments. Bilirubin toxicity.

Bilirubin is formed from the breakdown of hemoglobin in the cells of the reticuloendothelial system (RES), it is particularly active in the spleen and hepatic Kupffer cells. In the adult 250-350 mg of bilirubin is formed daily. Bilirubin is poorly soluble in water; in the plasma bilirubin appears primarily in the unconjugated form bound to albumin (indirect, unconjugated bilirubin). Unconjugated bilirubin cannot pass through the kidney barrier. In the liver bilirubin is transferred from albumin to the sinusoidal surface of hepatocytes. In liver cells; indirect bilirubin undergoes enzymatic conjugation with glucuronic acid and is converted into bilirubinmono- and bilirubindiglucoronide (conjugated, or direct bilirubin). Conjugated bilirubin is water soluble; it comes from the bile in the gall bladder, or directly into the intestine. It loses bilirubin glucuronic acid and is reduced to urobilinogen. A part of urobilinogen absorbed in the small intestine and the portal vein enters the liver again, where it is oxidized to dipyrrole. In the colon, bile bilirubin becomes stercobilinogen under the impact of the normal intestinal flora. In

the lower portion of the major part of the colon, colorless stercobilinogen is oxidized into brown stercobilin, and is excreted in the feces. A small part of stercobilinogen absorbed into the blood and through the hemorrhoidal veins and inferior vena cava enters the kidneys and then into the urine. Normal urine contains a minimal amount of conjugated bilirubin (7 - 20 mg / l) that are not detectable by qualitative methods.

Bile pigments are breakdown products of hemoglobin and other derivatives of porphyrin excreted in the bile, urine and feces.

Their greater portion is formed during the catabolism of hemoglobin in red blood cells in the the cells of mononuclear phagocytes system. Bile pigments are compounds containing four pyrrole groups linked by one-carbon bridges in the open, non-closed circuit (in contrast to the closed structure of the heme). As a result of a-methine bridge of heme breakdown in hemoglobin, verdoglobin (holeglobin) is formed, which is an iron-porphyrin pyrrole compound with an open structure, of green color. After release of globin protein and iron by the molecule of verdoglobin, green bile pigment biliverdin is produced. An alternative way of biliverdin formation is cleavage of the protein part of hemoglobin, formation of iron porphyrin hematin pigment and oxidation of hematin with a breakdown of methine bridge and loss of iron. In the cells of the mononuclear phagocyte system, biliverdin is reduced to bilirubin, which is delivered into the liver with the blood. A small amount of bilirubin is formed in the cells of the mononuclear phagocyte system of the heme that was not used for the synthesis of hemoglobin; also from the heme formed in the liver for catabolism of other heme-containing proteins (myoglobin, cytochromes, etc.), or from hemoglobin renovated in the process of maturation of erythrocytes. Bilirubin is the main and most diagnostically valuable bile pigment that occurs in human bile. Biliverdin is present in the bile in trace amounts (15-20% of its dry weight). In the liver, bilirubin forms pairs of compounds, or conjugates, mainly with glucuronic acid, and to a lesser extent - with sulfuric acid. About 300 mg of bilirubin is produced in the body daily. About 75% of bilirubin is formed from bilirubin glucuronide, and 15% - from bilirubin sulfate.

Newborn jaundice

Hemolytic disease of newborn

Causes. Incompatibility of the mother's and fetal blood in the group or Rh factor. Accumulation of hydrophobic forms of bilirubin in the subcutaneous fat causes yellowness of the skin. However, the real danger is posed by accumulation of bilirubin in the gray matter of nervous tissue and stem nuclei with development of "kernicterus" (bilirubin encephalopathy).

Clinical diagnosis. Its manifestations are drowsiness, poor sucking, mental retardation, stiff neck, tonic convulsions, tremor of limbs, changed reflexes; development of deafness and paralysis is possible.

Laboratory diagnosis. Severe anemia, reticulocytosis, erythropoietin and normoblastosis are detected in the blood. Hyperbilirubinemia due to indirect fraction from 100 to 342 mmol / l, in the future, and direct attached fraction. Bilirubin content in the blood increases rapidly reaching its peak by the 3-5 day of life.

Physiological (transient) neonatal jaundice

Causes:

- relative reduction of UDP-glucuronyl transferase activity in the first days of life associated with intensified degradation of fetal hemoglobin,
- an absolute decrease in the activity of UDP-glucuronyl in the first days of life,
- ligandin deficit,

- poor activity of the biliary tract.

Clinical diagnosis

- skin coloration for 3-4 days after birth,
- absence of hemolysis or anemia.

Symptoms disappear within 1-2 weeks after birth.

Laboratory diagnosis. Increased concentrations of free serum bilirubin to 140-240 mmol/l.

Jaundice of prematurity

Causes:

- relative reduction of UDP-glucuronyl transferase activity in the first days of life associated with increased degradation of fetal hemoglobin,
- an absolute decrease in the activity of UDP-glucuronyl in the first days of life,
- ligandin deficit,
- poor activity of the biliary tract.

Clinical diagnosis

- skin coloration for 3-4 days after birth,
- absence of hemolysis or anemia.

Symptoms disappear within 3-4 weeks after birth.

Laboratory diagnosis. Increased concentrations of free serum bilirubin reaching the peak on day 5-6 after birth, more pronounced in comparison with physiological jaundice.

Nonhemolytic neonatal hyperbilirubinemia caused by breast milk.

This condition develops in 1% of breast-fed infants.

Causes. Inhibition of UDP-glucuronyl presumably by milk estrogen.

Clinical diagnosis. Manifested as jaundice, sometimes with CNS symptoms.

Laboratory diagnosis. Increased concentrations of free bilirubin in the serum.

Reference values, differential diagnosis of liver diseases.
Fraction of bilirubin in the blood, urine and feces.

Liver disease

5% of healthy people may experience a slight elevation in liver enzymes without any signs of liver damage. A practical approach to screening patients with isolated increased aminotransferase is to repeat the test; further studies are only advisable when there is a 2-fold excess of the norm or identification of risk factors for liver disease are identified. These enzymes are not only found in the liver; ACT in cardiac muscle, skeletal muscle, kidney, brain, pancreas, lungs, leukocytes and erythrocytes; ALT in skeletal and cardiac muscle (although in much smaller amounts ACT); LDH in virtually all cells and body fluids; isozymes LDG5 and iLDG4 are more common to the liver.

Aminotransferase levels do not correlate with the outcome of liver disease, since acute hepatitis B with 20-fold and more increased enzyme content ends in complete recovery, whereas alcoholic hepatitis with a much lower elevation of aminotransferase can result in liver failure. Decreased ALT and AST content is often a sign of recovery, but a rapid decline in the number of cases may be a sign of massive death of hepatic cells.

Small (less than 3-fold) increase in aminotransferase occurs in fatty liver, nonalcoholic steatohepatitis and chronic viral hepatitis; their 3 to 20 fold increase is typical of acute viral hepatitis, autoimmune hepatitis, and alcoholic hepatitis (sometimes chronic viral hepatitis).

Finally, a more than 20 fold increase is more likely to be seen in acute viral hepatitis, drug-induced (or toxic) liver disease and ischemic hepatitis.

In alcoholic liver disease AST content is characteristically while ALT content is almost normal (or slightly elevated). The ratio of AST / ALT in 70% of patients exceeds 2.

Isolated increase in aminotransferase content is typical of non-alcoholic fatty liver disease (only if the content of hepatic markers is negative and no alcohol abuse in past history). An increase in this value can be observed in celiac disease, tuberculosis, sarcoidosis and amyloidosis with hepatic lesions and metastases into the liver.

Evaluation of liver synthetic function

Albumin. Albumin is synthesized in the liver only in an amount of about 150 mg / kg / day. The half-life of albumin is 20 days. Therefore, in acute liver failure it is not a very good indicator. In addition, the cause of decreased albumin may also be its transition into the third space (e.g. sepsis), malnutrition, loss through the kidneys (nephritis) and intestine (enteropathy with protein loss). If we see a decrease in albumin in liver disease, may be assured about the long-term course of liver failure in this patient.

Prothrombin time. This value is the most rapid indicator of the synthetic function of the liver as all clotting factors (except factor VIII) are synthesized in the liver, and the half-life of factor VII is just 3-5 hours. A number of coagulation factors (II, VII, IX, X) need vitamin K as a cofactor. Given the possible lack of this vitamin (in cholestasis for instance), one should administer it at a dose of 10 mg / day for 3 days to see whether its reduction is associated with a lack of PT or with impairment of the synthetic function of the liver. PT is a valuable prognostic factor in assessing liver failure and helps to determine the time when a patient should be referred for liver transplantation.

Urea and ammonia. These indicators do not play a major role in laboratory diagnosis of liver disease, but their correct interpretation may help in diagnostics and predict the possibility of complications such as liver failure. Take into account that the urea is synthesized by the liver from NH3, which, in turn, is largely a waste product of colonic bacteria. Plasma ammonia concentration may be increased in the following cases:

- Increase in its production (gastrointestinal bleeding, a large amount of protein in the diet).
- Portosystemic shunt.
- Liver failure.
- Congenital (genetic) defects.

Bilirubin fraction in the blood

- Total bilirubin.
- Direct (conjugated) bilirubin.
- Indirect (unconjugated) bilirubin.

Fraction of bilirubin in urine

Normal serum contains an average of 17 mmol / L of total bilirubin, of which only 10 - 15% is part of the direct fraction. Indirect bilirubin cannot pass through the renal corpuscles, and therefore healthy human urine does not contain this pigment. Presence of bilirubin in the urine indicates an increase of its direct fractions in the blood; it usually indicates an impairment of excretion of bile pigments into the intestines.

Fraction of bilirubin in the feces

In intestinal infections urobilinogen (urobilin) is formed in the upper part of the intestine (in the small intestine and in the beginning of the large intestine) from bilirubin-glucuronide arriving from the liver. Part of urobilinogen is resorbed through the intestinal wall, and the portal system carries the blood to the liver, where urobilinogen is cleaved completely, so that it does not enter the bloodstream and is not excreted with the urine. In impaired liver function urobilinogen enters the bloodstream and is excreted with the urine.

Stercobilinogen is also formed from of bilirubin-glucuronide arriving from the liver to the duodenum, and from there – to the large intestine. This bilirubin-glucuronide is restored to stercobilinogen with the help of anaerobic intestinal microflora. The colouring of stools in a healthy person is determined by the presence of stercobilinogena and sterkobilin, its oxidation product**.** Stercobilin in fecal matter is important for differential diagnosis of jaundice. In hemolytic jaundice stercobilin content in feces is significantly increased, while in obstructive jaundice and cholestatic form of viral hepatitis it is not detected.

Reference value of liver function tests

Test	Normal value
Protein fractions	
Total protein	65—85 g/l
Albumin	40—50 g/l
Globulin	20—30 g/l
Indicators of pigment metabolism	
Total bilirubin	11,12 (8,6-20,5) mcmol/l
Direct bilirubin	2,57 mcmol/l
Indirect bilirubin	8,6 mcmol/l
Enzymes	
ALT	0,1—0,68 mmol / (h • l)
AST	0,1—0,45 mmol / (h • l)
Alkaline phosphatase	1,0—3,0 mmol / (h • l)
Lipids and lipoproteins	
Total lipids	4,6—10,4 mmol/l
Triglycerides	0,565—1,695 % mmol/l
Total cholesterol	3,11—6,48 mmol/l
Indicators of nitrogen metabolism	
Urea	3,3—6,6 mmol/l
Creatinine	M - 0,088—0,177 mmol/l F - 0,044—0,141 mmol/l
Uric acid	0,12—0,38 mmol/l

Additional tests:

Test	Normal value
Lactate dehydrogenase LDH	0,8—4,0 mmol / (h • l)
Glutamyltransferase (GGTP)	0,6—3,96 mmol / (h • l)
Sorbitol dehydrogenase **(**SDH**)**	0—0,02 mmol / (h • l)
Glutamate dehydrogenase	M – 0-7 U / L F – 0-5 U / L
Cholinesterase	5300 — 12900 U / L
AFP	0-10 U / L

DISCUSSION

1. Types of jaundice: suprahepatic, hepatic, obstructive.
2. Hyperbilirubinemia and bilirubinuria.
3. Synthesis of bilirubin and its fractions in the blood, liver, intestines and kidneys.
4. Unconjugated (indirect) and conjugated (direct) bilirubin, urobilinogen and stercobilinogen, bile pigments.
5. Bilirubin toxicity.
6. Newborn jaundice.
7. Reference values, the differential diagnosis of liver diseases.
8. Fraction of bilirubin in the blood, urine and feces.

INDEPENDENT WORK OF STUDENTS

1. Write down practice session protocol indicating the purpose and objectives, list the major types of jaundice, their distinctive characteristics.

2. Write down synthesis mechanism of bilirubin, its main factions, and its role in laboratory research in diseases of the liver.

3. Write down features of formation of neonatal jaundice.

4. Write down the main reference value, the differential diagnostics of liver diseases.

5. Write down fraction of bilirubin in the blood, urine, feces.

5. Topic: **Blood plasma proteins, their functions. Protein synthesis in the liver, RES, cells of the immune system. Determination of total protein in the blood and urine. Characterization of protein fractions. Acute-phase proteins. Proteinogram types.**

Proteins are high polypeptides consisting of over 20 types of α-amino acids. There are simple and complex proteins. Simple proteins contain only amino acids, while complex ones contain, besides, other components: heme, vitamin derivatives, lipids, or carbohydrates, etc.

As in many diseases we see changes in the content of certain proteins, the study of their concentration in the blood is widely used for diagnostic purposes. In biological fluids we determine total protein, protein fractions and individual proteins.

Session Purpose: To know the protein composition of blood plasma, methods for the determination of total protein in biological fluids, to learn to interpret proteinogram in case of various pathological processes.

The student should know about:

- blood plasma proteins and their function;
- synthesis of proteins in the liver, RES, cells of the immune system;
- method of defining total protein content in the blood and urine;
- plasma protein fractions and their characteristics;
- acute phase proteins, their function and diagnostic criteria;
-normal proteinogram and its variations in case of various pathological conditions (acute and chronic inflammation, impairement of the renal filter, malignancies, hepatitis, cirrhosis of the liver, jaundice).

The student should be able to:

- interpret proteinogram results obtained in various pathological conditions;
- evaluate the outcome of the treatment of an inflammatory process according to the values of acute phase proteins.

Human plasma normally contains more than 100 types of proteins. Approximately 90% of the total blood protein is constituted by albumins, immunoglobulins, lipoproteins, fibrinogen, transferring; other proteins are present in the plasma in small amounts.

Functions of plasma proteins:

- maintaining constant colloid osmotic pressure of the blood;
- determining the viscosity of the blood and maintaining the stability of erythrocytes and leukocytes in the blood for normal blood flow in capillaries (rheological properties of blood);
- protein buffer system is involved in the regulation of acid-base balance;
- specialized proteins bind and transport carbohydrates, lipids, hormones, drugs, vitamins, and toxic substances;
- maintaining cations of calcium, magnesium, iron, copper and other ions in bound state and transporting them, preventing their loss with the urine;
- proteins are involved in blood coagulation (fibrinogen, prothrombin, antihemophilic globulin, etc.);
- immunoglobulins and the factors of the complement system, transferrin and properdin serve a protective function by preventing infection and maintaining the resistance of the body;
- proteins are reservoir of amino acids.

Synthesis of plasma proteins is carried out by:

- the liver: it synthesizes blood fibrinogen and albumin, most of the α-and β-globulin;
- reticuloendothelial system (RES) of the bone marrow and lymph nodes synthesizes a part of β-globulins and γ-globulins (immunoglobulins).

The state of protein metabolism in the body is estimated by determining the total protein, protein fractions and individual plasma proteins.

Methods of determining total protein

Among the methods of determining the concentration of total protein there are several main groups, based on different principles:

- nitrogenometric;
- gravimetric;
- precipitation-based
- spectrophotometric;
- refractometric;
- colorimetric;
- nephelometric;
- polarimetric.

Nitrogenometric methods

Nitrogenometric methods of determining total serum protein are based on the quantity of protein nitrogen produced upon the destruction of amino acids that make up proteins. The first method was proposed by Kjeldahl in 1883. In the Kjeldahl method, which is currently of historical interest only, the nitrogen contained in the protein is oxidized to ammonium ion and its exact amount is determined by titration with hydrochloric acid. In addition, the ammonium ion can be determined with the Nessler reagent, by the manometric method after conversion of ammonium to molecular nitrogen under the impact of hypobromite or by Warburg optical test with participation of glutamate dehydrogenase. Based on the fact that proteins from biological objects contain an average of 16% of nitrogen, the amount of nitrogen obtained in the test is multiplied by a factor of 6.25. For individual protein fractions in the serum or plasma the value of the factor ranges from 5.69 to 6.52.

The downside of nitrogenometric methods is that the procedure is lengthy and complicated, even though the ammonia formed in the reaction can be determined by an enzymatic method. Automation makes it possible to use this method in some cases as a method of comparison because of its reasonable accuracy and reproducibility.

Gravimetric methods

Gravimetric methods for determination of total protein in the serum are based on drying the proteins to their constant weight and weighing on an analytical balance. These methods are cumbersome and are not used currently to determine the total serum protein. The gravimetric method is still used in some laboratories to determine fibrinogen in the blood plasma.

Precipitation-based methods

In precipitation-based methods of determining total protein are based on the reduce of solubility of proteins so that a suspension of suspended particles is formed under the influence of various agents. The content of protein in the sample is judged either by light scattering intensity (nephelometric method of analysis), with determining the number of light-scattering particles, or by the way the resulting suspension reduces the light flux (turbidimetric method of analysis).

The outcomes of this group of methods depend on many factors: the rate of mixing the reagents, reaction temperature, pH of the medium, the presence of foreign compounds, methods of photometry. Careful compliance with reaction conditions promotes formation of a stable suspension where the particles have all a constant size and to obtain reproducible results. Precipitation-based methods for determination of protein in the blood serum did not receive recognition and are used to determine protein in urine, cerebrospinal fluid, and many individual proteins with specific antibodies.

Spectrophotometric methods

Spectrophotometric method is measuring the degree of light absorption in the ultraviolet region at two wavelengths with further calculation using special formulas (230 and 260 nm, 280 nm and 260, 235 and 280 nm, 215 nm and 225, 280 and 205 nm).

Refractometric method

Refractometric methods of determining the total serum protein solutions are based on the ability of the protein to refract the light flux. At a temperature of 17.5 ° C the refractive index of water is equal to 1.3332; at the same temperature, the refractive index of the serum is in the range of 1.3480-1.3505. Due to the fact that the concentration of electrolytes and non-protein organic compounds affecting its refractive power is low and fairly constant in the serum of a healthy person, the refractive index of serum depends primarily on its protein content. Calibration is carried out with known concentrations of serum protein. Refractometry is a convenient method of determining total protein in serum, although in a number of diseases, including diabetes mellitus, chronic renal failure, its use can result in significant errors.

Colorimetric (photometric) methods

Colorimetric methods of determining total protein are based on the color reaction of proteins with chromogen-forming reagents or non-specific binding of the dye.

The most common colorimetric method of determining total serum protein is the one based on the so-called "colored biuret reaction", in which proteins react with copper sulfate in alkaline medium to form compounds of purple colored, the color intensity depending on the concentration of total protein in the serum. The biuret method was approved as a unified method in 1972.

Colorimetric methods of determining total serum protein are rather simple and relatively cheap. The disadvantage of the method is the interfering effect of certain substances (including medications).

The normal amount of total protein in blood serum ranges from 60 to 80 g/l. The plasma contains 2.4 g / L more at the expense of fibrinogen, which is absent from the serum.

Decreased concentration of proteins in the blood is called **hypoproteinemia,** an increased concentration - **hyperproteinemia.**

Causes of hypoproteinemia:

- insufficient consumption of protein (prolonged starvation, protein-free diet);
- increased protein loss (in a variety of kidney diseases, blood loss, burns, tumors, ascites, diabetes);
- impairement of protein synthesis in the body: renal failure (hepatitis, cirrhosis, toxic damage), long-term corticosteroid therapy, malabsorption (enteritis, enterocolitis, pancreatitis).

Hyperproteinemia is observed due to:

- dehydration due to loss of intravascular fluid (upon a severe trauma, extensive burns, cholera);

- presence of paraproteins in the blood: pathological proteins produced in large quantities in multiple myeloma, Waldenstrom's disease.

With the help of electrophoresis **5 standard fractions** are distinguished on paper: albumin and four globulin fractions (α1-globulin, α2-globulin, β-globulin, γ-globulin).

The principle of electrophoresis of proteins is as follows:

Cellulose acetate film, gel, special paper (the medium) is placed on the frame so that the opposite edge of the medium dips in a pan with buffer solution. Blood serum is place on the starting line. The principle of the method is that the buffer moves on the surface of the medium under the influence of the electric field. While moving the buffer solution captures molecules of serum proteins. The molecules with the highest negative charge and the smallest size, i.e. albumin, move faster than others. The largest and most neutral molecules (γ-globulins) turn out to be the last.

The course of electrophoresis is influenced by the mobility of the separated substances which depends on the following factors: charge (usually depending on the pH), the size and and shape of molecular substances, the electric field, the buffer and medium (considering its hydrophilicity and adsorption capacity).

General view of the electrophoresis

The number of isolated fractions is determined by the conditions of electrophoresis. Electrophoresis on paper and cellulose acetate films in clinical diagnostic laboratories isolates 5 standard fractions, while electrophoresis in polyacrylamide gel - up to 20 or more fractions. When using more advanced methods (radial immunodiffusion, immunoelectrophoresis, and others), numerous individual proteins are distinguished in globulin fractions.

Electrophoregram (top) and the result of its graphical processing (bottom)

Only those proteins concentration of which is high enough can affect the proteinogram.

Normal proteinogram

Albumins	52-65%	3 5 - 50 g / l
α $_1$-Globulins	2.5-5%	1-3 g / l
α $_2$-Globulins	7 -1 3%	6-10 g / l
β-Globulins	1.8 4%	7.11 g / l
γ-Globulins	1 2 -2 2%	8-16 g / l

Albumin fraction includes albumin (the main part) and prealbumin; its share is more than 50% of the plasma proteins.

Globulin fractions are more diverse.

Alpha 1-globulin fraction contains the following proteins:

- α1-antitrypsin (the main component of this fraction) is an inhibitor of many proteolytic enzymes: trypsin, chymotrypsin, plasmin, etc.)

- α1-lipoprotein (HDL) is involved in lipid transport.

- α1-acid glycoprotein (orosomucoid). It increases in response to various acute and chronic inflammatory stimuli; it is used to indicate the acute phase response.

Alpha2-globulin fraction comprises:

- α2-macroglobulin (the main component fractions) is the regulator of the immune system and is involved in the development of infections and inflammatory reactions.

- Haptoglobin is a glycoprotein, which forms a complex with the hemoglobin released from red blood cells in intravascular hemolysis, and then utilized by the cells of the reticuloendothelial system, which is necessary to prevent a loss of iron and hemoglobin in kidney damage.

- Ceruloplasmin specifically binds copper ions; it is an oxidase of ascorbic acid, adrenaline, dihydroxyphenylalanine (DOPA); it is able to inactivate free radicals. When ceruloplasmin is low (Wilson's disease), copper is accumulated in the liver (causing cirrhosis) and in the basal ganglia of the brain (the cause of choreoathetosis). An increase in ceruloplasmin is specific for melanoma and schizophrenia.

- Apolipoprotein B is involved in lipid transport.

Beta-globulin fraction comprises:

- Transferrin: a protein that transports iron, thus preventing accumulation of iron in the tissues and its loss with urine.

- Hemopexin links the heme and prevents its excretion by the kidneys.

- Complement components are involved in the immune response.

- β-lipoproteins are involved in the transport of cholesterol and phospholipids.

Gamma globulin fraction consists of immunoglobulin, (IgG, IgA, IgM, IgE), that are antibodies functionally providing humoral immune defense against infections and foreign substances.

Disproteinemia is disruption of the normal ratio of plasma protein fractions; it is found in many diseases significantly more often than the change in total protein. Dysproteinemia is very dynamic which is associated with the phase of the process development, its duration and intensity of the therapeutic measures.

Types of proteinograms

In clinical practice there are 10 different types of electrophoregrams **(proteinograms)** corresponding to different pathological conditions:

Proteinogram type	Albumins	Globulin fraction				Examples of diseases
		α_1	α2	β	γ	
Acute inflammation	↓↓	↑	↑	-	↑	Initial stages of pneumonia, acute polyarthritis, pleural tuberculosis, acute infectious diseases, sepsis, myocardial infarction
Chronic inflammation	↓	-	↑	-	↑↑	Advanced stages of pneumonia, chronic pulmonary tuberculosis, chronic endocarditis, cholecystitis, cystitis and pyelitis
Renal filter impairement	↓↓	-	↑	↑	↓	Genuine, lipoid or amyloid nephrosis; nephritis, nephrosclerosis, toxemia of pregnancy, end-stage pulmonary tuberculosis, cachexia

Malignant tumor	↓↓	↑↑	↑	↑↑	↑↑	Metastatic tumors with various localizations of the primary tumor
Hepatitis	↓	-	-	↑	↑↑	Toxic damage of the liver, hepatitis, hemolytic processes, leukemia, malignant newgrowth in the hematopoietic and lymphatic system, some forms of arthritis, dermatitis
Necrosis of the liver	↓↓	-	↓	↑	↑↑	Cirrhosis of the liver, severe pulmonary, some forms of chronic arthritis and connective tissue
Obstructive jaundice	↓	-	↑	↑	↑	Obstructive jaundice, jaundice caused by cancer of the biliary tract and pancreas head
α_2-globulin plasmacytoma	↓	↓	↑	↓	↓	α_2-plasmacytoma
β-globulin plasmacytoma	↓	↓	↓	↑↑	↓	β_1-plasmacytoma,β_1-plasma cell leukemia and Waldenström's macroglobulinemia
γ-globulin plasmacytoma	↓	↓	↓	↓	↑↑	γ-plasmacytoma, macroglobulinemia and some forms of reticulosis

For an integrated assessment of a proteinogram we use A/G ratio (albumin-globulin ratio), representing a rate of 1 - 2 relative units.

Acute phase proteins (APP) are a large group of serum proteins synthesized in the liver, concentration of which increases in the presence of inflammation, compression, burns, bacterial or viral infection.

These proteins start a cascade of reactions for isolating the inflammatory focus from intact tissue, restoring the impaired structure and function.

Synthesis of acute phase proteins is activated by pro-inflammatory cytokines (IL - 1, 6, 11, tumor necrosis factor, γ-interferon).

APP are specific; their concentration in the blood strongly correlates with the intensity of a disease, the stage of the process and the scope of lesion, which makes these tests valuable in monitoring of the disease and the effectiveness of treatment. The concentrations of different proteins in lesion and inflammation vary widely. The following is a classification of acute phase proteins depending on the increase in their concentration in trauma.

The basic methods used to determine APP are the following:

1. Instrumental: nephelometry, immunoturbidimetry.

These two methods are approximately equal in sensitivity, specificity, complexity and cost of the study. Standard equipment and automation of research makes them optimal methods for large and medium-sized laboratories that perform hundreds of tests per day.

2. Methods that do not require equipment: radial immunodiffusion. Ready-to-use immunodiffusion plates allow for quantitative analysis of C-reactive protein and other APP proteins without instruments or accessory reagents. They are recommended for small laboratories that perform a limited number of studies (from one to 20 tests) per day.

3. Latex can be used as a quick method for semi-quantitative determination of C-reactive protein. It screens for increased concentrations, after which quantitative methods should be used.

APP tests used in clinical practice

1. In acute disease. In all cases, determine the C-reactive protein which concentration is increased as early as 6-8 hours after the onset. If untreated it reaches a maximum within 2-3 days. The highest levels of C-reactive protein are observed in bacterial infection (100 mg / L and more). An effective therapy reduces C-reactive protein concentration on the next day, but if it does not, one should take into account the changes in C-reactive protein and consider adequate antibiotic therapy. In viral infection C-reactive protein concentration may increase only slightly (less than 20 mg / l), which is used to differentiate viral infections from bacterial ones.

2. When there is an accompanying bacterial infection. In any diseases or after surgery a developing bacterial infection, be it a local process or sepsis, is accompanied by increased APP levels.

3. In tissue necrosis. Necrosis causes acute-phase response, similar to that of bacterial infection. This is possible in myocardial infarction, tumor necrosis of kidney, lung, and colon. If there is an increase in the APP concentration, but you cannot see any clear signs of inflammation, the patient should be evaluated for the presence of malignant disease.

4. For monitoring the effectiveness of treatment of chronic diseases. There is a correlation between the activity of inflammation, tissue damage and the level of APP concentration. In this case several APP concentrations should be tested dynamically, which will quickly detect response to treatment. For example, in systemic vasculitis a control C-reactive protein is used as an objective test to minimize the dose of steroids. Acute phase response rejection is one of early signs of renal transplant rejection.

DISCUSSION

1. Structure of blood plasma proteins.
2. Functions of blood proteins.
3. Proteins synthesis in the liver, RES, cells of the immune system.
4. Total protein in the blood serum, hypo-and hyperproteinemia.
5. Methods determining total protein in the blood.
6. Determination of total protein in the urine.
7. General characteristics of protein faction.
8. Albumin, hyper- and hypoalbunemia.
9. α1-Globulins: α1-proteinase inhibitor, α1-acid glycoprotein.
10. α2-globulins: α2-macroglobulin, haptoglobin, ceruloplasmin.
11. β-Globulins: transferrin, hemopexin.
12. γ-Globulins: immunoglobulins, hyper-gammaglobulinemia.
13. Acute-phase proteins, their classification and characteristics.
14. Tests for acute phase proteins used in clinical practice.
15. Proteinogram types.
16. Proteinogram in acute and chronic inflammation.
17. Proteinogram in renal filter impairement and malignancies.
18. Proteinogram in hepatitis, cirrhosis of the liver, jaundice.

INDEPENDENT WORK OF STUDENTS

1. Write down the protocol of your practical class indicating its objectives and outcomes, schemes and methods for determining the total protein of blood and urine, the classification of protein fractions, APP proteins according to the degree of their concentration in the blood serum, tables of normal proteinogram.

2. Interpret proteinograms in various pathological conditions. Give your opinion, write into the protocol.

3. Write down tests for acute phase proteins used in clinical practice. Give your opinion, write into the protocol.

6. Topic: Biochemical research of diseases of the pancreas. The enzyme activity in duodenal juice. Pancreatitis, the diagnostic value of determining the activity of α-amylase in the blood and urine. Trypsin activity, α1-proteinase inhibitor, α2-macroglobulin in the blood.

The pancreas is an elongated organ located in the abdomen. It plays an essential role in converting the food that we eat into fuel for the body's cells. The pancreas has two main functions: an exocrine function that helps in digestion and an endocrine function that regulates blood sugar level.

The pancreas is located behind the stomach and is surrounded by other organs including the small intestine, liver, and spleen. It is about fifteen cm long and is shaped like a flat pear. The wide part, called the head of the pancreas, is positioned toward the center of the abdomen; the middle section is called the neck and the body of the pancreas; the thin end is called the tail and extends to the left side.

Session purpose: To know the biochemical diagnostics of pancreatic diseases, the diagnostic value of the determination of enzyme activity in the blood and urine.

The student should know about:

- pancreatic enzymes;
- diagnostic value of determination of the activity of α-amylase in the blood and urine;
-diagnostic value of determining the activity of trypsin, α1-proteinase inhibitor, α2-macroglobulin in the blood.

The student should be able to:

- interpret the data of the content of pancreatic enzymes in the blood and urine.

Functions of pancreas:

1. *Exocrine Function*: The pancreas contains exocrine glands that produce **enzymes** important to digestion. When food enters in the stomach, these pancreatic juices are released into a system of ducts that culminate in the main **pancreatic duct**. The pancreatic duct joins the **common bile duct** to form the **ampulla of Vater** which is located at the first portion of the small intestine, called the **duodenum**. The common bile duct originates in the liver and the **gallbladder** and produces another important digestive juice called **bile**. The pancreatic juices and bile that are released into the duodenum help the body to digest fats, carbohydrates, and proteins.
2. *Endocrine Function*: The endocrine component of the pancreas consists of islet cells that create and release important **hormones** directly into the bloodstream. Two of the main pancreatic hormones are **insulin**, which acts to lower blood sugar, and **glucagon**, which acts to raise blood sugar. Maintaining proper blood sugar levels is crucial to the functioning of key organs including the brain, liver, and kidneys.

Inflammation of the pancreas is called *pancreatitis.* This disease has two forms: acute and chronic. Either form is a serious condition and can lead to complications. In severe cases bleeding, infection, and permanent tissue damage may develop. Both forms of pancreatitis occur more often in men than women.

Acute pancreatitis is inflammation of the pancreas that has a sudden onset and, if treated, usually resolves within a few days. Acute pancreatitis can be a dangerous illness with severe complications. The most common cause of acute pancreatitis is the presence of gallstones - small, pebble-like substances made of hardened bile - that cause inflammation in the pancreas as they

pass through the common bile duct. Chronic, heavy alcohol use is also a common cause. Acute pancreatitis can occur within hours or as long as 2 days after consuming alcohol. Other causes of acute pancreatitis include abdominal trauma, medications, infections, tumors, and genetic abnormalities of the pancreas.

Chronic pancreatitis is inflammation of the pancreas that does not heal or improve - it gets worse over time and leads to permanent damage. Chronic pancreatitis, like acute pancreatitis, develops when digestive enzymes attack the pancreas and nearby tissues causing episodes of pain. Chronic pancreatitis often develops in people who are between the ages of 30 and 40.

The most common cause of chronic pancreatitis is many years of heavy alcohol use. The chronic form of pancreatitis can be triggered by one acute attack that damages the pancreatic duct. The damaged duct causes the pancreas to become inflamed. Scar tissue develops and the pancreas is slowly destroyed.

Laboratory tests:

1) *Serum amylase*. An increase of amylase in the blood usually indicates pancreatitis.

2) *Serum lipase*. Sudden (acute) pancreatitis almost always raises the level of lipase in the blood.

3) *Complete blood count (CBC).* The number of white blood cells rises during an attack of pancreatitis, sometimes dramatically.

4) *Liver function tests*. Elevated content of liver enzymes, particularly of alkaline amino transferase and alanine phosphatase, can be a sign of sudden pancreatitis caused by gallstones.

5) *Bilirubin.* The content of bilirubin in the blood may increase if the common bile duct is blocked.

6) *Trypsin*. This is a pancreatic enzyme that, along with bile from the liver, digests fats. Measurement of serum trypsin is thought to be the most sensitive blood test for pancreatitis, particularly chronic pancreatitis, but is not widely or routinely used.

Other tests that may be used to check for complications of acute pancreatitis include:

-*glucose*
-*calcium*
-*magnesium*
-*C-Reactive protein* (a measure of inflammation)

Other tests that may be used to help diagnose and evaluate chronic pancreatitis include:

-*Fecal fat*
-*Fecal pancreatic elastase*
-*Molecular biology tests* for genetic mutations such as those associated with cystic fibrosis

α-amylase is major enzyme that participate in hydrolysis of carbohydrates that is decomposition of starch and glycogen to dextrins, maltose and glucose. This enzyme is produced in the salivary glands and pancreas. Thus, in serum there are 2 types of this of alpha-amylase: P-type (pancreatic) and S-type (salivary). Alpha-amylase activity test is primarily used in diagnostics of pancreatic diseases. In acute pancreatitis we may observe an increase in serum alpha-amylase activity within 2-12 hours before onset; the level of enzyme returns to normal within three to four days. Usually, increases the level in 4-6 times with maximum in period 12-72 hours before onset. Alpha-amylase is excreted by the kidneys, thus intensified serum enzyme activity leads to an increase in alpha-amylase content in the urine.

The diagnostic significance of the analysis

The main diagnostic significance of P-type alpha-amylase detection is that its increased activity is highly specific for pancreatic diseases. Pancreatic amylase elevates in acute pancreatitis. In that case, total amylase activity is increased by pancreatic fraction. Diagnostic sensitivity of pancreatic fraction in serum for acute pancreatitis is 92%, specificity - 85%.

Alpha-amylase in pancreatitis

P-type amylase activity detection is especially important for chronic pancreatitis patients with normal level of total amylase. In patients with chronic pancreatitis the level of p-type amounts to 75-80% of the total amylase level. Elevation of p-type means an attack of *chronic pancreatitis*, but its decrease – *exocrine failure* of pancreas in acinar tissue atrophy and organ fibrosis in patients long suffering from this disease.

P-type amylase in urine increases in acute pancreatitis, and it mainly includes total amylase, as it is excreted with urine better than the salivary fraction.

In contrast to total amylase, P-type amylase activity does not increase in parotitis, diabetic ketoacidosis, lung cancer, acute gynecologic diseases. What is more, in other pancreatic diseases the test may be false-positive

Elevation of serum amylase is observed in:

1. Acute pancreatitis
2. Chronic relapsing pancreatitis
3. Pancreatic carcinoma – mainly of the head of the pancreas
4. Obstruction of pancreatic duct
5. Diabetic ketoacidosis
6. Cholecystitis
7. Peptic ulcer
8. Gastric resection
9. Intestinal obstruction
10. Mesenteric thrombosis
11. Peritonitis
12. Abdominal surgery
13. Ectopic pregnancy and salpingitis (Fallopian tube amylase)
14. Treatment with morphine, codeine, (Oddi's sphincter spasm)
15. Renal failure
16. Ectopic production (malignancies)
17. Post-traumatic

Alpha-1-antitrypsin (A1A) is a glycoprotein synthesized in the liver, monocytes, macrophages, and the cells of intestinal mucosa. It acts as an inhibitor for most proteolytic enzymes that contain the serine amino acid (trypsin, chemotrypsin, elastase and other tissue proteases). The most important physiological role of A1A is to suppress proteases, especially elastases that are release from leucocytes during phagocytosis. Having a small size of molecule it is easily diffused from the plasma to other body liquids including bronchial secret.

A1A is an acute phase protein. An increase in its activity may be evidence of inflammation processes: acute, subacute and chronic infections, acute hepatitis and cirrhosis of the liver in active form, acute and chronic. The concentration of A1A in serum increases in malignant tumors: cancer and metastasis, lymphoma, lung diseases.

Deficiency of A1A leads to reduced activity of these enzymes. It is accompanied by intensified cellular destruction and generation of fibrosis tissue. Insufficient concentration of A1A is caused by its damage or gene mutation. Serious native deficiency accompanies liver diseases, especially in childhood (neonatal hepatitis syndrome, infantile cirrhosis) and chronic lung diseases in adults (emphysema and chronic bronchitis). The rate of hepatoma detection also increases in deficiency of A1A populations. Acquired deficiency of A1A occurs in nephrotic syndrome, gastroenteropathy with a loss of proteins, acute phase of thermal burns. It is recommended to screen all patients for A1A concentration routinely as it is impossible to make diagnosis based on clinical data only.

Alpha2 Macroglobulin is a high molecular blood protein found in the serum and other extravascular fluids; it is synthesized in the liver and pancreas. The concentration of A2M in serum is 2-4mg/ml depending on the patient's sex and age. In vertebrates, A2M is one of two main plasma proteases inhibitors. A2M have a wide activity spectrum that inhibits bacterial and eukaryotic endopeptidase. A2M is a unique endogenous proteinase inhibitor that interacts with enzymes and eliminates the proteinase activity but keeps their ability to hydrolyze peptides. In humans it is a large tetramer glycoprotein that weighs 725kD and is involved in pathogenesis of many diseases. Interactions between A2M and proteinases lead to conformational changes of the inhibitor molecule. It leads to quick elimination of A2M from bloodstream by absorption of hepatocytes, macrophages, fibroblasts. It is established that different forms of A2M bind such cytokines as IL-1, 2, 6, 8, TNF-a PDGF, FGF, NGF, TGF and others.

DISCUSSION

1. Functions of the pancreas.
2. Concept and forms of pancreatitis.
3. Laboratory tests for acute and chronic pancreatitis.
4. Diagnostic value of determination of α-amylase in diseases of the pancreas.
5. Diagnostic value of determination of α-1-antitrypsin in diseases of the pancreas.
6. Diagnostic value of determination of α-2-macroglobulin in diseases of the pancreas.

INDEPENDENT WORK OF STUDENTS

1. Write down practice session protocol indicating the purposes and tasks, the main functions of the pancreas.
2. Write down basic forms of pancreatitis and their characterization.
3. Write down laboratory tests for the diagnosis of pancreatic diseases.
4. Write down the diagnostic value of determining α-amylase, α-1-antitrypsin and α-2-macroglobulin with pancreatic diseases.

7. Topic: Diabetes. Its definition, classification and clinical features. Absolute and relative insulin deficiency. The effect of insulin on metabolism. Glucose in whole blood and plasma. Diagnostic criteria of diabetes mellitus type I and II. Hyperglycemia and glycosuria. Impaired fasting glucose, impaired glucose tolerance, postprandial hyperglycemia

Diabetes mellitus (DM) is a chronic metabolic syndrome characterized by hyperglycemia, glycosuria and related metabolic disorders. It develops as a result of absolute or relative (disturbance of interaction with target cells) failure of insulin hormone and leads to disruption of carbohydrate, fat and protein metabolism.

Session Purpose: To study the basic forms of diabetes, to be able to differentiate them according to the main symptoms and clinical manifestations, to properly evaluate the results of the glycemic profile, glucose tolerance test.

The student should know about:

- classification of diabetes and its shape;
- the main symptoms and clinical manifestations;
- difference between absolute and relative insulin deficiency;
- differences in glucose content in whole blood and plasma;
- the principle of oral glucose tolerance test

The student should be able to:

- interpret the results oral glucose tolerance test;
- to evaluate the results of glycemic profile

The main symptom determining the pathogenesis and clinical presentations of diabetes is hyperglycemia. Normal fasting glucose values range 3.3-5.5 in children under 14 years, and 3.8-5.8 in adults. Blood glucose undergoes complete ultrafiltration in the glomeruli of the kidneys, and is then completely reabsorbed in the renal tubules. However, the ability of tubular epithelium to reabsorb glucose has a quantitative limit (renal threshold for glucose is 8.9-10 mmol / L). Thus, as soon as blood glucose and glucose in primary urine exceeds this limit, glycosuria develops.

Classification of Diabetes Mellitus

Currently proposed classification of diabetes mellitus using an etiological principle

Etiological classification of diabetes mellitus (WHO, 1999)

I. Diabetes mellitus type 1 (destruction of beta cells, absolute insulin deficiency)

A. Hashimoto diabetes

B. Idiopathic diabetes

II. Diabetes mellitus type 2 (development of primary insulin resistance with relative insulin deficiency to predominantly secretory defect of insulin in combination with peripheral insulin resistance)

III. Other specific types of diabetes

A. Genetic defects of beta-cell function

B. Genetic defects in insulin action

C. Diseases of the exocrine pancreas

D. Endocrinopathy

E. Diabetes induced by chemicals and drugs

F. Infection (congenital rubella, cytomegalovirus)

G. Unusual forms of immune-mediated diabetes

H. Other genetic syndromes sometimes combined with diabetes (Down's syndrome, Klinefelter's syndrome, Turner's syndrome, etc.)

IV. Gestational diabetes

Diabetes is of two types. Clinical manifestations of diabetes usually present two groups of symptoms: main and secondary ones.

The main symptoms of diabetes are:

Polyuria, that is, increased urine output, which is caused by increasing its osmotic pressure due to the presence of dissolved urine glucose (normal glucose in urine should not be present). Fairly frequent urination during the day and at night is noted.

Polydipsia, which is unquenchable thirst due to substantial loss of water in the urine, as well as an increase in the osmotic pressure of blood. Patient drinks 3-5 liters of fluid and more a day.

Polyphagia, which is constant insatiable hunger. This symptom is caused by metabolic disorders accompanying diabetes, the inability of cells to absorb and process glucose without insulin.

Signs of the first type of diabetes are: thirst, frequent urination, great weight loss, dry mouth, irritability, fatigue, nausea and sometimes vomiting. Minor signs of this type of diabetes are: heart pain, pain in the calf muscles and cramps in them, abrasions, itching, headaches, irritability and sleep disturbances. Speaking of the secondary symptoms of diabetes type 1 in children, we should note the emergence of previously unobserved bedwetting and rapid deterioration of health.

The second type of diabetes is characterized by numbness, leg cramps, pain in the feet and hands, sensation of constant thirst, itching, blurred vision, poor wound healing, presence of skin infections, fatigue and drowsiness, decreased sensitivity to pain, frequent infectious disease, gradual weight gain, reduced male potency, and etc. In addition, in diabetes type 2 we observe loss of hair growth, appearance of small yellow body growths called xanthomas. Balanitis associated with frequent urination is also one of the first signs of diabetes type 2.

The effect of insulin on metabolism

In almost all tissues of the body insulin affects the metabolism of carbohydrates, fats, proteins and electrolytes, increasing the transport of glucose, proteins and other substances through the cell membrane.

The main action of insulin is to increase glucose transport across the cell membrane. Glucose in the blood serum reflects the ever-changing state of the two processes under permanent control of insulin: glucose utilization and release of glucose into the bloodstream.

Its biological effect on the cellular level is exercised through the appropriate insulin receptor in tissues. Stimulation of insulin increases the rate of glucose transport in cells 20-40 times, where proteins are the transporters. With stimulation of insulin, the content of glucose transporter proteins in the plasma membranes increases 5-10 times, while their content in the intracellular pool reduces by 50-60% of. Stimulation of glucose increases energy expenditure 20-30 times.

Most of the insulin is metabolized in the liver, in one passage it retains 40-60% of the hormone coming from the portal vein system. After binding to insulin the receptor of hepatocytes is exposed to proteolysis accompanied by inactivation of the hormone. About 40% of insulin is inactivated by the kidneys. It should be noted that renal uptake and inactivation of insulin by the kidneys is reduced to 9.10%, in diabetic patients with renal failure insulin requirements are decreased (Zubrody Dan syndrome).

Absolute and relative insulin deficiency

The basis of the disease is *absolute* and *relative* insulin deficiency.

Absolute failure develops due to a decrease in insulin B-cells of Langerhans islets of the pancreas as a result of degenerative changes or necrosis under the influence of damaging factors or impaired insulin synthesis, resulting in incretion hormone with reduced biological activity.

Absolute insulin deficiency contributes to autoimmunity (disturbed immunogenesis system causing development of auto-immune aggression with selective lesion of B-cells), viral infection, inflammatory disease, fibrosis or calcification of the pancreas, circulatory changes (atherosclerosis), cancer.

Absolute insulin deficiency is the cause of diabetes in only 10% of patients. In most cases, development of the disease occurs in normal or even high concentrations of endogenous insulin in the blood. The cause of metabolic disorders in these cases is ***relative insulin deficiency,*** which is based on reduced sensitivity of insulin-dependent tissues to the action of endogenous insulin: tissue insulin resistance.

Glucose in whole blood and plasma

Normal fasting glucose values range between 3.3-5.5 mmol / l in children under 14, and 3.8-5.8 mmol / l in adults. In whole blood glucose concentrations are lower than in plasma. The cause of this discrepancy is the lesser water content in the whole blood.

Hyperglycemia and glycosuria

Hyperglycemia is a clinical symptom indicating high blood glucose in the blood serum. Hyperglycemia appears mainly in diabetes or other diseases of the endocrine system.

Diagnosis of diabetes and other categories of hyperglycemia (WHO, 1999)

	Glucose concentration, mmol / L			
	whole blood		plasma	
DM	venous	capillary	venous	capillary
Fasting	> 6.1	> 6.1	> 7.0	> 7.0
2 hours after glucose load	> 10.0	> 11.1	> 11.1	> 12.2
Impaired glucose tolerance				
Fasting (if defined)	<6.1	<6.1	<7.0	<7.0
2 hours after glucose load	> 6.7 and <10.0	> 7.8 and <11.1	> 7.8 and <11.1	> 8.9 and <12.2
Impaired fasting glycemia				
Fasting	> 5.6 and <6.1	> 5.6 and <6.1	> 6.1 and <7.0	> 6.1, and 7.0
In 2 hours	<6.7	<7.8	<7.8	<8.9

There are several conventional degrees of severity of symptoms:

- Slight hyperglycemia (blood sugar is 6 - 10 mmol / l).
- Moderate hyperglycemia (10 - 16 mmol / l).
- Severe hyperglycemia (more than 16 mmol / l).

In people with diabetes mellitus, there are two types of *hyperglycemia:*

- Fasting hyperglycemia (if the person was not eating for about 8 hours, the level of blood sugar rises above 7.2 mmol / l).
- Postprandial hyperglycemia (after meals blood sugar rises above 10 mmol / L).

Glycosuria means identification of glucose in the urine. In the urine of healthy humans glucose is contained in a very low concentration (0.06-0.083 mmol / L). Therefore, and due to low sensitivity of the method, it is not revealed in urine tests in clinical diagnostic laboratories.

Detection of glucose in the urine indicates pathology. Glycosuria depends on three factors:

- concentration of glucose in the blood,
- renal glomerular filtrate in 1 minute,
- reabsorption of glucose in the tubules in 1 ml.

Glycosuria often precedes hyperglycemia. After filtration in the renal glomeruli glucose is reabsorbed in the proximal tubule.

With normally functioning kidneys glycosuria develops when the level of glucose in the blood exceeds 8.8-9.9 mmol / L, the so-called "renal threshold" or glomerular glucose clearance. The concept is relative, as the "renal threshold" is defined by the enzyme system of renal epithelium. The "renal threshold" in children is over 10.45-12.65 mmol / L.

The scope of glomerular filtration rate also affects the level of glycosuria. Its decline does not always cause glycosuria. Therefore, in some chronic kidney diseases the threshold for glucose increases. In the case of kidney disease associated with impaired glucose resorption (renal diabetes) we may note glycosuria and normal or low levels of blood glucose.

Glucose tolerance

Glucose intolerance is a condition which precedes diabetes. In this condition, the patient's blood glucose level is higher than normal but lower than that justifying the diagnosis of diabetes according.

Diagnostic criteria for assessing glucose tolerance test (WHO Expert Committee on Diabetes Mellitus, 1999)

Evaluation results	Capillary blood glucose, mmol / l	
	Fasting	In 2 hours
Strong	<5.	<7.8
Impaired glucose tolerance	<6.1	> 7.8 <11.1
Diabetes mellitus	> 6.1	> 11.1

Diagnostic importance of this condition is that at this stage it is already possible to identify the risk of type 2 diabetes and to prevent it just in time. It was found that 10 years after

the detection of impaired glucose tolerance, one third of patients develops diabetes, and a third of patients normalizes metabolism!

Therefore, an oral glucose tolerance test can identify at-risk patients who could potentially suffer serious illness, advance to make recommendations to prevent and thus preserve their health and extend their life. That is why now we call it "pre-diabetes", while earlier we called it glucose intolerance.

How to determine if there is glucose intolerance?

The presence of impaired glucose tolerance is determined by the same test for glucose in the blood. The so-called glucose tolerance test helps significantly to confirm or exclude the presence of impaired glucose tolerance. To do this, after determining the level of fasting blood glucose, the patient is given a drink of 75 g of glucose dissolved in 250-500 ml of water for 5 minutes (for the kids - 1.75 g per 1 kg of body weight). During the test in very obese patients glucose is added at the rate of 1 g per 1 kg of body weight, but not more than 100 g. After that capillary blood sampling is made in 1 and 2 hours.

In a healthy person after taking the glucose there is a rapid rise in blood sugar for 20-60 minutes (slightly different rates in venous and capillary blood) due to the absorption of glucose in the intestine. After this comes its decline due to the reaction of the regulatory system (insulin), down to the original level between 1.5 - 2 hours after glucose intake. Between 2 and 2.5 hours, there is a decline below the initial value of fasting plasma glucose, the more, the higher the initial level. Between 2.5 and 3 hours, blood sugar returns to normal. In patients with impaired glucose tolerance, fasting blood sugar is somewhat higher and in two hours it does not fall to the initial value.

Interpretation of the results of this test is of great clinical importance, so it is important to know the following:

• For a few days before the test a normal diet should be maintained with carbohydrates at least 125-150 grams per day. It is important to know that if the day before the patient is not getting enough carbohydrates, the increase in blood glucose levels will be higher and the fall of it is later, which greatly distorts the interpretation of results.

• For a few days before the test the patient should follow the usual physical activities. Significant physical activity before the test can cause increased growth of blood glucose levels, and physical strain after taking glucose can give more pronounced and longer lasting wave of hypoglycemia.

• The test is carried out on an empty stomach in the morning after a night sleep for 10-14 h

• Before the test, in the evening the patient should refrain from smoking and drinking alcohol.

• During the test (2 h after glucose administration), the patient should lie down or sit back, temperature fluctuations should be excluded (for example, when leaving the room), as well as physical activity. ***Eating, drinking and smoking are not allowed during the test!***

• The test is not recommended during and after stress effects, debilitating diseases, after surgery and childbirth, inflammation, alcoholic cirrhosis of the liver, hepatitis, during menstruation, diseases of the gastro-intestinal tract, malabsorption of glucose, malignant diseases.

• Before the test we discontinue medical treatments and medications (epinephrine, corticosteroids, contraceptives, caffeine, carbonic anhydrase inhibitors (acetazolamide, diamox), phenytoin (difenin) diuretin, morphine, tiazidine drugs. False-positive results are observed in hypokalemia and some endocrine disorders (acromegaly, Cushing's syndrome, hyperthyroidism).

• If the function of the gastrointestinal tract is in any way impaired (gastric surgery, peptic ulcer), you must perform the test with intravenous glucose.

• Glucose tolerance test may be false negative (blood glucose within the normal range) when there is any form of malabsorption, weight-reducing diet, intense exercise on the eve of the procedure.

Postprandial hyperglycemia

As we know, chronic hyperglycemia is the cause of development and progression of complications of the disease, and macroangiopathypathic complications are the main cause of death in patients with diabetes.

A recent analysis by the scientists confirmed that improved glycemic control significantly reduces the incidence of macroangiopathypathic complications in patients with diabetes type 1 or type 2. Until recently, the dominant focus of therapy has been to reduce HbA1c levels, with particular emphasis on the performance of fasting glucose. However, despite the fact that the control of fasting plasma glucose is required, maintaining optimal glycemic control is usually enough. Currently, there has been a sufficient amount of data showing that the decline in postprandial (after meals) plasma glucose plays the leading role and is equally important in achieving the targets of glycated hemoglobin (HbA1c).

As a result, it is fairly universally recognized that postprandial hyperglycemia is an independent risk factor for macroangiopathypathic complications.

Thus, postprandial blood glucose causes severe complications and should be monitored.

Numerous studies have shown that the use of drugs that reduce postprandial plasma glucose helps to reduce the incidence of vascular complications. Thus, therapy aimed at reducing both fasting plasma glucose (FPG) and postprandial glucose, is strategically important to achieve optimal glycemic control in the light of the prevention of diabetic complications. It is clear that implementation of a strategy aimed at normalization of postprandial glucose, is absolutely necessary.

DISCUSSION

1. Diabetes, definition.
2. Classification of diabetes mellitus.
3. The main forms of diabetes.
4. Diagnostic criteria for diabetes mellitus type I and II.
5. Main symptoms and clinical manifestations.
6. Insulin effect on metabolism.
7. Hyperglycemia and glycosuria.
8. Glucose in whole blood and plasma, the difference.
9. Impaired glucose tolerance.
10. Diagnostic criteria for assessing glucose tolerant test.
11. Impaired fasting glycemia.
12. Absolute and relative insulin deficiency.
13. Postprandial hyperglycemia

INDEPENDENT WORK OF STUDENTS

1. Write down practice session protocol indicating the purposes and tasks, forms of diabetes, major clinical symptoms, normal blood glucose, in contrast to its content in whole blood and plasma, the principle of glucose tolerant test, absolute and relative insulin deficiency.

2. Interpret results of glucose tolerance test. Give conclusion writing into the protocol.

8. Topic: Diabetes. Methods for determination of glucose. Early diagnosis of diabetes mellitus: identification of antibodies to β-cells of the pancreas, proinsulin and C-peptide. Compensation of diabetes. Effective glycemic control: determination of glycated hemoglobin, fructosamine. Assessment of cardiovascular risk: HbA1C, fasting venous plasma glucose, capillary blood glucose before meals, postprandial hyperglycemia, and lipid profile. Hypoglycemic coma.

Now the world has accumulated evidence that the effective control of diabetes can minimize many of the complications associated with it. Thus, improved control of blood glucose levels can significantly reduce the risk of both microangiopathy and macroangiopathy. For each decreased percent of glycated hemoglobin risk of microvascular complications (retinopathy, nephropathy) is reduced by 35%.

Session Purpose: To investigate the methods for the determination of glucose, markers of early diagnosis of diabetes, how to identify markers of cardiovascular risk.

The student should know about:

- methods for the determination of glucose;
- key markers of early diagnosis of diabetes;
-glycosylated hemoglobin and fructosamine – as an effective exposure controls hyperglycemia;
- an assessment of cardiovascular risk in diabetes mellitus;
- the concept of hypoglycemic coma.

The student should be able to:

- interpret the results of glycosylated hemoglobin content;
-evaluate the lipid profile.

Methods for determination of glucose.

Determination of the concentration of glucose in the blood – one of the most frequently performed biochemical studies in the clinical diagnostic laboratory. The reason for the exceptional popularity of the test due to the high incidence of diabetes. This test is performed in the hospital and outpatient settings. Diabetics have to investigate the level of blood glucose at home, because without this information it is difficult to adjust their diet, exercise, the use of insulin and other glucose-lowering drugs. The extreme importance of the test and carried large amounts of research stimulated developers to create various types of instruments and methods for determining the concentration of glucose in the blood.

At present there are many methods for determining glucose. They can be classified as follows:

1. Reductometric. Hardly used
2. Colorimetric. Hardly used
3. Enzymatic:
   a) glucose oxidase
      - Photometric end-point.
      - Photometric kinetic.
      - Reflectance photometry - dry chemistry.
      - Electrochemical.
   b) hexokinase.

The first two methods are extremely inconvenient, toxic and with low accuracy, so we are not going to stop.

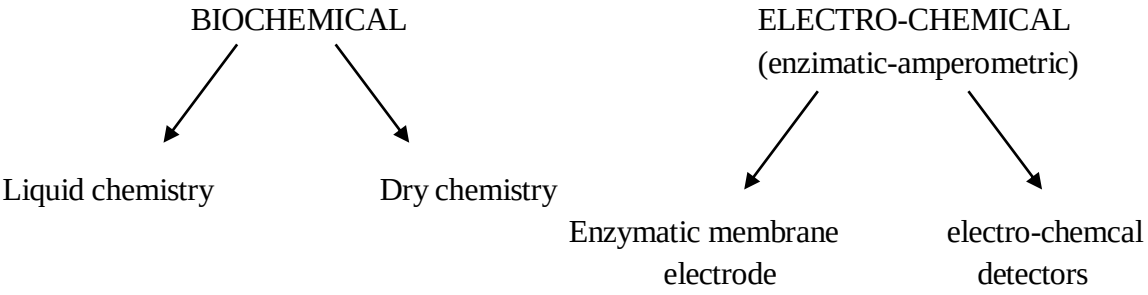
Oxidase method

Today, the most widely used methods based on the use of enzyme - glucose oxidase. Method is based on the following reaction:

$$GLUCOSE + H_2O + O_2 \xrightarrow{GLUCOOXIDASE} GLUCURONIC\ ACID + 2H_2O_2$$

Glucose oxidase catalyzes the transfer of two hydrogen atoms on the first carbon atom of glucose on the oxygen dissolved in the liquid reagent. In the course of the reaction hydrogen peroxide is formed in equimolar amounts. Ie the concentration of the hydrogen peroxide formed is exactly determined by the concentration of glucose. Consequently, the use of the glucose oxidase reaction, transformed the task of determining the concentration of glucose in the task of determining the concentration of hydrogen peroxide, which, as will be shown below, is much simpler than the first. And there are several ways that are widely used today in the laboratory (see diagram).

METHODS FOR REGISTRATION OF GLUCOOXIDASE REACTION



Among the above methods of registering the most widely used photometric biochemical method in which molecules of hydrogen peroxide by the enzyme peroxidase is cleaved to form of an active oxygen - superoxide anion radical – O2, which oxidizes the chromogen, whith results in a significant change in the absorption spectrum of the chromogen.

$$H_2O_2 \xrightarrow{PEROXIDASE} O_2 + CHROMOGEN \longrightarrow COLORATED\ PRODUCT + H_20$$

The great popularity of this method of determination of glucose is due to its high specificity and ease of implementation. The method can be implemented as a conventional photometer, and with the help of automatic biochemical analyzers.

Oxidase method is recognized today as one of the most accurate methods for the quantitative determination of glucose. As biological material serum and whole blood may be used. When working with the whole blood should take into account the fact that the capture of the proportion of capillary blood serum (plasma) depends on hematocrit values, which can lead to inaccurate results. Therefore, in determining glucose method described above is preferred to use a patient's blood serum.

Along with the method of photometric end-point, a few years ago, there were sets that implement the kinetic photometric method. The method states that the rate of colorating of a probe after addition some reagents will be proportional with the concentration of glucose in the serum (in the constant activity of both enzymes glucooxidase and peroxidase). The advantage of this method is that the result does not depend on the presence of other compounds in the sample, because the absorption of these compounds is stable over time. This method requires the application of the kinetic photometer, semi-automatic biochemical analyzer or analyzers. Measurement of the concentration of glucose from whole blood is conveniently performed using the instruments, which are based on the amperometric measuring principle, with special enzyme sensors. Hydrogen peroxide is a highly unstable chemical compound, and it can be a source of charged particles. This is what is used in enzyme membrane type sensors or electrochemical cell portable blood glucose meters.

Finally, we should mention the shortcomings of the glucose oxidase method. The resulting hydrogen peroxide and superoxide anion radical can oxidize not only chromogen, but other substances in biological fluids: ascorbic acid, uric acid, bilirubin. In this case, therefore, the percentage of peroxide, taking part in the oxidation of the chromogen is reduced, leading to lower results for glucose. This method is linear, usually up to 20–30 mmol / l glucose.

Hexokinase method

Hexokinase method consists of two consecutive reactions, but is quite different:

$$\text{GLUCOSE + ATP} \xrightarrow{\text{HEXOKINASE}} \text{GLUCO-6-PHOSPHATE + ADP}$$

$$\text{GLUCO-6-PHOSPHATE + NADP} \xrightarrow{\text{DEHIDROGENASE}} \text{6-PHOSPHOGLUCONATE + NADP}\times H_2$$

Registration is carried out at a wavelength of 340 nm absorption by NADH. This method is highly specific and does not react with other components of blood serum. Hexokinase method is considered the reference for determination of glucose. As a rule, it is linear up to 50 mmol / L, which allowed his widely recommended for clinics with endocrine compartments.

Early diagnosis of diabetes mellitus: identification of antibodies to β-cells of the pancreas, proinsulin, C-peptide

Antibodies to beta-cells of the pancreas (anti-islet cells, ICA) – it is a marker of autoimmune destruction of beta cells in the pancreas that produce insulin. Main indications are: diagnosis of diabetes of the first type, the assessment of risk of diabetes of the first type in individuals with family history of diabetes.

This type of autoantibodies (antibodies produced in the body to its own antigens, proteins and other substances in the body) to antigens produced by the islet cells of the pancreas which secrete insulin. Test, in fact, refers to the process of destruction of islet cells. A characteristic feature of this group of antibodies is their early appearance in the blood serum, in the few years before the development of the clinical form of diabetes. These antibodies appear in patients before the clinical development of diabetes after infectious diseases caused by Coxsackie B4 virus, mumps and other viruses. Determination of these antibodies can be used to identify the risk of developing insulin-dependent diabetes mellitus.

Marker of autoimmune destruction of beta cells of the pancreas are autoantibodies to islet cells – ICA. These are antibodies to antigens that are in the cytoplasm of cells of islets of

Langerhans. They can be defined in the serum of healthy individuals (0.5%), in patients without diabetes, who are relatives of patients with diabetes of the first type (2–6%) and are found in patients with diabetes in 70–80% of cases. Found a pattern: the younger the patient with known antibodies ICA and the more their titer, the higher the probability of diabetes of the first type outcome. Antibodies are found not only in patients with diabetes, but also for the relatives of patients, more frequently in those who have identical genes of HLA.

Keep in mind that antibodies to pancreatic islets are not specific to the antigens of the beta cells, although there is a small cross-reaction between the two. Feature of antibodies to antigens of the islets is a decrease in their content with increasing time from the start of the first type of diabetes. In the first months of the manifestation of the disease they are found in 70–90% of those 1–2 years only 20%. 15–20 years cytoplasmic antibodies (ICA) can be found only in 5% of patients.

C-peptide – is an indicator of insulin synthesis and carbohydrate metabolism. The main indications for use: Diabetes Type I and II, insulinoma, assessment of insulin secretion in liver diseases, assessment of insulin therapy.

C-peptide is a protein part of proinsulin, which is formed during the synthesis of insulin. In response to increasing glucose proinsulin is divided to insulin and C-peptide and secreted into the blood in equimolar amounts. As about formation of C-peptide, proinsulin represented by one large polypeptide chain containing 84 amino acids, it has low biological activity. Site of synthesis of proinsulin is microsomal fraction of the beta cells of pancreatic islets, proinsulin conversion to active insulin by partial proteolysis occurs when proinsulin moves from ribosomes to secretory granules (cleavage of the C-terminus polypeptide chain containing 33 amino acids and called “joining” or C-peptide). Length and primary structure of the C-peptide is more variable in different animal species than A and B chains of insulin. C-peptide does not possess biological activity, but it reflects the rate of insulin synthesis. However, the half-life of insulin and C-peptide levels are different, there is a strong correlation between their presence in the blood, but concentrations in the serum does not match. The ratio of C-peptide to insulin is usually 5: 1. Determination of C-peptide can evaluate the content of synthesized insulin, because insulin used in the treatment, does not contain the C-peptide.

Proinsulin - the precursor of insulin, synthesized by beta-cells of the islets of Langerhans of the pancreas. The main cases for use: clinical signs of insulinoma, identifying reasons for hyperinsulinism.

3–10% of the proinsulin enters the bloodstream, the rest is converted into insulin by cleavage of C-peptide. Insulin and C-peptide enter the blood in equimolar amounts. Proinsulin has virtually no metabolic activity of insulin (glucose-lowering activity). Its activity is more than 10 times less than the activity of insulin, but a significant increase in its concentration can lead to hypoglycemia.

Proinsulin is a key marker for the diagnosis of tumors in the beta cells of the pancreas (insulin). This test may be important in the differential diagnosis of conditions associated with hyperinsulinemia.

Criteria for diabetes compensation

The patient's satisfactory state, a stable course of the disease (daily glycemic values within normal) and normal levels of **glycated hemoglobin** are considered to be the criteria allowing the physician to regard the diabetes as compensated.

A long-term objective indicator of the degree of compensation of diabetes is glycosylated (glycated) hemoglobin (or glycohemoglobin or HbA1c test, where Hb – hemoglobin, Al c – bound glucose). Hemoglobin and other proteins bind with glucose in a slow non-enzymatic

reaction, which depends on the concentration of glucose. The more glucose in the blood, the more glycated hemoglobin accumulates in erythrocytes. Determination of glycosylated hemoglobin test reflects the average blood glucose over the past 2-3 months.

Normally HbAIc content in the blood is 5–7% of total hemoglobin.

Fructosamine – a product of protein glycosylation of blood plasma (the compound of glucose and proteins). Albumin gives more than 60% of all proteins that react with glucose. The degree of glycosylation of plasma proteins depends on the concentration of glucose in the blood and the length of the proteins half-life period. Number of fructosamine in the blood is a good indicator for retrospective monitoring of blood glucose in patients with diabetes and it evaluates the effectiveness of the treatment without laborious daily monitoring of blood glucose.

The half-life of serum proteins is less than the life of red blood cells. Therefore, in contrast to the glycosylated hemoglobin, fructosamine levels reflect the degree of permanent or transient increase in blood glucose for 1–3 weeks before the study.

Lipid profile in diabetes

The **lipid** spectrum in diabetes type 2 is characterized by the "lipid triad", which includes:

- increase in the concentration of triglycerides,
- reduction in high density lipoprotein cholesterol (HDL),
- predominance of small dense particles of blood low-density lipoprotein (LDL).

This condition can lead to the development of **metabolic syndrome.**

The mechanism of occurrence and development of the metabolic syndrome:

- When insulin resistance takes place, beta cells of the pancreas increase the synthesis and secretion of insulin to compensate for the disturbed insulin sensitivity and maintain normal glucose tolerance, and hyperinsulinemia develops.
- Chronic hyperinsulinemia causes paradoxical vasoconstriction and increased cardiac output blood volume, resulting in hypertension.
- Insulin regulates the rate of synthesis of VLDL in the liver. It leads to the increase of VLDL and decrease of HDL.

Thus hyperinsulinemia is a compensatory response that provides for normal transport of glucose into cells, accompanied by a number of pathological disorders. This process continues as long as the pancreas is still able to increase the secretion of insulin. But, at a certain point, the secretion of insulin is not sufficient to maintain normal tissue glucose tolerance, and glucose tolerance becomes impaired followed by decompensation of the pancreas, respectively insulin in the blood begins to fall when the patient is fasting. Diabetes develops.

Clinical presentations

The main symptoms and signs of the metabolic syndrome:

- abdominal visceral obesity
- insulin resistance and hyperinsulinemia
- dyslipidemia
- hypertension
- impaired glucose tolerance / diabetes type 2
- early atherosclerosis / CHD
- microalbuminuria

Treatment

- weight-reducing diet

- increasing physical activity
- eliminating smoking and alcohol consumption as factors in the development of disease

Hypoglycemic coma

The increase of blood sugar level is dangerous, but sudden decreasing of glucose concentration is more severe. In concludion, one of the most common acute complications of diabetes is hypoglycemic coma.

In the basis of this condition is hypoglycemia, that is, drop in blood sugar. The disease occurs when the blood glucose is between 3 and 3.5 mmol / l or less, although in some situations, such as an active physical work, a number of features can be observed at 4 mmol / l.

Hypoglycemia is often a consequence of a breach of reception of tablets sugar-reducing drugs or insulin. Depending on the severity can distinguish mild, moderate and severe hypoglycemia. Most often this condition can occur in diabetes l-type, being just the result of violations of the dosage of insulin, but often can occur in elderly patients with diabetes mellitus type 2.

Hypoglycemic coma - is severe manifestations of hypoglycemia.

DISCUSSION

1. Diabetes mellitus and its definition.
2. Methods for determination of glucose in the blood.
3. The principles of the glucose oxidase and hexokinase methods.
4. The methods of early diagnosis of diabetes.
5. Detection of antibodies to the β-cells of the pancreas, the role in the diagnosis of diabetes.
6. Determination of proinsulin and C-peptide, role in the diagnosis of diabetes.
7. Compensation criteria of diabetes mellitus.
8. Glycosylated hemoglobin, the concept.
9. Early diagnosis of diabetes: glycosylated hemoglobin and fructosamine.
10. Lipid profile in diabetes mellitus.
11. Postprandial hyperglycemia, the concept.
12. Hypoglycemic coma and its causes.

INDEPENDENT WORK OF STUDENTS

1. Write down practice session protocol indicating its purposes and tasks, basic principles for determining glucose in blood, early diagnosis of diabetes, the concept of glycosylated hemoglobin and its role in the diagnosis of diabetes, hypoglycemic coma

2. Interpret the results of the content of glycated hemoglobin. Give a conclusion in written form into the protocol.

3. Write downthe main changes in the lipid profile. Give a conclusion in written form into the protocol.

9. Topic. Cardiovascular system diseases. Atherosclerosis, the stages of development. Disorders of lipid metabolism. Diagnostic value of the determination of the cholesterol content and its fractions in the structure of lipoprotein blood. Hypercholesterolemia. Main indicators of atherosclerosis: total cholesterol, α-cholesterol (HDL), atherogeneity index. Recommended and border values of total cholesterol, moderate and severe hypercholesterolemia.

Lipid metabolism disorder is the main reason of cardiovascular diseases. And it's important to diagnose dislipidemia.

Session Purpose: To estimate the indicators of lipid metabolism and calculate the risk of cardiovascular diseases development.

The student should know about:

- structure and functions of different classes of lipids;
- features of lipids spectrum research;
- dislipidemia diagnosis algorithm;
- rules of blood sampling for lipids spectrum;
- the mechanism of atherosclerosis development and its complications.

The student should be able to:

Interpret result of lipids spectrum research in case of different pathological conditions.

Lipids are organic compounds, water-insoluble, but soluble in organic solvents (ether, gasoline, chloroform).

Classification

- Fatty acids.
- Phospholipids.
- Cholesterol.
- Triglycerides.
- Glycolipids.
- Lipoproteins.

Functions of lipids

1. Structural: phospholipids, glycolipids, cholesterol are as a part of membranes
2. Power: when splitting 1g of fat, 38,9 kJ of energy is formed.
3. Reserving: a reserve energy source (a fat drop in a cell, a fatty body of insects, a hypodermic fatty tissue).
4. Protective:

- physical protection against bruises;
- water repellency: wax: cuticle, feathers, wool;
- electric isolation: glycolipids (myelin);
- prostaglandins (causing fever, stimulate reduction of muscles of an internal).

5. Thermoregulatory:

- thermal isolation (hypodermic fat);
- «brown fat» is a biological heater.

6. Source of endogenous water: oxidation of 100 g of fat gives 107 ml of water.
7. Regulatory: lipids are predecessors of synthesis of steroid hormones, liposoluble vitamins A, D, E.

Cholesterol

Daily consumption of cholesterol is in a range from 0,2 to 0,5 g. In an organism more than 1 g is daily synthesized. All cells of an organism contain it as a part of the membranes and are theoretically capable to synthesize it. Cholesterol total in a body of the person hugely is more than 300 g.

Cholesterol in a form bound to fatty acids contains in adrenal glands, gonads (83%), in a blood plasma (70%).

Cholesterol functions:

• lowers fluidity and a transitivity of biological membranes,

• participates in ensuring barrier function of membranes,

• influences activity of membranous enzymes,

• excess of cholesterol in a cytoplasmatic membrane complicates operation of calcium pumps,

• is the predecessor of steroid hormones of adrenal glands and sexual hormones, vitamin D,

• being oxidized, turns into bilious acids and eliminates from an organism,

• the lack of a cholesterol in the organism promotes the increased risk of development of tumoral and viral diseases.

Lipoproteins

Particles of lipoproteins have a spherical form and consist of a hydrophilic envelope and a hydrophobic core. The hydrophobic core is presented non-polar triacylglycerides and cholesterol ethers. The hydrophilic envelope is the top tessellated monolayer consisting of phospholipids, cholesterol and apoproteins. The hydrophilic envelope provides solubility of lipoproteins and defines paths of a metabolism and destiny of each lipoproteins (due to apoproteins).

In accordance with hydrated density LP can be divided into five classes:

- chylomicrons,
- very low density lipoproteins (VLDL),
- intermediate density lipoproteins,
- low-density lipoproteins (LDL),
- high density lipoproteins (HDL).

Characteristics of lipoproteins

Class of LP	Density	Size, nm	Compounds, %				Apo	Synthesis	Functions
			Protein	TG	chol	PhL			
Chylomicrons	<0,960	500-700	4	90	1	5	A-IV, B-48, C, E	small intestine	transport of exogenous TG
VLDL	0,960-1,006	30-70	10	65	15	10	B-100, C, E	liver	transport of endogenous TG
Intermediate density LP	1,007-1,019	15-25	10	35	40	15	B-100 C, E	metabolism of VDL	precursor of LDL
LDL	1,020-1,063	15-30	20	5	50	25	B-100	metabolism of VDL	transport of chol
HDL	1,064-1,210	7,0-13	45	5	25	25	A-I, A-II, C, E	metabolism of VDL, small intestine, liver	return transport of chol

Apo – apoproteins,
TG – triglycerides,
chol – cholesterol,
PhL – phospholipids.
The less the size of the LDL particle is the higher is the atherogenesis.

The size of LDL is a predictor of acute coronary diseases.

• Increase of LDL level for 10% is accompanied by increase in risk of an ischemic heart disease for 20%

• The integral risk of an ischemic heart disease increases at a combination to other risk factors:

– low level of the HDL
– smoking
– arterial hypertension
– diabetes

HDL deletes an excess cholesteriol from tissues and from a bloodstream and promotes its transportation in a liver.

- HDL are anti-atherogenous, because of decreasing the risk of atherosclerosis.
- The lower is HDL level the higher is the risk of atherosclerosis (low level of the HDL < 0.9 mmol/l)
- HDL level is lowered at high TG
- HDL level is lowered during the smoking, an obesity, a hypodynamia.

Triglycerides

• Correlation between TG levels and increased risk of an ischemic heart disease is proved.

• This correlation may be caused by:

– low level of the HDL
– persistanse of the LDL high-atherogenous forms (small particles).

Common cholesterol = LDL + VLDL + HDL
Calculation of the LDL
VLDL=TG/2,2, mmol/l
VLDL=TG/5, mg/dl
LDL=cholesterol-TG/2,2-HDL, mmol/l
LDL=cholesterol-TG/5-HDL, mg/dl

LDL = chol – (HDL +TG*/2,2)
*if level of TG doesn't exceed 4,5 mmol/l (Fridvald's Formula)

Research of a lipids exchange

Rules of a blood capture for research of a lipids exchange:

1. Blood should be taken in the morning on an empty stomach (for TG and LDL research) in 12–14 h after food intake.

2. Patient should follow his routine diet during 2 weeks before blood capture.

3. Alcohol intake should be avoided in the evening before blood capture: presence of alcohol in the blood is the widespread reason of identification of a hypertriglyceridemiya, even at starving patients.

4. If patient undergone a myocardial infarction, blood should be taken within 24 h after a heart attack, or after 3 months because during convalescent period lipids metabolism is broken.

5. Don't allow a blood stasis, don't press vessels more than 1 minute long.

6. The pose of the patient at a blood capture should be standardized.

7. It is necessary to use only one type of a blood sample: capillary blood, serum or blood plasma.

8. Separation of serum (plasma) from formed elements of blood should be carried out during the first 3 h after the moment of a blood capture.

9. Samples should be stored at temperature of 0–4 °C no more than 3 days.

10. In the course of test storage concentration of thriglycerides changes under the influence of endogenous lipases. Concentration of TG decreases, and the content of the free glycerin grows; severety of these changes is individual and it isn't associated with initial level of TG.

11. Hemolysis prevents from the measurement of lipids and LP.

The level of lipids and LP	The concentration of lipids and LP, mmol / L				Atherogenic index
	chol	LDL	HDL	TG	
Desired	<5,2	<3,36	>1,0	<2,0	<3,0
Borderline-high	5,2-6,5	3,36-4,14	0,9-1,0	2,0-2,5	3,0-4,0
High	>6,5	>4,14	<0,9	>2,5	>4,0

Target levels of the maintenance of lipids in blood according to the European recommendations, 2003.

Indicator	Patients without coronary heart disease and diabetes	Patients with coronary heart disease and diabetes
Cholesterol	< 5 mmol/l	< 4.5 mmol/l
LDL-cholesterol	< 3 mmol/l	< 2.5 mmol/l

Markers of increase in risk of death from cardiovascular diseases are also:
- HDL <1.0 mmol/l at men and <1.2 mmol/l at women,
- TG >1.7 mmol/l

Low cholesterol: anemias, oncological diseases, hyperthyroidism, necrosis of hepatocytes.

Level of lipids in blood serum changes also at pregnancy, physical activity, infectious diseases, surgical interventions, a myocardial infarction, hormonal therapy.

Dislipoproteinemia is a condition, which is characterized by increase, decrease or the total absence of one or two classes LP.

• Abetalipoproteinemia

• Hipobetalipoproteinemia
• Hyperalfalipoproteinemia
• Analphalipoproteinemia

Hyperlipoproteinemia

Characteristics of hyperlipoproteinemia (HLP).

HLP types	Increase	The content of cholesterol	The content of TG	Atherogenicity	Prevalence
I	chylomicrons	N	↑↑↑↑	???	<1%
IIA	LDL	↑↑	N	+++	10%
II	LDL and VLDL	↑↑	↑↑	+++	40%
III	intermediate density LP	↑↑	↑↑	+++	<1%
IV	VLDL	N or ↑	↑↑	+	45%
V	VLDL and chylomicrons	↑↑	↑↑↑↑	+	5%

Clinical classification of hyperlipoproteinemia

Primary HLP	**Secondary HLP**
Polygenetic HLP	Diabetes mellitus
Monogenetic HLP	Chronic alcoholism
Familial hypercholesterolemia	Hypothyroidism
Familial combined hyperlipidemia	Obstructive liver disease
Disbetalipoproteinemia	Nephrotic syndrome
Family endogenous hyperglyceridemia	Beta-blockers, diuretics
Family chylomicronemia	

Diagnostics of violations of a lipids exchange

The main purpose of a lipids exchange research is identification of some disorder in a lipids metabolism as a risk factor of cardiovascular diseases. That is why it is necessary to carry out the lipids exchange research in patient with:

• An ischemic heart disease with cerebral circulation disorders and with decreased blood flow in large arteries;

• family predisposition to early development of an ischemic heart disease (at persons younger than 60 years);

• other risk factors: diabetes mellitus, arterial hypertension, etc.; local lipid deposits (xanthomas, lipide striya, a lipide arch of a cornea) in age less than 50 years.

It is effective to perform the diagnostics of a lipid exchange disorders with three stages:

1. The first stage is definition of the maintenance of the common cholesterin and triglycerides. In case of hypercholesterolemia or hypertriglyceridemia it is necessary to carry out the second investigation phase.

2. The second stage is measurement of a lipid range: cholesterol, TG, HDL-cholesterol, LDL-cholesterol; LP electrophoresis; calculation of an index of an atherogeneity (AI) and level of the LDL if it wasn't measured.

Level of the LDL count on Fridvald's formula:

LDL-cholesterol = cholesterol – (HDL-cholesterol + TG/2,2).

The formula can be used, if concentration of TG less than 4,5 mmol/l, and if blood for research is taken on an empty stomach.

The atherogeneity index for an assessment of a ratio of atherogenous and anti-atherogenous LP pays off on a formula:

AI = (cholesterol – HDL-cholesterol) / HDL-cholesterol

The index of an atherogeneity is theoretical at newborns (no more than 1), reaches 2,2–2,5 at healthy men and women at the age of 25–30 years and increases to 4–6 units at persons with an ischemic heart disease.

3. The third stage is differentiation between primary and secondary HLP performing by excludion all diseases with secondary HLP : a diabetes mellitus, a nephrotic syndrome and other renal parenchyma damages, liver pathology with the cholestasis phenomenon, decrease of albumin level in the blood, presence of a acute or chronic phase of inflammatory process, etc.

HLP identification is usually carryed out at level of cholesterine and TG exceeding 6,2 and 2,3 mmol/l, respectively. Complex laboratory research allows to diagnose primary HLP and further to be engaged in clarification of concrete mechanisms of metabolic disorder of lipoproteins for the purpose of their correction.

Cardiovascular diseases are consequences of a lipide exchange disorders

Ischemic heart disease is atherosclerotic damage of the coronary arteries, conducting to a coronary failure and being shown in the form of angina pectoris, dystrophy, necrosis (heart attacks), a myocardium sclerosis, and also their consequences and complications, including sudden death.

Hyperlipoproteinemia is a major risk factorof the ischemic heart disease, characterized by the raised maintenance of lipids and LP in blood serum.

Atherosclerosis is result of disorder of the active transport of polynonsaturated fatty acids, development of a syndrome pathological compensation, the broken synthesis of eicosanoids, the chronic progressing disease of large and average elastic and is muscular-elastic arteries characterized by the proliferative-synthetic response of some endotheliocytes and blood cells on pathological lipoproteins, with formation of atheromas in intima (is fibrous-lipide plaques). Progressing of atheromas leads to involving media and to complications (an ulceration, a calcification, a thrombosis and an embolism, aneurysms, bleedings).

Primary factors of risk of development of an atherosclerosis

- Dislipoproteinemia (both hereditary, and non-hereditary)
- Hypertensia (especially at persons elder than 50 years)
- Smoking
- Diabetes
- Accessory to a male (except age groups after 75 years)

The "soft" risk factors of development of an atherosclerosis

- Adiposity (especially abdominal type)
- Hypodynamia
- Chronic stress
- Hyperuricemia
- Hyperhomocisteinemia
- Hypervitaminosis D
- Use of peroral contraceptives

The basic theories of an atherosclerosis

• Thrombogenic (Rock Itansky, 1852; Djugid Z.B., 1949)
• Parenchymatous inflammation (Virhov, 1856)
• Arteriomalacion (Thom, 1883)
• Infiltracional and combinational (Anichkov N.N., Halatov S.S., 1946)
• Protein (Ignatovsky, 1908)
• Endothelium damages (Ross, 1976)
• Tumoral (Benditt, 1973, 1988)
• Infectious (Manufacturer К.Г., 1985)
• Infringements of the active transport and deficiency in cages fatty acids (Titov V. N, 1995)

Because of fatty acids deficiency, mainly Ω-3 fatty acids 2 processes dominate:

• structural and functional changes in membranes of cells and changes in chemical structure of biologically active eicosanoids

• the pathological compensation directed on creation non-physiological active transport of fatty acids into the cell.

The necrosis of mesenchymal cells starts an inflammation syndrome.

Stages of formation of a pathological compensation syndrome:

1. Neoantigen formation (formation modified LDL-cholesterol)
2. Activation of cellular and humoral parts of immune system
3. An inflammatory syndrome.

DISCUSSION

1. Structure, classification, function of lipids.
2. Atherogenic lipoproteins, markers of increased mortality from cardiovascular disease.
3. Cholesterol levels (normal, borderline-high, high).
4. Rules of taking blood for studies of lipid metabolism.
5. Dyslipidemia, characteristic, classification.
6. Primary hyperlipoproteinemia.
7. Secondary hyperlipoproteinemia.
8. Diagnostic phases of the lipid metabolism.
9. Coronary heart disease, atherosclerosis, the concept of cause-and-effect relationships.
10. Theory of atherosclerosis, the mechanism of atherosclerosis.

INDEPENDENT WORK OF STUDENTS

1. To write down the protocol of the practical class indicating its objectives and outcomes, lipoproteins classification and table «Interpretation of lipid spectrum investigation».

2. To examine clinical causes. To make the conclusion in the protocol.

10. Topic. Diseases of the cardiovascular system. Myocardial infarction. Disturbance of oxygen supply of heart at coronary heart disease. Basic metabolic disorders in acute myocardial infarction. Invertibility conditions of myocardial changes. Irreversible changes in the heart muscle. Marker enzymes of myocardium. Enzyme diagnostics of myocardial infarction. Time of changes in the activity of enzymes. Non-enzymatic markers of myocardial infarction.

Cardiovascular diseases are the main cause of death worldwide. Diagnosis and monitoring of treatment of cardiovascular diseases are important in the clinical laboratory. Special attention is paid to rapid diagnosis of diseases such as myocardial infarction.

Session Purpose: To learn the usage of laboratory data in the diagnosis of cardiovascular diseases.

The student should know about:

- biochemical markers of myocardial infarction, time of change of their activity in the blood;
- basic metabolic disorders in acute myocardial infarction;
- basic and additional studies carried out in the differential diagnosis of cardiovascular diseases.

The student should be able to:

- to interpret the results of laboratory studies of markers of cardiovascular diseases.

Coronary heart disease is the narrowing or blockage of the coronary arteries, usually caused by atherosclerosis.

Risk factors

Biological factors:

- old age;
- male;
- genetic factors.

Anatomical, physiological and metabolic factors:

- dyslipidemia;
- arterial hypertension;
- obesity;
- diabetes.

Behavioural factors:

- dietary habits;
- smoking;
- alcoholism;
- low physical activity.

Disorder of the balance between the real blood supply of the myocardium and its need of this may occur because of the following facts:

Intravascular reasons:

- atherosclerosis narrowing of the coronary arteries;
- thrombosis and embolism of coronary arteries;
- spasm of the coronary arteries.

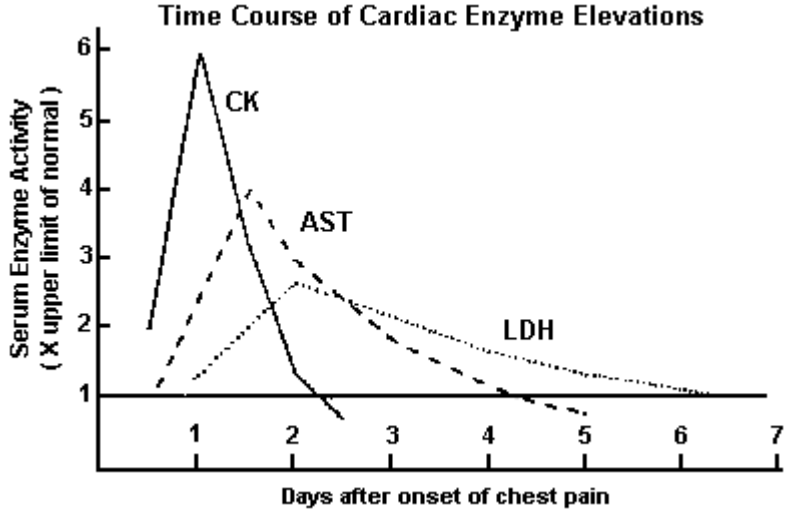
Outvascular reasons:

- tachycardia;
- myocardial hypertrophy;
- arterial hypertension.

Diagnosis of heart attack is based on symptoms of disease, ECG diagnostics, laboratory diagnostics (increase of the enzymes level in the blood).

The development of the myocardial ischemia causes suppression of oxidative phosphorylation, activation of glycolysis and declining of the assimilation of glucose.

As a result of defects arising in the cytoplasmic membranes of myocardiocytes, proteins and enzymes of the cytoplasm enter the blood. It depends on molecular size and the speed of their elimination from the blood flow.

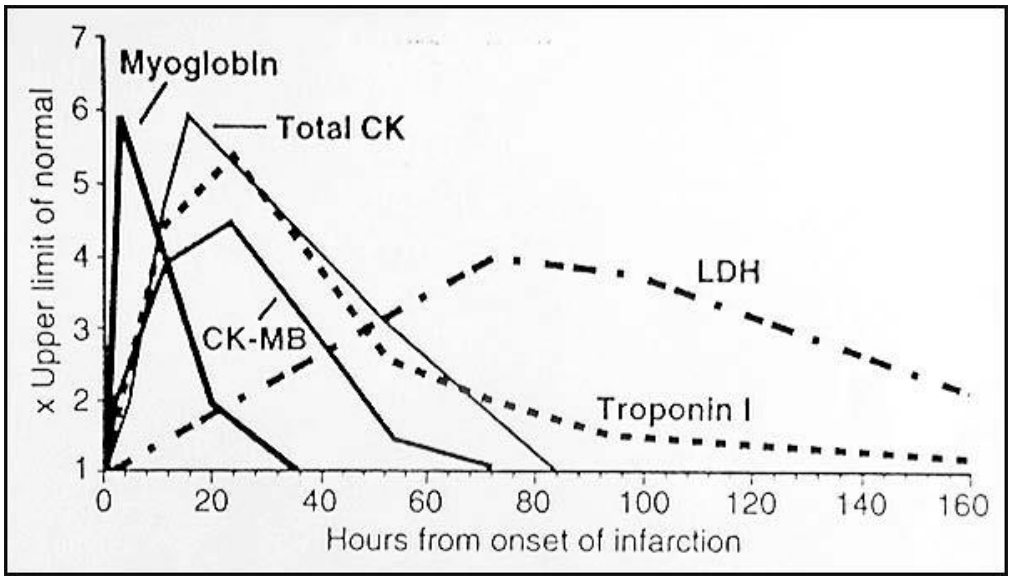


The sensitivity and specificity of heart attack markers

Marker	**Sensitivity**			**Specificity**
	3 hours	**6 hours**	**12 hours**	
Myoglobin	69 (48-86)	100 (87-100)	100 (87-100)	46 (33-60)
Troponin I	54 (33-73)	81 (61-93)	100 (87-100)	90 (80-96)
Troponin T	51 (26-70)	78 (58-89)	100 (82-96)	89 (78-95)
CK-MB	46 (27-67)	88 (70-97)	100 (87-100)	78 (66-88)

Modern requirements for markers of myocardial necrosis

The ideal biochemical marker should have the highest specificity and sensitivity with regard to myocardial necrosis, in a short time after the onset of symptoms of myocardial infarction (MI) as high blood diagnostically significant level, this level should be maintained for several days. At present marker meets all these requirements does not exist, so it is recommended for the diagnosis of myocardial infarction in parallel using two markers – the "early" and "late." The content of the "early" marker in MI diagnostically significant increases in blood during the first hours of the disease, "late" – reaches diagnostically significant levels only after 6-9 hours, but it has a high specificity for myocardial necrosis.



Markers of heart attack

1.**Creatinekinase (CK-MB)**

3 isoenzymes: MM (muscle), BB (brain), MB (cardiomyocytes).

Diagnostic value: increase of the CK-MB level is 4–8 hours after the acute attack and lasts 12–24 hours, on the third day the activity of the enzyme returns to normal values. The magnitude of improvement is corresponding to the affected areas of the myocardium.

Increased CK-MB in the blood can indicate pathologies:

- heart attack,
- the heart operation,
- myocarditis and myocardiodystrophy,
- chest radiotherapy,
- stress,
- muscle damages.

2.**Troponin I**

Contractile protein.

Diagnostic value: increase of the troponin I level in the blood is 4–6 h after acute attack, it reaches its maximum on the 2-nd day and returns to normal between 6 and 8 days.

Analysis of the increase of troponin blood is used for:

- heart attack diagnostic,
- assessment of the reperfusion after thrombolysis treatment,
- selection of high coronary risk groups among patients with acute coronary syndrome without ST segment elevation.

3.**Myoglobin**

Chromoprotein, the transporter of oxygen in the muscles.

Diagnostic value: increase of the protein level in the blood is 2–3 h after the pain and lasts 2–3 days. Repeated increase of the myoglobin level in the blood may indicate the expansion of the heart damages zone.

Increased myoglobin in the blood can indicate pathologies:

- heart attack,
- electric shock,

- crash-syndrome,
- muscle damages,
- burn.

4.**Lactatedehydrogenase (LDH)**

Isoenzymes: LDH 1, 2 – heart, LDH 3, 4 – lung, LDH 5 – liver.

Diagnostic value: level of enzyme increases rapidly in 2–4 hours, it reaches its maximum on the 2-nd day and is normalized by only 2–3 weeks.

Increased LDH in the blood can indicate pathologies:

- cardiovascular diseases,
- diseases of the liver,
- anemia,
- oncology diseases.

5.**Aminotransferases (ALT, AST)**

Aminotransferases of liver, heart, muscles.

Diagnostic value: level of enzymes increases in 6–12 hours, and is normalized by only 5–7 days.

Increased ALT, AST in the blood can indicate pathologies:

- damages of heart and muscles. De Ritis coefficient = AST/ ALT (1,33±0,42)

6.**C-reactive protein (CRP)**

Protein of inflammation acute phase.

Diagnostic value: the concentration of CRP in the blood serum or plasma increases within 24–48 h after acute tissue damage, reaches a peak in the acute phase and is reduced after the resolution of inflammation or injury, a basic level of C-reactive protein reflects inflammation in vessel's intima and prospectively determines the risk of the development of vascular complications.

Increased CRP in the blood can indicate pathologies:

- tissue damage (inflammation, trauma)

Other markers:

• Natriuretic peptides (brain, atrial).
• Protein that binds fatty acids, the cardiac form of (H-FABP).
• Homocysteine.
• Cytokines.
• Hemostatic factors.
• Adhesion molecules.
• Caspase.
• Lipid spectrum.

Obligatory and additional investigations in cases of suspected diseases of the cardiovascular system

	Obligatory investigations	**Additional investigations**
Angina pectoris	Cholesterol and its fractions, lipoproteids, atherogeneity index, fractions of lipoproteins, CK, LDH and its isoenzymes, aminotransferases, coagulogram.	Glucose, glucose tolerance test, electrolytes (K, Na, Ca, Cl), phospholipids, and the acid-basic state.

Myocardial infarction	Troponin, CK, LDH, mioglobin, aminotransferases, acute phase proteins, uric acid, urea, cholinesterase.	Apolipoprotein A, myoglobin in the blood and urine, blood glucose, glucose tolerance test, electrolytes (K, Na, Ca, Cl), glycoproteins, sialic acids, acid-basic state.
Hypertension	Urea, creatinine, uric acid, cholesterol and its fractions, atherogeneity index, cholinesterase, threeglycerides, electrolytes (K, Na).	Phospholipids, renin, aldosterone, fractions of lipoproteins.
Symptomatic hypertension	Primary aldosteronism: K, Na, aldosterone and rennin.	Angiotensin I, 17-oxicorticosteroids
	Feochromotsytoma: adrenalin, noradrenalin (in the blood and urine), vanililmandelic acid in the urine.	Free fatty acids, glucose (blood and urine), renin
	Diseases of the kidney: urea, glomerular filtration rate, creatinine, renin, aldosterone.	Adrenalin, noradrenalin (in the blood and urine), vanililmandelic acid in the urine.
	Cushing's syndrome (polyglandular syndrome): adrenocorticotropic hormone.	Glucose, glucose tolerance test, renin, angiotensin I
Arterial hypotension	Hydrocortisone	
Cardiomyopathy	CK, LDH, aminotransferases, creatinine	Sialic acids, aldolaza.
Atherosclerosis	Cholesterol and its fractions, lipoproteids, atherogeneity index, fractions of lipoproteins.	Phospholipids, blood glucose, glucose tolerance test.
Endomyocarditis	LDH, CK, sialic acids, protein profile.	

DISCUSSION

1. Coronary heart disease, concept, causes, risk factors.
2. The diagnosis of myocardial infarction, the enzyme diagnosis, markers of high and low specificity.
3. Creatinekinase MB, structure, diagnostic significance in myocardial infarction.
4. Myoglobin, structure, diagnostic significance in myocardial infarction.
5. Troponin I, structure, diagnostic significance in myocardial infarction.
6. Aminotransferases, structure, diagnostic significance in myocardial infarction.
7. Lactatedehydrogenase, structure, diagnostic significance in myocardial infarction.
8. C-reactive protein, structure, diagnostic significance in myocardial infarction
9. Laboratory diagnosis of angina pectoris.
10. Laboratory diagnosis of hypertension.
11. Laboratory diagnosis of hypotension.
12. Laboratory diagnosis of myocarditis.
13. Laboratory diagnosis of atherosclerosis.
14. Laboratory diagnosis of cardiomyopathies.

INDEPENDENT WORK OF STUDENTS

1. To record the protocol of your practical class indicating its objectives and outcomes, table «The main markers of heart attack and their diagnostic value».
2. To examine clinical causes. To make the conclusion in the protocol.

11. Topic: Renal diseases. Filtration, reabsorption and secretory functions of the kidneys. Clinical and biochemical analysis of urine. Clearance, transport maximum renal threshold, the functional indicators of kidneys. Diuresis and its disorders. Physiological and pathological components of urine. Methods of their identification. Glomerular, tubular, extrarenal proteinuria.

Renal and urinary system diseases are important in the structure of morbidity. According to the WHO, about 7–10% of the adult population of industrialized countries have various nephrological diseases. Numerous kidney diseases commonly include glomerulonephritis, pyelonephritis, polycystic disease, hydronephrosis, urolithiasis. In 2002, the National Kidney Foundation U.S. proposed the term *chronic kidney disease* uniting different clinical forms of kidney disease. Chronic kidney disease is lesion of the kidneys or decrease in their function for 3 months or more. A significant impact on the development and progression of chronic kidney disease in a given population may be due to a number of factors. These include prevalence of certain infections, certain medications, alcohol consumption and smoking, environmental conditions, the climate, the diet and eating habits, genetic characteristics of the population, etc. At the same time, hypertension, diabetes, autoimmune disease, dyslipidemia, obesity and metabolic syndrome are factors associated with the development of renal dysfunction.

Session Purpose: To learn about the basic kidney diseases: glomerulonephritis, pyelonephritis, renal failure, nephrotic syndrome, nephrolithiasis. Have an understanding of filtration, reabsorption, secretion, and physiological and pathological components of urine, urine output disorders, clinical, and biochemical analysis of urine.

The student should know about:

- key kidney diseases: glomerulonephritis, pyelonephritis, renal failure, nephrotic syndrome, nephrolithiasis;
- the concepts of filtration, reabsorption, clearance, renal threshold;
- disturbances of diuresis: polyuria, oliguria, anuria, nocturia;
- normal values of physiological components of urine: urea, creatinin, creatine, uric acid;
- pathological components of urine: glucosuria, proteinuria and its types.

The student should be able to:

- assess the clinical and biochemical analysis of urine in major renal diseases;
- evaluate the disturbance of diuresis;
- evaluate the concentration of physiological components of urine;
- make a diagnostic assessment of the pathological components of urine/

Correct interpretation of laboratory test results is only possible if you have a clear understanding of the structure and function of the kidneys, and the process of urine formation. Kidneys are paired parenchymal organs. The basic functional unit of the kidney is the nephron. Groups of nephrons give rise to the collecting tubes which open out into the apex of the renal papilla. Papilla opens into the renal calyx, which passes into the renal pelvis which is the continuation of the ureter. A nephron consists of vascular glomeruli, its capsule (Bowman's capsule) and tubular apparatus (proximal tubule, loop of Henle, distal tubule and collecting tube). Each department of the nephron has a high structural and functional specialization.

Glomerular filtration is a passive process of transition of the liquid part of blood plasma from the lumen of the glomerular capillaries into the capsule through the renal glomerular filter (capillary endothelium, basement membrane, the epithelium of the capsule). Low molecular

substances are thus filtered together with blood plasma. Glomerular filtration rate is determined by: 1 – the extent of renal blood flow, 2 – intraglomerular hydrostatic pressure and 3 – surface area of filtration. Consequently, an increase in renal blood flow, increased intraglomerular pressure and glomerular hypertrophy (increase in the surface area of filtration) increase the glomerular filtration rate.

In clinical practice, the rate of glomerular filtration is measured by the Rehberg-Tareev method based on creatinine clearance. Creatinine is a metabolite normally excreted by the kidneys. There are several options for Rehberg assay, the most common ones being daily and single assays. Rehberg's daily assay determines the concentration of creatinine in the blood serum and urine collected per day (24h). Minute dieresis is calculated: total urine per day (ml) divided by 24 (h) and 60 (minutes). Next, the glomerular filtration rate is calculated as:

GFR = urine creatinine (mol / L) x diuresis minute (ml / min) / serum creatinine (mol / L)

Rehberg's single assay is done in the morning before fluid intake, after urination. Next the patient drinks half a liter of water and in half an hour the blood is sampled. Half an hour later urine is collected. The volume of collected urine is measured. Minute dieresis is calculated: the amount of collected urine (ml) divided by 60 (minutes). GFR is calculated earlier with this formula, too.

The process of reabsorption proceeds in the proximal tubule, loop of Henle and the distal tubule. Reabsorption is the ability of cells of the renal tubules to reabsorb substances from the tubular lumen into the blood. All biologically important organic compounds (glucose, amino acids, protein, urea), and lactate, bicarbonate, inorganic phosphorus, chlorine, potassium, sodium are reabsorbed in the tubular lumen. Inorganic components of tubular fluid: potassium, sodium, magnesium, calcium, are reabsorbed in the loop of Henle and the distal tubule.

Secretion proceeds along with the reabsorption in the tubules. Tubular secretion is characterized by the ability of cells of renal tubules to transport electrolytes and various substances (organic, foreign substances formed during metabolism and synthesized in the cells of the tubules) to be eliminated from the blood into the lumen of the tubules. Secretion of organic acids and bases, end products of metabolism of foreign substances takes place in the proximal tubules. Secretion can be passive and active (with the expenditure of energy). In the distal tubule secretion of potassium, hydrogen and ammonia ions takes place. The ability of the kidney to secrete hydrogen ions and ammonia provides for regulation of acid-base balance; their ability of secrete potassium ions - fluid and electrolyte homeostasis.

Changes in urine are an important sign of kidney and urinary tract lesion, so urinalysis remains a conventional laboratory investigation of kidney function. General urine analysis includes determination of physical and chemical properties (the color, transparency, reaction, relative density, protein, glucose, ketones, bilirubin, urobilinogen) and urine microscopy. Physical parameters of urine largely depend on the particular diet, drinking regimen, medication intake, age, and therefore may be of diagnostic value in combination with other parameters of urine.

The change in urine color is a sign that will prompt the patient to see a doctor. It should be remembered that the intensity of the color depends on the concentration of urine urochrome (pigment metabolism products). Food intake affects the coloration of urine. Beetroot yields a red tint, and rhubarb – green. Medications can change the color of urine. Aspirin yields pink hue, aminopyrine – red, and 5 furagin NOC – yellow-brown, vitamins – pronounced opalescent yellow.

Urine coloration	Pathological conditions	Cause
Tawny	Congestive kidney, swelling, burns, diarrhea, vomiting,	Increased concentration of dyes
Pale	Diabetes mellitus and diabetes insipidus, renal glucosuria, renal failure	Low concentration of dyes
Dark brown	Congenital anemia	Urobilinogenuria
Dark (black)	Acute hemolytic kidney, melanocarcinoma	Hemoglobinuria, melanin
Red	Nephrolithiasis, renal infarction, lead anemia	Hematuria, uroporphyrinuria
"meat slops"	Acute glomerulonephritis and exacerbation of chronic condition	Hematuria
Beer color, greenish-brown	Parenchymal jaundice	Bilirubinuria, urobilinogenuria
Greenish-yellow, brown	Obstructive jaundice	Bilirubinuria
Whitish	Fatty degeneration	Lipuria, pus, phosphate crystals
Milk	Kidney lymphostasis	Chyluria

Changes in the color of urine are often dependent on the presence of salts. Thus, uric acid yields a vivid yellow color, urates – brick red; phosphates form a white precipitate.

The amount of urine depends on the drinking regimen. Healthy adults excrete from 0.6 to 2.0 liters of urine during the day. The ratio of the volume of day to night urine output is 3–4:1. An increase in nocturnal urine is called nocturia. It is seen in prostate hypertrophy, diabetes, cardiovascular conditions, severe kidney diseases. A condition when the daily volume (diuresis) exceeds 2 liters of urine is called polyuria. It is seen in excessive drinking, diabetes mellitus and diabetes insipidus, nephrosclerosis, endocrine disturbance of urine formation.

Oliguria is a condition when one excretes under 500 ml of urine a day. Oliguria is divided into prerenal, renal and postrenal. Prerenal oliguria develops due to insufficient blood supply to the kidney (decrease in the volume of circulating blood, reduced vascular tone, bleeding, stenosis of the renal vessels). Oliguria develops due to impaired renal filtration of urine, due to inflammatory changes in the glomeruli of the kidneys (glomerulonephritis, viral and bacterial infections, tubulointerstitial necrosis). Postrenal oliguria is associated with occlusion of the urinary tract by a stone, blood clot, or tumor.

Complete cessation of urination is called anuria (physiological condition in newborns during the first hours of life). It is observed in severe renal disease, acute renal failure, progressing peritonitis, poisoning.

Dysuria means disturbed urination; there can be pollakiuria – frequent urination, ollakiuria – rare urination, and enuresis – incontinence.

Relative urine density depends on the concentrative capacity of the kidneys. Besides, the density of urine depends on glucosuria and proteinuria. Relative density can vary from 1.003 to 1.028 within a day. Causes of disturbed concentrative renal function had better be analyzed in dynamic observation. Zimnitsky's test is made for this purpose: measuring the relative density of urine in 8 separate samples collected during the day every three hours (at 6, 9, 12, 15, 18, 21, 0, and 3 o'clock). The greater the difference between the maximum and minimum value of the relative density, the higher the functional capacity of the kidneys. Normally, it should not fall below 0.007. First of all, this test is more sensitive in identifying diseases of tubules. Hypostenuria is disturbance of concentration, while maintaining the primary ultrafiltrate dilution of urine.

The reaction of the urine is an indicator of normal diet. With a mixed diet there is commonly predominance of acidic products in the food, so the urine pH is 5.5–6.5. Neutral or alkaline reaction

is typical of vegetarians. With a mixed diet alkaline urine can be a sign of infection of the urinary tract, as microflora converts urea (a component of urine) to ammonium alkalizing the urine. In clinical practice, the definition of pH is important in view of the fact that some drugs used in nephrology are effective in an acidic medium, while others – in an alkaline medium.

Glucosuria is regarded as a pathological phenomenon. Glucose is easily filtered by renal glomeruli and is normally completely reabsorbed by the proximal tubular cells. Transfer of glucose from the lumen of the tubule in the brush border membrane is effected by a carrier. The maximum amount of glucose molecules reabsorbed from the tubular fluid in the blood depends on the number of glucose transporters. If the amount of reabsorbed glucose exceeds the capacity of transporters, glucose is detected in the urine. The maximum concentration of glucose in the blood without glucosuria is called renal threshold. Normal renal threshold is 10 mmol / L of glucose in the blood. With age, renal threshold for glucose increases. The number of glucose transporters is reduced in chronic kidney disease, hypertension, and diabetic nephropathy. This means that in these diseases glucosuria can occur if the blood glucose concentration is less than the threshold concentration (<10 mmol / l).

Ketonuria means that acetone, acetoacetate or beta-hydroxybutyric acid are detected in the urine. This condition is a disturbance of carbohydrate, protein and fat metabolism, which leads to intensified ketogenesis (diabetes, fasting, fever during an infectious process, poisoning, alcohol intoxication, elevated catabolism). Ketonuria does not mean an immediate lesion of the kidneys.

Proteinuria (detection of protein in the urine) is an important and practically significant symptom of renal and urinary tract lesion. Protein penetrating through the filter into the lumen of the renal tubules of the kidney depends on the condition of the basal membrane of the glomerular capsule, the shape and size of the protein molecule, the amount of protein in the plasma. Normally, proteins with a molecular mass of 70 kDa (albumin, immunoglobulin light chains, many enzymes) can pass through the renal filter. Normally, the protein concentration in a single portion should not exceed 0.033 g / l. In a daily portion of urine, up to 0.15 g / day is admissible. In the urine of healthy individuals over two hundred proteins of different origins have been found. Some are filtered from the blood plasma, others are of renal origin or are secreted by the urinary tract epithelium.

Protenuria can be functional or organic. Functional proteinuria is associated with hemodynamic stress and can be observed in fever, emotional stress, physical stress, or after cooling. An increase in urinary protein excretion upon a change of body position (horizontal to vertical), most commonly seen among teenagers, is called orthostatic proteinuria.

Organic (pathological) proteinuria may be prerenal, renal and postrenal. Prerenal (overload) proteinuria is not associated with renal disease. It develops as an outcome of diseases associated with increased synthesis of low molecular weight proteins (multiple myeloma). Renal proteinuria develops due to renal glomerular and tubular lesions of the kidneys. Thus disorders of filtration develop (proteinuria of glomerular type) or disturbed protein reabsorption in the proximal tubule (tubular type of proteinuria). When the glomerular barrier (proteinuria of glomerular type) is disturbed, we distinguish highly selective, selective and non-selective proteinuria. In the highly selective type proteins (albumin) of low molecular weight under 70 kDa are found in the urine. In selective proteinuria proteins up to 150 kDa are detected in the urine. In non-selective proteinuria proteins of high molecular weight of 830–930 kDa (immunoglobulins) are detected in the urine. Postrena proteinuria develops when inflammatory exudate rich in protein is present in the urine (cystitis, prostatitis).

A microscopic examination of urine sediment distinguishes between its organic and inorganic parts. The organic part is represented by red blood cells, white blood cells, cylinders and epithelium. Erythrocyturia is a pathological urinary syndrome.

Causes of erythrocyturia

Prerenal causes	
Overdose of anticoagulants Hemophilia Hypo-and afibrinogenemia Thrombocytopenia and thrombocytopathy Severe liver disease with disturbed synthesis of coagulation factors DIC	
Renal causes	
Glomerular	Proliferative (primary and secondary glomerulonephritis) Nonproliferative (hereditary nephritis, membranous nephropathy, nephrosclerosis, vascular lesions)
Non-glomerular	Polycystic kidney disease, tubulointerstitial renal disease, cancer, infectious disease and renal disease
Postrenal	
Lesion of the pelvis and ureter	Blockage, infection, stones, tumors, vascular malformations, tuberculosis, kidney
Lesion of the bladder	Blockage, infection, tumors, vascular malformations, trauma
Others	
Hematuria caused by exercise Nephroptosis Hypertrophy or adenocarcinoma of the prostate Endometriosis Pseudohematuriya	

Leucocyturia. A microscopy of a healthy human's urine sediment microscopy reveales single leukocytes in each field of vision. Leucocyturia commonly develops in kidney and urinary tract infections.

Cylindruria means that cylinders are found in the urine. Cylinders are tubular casts composed of proteins and glycosaminoglycans (hyaline). Normally hyaline is secreted by the renal epithelium of the distal tubule and excreted in the urine in a dissolved form. Elevation of protein in the urine, acidification of urine, presence of inflammation in the tubular part of the nephron, slow urine flow to the distal tubule promote precipitation of hyaline and protein aggregation.

In order to diagnose latent forms of kidney and urinary tract inflammation, the methods of quantitative estimation of erythrocytes, leukocytes, and cylinders are used. These are Addis-Kakovsky method (red cell count, white blood cells and the cylinders in the volume of daily urine) and the Nechiporenko method (in 1 ml of urine). Normally, adults have $1x10^6$ / L red blood cells, 2 to $4x10^6$ / l leukocytes and 0.02×10^6 cylinders.

Epithelial cells in the urine are of different origin; they appear in the urine as it passes along the urinary tract.

Unorganized urine sediment is represented by salts of varying chemical nature.

Physiological components of urine are urea, creatinine, creatine and uric acid. Urea is a product of the metabolism of proteins, normally excreted by the kidneys. Blood urea concentration of 2.5 to 8.3 mmol / L is considered to be physiologically acceptable. An increase in the concentration of urea in the blood accompanied by pronounced clinical intoxication

syndrome is called uremia. By itself, urea is of little toxicity, but toxic substances accumulate along with it. Therefore, urea is considered as a marker of toxicity. In patients with uremia we observe reduced concentrations of urea in the urine.

Creatine is produced in the liver; the blood flow carries it to the muscular tissue, where it is phosphorylated with formation of creatine phosphate. Creatine phosphate is a macroerg utilized in the contraction of muscle fibers. It is destroyed in myofibrils and energy is released. Creatinine formed in this reaction is a nonthreshld substance and is excreted with the urine. Its concentration in the blood and urine is mainly determined by muscle mass and secretory capacity of the kidneys. Daily excretion of creatinine in the urine is relatively constant, so tests for its concentration in the blood and urine are widely used to assess renal function.

As already mentioned, the basis of urine production is constituted by the processes of filtration, reabsorption and secretion; they generally determine the ability of the kidneys to "cleanse" various substances. There are methods determining the cleansing ability of the kidneys (clearance) based on comparing the content of certain (creatinine) substances in the blood and urine. Glomerular clearance means glomerular filtration, it corresponds to the amount of the first excreted urine (ml) within 1 min. In clinical practice, we use Rehberg's method described above.

Uric acid is the end product of nucleoprotein metabolism. In kidney disease excretion of uric acid in the urine is disturbed.

From a clinical point of view the process of diagnosis of kidney disease should be based on the syndrome-nosology principle. There are the following symptoms of kidney disease:

1. Urine sign.
2. Nephrotic.
3. Hypertonic.
4. Acute nephritic.
5. Acute renal failure.
6. Chronic renal failure.
7. Tubular dysfunction syndrome.

Urinary syndrome is the most constant sign of urinary tract infection. The notion of urinary syndrome includes proteinuria, hematuria, leukocyturia and cylindruria. If extrarenal symptoms (edema, hypertension) are absent, changes in the urine are the only diagnostic criterion of renal disease. For example, glomerulonephritis with an isolated urinary syndrome, chronic pyelonephritis with a latent course, the initial stage of renal amyloidosis.

Nephrotic syndrome is a condition characterized by generalized edema, massive proteinuria (over 3.5 g / day), hypoproteinemia and hypoalbuminemia (under 20 g / l), hyperlipidemia (cholesterol above 6.5 mmol / l).

Hypertensive syndrome is associated with diffuse renal disease. Its clinical manifestation is elevated blood pressure; laboratory sign – reduced glomerular filtration rate.

The clinical and laboratory complex of acute nephritic syndrome consists of oliguria, proteinuria, hematuria, progressing edema and hypertension. Development of acute nephritic syndrome is most characteristic of acute nephritis.

Acute renal failure is a syndrome characterized by a sudden onset of azotemia, changes in water and electrolyte balance and acid-base status, i.e., rapidly emerging major violations, primarily of excretory kidney function. These changes are a result of the severe acute renal lesion of the blood flow, glomerular filtration and tubular reabsorption, usually occurring at the same time. Many causes may trigger the development of acute renal failure, first of all

exogenous ones (toxic exposure, infection), as well as renal vascular obstruction, obstruction of the urinary tract, injury of interstitial tissue.

Chronic renal failure is a concept that involves a gradual and permanent impairment of glomerular and tubular renal function to such an extent that the kidney can no longer maintain the normal composition of the internal environment. Clinical and laboratory symptoms of developing chronic renal failure, taken together, are called uremia. CRF represents the final phase of any progressive renal failure. The most common causes of chronic renal failure include chronic glomerulonephritis, chronic pyelonephritis, amyloidosis, polycystic disease.

Tubular dysfunction (tubulopathy) is composed of a group of renal diseases characterized by early partial or generalized lesion of tubular function at normal or somewhat reduced glomerular filtration. Tubular changes are primary; glomerular lesions may develop in the later stages of the disease and be of secondary nature.

It should be mentioned that the understanding of pathophysiological processes in the kidneys can clearly identify the need for this or that laboratory investigation. In this case, a proper patient preparation prior to the test, compliance with the rules of sampling biological material for investigation is the key to obtaining results that do not cause problems in interpretation.

DISCUSSION

1. Filtration, reabsorption, clearance, renal threshold.
2. Normal levels of physiological components of urine: urea, creatinin, creatine, uric acid.
3. Key kidney diseases:
    a) glomerulonephritis,
    b) pyelonephritis,
    c) renal failure,
    d) nephrotic syndrome,
    e) nephrolithiasis.
4. Disturbances of diuresis: polyuria, oliguriya, anuria, nocturia.
5. Pathological components of urine: glucosuria, proteinuria and its types.
6. Kidney damage syndromes:
    a) uric syndrome;
    b) nephritic;
    c) hypertonic;
    d) acute nephritic syndrome;
    e) acute renal failure;
    f) chronic renal failure;
    g) tubular dysfunction syndrome.

INDEPENDENT WORK OF STUDENTS

1. Write down the practice session protocol indicating its objectives and outcomes, schemes and methods of determining the urinalysis.

2. Interpret the urinalysis in various pathological conditions of the human body. Make a conclusion, enter it in the protocol.

3. Write down the tests assessing kidney function used in clinical practice. Make a conclusion, enter it in the protocol.

12. Topic: Exchange of body fluids. Swelling. Exchange of sodium. Hypo-and hypernatremia. Methods of diagnosis of water- electrolyte balance.

Water-salt metabolism – the processes of the flow of water and salts (electrolytes) in the body, their absorption, distribution in the internal environment, and excretion. Daily human consumption of water is about 2,5 liters, of which about 1 liter it gets from food. In humans, two thirds of the total water falls on the intracellular fluid and the third – in the extracellular. The system of regulation of water-salt metabolism are maintaining the total concentration of electrolytes (sodium, potassium, calcium, magnesium) and ionic composition of intracellular and extracellular fluid at the same level. The precise regulation of water-salt metabolism in a healthy person can support not only the permanent composition, and a constant volume of body fluids, keeping almost the same concentration of osmotically active substances and acid-base balance. Disregulations of water-salt metabolism, appeared as accumulation of fluid in the body, leading to swelling or fluid deficit, decrease or increase the osmotic pressure of blood, electrolyte disturbances, i.e. decrease or increase in the concentration of individual ions (hypokalemia and hyperkalemia, hypocalcemia and hypercalcemia, etc.), changes in acid-base balance – acidosis or alkalosis. Knowledge of pathological conditions in which changing the ionic composition of the blood plasma or the concentration of the individual ions is important for the differential diagnosis of various diseases.

Session purpose: To know the basics of water-salt metabolism. To have an understanding of the positive and negative water balance. To understand the mechanisms of edema in various diseases. To know the exchange of sodium, its regulation and the types of disregulations. To have an understanding of methods for diagnosis of disregulations of water-salt metabolism.

The student should know about:

- positive and negative water balance;
- mechanisms of edema in cardiovascular and renal diseases;
.- hypernatremia, its types and mechanisms of progression;
- relative and absolute hyponatremia;
- hormonal regulation of sodium excretion by the kidneys.

The student should be able to:

- conduct a diagnostic assessment of the electrolyte composition of the blood and urine;
- evaluate disorders of water-electrolyte balance.

Water - the basis of the internal environment, the universal solvent and carrier of almost all substances. Water connects the body and the environment, providing intake and excretion of solutes involved in biochemical reactions (hydrolysis, dehydration, etc.) involved in thermoregulation (heat transfer), the humoral regulation of metabolism (transport of hormones, etc.), etc., allowing the body to functioning as a unit.

Body water content is much higher than all other substances. In adult males the water is about 52% of total body weight, and women – 46%. Approximately 75% of the weight of a newborn baby falls on the water. The total volume of the body fluid is divided into the intracellular (~ 40% of body weight) and extracellular (20–25% of body weight) of liquid. Extracellular fluid is divided into cell-cell or interstitial (~ 15% by weight), intra-vascular (plasma and lymph, about 5% by weight), and intracavitary (pleural, peritoneal, cerebrospinal, pericardial, joint fluid, 1–2%).

In a number of physiological and pathological conditions it is often necessary to determine the amount of the circulating fluid. For this purpose special products are injected into the blood

stream (e.g., Evans blue dye or 131I-labeled albumin). Knowing the amount of material injected into the blood stream, and determining its concentration in the blood, the amount of circulating fluid is calculated.

The content of the extracellular fluid is determined by substances that do not penetrate into the cells (inulin, mannitol, thiosulfate).

The total volume of water in the body is measured by the distribution of deuterium oxide (D2O) and tritium oxide (T2O). Water, which includes tritium or deuterium, evenly mixed with all the water contained in the body. Intracellular water volume equal to the difference between the total volume of water and the amount of extracellular fluid.

Different fluid spaces of the body are separated by cell membranes, vascular and epithelial walls, serous and synovial membranes, which have a selective permeability. Fluid and solutes move through biological membranes by various driving forces. Transport of substances can be either active or passive. Cell membranes have relatively high permeability for water. Their permeability to solutes is much lower and it depends on the molecular properties of a substance.

The main factor leading to the necessary balance between the extracellular and intracellular fluid volume, is the osmotic pressure of the blood, which plays a crucial role in maintaining metabolic homeostasis and blood pressure levels. Osmotic pressure is indirect characteristic of water and the concentration of substances in solution. The higher it is, the lower the water content in the solution and the higher the concentration of dissolved substances. The osmotic pressure of the solution is directly proportional to the concentration of dissolved particles. Osmotic pressure is proportional to the osmolality. Osmolarity – sum of the concentrations of anions and cations, non-electrolytes, i.e. all kinetically active particles in 1 liter of solution. It is expressed in milliosmol per liter (mOsm / L). In osmotic pressure and regulate it through the exchange of water and salt between different tissue compartments is set colloid osmotic (oncotic) pressure of proteins, which in healthy is about 0.03–0.04 atm. Its decreasing is one of the major mechanisms of edema syndrome.

Osmolarity values in normal

Blood plasma	Urine	Cerebrospinal fluid	Osmolarity index	Free water clearance
280-300 mOsm / L	600-1200 mOsm / L	270-290 mOsm / L	2,0-3,5	(-1,2) – (-3,0) ml/min

The average person consumes about 2,5 liters of water per day. About half of this amount comes from the drink, and the other half falls on the water in the food and so-called "metabolic" water produced by the digestion of organic products. Excretion of water is performed by the kidneys, intestines, lungs and skin. On average about 1,4 liters of water in day is excreted in the urine, 100 ml in feces and 900 ml is removed as a vapor from the skin and the lungs.

The normal flow of water in the body is equal to its loss, to produce "zero" the daily water balance. With positive balance develops hyperhydration (excessive water, high blood pressure, edema), and a negative – dehydration (lack of water).

Thus, many diseases are characterized by an excess of water and electrolytes in the body, which clinically manifests as edema syndrome (nephrotic syndrome kidney disease, heart failure, in diseases of the circulatory system, liver cirrhosis, etc.). The patient can observe weight gain, nausea, vomiting which does not bring relief. Skin and mucous membranes are moist, body

temperature often decreases. Peripheral edema means fluid in the abdominal and thoracic cavity. Urine output is reduced. We have the apathy, drowsiness, headache, seizures are possible.

In cases involving the loss of fluids (hyperthermia syndrome, kidney disease, with polyuria, diabetes and diabetes insipidus, adrenal insufficiency syndrome, hypersecretion of parathormone, hypervitaminosis D, uncontrollable vomiting, digestive diseases with diarrhea, inflammatory disease with involvement in the pathological process of serous membranes, etc.), as well as irrational and uncontrolled use of powerful loop diuretic, a negative fluid and electrolyte balance is developing. Clinically it is manifested only when the loss of extracellular water is at least 1/3 of the total. Negative signs of fluid and electrolyte balance - severe headache, drop in body weight, thirst, dryness and hardening of the skin and mucous membranes, the increase in average body temperature, impaired breathing rhythms and heart rate, drop in blood pressure, in severe cases, up to the collapse, confusion consciousness, seizures, an increase in hematocrit.

Regulation of water-salt metabolism in healthy people can support not only the permanent composition, and a constant volume of body fluids. Regulation of water-salt metabolism conducted with the participation of several physiological systems. The signals coming from special receptors that respond to changes in the concentration of osmotically active substances, ions, and the volume of liquid are transferring to the central nervous system, and then excretion of body water and salt and its consumption is changing. Thus, with increasing electrolyte concentration and decreasing the volume of circulating fluid (hypovolemia) a sense of thirst is appeared, while increasing the volume of circulating fluid (hypervolemia) decreases it . Increasing of the volume of circulating fluid due to higher content of water in the blood (dilution anemia) may be compensatory, arising after massive blood loss. Dilution anemia is one of the mechanisms to re-establish conformity of the circulating fluid volume. Pathological polyplasmia is a result of water-salt metabolism disorder, for example in kidney failure, etc. A short-term physiological dilution anemia after ingestion of large amounts of fluid can be developed in healthy person. Excretion of water and electrolyte ions by the kidneys is controlled by nervous system by number of hormones. In the regulation of water-salt metabolism also involved the kidney-produced physiologically active substances - derivatives of vitamin D3, renin, kinins, etc.

The following **types of disorders of water-electrolyte balance (the total amount of water and sodium in the body):**

1. **Hypoosmotic hyperhydration.** This condition occurs when the intake of water (without electrolytes) exceeds its excretion. There is a "dilution" of the extracellular fluid and reduced osmotic pressure. The osmotic pressure inside the cell is higher than in the extracellular fluid and the water goes into the cells against an osmotic gradient. This leads to swelling of cells and disruption of their function. So-called water intoxication is developing. Clinically it is manifested by nausea and vomiting, moist mucous membranes , drowsiness, headache, muscle twitching, seizures. In severe cases – by pulmonary edema, ascites, hydrothorax. Water intoxication can be eliminated by intravenous administration of hypertonic solution of sodium chloride and sharp limitation of water consumption.

2. **Hyperosmolar hyperhydration.** This kind of disruption in water-salt metabolism occurs while the introduction of a large amount of body water and electrolytes. Osmotic pressure in the extracellular space increases dramatically, the water comes out of the cells, it's their dehydration occurs. This is manifested by severe thirst with an excess of water in the body. Dehydrated cells die. This condition occurs when drinking sea water.

3. **Hypoosmolar hypohydration** observed in cases where the body loses large amounts of water and electrolytes (uncontrollable vomiting of pregnancy, profuse diarrhea, sweating,

diabetes and diabetes insipidus), but restores it by introducing water without salt. In this case, the osmotic pressure in the cells is higher than the osmotic pressure in the extracellular space, the water goes into the cell and its swelling occurs. As with the above pathological conditions the water loss is still greater than its uptake, so blood clots occurs, increasing its viscosity, which can lead to serious disorders of blood circulation. Thus, at high water losses the thirst should quench by low salted water to enter electrolytes into the body.

4. **Hyperosmolar hypohydration** occurs when water loss exceeds its introduction into the body and its endogenous formation. In this case, lost of a small amount electrolytes occurs. If this water lost is not compensated by drinking, the osmotic pressure in the intercellular spaces is higher than the osmotic pressure in the cells and the water starts to go in an area with a high osmotic pressure, dehydration and cell death occrus. A similar situation occurs when the water do not come in the body, for example, with an absolute "dry" fasting. Shortage of water with relatively little loss of electrolytes is due to sweating during hyperthermia or hard physical labor. Water is lost during prolonged hyperventilation, and after taking some diuretics.

One of the most common diseases of water-salt metabolism is swelling. Accumulation of water and electrolytes in body cavities and in the intercellular spaces is called edema. The main reason for their occurrence is excess sodium in the intravascular and interstitial spaces, often with kidney disease, chronic liver failure, increased permeability of the vascular walls. In heart failure, excess of sodium in the body can exceed the excess of water. There are following forms of edema depending on in which parts of the body fluid retention and salt occurs. When fluid and electrolytes accumulate in the subcutaneous tissue - this type of edema is called **anasarca**, when in the chest cavity – **hydrothorax**, when in the pericardium – **hydropericardium**, when in the abdomen - **ascites**, when in the scrotum - **hydrocele**.

Distinguish intracellular and extracellular edema.

Intracellular edema contribute to the oppression of the metabolic processes in tissues and worsening nutrition of cells. For example, at decreasing of blood supply to the tissues the delivery of oxygen and nutrients to the tissues is reduced. If blood flow becomes so small to sustain metabolic processes, the oppression of ion pumps of the cell membrane will happens. Leakage of sodium ions from the environment into the cell, compensated by pumps, leads to increased concentrations of sodium and water movement into the cell.

Accumulation of fluid in the extracellular space leads to extracellular edema and occurs in different states. Swelling caused by the problem of increase in the capillary filtration or by disorder of lymph drainage, which helps to return fluid from the intercellular space into the blood.

The causes of edema are:

1. **Increase in pressure in the capillaries.** When the pressure in the arterial part of the capillary increases, the liquid transition from the vascular bed into the tissues is more intensive. Increased pressure in the venous part of the capillary is not changing the fluid flow from the tissue into the blood vessels. Increasing the pressure in the arterial part of the capillaries is extremely rare and may be associated with a general increase in blood volume. Increased pressure in the venous part often happens in terms of pathology, for example, venous congestion, with a total venous congestion associated with heart failure. In these cases, fluid is retained in tissues and edema occurs.

2. **Increased permeability of vascular-tissue membranes.** This causes an increase in the circulation of the fluid between the bloodstream and tissues. Increase the permeability of membranes can occur under the influence of biologically active substances (histamine), toxic factors (chlorine ions), the enzyme hyaluronidase microorganisms, which, acting on hyaluronic acid, leading to depolymerization of mucopolysaccharides cell membranes and causes an increase in their permeability.

3. **Change in osmotic pressure.** Accumulation of electrolytes in the intercellular spaces and cavities of the body leads to an increase of the osmotic pressure in these areas, which causes the flow of water.

4. **The decrease in protein content in the plasma.** Under certain pathological conditions the oncotic pressure in the tissues may become larger than in the bloodstream. In this case, the liquid will seek from the vascular system into the tissue, and edema develops. This occurs when the loss of protein in the urine (nephrotic syndrome), protein loss through the areas of damaged skin for burns, wounds, abuse of protein synthesis (liver disease, expressed malnutrition or lack of protein in the diet).

5. **Disorder of lymph drainage.** When the pressure in the lymphatic system increases the water goes into the tissues, resulting in edema. This observed in cancer, some types of parasitic infections (e.g., filariasis), surgery, atresia or congenital pathology of lymphatic vessels.

6. **Reduction of tissue mechanical pressure**, when the mechanical resistance to the current fluid from the vessels into the tissues decreases, as, for example, the depletion of tissue collagen, improve their looseness in the amplification of hyaluronidase activity, which is observed in inflammatory and toxic edema.

Edema caused by heart failure. One of the most common and dangerous causes of edema is heart failure in which the heart is unable to pump blood, which is normally coming from the veins into the arteries. Venous pressure increases, with increasing in capillary filtration. Besides, there is a tendency to lower blood pressure, which reduces the release of salt and water by the kidney and, in turn, increases the amount of blood and leads to a further increase in hydrostatic pressure in the capillaries, further contributing to the development of edema. Reduced blood flow in the kidney also increases renin secretion, resulting in the formation of angiotensin II, which stimulates the secretion of aldosterone. Both of these factors cause an additional delay of salt and water by the kidneys. Thus, if no action is taken for the treatment of heart failure, the summation of all these factors leads to the development of generalized extracellular edema.

In patients with **left ventricular failure** without significant lesions of the right half of the heart blood flow to the lungs is not broken, but the flow through the pulmonary veins is obstructed by the pronounced weakness of the left heart. As a result, the pressure in the vessels of the lungs, including capillaries, much higher than normal, causing a life-threatening condition - pulmonary edema, which can progress rapidly and if not treated, lead to accumulation of fluid in the lungs, and death within a few hours.

Swelling caused by decreasing excretion of water and salt by the kidneys. As noted earlier, most of the NaCl, which got into the blood stream, remains in the extracellular fluid, and only a small amount enters the cell. So in renal pathology, when the excretion of salt and water is broken, they are added in large amounts to the extracellular fluid. Most of the water and salt enters the intercellular fluid, and a small amount remains in the blood. The main results of the changes are: generalized increase in extracellular fluid volume (extracellular edema), an increase in pressure due to the increased blood volume. For example, in children with acute glomerulonephritis (kidney due to inflammation in the glomerulus can not filter required amount of liquid), there was a large extracellular edema in all areas of the body, and is usually accompanied by severe hypertension.

Edema, caused by a lower protein concentration in plasma. Reducing the concentration of protein in the plasma due to a disorder of its synthesis in sufficient amount and leakage of protein from the plasma leads to a decrease in oncotic pressure, which, in turn, generally increases fluid filtration in the capillaries, leading to extracellular edema.

One of the most important reasons for the **decline of the protein in plasma** is protein loss in the urine in certain diseases of the kidneys, named as the nephrotic syndrome. In various diseases of

the kidneys glomeruli are damaged, and their membranes become permeable to plasma proteins, which often results in a large amount of protein in the urine. When losses begin to exceed the amount of protein synthesized by the body, its concentration in the plasma is reduced. Significant generalized edema were observed at concentrations of the protein in plasma below 25 g/l.

The system of regulation of water-salt metabolism are maintaining the total concentration of electrolytes (sodium, potassium, calcium, etc.), and the ionic composition of intracellular and extracellular fluid at the same level. In human plasma ion concentration is maintained with a high degree of permanence and is (in mmol / l) 136–145 for sodium , 3.5–5.1 for potassium , and 2.3–2.75 for calcium (including ionized not bound to proteins – 1.12–1.32). Compared with blood plasma and interstitial fluid cells have higher content of potassium ions and a lower concentration of sodium ions.

Sodium is the main ion of the extracellular fluid, it contains 96% of the total amount of sodium in the body (90–100 g). The normal concentration of Na in plasma is 136–145 mg / dL, and it is maintained with high accuracy, because it determines the osmolality of plasma and water exchange.

The most important biological functions of sodium:

1. Major role in maintaining the osmolarity of blood plasma and extracellular fluid .

2. Participation (with potassium) in the occurrence of the electrochemical potential of the plasma membrane of cells, ensuring their excitability and membrane transport.

3. Stabilization of protein molecules and enzymes, ensuring the number of enzymatic reactions.

Determination of the concentration of sodium in plasma is essential in the diagnosis of disorders of water balance in the body. Osmolarity of plasma is directly related to the level of sodium because sodium and related ions (usually chlorine) represent about 90% of the substances dissolved in the extracellular fluid. Therefore, Na is an indicator of osmolarity at various conditions. Reducing its concentration in the plasma for a few milli-equivalents (below 142 mEq/L) indicates the disease called hyponatremia. The excess of the normal range is called hypernatremia.

Hyponatremia – reducing sodium concentration below 134 mmol / l. Characterized by the expression of apatite, loss of appetite, nausea, vomiting, abnormal reflexes, tachycardia, anuria, hypotension, loss of consciousness, psychoses. Reducing the concentration of sodium in the plasma can occur for two reasons: the loss of sodium from the extracellular fluid or excess water in the extracellular fluid.

Absolute hyponatremia occurs when sodium reduced entry into the body (for example, in patients with heart failure who have to comply with a salt-free diet) and a loss of sodium through the gastrointestinal tract, urine, blood, lots of edema fluid, abusing diuretics, primary and secondary hypocorticizm, chronic heart failure, cirrhosis, liver failure, nephrotic syndrome, malnutrition. Sodium loss is usually associated with hypoosmolar dehydration due to decreased extracellular fluid volume that occurs with diarrhea and vomiting. Abuse of diuretics, which reduce the sodium-saving ability of the kidneys, as well as Addison's disease, in which the result of decreased production of the hormone aldosterone impaired ability of the kidneys reabsorb sodium, can also lead to moderate hyponatremia.

Relative hyponatremia is associated with delayed or excessive flow of fluid in the body, leading to dilution of the extracellular fluid and reduce the concentration of sodium. Hyponatremia is most often formed when fluids that do not contain electrolytes (eg, infusion of isotonic glucose) introduced into the body, which contributes to the dilution of the plasma. It leads to excessive production of antidiuretic hormone, which promotes more water reabsorption in the collecting tubules of the kidneys, leading to fluid overload and hyponatremia. Syndrome

of inappropriate secretion of antidiuretic hormone is observed not only in the CNS, but also in patients with lung cancer, pancreatic cancer, inflammatory diseases of the lung.

Hypernatremia – increasing the concentration of sodium above 145 mmol / l. Accompanied by severe general condition of patients, fever, tachycardia. The increase in plasma sodium concentration, which increases its osmolarity, may arise either as a result of the loss of extracellular fluid of water, or when hit by the excess ions Na +.

Absolute hypernatremia – may be delayed due to the electrolyte ions in the plasma of patients with increased function of the adrenal cortex (with hyperaldosteronism, syndrome or Cushing's disease), increased sodium excretion from the tissues into the plasma during activation of metabolism in patients suffering from purulent-septic diseases, convulsions , fever, with excess saline therapy. Hypernatremia can also occur with an increase in revenues of sodium into the extracellular fluid. This often leads to a hyperosmolar dehydration, as excessive content of NaCl in the extracellular fluid usually leads to low water retention by the kidneys. For example, the hypersecretion of the hormone aldosterone, which causes sodium retention, may cause mild dehydration and hypernatremia. Moderation hypernatremia due to the fact that the increase in production of aldosterone leads to increased sodium and water reabsorption in the kidneys.

Relative hypernatremia – caused by excessive loss of water through the skin (profuse perspiration), lungs (long hyperventilation), gastrointestinal tract (severe vomiting or diarrhea of various etiologies), kidneys (polyuretic state typical for diabetic acidosis).

When the reason is the loss of water, hyperosmolar dehydration occurs. This condition occurs when the body cannot secrete antidiuretic hormone (ADH), which is needed for kidney reabsorption of water. Decrease in the production of ADH promotes the release of large amounts of dilute urine (diabetes insipidus), which in turn leads to dehydration and increase the concentration of NaCl in the extracellular fluid. In some diseases, the kidneys become immune to ADH, which leads to a variant of nephrogenic diabetes insipidus. A common cause of hypernatremia associated with a decrease in extracellular fluid volume is dehydration (dehydration), when water loss exceeds its delivery. An example of this condition may be a significant sweating under heavy physical stress.

Thus, the first step in the analysis of the sodium content in the plasma and in the correct treatment is to determine the cause: if disorder caused by loss (excess) of sodium or by loss (excess) of water in the body.

Specialized systems of regulation of water-mineral metabolism:

- nervous control through the central nervous system;
- organ regulation by changing the functioning of the kidneys, which provide active processes of filtration and reabsorption of water and ions, and the intestine, salivary glands, lungs, and skin with sweat glands;
- hormonal regulation.

Water-salt metabolism is regulated by antidiuretic and antinatriuretic systems that support, respectively, the level of water and salts, especially sodium ions in the body. Efferent link of these systems is presented by osmo- and volyumo -receptors, most of which are located in the atria, in the mouths of some of the pulmonary veins and in arteries.

The central organs of regulation are neurosecretory supraoptic and paraventricular nucleus of the hypothalamus that control the synthesis of anti-diuretic hormone (vasopressin).

Hormone reduces urine output through enhanced water reabsorption in the renal tubules. A thirst also associated with vasopressin, which requires taking of new portions of the liquid. With excess of water hormone synthesis is inhibited with urine output increasing.

Water retention is also due to sodium retention. The process is regulated by aldosterone adrenal cortex, the synthesis of which is under control of the nuclei of the hypothalamus, the anterior part of the mid-brain, and the pineal gland. Water-salt exchange with the environment is carried out not only by the kidney, and by skin, lungs, and gastrointestinal tract.

The major hormones that affect the exchange of water and sodium, are:

- pituitary antidiuretic hormone (ADH or vasopressin) – increases the reabsorption of water in the distal tubules of the kidneys, "diluting" the blood, and reducing its osmolality and increasing volume;

- aldosterone – a hormone of the adrenal cortex- increases renal reabsorption of sodium and water, increases plasma osmolality and increases circulation volume;

- atrial and brain natriuretic factor (NUF) – released in response to stretching of the atria and blood vessels, is an aldosterone antagonist, increasing the loss of water and sodium by the kidneys and reducing the circulating blood volume.

The sodium content of the body is regulated mainly by the kidneys under control of CNS through specific natrioreceptors responsive to changes in the of sodium content in the body fluids, and by volyumoretseptors and osmoreceptors responsive to changes in the volume of circulating fluid and the osmotic pressure of the extracellular fluid, respectively. Sodium balance in the body is also controlled by renin-angiotensin system, aldosterone, natriuretic factor. With decreasing water content in the body and increasing the osmotic pressure of the blood the secretion of vasopressin (antidiuretic hormone) increase, which causes an increase in water reabsorption in the renal tubules. Increased sodium retention by the kidneys express aldosterone, and increased excretion of sodium – natriuretic hormones, or natriuretic factors. These include atriopeptides with diuretic and natriuretic effect, which synthesized in the atria, and some prostaglandins, ouabain-like substance formed in the brain, etc.

Disorders of water-salt metabolism lead to disturbances in the metabolic pathways, thereby reducing their reserves of compensatory – adaptive processes. Thus it decreases level of human health in general, and also modifying and complicating disease.

DISCUSSION

1. Distribution of water in the body. Intracellular fluid. extracellular fluid. Liquid space.
2. Negative water balance. Positive water balance.
3. Methods for assessing the water balance.
4. Osmotic and oncotic pressure. Determination of osmolarity.
5. Types of disorders of water-electrolyte balance.
6. Swelling. The causes of edema. Swelling in diseases of the cardiovascular system. Swelling in renal disease.
7. Exchange regulation of sodium and water.
8. Types of disorders of sodium metabolism. Hyponatremia. Hypernatremia.

INDEPENDENT WORK OF STUDENTS

1. Write down practice session protocol indicating its goals and objectives, schemes and methods for determining the water-electrolyte balance.
2. Decrypt of water-electrolyte balance analysis in various pathological conditions of the human body. To give an opinion with recording in the protocol.
3. Write tests to determine the water-salt metabolism, which is using in clinical practice. To give an opinion with recordinf in the protocol.

13. Topic: The biological role of potassium, calcium and phosphorus. The clinical significance of diselectrolytemia. Methods for determining the parameters of mineral metabolism.

Potassium – the main intracellular cation. It regulates fluid exchange and blood pressure. Potassium takes part in the maintenance of osmotic pressure and acid-base balance inside the cells. Sodium and potassium create voltage on both sides of the cell membrane that provides energy to the physiological processes that occur in membranes. It is involved in the biosynthesis of protein, glycogen. ATP, creatine phosphate, acetylcholine and also involved in the transfer of electric potential from nerves to muscles.

Calcium ions are important for the course of many processes of neuromuscular excitation, muscle contraction, blood clotting, cell membrane permeability, activity of many enzymes and lipid peroxidation.

Phosphates are the building blocks of bone tissue, are involved in the transfer of energy in the form of high-energy bonds (ATP, ADP, creatine phosphate, guaninfosfat and others). Phosphoric acid takes part in glycolysis, glycogenesis, fat metabolism. Phosphorus is a part of the structure of DNA, RNA, which provide protein synthesis. It is involved in oxidative phosphorylation, phosphorylation of certain vitamins (thiamine, pyridoxine, and others). Phosphorus is also important for the functioning of muscle tissue (skeletal muscle and cardiac muscle). Inorganic phosphates are part of the buffer systems of plasma and interstitial fluid. Phosphorus enables absorption of calcium in the intestine.

The plasma concentration of electrolytes (potassium, calcium, phosphorus) is the result of dynamic equilibrium between them, to the intracellular content and clearance from the body. State electrolytes assessed by determining their concentration in plasma (serum), urine and other biological fluids. Knowledge of pathological processes that lead to exchanges in the ionic composition of the blood plasma or the concentration of the individual ions in it, is important for the differential diagnosis of various diseases.

Session Purpose: To know the biological role of potassium and cause of hyper-and hypokalemia. To learn the basic statements of calcium metabolism. Clinical manifestations of disorders in calcium. Know the exchange of phosphorus, the clinical significance of hyper-and hypo-phosphatemia. Have an idea on how to identify indicators of mineral metabolism.

The student should know about:

- the role of potassium ions in muscle contraction, maintaining the cardiovascular system and kidneys;
- hyper-and hypokalemia, clinical manifestations;
- calcium, hyper-and hypocalcemia in children and adults;
- phosphorus, acid soluble and acid-fraction;
-the clinical significance of serum phosphorus disorders;
- methods for determining the components of mineral metabolism.

The student should be able to:

- conduct a diagnostic assessment of the electrolyte composition of the blood and urine;
- to evaluate the performance of mineral metabolism disorders.

Hyper-and hypokalemia, clinical manifestations

The human body contains 150 g of potassium, and 98% of this amount is in the cells, and 2% – outside the cells. Most of potassium found in muscle tissue – 70% of its total amount in the body, in terms of concentration it's about 100 mmol / kg of muscle tissue. Specific content of potassium in erythrocytes is no more than 87% mmol / kg red blood cells. About 7.5% of the total amount of potassium is in the bones. Almost all potassium is actively involved in the exchange.

The balance of potassium in the body is made up of a balance between its introduction, deposition and removal. Intake of potassium into the cell is controlled by insulin, catecholamines, aldosterone, the hydrogen ion concentration, the intensity of aerobic processes. Redistribution of potassium between the extra-and intracellular fluid is dependent on the pH of the extracellular fluid: lower pH – acidosis, leads to increased levels of potassium in plasma, stimulating exchange of hydrogen ions into the intracellular potassium, on the contrary, an increase in pH – alkalosis promotes outflow of potassium from the extracellular fluid into the cells and at the same time hydrogen ions moves out of the cells.

Kidneys are an integral and essential functional unit of the system stabilization of potassium. They account for 90% of the excretion of potassium removed from the body during the day. When excess potassium content in the extracellular fluid it leads to increasing potassium excretion, however, with insufficient intake of potassium in the body or in increased levels of intracellular potassium consuming renal mechanisms enable. Renal handling processes potassium include its filtration, reabsorption and secretion.

Determination of the concentration of potassium in plasma (serum) is necessary to evaluate the depth of potassium metabolism disorders, which may lead to increased levels of potassium in the blood **(hyperkalemia),** or to a reduction in levels **(hypokalemia).**

Hyperkalemia is characterized by metabolic disorders of potassium, which lead to a transient or sustained increase in its level in the blood plasma of more than 5.1 mmol / l. Hyperkalemia occurs due to excessive intake of potassium in the blood (with nutritional stress, mobilization of cells, regulation of potassium disorders) or inadequate removal of potassium by the kidneys.

Excessive intake of potassium in the blood may be due to nutritional stress by excessive potassium or parenteral administration. With normal or increased diuresis hyperkalemia is temporary. Hemolysis, heavy massive tissue damage contribute to the development of hyperkalemia due to release of potassium from the red blood cells and cells of the affected tissue. Duration and severity of hyperkalemia in hemolysis and tissue damage will be determined by the functional state of the kidneys. Loss of intracellular potassium stimulates alcohol, fluoride, depolarizing muscle relaxants, cardiac glycosides. Muscle cells lose significant amounts of potassium in the maximum short-term or long-term physical activity. Acidosis, insulin deficiency, the use of beta – blockers lead to hyperkalemia, the development of which is due either to the release of potassium from the cells, or a reduced supply of potassium into cells.

Amount of potassium excreted by the kidneys depends on the number of functionally active nephrons, the ability of the epithelial cells of the distal tubule and the cortical part of the collecting tube to remove potassium, normal aldosterone secretion and sensitivity of receptors. Each of these factors alone or in combination with others can lead to severe hyperkalemia. Hyperkalemia cause heparin, nonsteroidal analgesics, spironolactone.

When hyperkalemia potassium concentration in blood plasma above 5.0 mmol / l. If the level of potassium 5.5–7.0 mmol / L common therapeutic methods are used , because the

concentration of potassium in the blood is life threatening. When potassium levels in the blood plasma of 7.0–8.0 mmol / L dialysis must be performed. Potassium concentration in the 10.0–12.0 mmol / L can cause cardiac arrest. Hyperkalemia accompanies metabolic acidosis.

Wrong blood sampling (long imposition of tourniquet), stored in the refrigerator, provoking hemolysis and other effects that cause the output of potassium from the red cells, given the analysis of the high content of potassium in the blood, which is referred to as "false" hyperkalemia. In this case, repeat the study, in compliance with the correct blood obtaining, and the preparation of blood samples.

Hyperkalemia often causes no symptoms and is found in a laboratory study. When symptoms do occur, they are not specific and are mainly related to impaired muscle function (paresthesia, muscle weakness, fatigue) or disfunction of the heart. Severe hyperkalemia is a life-threatening condition because it can cause serious heart and neuromuscular complications, which include heart failure and paralysis of the respiratory muscles.

Hypokalemia, metabolic disorders characterized by potassium, which lead to a transient or sustainable reduction of its level in the body as a whole. The concentration of potassium in the blood plasma of less than 3.5 mmol / L

Potassium deficiency observed in the negative ion balance, that is, the imbalance between the ion and the food intake and selection. Increase the excretion of potassium from the body promotes catabolic metabolism orientation at which the active disintegration of the cellular protein and decreased production of cell energy, which contributes to the exit of potassium from the cells even in the intact membrane.

Causes of hypokalemia

Not enough (less than 10 mEq / day) intake of potassium in the diet	Fasting or limitation of taking any products containing potassium compounds, – vegetables, dairy products
Excess excretion of potassium from the body	-Chronic diarrhea. - Repeated vomiting. - Improper use of diuretics. - Hyperaldosteronism: Primary (in patients with tumors or hypertrophy of the adrenal cortex). Secondary (renal ischemia and improve formation in their renin in heart failure, hepatic failure). - Defects in the renal tubule – membrane-and fermentopathy (Barttera syndrome), renal tubular acidosis. - Damage to the kidney nephrotoxic agents, including drugs (some antibiotics: penicillin, gentamicin or certain antifungal agents, including amphotericin B).
Redistribution of K + from the blood and / or the extracellular fluid into the cells	- Increase the level of insulin in the blood (with an overdose of insulin or islet adenoma). - Hypercateholaminemiya (due to drug epinephrine, norepinephrine, dopamine, or pheochromocytoma). - An overdose of folic acid or vitamin B12 (for the treatment of patients with megaloblastic anemia).

Due to potassium deficiency affects all muscle: striated, smooth and cardiac muscle, and kidney. These changes determine the main clinical symptoms – severe muscle weakness, paralysis, absence of reflexes, bloating, constipation, paralytic ileus, ECG changes, symptoms of myocarditis, dilatation of the heart, increased sensitivity to digitalis, a cardiac arrest, disturbances of breathing, paralysis of the respiratory muscles to the development of asphyxia , nephropathy with polyuria, gipostenuria, paralysis of the bladder.

Serum potassium is uninformative, since 98% of potassium is intracellular. Despite the lack of potassium in the cells, its concentration in the blood may be high or normal (at stress, oliguria, or acidosis). The diagnosis of potassium deficiency most objectively confirmed its intracellular level, which in clinical practice corresponds to the analysis of K + concentration in erythrocytes. Most essential for cell function are not absolute values of K + concentration inside and outside the cells, but their ratio (normally 1:20–1:30).

Elevated levels of potassium ions in the plasma can take place, despite the deficit in the cells during the compensated metabolic or respiratory alkalosis, when instead of lost potassium, sodium ions and hydrogen flows into the cell. In this case we should assess acid-base status and exclude compensated metabolic or respiratory alkalosis.

Calcium, hyper-and hypocalcemia in children and adults

The functions of calcium in the body are structural (bone, teeth) signaling (intracellular second messenger) enzymatic (coenzyme blood clotting factors: integer), neuromuscular (control excitability, neurotransmitter secretion, initiation of muscle contraction).

The main role in the metabolism of calcium in the human body belongs to the bone. In the bones of calcium phosphates represented – $Ca_3(PO_4)_2$ (85%), carbonate – $CaCO_3$ (10%), salts of organic acids – citric and lactic (about 5%). Outside skeletal calcium is found in the extracellular fluid and virtually absent in cells. An adult human body contains 2,1 kg of calcium, 98% of which is composed of a skeleton. It is about 2% of body weight. The blood level of calcium – 2,2–2,8 mmol / l.

Regulation of calcium between extra-and intracellular fluid by parathyreothropic hormone, calcitonin, 1,25–dioksiholekaltsiferol. When the concentration of calcium ions increases the secretion parathyreothropic hormone (PTH), and osteoclasts increase the dissolution contained in bone mineral compounds. PTH increases the reabsorption of Ca^{2+} in the renal tubules. As a result, the level of calcium in the blood serum increase. With an increase in calcium ions calcitonin is secreted, it decreases the concentration of Ca^{2+} due to calcium deposits in the result of the activities of osteoblasts. Vitamin D participates in this regulation. Changes in the level of calcium in the blood can cause thyroxine, androgens increase the content of $Ca^{2+,}$ and glucocorticoids, reduce it. Ca^{2+} bind many proteins, including certain blood clotting proteins. The proteins of the coagulation system have calcium-binding sites, the formation of which depends on vitamin K.

The plasma fraction contain protein-bound (nondiffusing) calcium (0.9 mmol / L) and diffusing: ionized (1.1–1.4 mmol / l) and unionized (0.35 mmol / L). Biologically active is the ionized calcium, it enters the cell through the membrane, non-ionized form is bound to proteins (albumin), carbohydrates and other compounds. Free calcium concentration is low inside the cells. Hypoalbuminemia does not affect the level of ionized calcium, which varies in a narrow range and provides the normal functioning of the neuromuscular system. When pH increases the part of bound calcium increases too. When alkalosis hydrogen ions dissociate from the albumin molecule, which leads to a decrease in the concentration of calcium ions. This can cause clinical

symptoms of hypocalcemia, despite the fact that the concentration of total calcium in the plasma is not changed. Contrary situation (increased concentration of calcium ions in the plasma) is observed in acute acidosis. Globulins also bind calcium, although lesser than albumin.

The daily requirement for calcium in adult is 20–37,5 mmol (0.8–1.5 g), pregnant and lactating – two times higher. Every day 35 mmol of calcium consumes with food, but absorbed only half of this amount. Absorption occurs in the small intestine (maximum is in the duodenum). It is best absorbed calcium gluconate and calcium lactate. Optimal absorption was observed at pH = 3.0. Calcium combines with fat and bile acids, and through the portal vein flows into the liver. Calcium is usually excreted through the intestine. The smaller excretion is observet with urine. Renal excretion of calcium increases in case of high blood concentration. It leads to kidney stones formation. The daily excretion ranges from 1.5 to 15 mM, depending on the circadian rhythms (maximum in the morning), hormone levels, acid-base status, the nature of food (carbohydrates increases the excretion of calcium). Bones serves as reservoir of calcium: while hypocalcemia calcium comes from the bones and, conversely, when hypercalcemia it is deposited in the skeleton. Lack of calcium in the body is often associated with the low solubility of most of its salts. Calcification of the arteries, formation of stones in the gall bladder, renal pelvis and tubules are associated with the poor solubility of calcium. Calcium phosphates are easily soluble in gastric contents. Maximum calcium absorption occurs in the proximal small intestine and decreases in the distal. The proportion of calcium absorption is more significant in children (compared to adults), pregnant and lactating. Calcium absorption decreases with age and with a deficit of vitamin D.

Components of the regulation of calcium in the blood plasma include:

- skeleton (the reservoir of calcium);
- kidney;
- calcium excretion through the intestine with the bile;
- parathyroid hormone, calcitonin (its secretion is determined by the level of calcium in the plasma);
- 1.25 dioksiholekaltsiferol.

Disorders of calcium and phosphate exchange are accompanied by metabolic disorders, and clinically manifested by changes in skeleton and neuromuscular excitability.

There is an inverse relationship between calcium and phosphorus in the blood serum (simultaneous increase observed in hyperparathyroidism, decrease - with rickets in children). When there is high content of phosphorus in the food nonabsorbable calcium phosphateis formed in the gastrointestinal tract.

Hypercalcemia - the result of increased calcium entry into the extracellular fluid of resorbable bone or food in case of declining renal reabsorption. The most common causes of hypercalcemia (90%) are primary hyperparathyroidism, malignancies. Hypercalcemia often clinically manifested. Rare causes of hypercalcemia include granulomatous disease (including sarcoidosis), hypervitaminosis D, hyperthyroidism, use of thiazide diuretics, lithium, milk-alkali syndrome, prolonged immobility, renal failure. The clinical symptoms of hypercalcemia include:

- loss of appetite, nausea, vomiting, abdominal pain (developing stomach ulcers and duodenal ulcer, pancreatitis), and constipation;
- weakness, fatigue, weight loss, muscle weakness;
- personality changes, impaired concentration, drowsiness, coma;
- fibrillation, shortening of the QT interval on the ECG;

- nephrocalcinosis, kidney stones, calcification of blood vessels, cornea;
- polyuria, dehydration, kidney failure.

The most common cause of lower total serum calcium concentration (hypocalcemia) is hypoalbuminemia. Calcium metabolism is not affected if the amount of free calcium is normal. The concentration of free calcium in serum decreases with hypoparathyroidism, resistance to parathyroid hormone (pseudohypoparathyreosis), avitaminosis D, kidney failure, severe hypomagnesemia, hypermagnesemia, acute pancreatitis, rhabdomyolysis (rhabdomyolysis), the decay of tumors, multiple transfusions of citrated blood. The clinical manifestations of hypocalcemia include paresthesias, numbness, muscle cramps, spasm of the larynx, behavior problems, stupor, prolongation of the QT interval on an electrocardiogram, a cataract. Mild hypocalcemia may be asymptomatic.

Hypercalciuria develops when excessive calcium intake with meals, overdoseage of vitamin D (increased reabsorption in the intestine), tubular disorders (idiopathic hypercalciuria, renal tubular acidosis), and increased breakdown of bones (multiple myeloma, a tumor of bone, phosphate diabetes, osteoporosis, hyperparathyroidism) .

Hypocalciuria is observed in hypoparathyroidism, hypovitaminosis D, hypocalcemia, reduced glomerular filtration.

Phosphorus, acid soluble and acid-insoluable fraction. Hyper-and hypophosphatemia in children and adults

The greatest amount of phosphorus is in the bone and inside the cells. This element in the body is found in two main forms: as free or inorganic phosphorus (phosphoric acid ions), 80% of which is disubstituted phosphate and 20% is monosodium phosphate and bound phosphorus presented in different ethers of phosphoric acid.

1. Acid-soluble phosphorus (remaining in the filtrate after precipitation of plasma proteins with trichloroacetic acid):

a) inorganic phosphorus (free);

b) organic phosphorus compounds (etherificated phosphorus, pyrophosphate: ATP, ADP, and others, geksozofosfates, pentozofosfates etc. glycerophosphates other phosphates).

2. Acid-insoluble phosphorus, the precipitated in the precipitation of plasma proteins with trichloroacetic acid (phosphorus from phospholipids – soluble in alcohol and ethyl ether, nucleic acid or protein phosphorus – insoluble in alcohol, is part of the nucleo- or phosphoproteins).

Phosphates are the building blocks of bone tissue, are involved in the transfer of energy in the form of high-energy bonds (ATP, ADP, creatine phosphate, guaninphosphate and others). With the participation of phosphoric acid by glycolysis, glycogenesis, fat metabolism. Phosphorus is a part of the structure of DNA, RNA, providing protein synthesis. It is involved in oxidative phosphorylation, resulting in the formation of ATP, the phosphorylation of certain vitamins (thiamine, pyridoxine, and others). Phosphorus is also important for the functioning of muscle tissue (skeletal muscle and cardiac muscle). Inorganic phosphates are part of the buffer systems of plasma and interstitial fluid. Phosphorus enables absorption of calcium in the intestine. The daily requirement for phosphorus is 30 mmol (900 mg), in pregnant women, it increases by 30–40% during lactation – twice. Need for phosphorus in adults – 1600 mg per day in children – 1500–1800 mg per day.

Rate of phosphorus in the blood

Age	**phosphorus, mmol / L**
Up to 2 years	1.45 – 2.16
2 years - 12 years	1.45 – 1.78
12 - 60 years	0.87 – 1.45
Women over 60 years	0.90 – 1.32
Man over 60 years	0.74 – 1.2

In the human phosphorus comes from plant and animal food in the form of phospholipids, phosphoproteins, and phosphates. 70–90% of the phosphorus absorption occurs in the small intestine. It depends on the concentration of phosphorus in the intestinal lumen, alkaline phosphatase activity (inhibition of this enzime reduces the absorption of phosphorus). Alkaline phosphatase increases vitamin D, and the absorption of phosphates – parathyroid hormone. Absorbed phosphorus goes to the liver, is involved in the processes of phosphorylation partially deposited in the form of mineral salts, which then pass into the blood and used in the bone and muscle tissue (synthesized phosphocreatine). The normal course of ossification and the maintenance of normal bone structure depends on the exchange of phosphate between the blood and bones.

The blood phosphorus presents in the form of four compounds: inorganic phosphate, organic phosphate esters, phospholipids and free nucleotides. The plasma inorganic phosphorus present in the form of orthophosphate, but its concentration in the serum evaluate directly (1 mg% P = 0.32 mmol / L phosphate). It penetrates the semi-permeable membrane, filtrated in the renal glomeruli. The concentration of inorganic pyrophosphate in plasma is 1–10 mmol / l. The inorganic phosphorus in the blood plasma of adults – 3.5–4 mg /100 ml, it is slightly higher in children (4–5 mg/100ml) and in women after menopause. The skeleton is a reservoir of inorganic phosphorus at reducing its content in the plasma it comes from the skeleton and, conversely, is deposited in the skeleton with increasing plasma concentrations.

Concentration of phosphorus in the blood serum is recommended to determine after fasting: the food, which contains a lot of phosphorus increase its level, and carbohydrates, infusion of glucose - is reduces. Phosphorus is removed from the body through the intestines and kidneys in the form of calcium phosphate. 2/3 of soluble single-and disubstituted sodium and potassium phosphates and 1/3 phosphates of calcium and magnesium are excreted with urine.

Parathyroid hormone reduces the amount of phosphorus in the blood serum by inhibiting the reabsorption of its proximal and distal tubules, increasing urinary excretion. Calcitonin has hypophosphatemic effect, reducing reabsortion and enhancing excretion. 1,25 (OH) 2D3, increases the absorption of phosphate in the intestine, and increases its level in the blood, promotes the fixation of calcium phosphate salts in the bone tissue. Insulin stimulates the flow of phosphate in the cells, thus reducing its concentration in the blood serum. Growth hormone increases the reabsorption of phosphate, vasopressin – excretion.

Exchange of phosphorus and calcium are closely related. It is believed that the best co-digestion of food, is the relationship between phosphorus and calcium equal 1:1–1,5. Hypercalcemia, reducing the secretion of parathyroid hormone, stimulates the reabsorption of phosphate. Phosphate can combine with calcium and lead to the deposition of calcium in tissues and hypocalcemia.

Hyperphosphatemia is frequently observed in patients with renal failure, occurs in hypoparathyroidism, pseudohypoparathyreosis, rhabdomyolysis, tumors decay, metabolic and

respiratory acidosis. Hyperphosphatemia inhibits 25-hydroxylation in the kidney gidroxicalciferola.

Moderate hypophosphatemia is not accompanied by significant impacts. Severe hypophosphatemia (less than 0.3 mmol / L (1 mg%) is accompanied by impairment of red blood cells, white blood cells, muscle weakness (broken formation of ATP, 2,3-diphosphoglycerate). It occurs when alcohol abuse and abstinention, respiratory alkalosis, malabsorption in intestine, connecting phosphate resume eating after fasting, with overeating, severe burns, treatment of diabetic ketoacidosis. When diabetic ketoacidosis hypophosphatemia is not a sight of depletion of phosphate. Moderate hypophosphatemia (1.0–2.5 mg%) can be observed with glucose infusion, vitamin D in the diet or reducing its absorption in the intestine, with hyperparathyroidism, acute tubular necrosis after kidney transplantation, the hereditary hypophosphatemia, Fanconi syndrome, paraneoplastic osteomalation, increasing the volume of extracellular fluid. Respiratory alkalosis can cause hypophosphatemia, stimulating activity phosphofructokinase and formation of phosphorylated intermediates of glycolysis. Chronic hypophosphatemia leads to rickets and osteomalacia. Hypophosphatemia manifested by loss of appetite, malaise, weakness, paresthesias in the extremities, pain in the bones.

Hypophosphaturiya develops in osteoporosis, renal hypophosphatemic rickets, infectious diseases, acute yellow atrophy of the liver, reduced glomerular filtration rate, increased reabsorption of phosphorus (with hypo-secretion of PTH).

Hypophosphaturiya observed at high filtration and decreased reabsorption of phosphorus (rickets, hyperparathyroidism, tubular acidosis, phosphate diabetes), hyperthyroidism, leukemia, poisoning by salts of heavy metals, benzene, phenol.

Calcium and phosphate homeostasis

Hypocalcemia stimulates the secretion of parathyroid hormone and thus increases the production of calcitriol. This increases the mobilization of calcium and phosphate from the bones and its intestinal absorption. Excess of phosphate is excreted with the urine (PTH has phosphaturic action), and calcium reabsorption in the renal tubules increases, and its concentration in the blood comes to normal. Hypophosphatemia is accompanied by increased secretion only calcitriol. Increase the action of calcitriol concentrations in plasma decreases the secretion of parathyroid hormone. Hypophosphatemia leads to stimulation of phosphate and calcium absorption in the intestine. Excess calcium is excreted in the urine, as calcitriol increases the reabsorption of calcium in the small extent (compared to PTH). As a result of the processes described normal concentration of phosphate in the blood plasma is restored regardless of the concentration of calcium.

Methods for determining the parameters of mineral metabolism

Determination of potassium

In biochemical laboratories measuring the concentration of potassium and sodium in biological fluids is carried out simultaneously. Currently, there are two main methods of analysis - flame photometry and ionometry.

Flame photometry is one of the types of emission spectral analysis, based on photometric light elements in the flame, which allows to determine their concentrations to within 2–4%. In recent decades, due to the invention of reliable and stable working ion-selective electrodes have appeared based on them ion-selective analyzers that allow the direct determination of the concentration of ions.

Ion-selective analyzers favorably with flame photometry compact, quiet performance, security (no need for a combustible gas), speed (sample analysis for 30–90 seconds), the

presence of auto-calibration at regular intervals. A distinctive feature is the ability of modern ionometry measurement of electrolytes in whole blood, which is not possible using flame photometry.

Determination of calcium

For the measurement of blood ionized calcium ion selective analyzer used. The most sensitive method for accurate measurement of total calcium in body fluids is atomic absorption spectrophotometry. This method is highly specific and it is a type of spectrum analysis.

Biochemical methods for the determination of phosphorus

For the determination of inorganic phosphorus colorimetric methods are used, the most common is the way Fiske C., Subbarow Y. in various versions. The method allows to measure the concentration of inorganic phosphorus and total phosphorus in the blood and urine inorganic phosphorus. Plasma proteins are precipitated with trichloroacetic or perchloric acid and the concentration of inorganic phosphorus in the protein-free filtrate is determined. The determination of the acid-soluble or lipid phosphorus we firstly should perform mineralization of the probe (that means burning).

All colorimetric methods for measuring the concentration of inorganic phosphorus are based on the formation of phosphomolybdic acid, the amount of which is determined by its reduction to molybdenum blue, which has a bright color. This method gives the most accurate results.

DISCUSSION

1. The balance of potassium. The role of potassium ions in muscle contraction, maintaining the cardiovascular system and kidneys.
2. Hyper-and hypokalemia, clinical manifestations, diagnosis.
3. Calcium metabolism. Regulation of calcium metabolism.
4. Hyper-and hypocalcemia in children and adults.
5. Phosphorus, acid soluble and acid-fraction.
6. The clinical significance and diagnosis of the level of phosphorus.
7. Methods for determining the parameters of mineral metabolism.

INDEPENDENT WORK OF STUDENTS

1. Record practice session protocol indicating its goals and objectives, schemes and methods for determining mineral metabolism.
2. Decipher analysis of electrolyte composition of the blood and urine in various pathological conditions of the human body. To give an opinion, subject to the protocol.
3. Write tests to determine the mineral metabolism, used in clinical practice. To give an opinion, subject to the protocol.

14. Topic: Acid-base balance. Types of acid-base balance (ABB) disturbances. Clinical and diagnostic value of changes in ABB indicators. Diagnosis of emergency conditions in anesthesiology and urgent medicine.

Homeostasis maintenance is extremely important to a living organism. Homeostasis is maintained by a number of parameters, including the concentration of hydrogen ions (pH). Acid-base balance is a state provided by physiological and physical-chemical processes that constitute a single system of functional stabilization of the concentration of H^+. The normal value of H^+ concentration is 40 nmol / L, which is 106 times less than the concentration of many another substances (glucose, lipids, minerals). The buffer systems (bicarbonate, phosphate, hemoglobin, etc.) maintain the pH of arterial blood in the normal range stabilizing the acid-base balance of the body. Bicarbonate system is the most capacious; it is a mixture of a weak carbonic acid (H_2CO_3) and its monosubstituted salts, bicarbonates. Buffer systems are able to resist changes in pH by adding acid or base. A reduction of $NaHCO_3/H_2SO_3$ leads to acidosis, its increase – to alkalosis, and the pH is within the normal range (from 7.35 to 7.45). Reduced arterial pH below 7.35 called acidemia, an increase of more than 7.45 – alkalemia.

Session Purpose: To know the fundamentals of acid-base balance of the body. Understand the role of buffer and physiological systems in maintaining the acid-alkaline balance. Know the types of disturbances of acid-base balance. Have an idea of diagnosis of acid-base balance disturbances and urgent conditions in critical care medicine and anesthesiology.

The students should know:

- acid-base balance;
- the mechanism of the buffer system of hemoglobin;
- physiological systems: the role of the lungs, kidneys, liver in maintaining the acid-alkaline balance;
- forms of disorders of acid-base balance. Alkalosis and acidosis: respiratory, metabolic, compensated, uncompensated conditions;
- clinical and diagnostic value of changes in ABB indicators;
- emergency conditions in anesthesiology and critical care medicine, general clinical tests, rapid diagnosis.

The students should be able to:

- evaluate disorders of acid-base balance;
- conduct diagnostic assessment of emergency conditions.

Acid-base balance is a condition provided by physiological and physical-chemical processes that constitute a single system of functional stabilization of the concentration of hydrogen ions. The metabolic activity of the cells, the function of enzymes and stability of the membrane depends on the pH, which is a key indicator of ABB. Most enzymatic reactions in the body take place at a narrow range of pH (7.30–7.50). Shifts in concentration of H^+ ions lead to a change in the activity of intracellular enzymes even at physiological values. For example, the enzymes of gluconeogenesis are more active during acidification of the cytoplasm, which is important in starvation or muscular exercise, the glycolytic enzymes - at normal pH.

In normal metabolism of about 15,000 mmol of hydrogen ions (15 billion nmol) are formed in the body daily. In the extracellular fluid – about 100 nmol / L.

ABB is characterized by the concentration of hydrogen ions, which are indicated by the pH symbol. The pH is a decimal logarithm of the concentration of hydrogen ions in solution taken with the opposite sign.

Normal levels of acid-base balance

Blood:	pH	pCO_2, mmHg	HCO_3-, mEq / L
Arterial blood	7.37-7.43	36-44	22-26
Venous blood	7.32-7.38	42-50	23-27

When the concentration of H^+ ions in the blood changes, compensatory activity of two major systems is activated:

1. System of chemical compensation:

• effect of extracellular and intracellular buffer systems;

• the intensity of intracellular formation of H^+ and HCO_3^-.

2. Physiological compensation system:

• pulmonary ventilation and elimination of CO_2;

• renal excretion of H^+ (acidogenesis, ammonium genesis), reabsorption and synthesis of HCO_3^-.

The buffer system is a combination of a weak acid and a salt formed by the acid, and a strong base. When the buffer systems are activated, the strong acid (or base) is replaced by a weak one, and the number of free ions [H^+] decreases. For example:

$$HCl + NaHCO_3 \rightarrow H_2CO_3 + NaCl$$

In plasma the most significant buffer systems are those of bicarbonate and protein; their weak acids are mostly balanced by sodium salts of these acids. In the cells, the phosphate and protein (hemoglobin in RBCs) buffering systems are of primary important; the buffer bases are mostly potassium salts of phosphoric acid and proteins.

The pH of the blood is significantly affected by pCO_2, which can be considered a component of the respiratory ABB. The mechanism of its effect is as follows:

$$CO_2 + H_2O \rightarrow H_2CO_3\ H_2CO_3 \rightarrow H^{++}\ HCO_3^-$$

If there is an excess of CO_2 in the equations, a shift to the right takes place with consideration to the dissociation (association) coefficient; carbonic acid and H^+ are formed and acidosis ensues. The shortage of CO2 is a shift to the left resulting in alkalosis.

The capacity of bicarbonate buffer system accounts for the greater part of the buffer capacity of the blood. It consists of a weak acid (H_2CO_3), and a salt of a strong base ($NaHCO_3$) at a ratio of 1 to 20. The mechanism of action of this system is as follows: when relatively large amounts of acidic products are released into the blood, hydrogen ions react with bicarbonate ions (HCO_3^-) and the carbonic acid H_2CO_3. A reduced concentration of carbonic acid is achieved by excretion of CO_2 through the lungs as a result of hyperventilation. When the number of bases in the blood increases, they interact with a weak carbonic acid to form bicarbonate ions and water. In this case, significant changes in pH take place. The great importance of bicarbonate buffer is

determined by the fact that excessive CO_2 and H_2O are rapidly excreted by the kidneys and lungs, respectively.

The phosphate buffer system is most important in the regulation of renal and tissue ABB. In the blood its role is to maintain the stability and reproduction of bicarbonate buffer. The system is represented by one-based phosphate NaH_2PO_4 (weak acid) and dibasic Na_2HPO_4 (weak base).

The buffer system of blood proteins functions depending on the pH. In alkaline media proteins dissociate releasing [H^+] ions; in acid media they act as acceptors of [H^+] ions. The hemoglobin buffer has the greatest capacity; it can be considered as a part of the protein buffer system. It accounts for 30% of the buffer capacity of the blood. Histidine plays an important role in the hemoglobin buffer system; it is contained in the protein in large quantities. The isoelectric point of histidine is 7.6, which makes it possible for hemoglobin take and give hydrogen ions easily upon the slightest shift in the physiological pH of the blood (7.35–7.45 normally).

The buffer system of hemoglobin-oxyhemoglobin plays an important role in the regulation of hemoglobin (weak base) - oxyhemoglobin (weak acid) ratio, as well as in the transformation of dissolved carbonic acid into carbon dioxide and its elimination through the lungs. Carbon dioxide formed in the tissues enters the red blood cells and is transformed into carbonic acid (H_2CO_3). Under the influence of carbonic anhydrase enzymeof RBCs, H_2CO_3 dissociates into H^+ ion and HCO_3^- anion. The hydrogen ion binds to hemoglobin and phosphate, and the bicarbonate anion returns to the blood plasma. Electrochemical neutrality is maintained due to the movement of chlorine ions into red blood cells of. In RBCs the chlorine anion binds to potassium cation.

In physiological conditions, an increase in pCO_2 in venous blood flowing from the tissues, stimulates the formation of HCO_3 in red blood cells. On the contrary, a decrease in arterial blood pCO_2 inhibits the formation of bicarbonate. This provides a relatively constant arteriovenous difference between HCO_3/CO_2 and, therefore, the stability of the pH value.

Reduced hemoglobin in the tissues is an acceptor of [H^+] ions and thus prevents acidification of the tissues.

Oxyhemoglobin formed in the lungs acts as an acid, because it is the donor of the [H^+] ions. Therefore, no pH shift to the alkaline side takes place. In the tissue capillaries, HbO_2 giving oxygen, loses some of its acidic properties.

The resulting reduced hemoglobin in the form of potassium salt has a high affinity to hydrogen ions and binds them freeing the potassium ions, which leave the RBCs upon an aggression of acids thus causing hyperkalemia and are easily excreted by the kidneys.

In the lungs, the formed oxyhemoglobin binds most of potassium, so that the chlorine anion is pushed out of the RBCs and binds to the sodium cation released upon elimination of carbon dioxide. As a result, there is an intensive formation and storage of HCO_3^- anion (a base) and elimination of carbon dioxide.

The extent to which oxygen binds to hemoglobin depends strongly on the shifts of blood plasma pH: if there is a shift to the acid side (acidosis, pH decreases), the affinity of hemoglobin to oxygen is reduced and the saturation of hemoglobin with oxygen reduces, accordingly; if there is a shift to the alkaline side of the pH (alkalosis increases), an inverse relationship is noted: the affinity of hemoglobin to oxygen and its saturation with oxygen increases.

The function of this system depends on the concentration of hemoglobin in the blood and the availability of sufficient amounts of oxygen: in hypoxia, anemia its power is significantly reduced.

In the lungs, after elimination of CO_2 (carbonic acid) the blood is alkalized. O_2 joins deoxyhemoglobin H-Hb forming H-HbO2 acid that is stronger than carbonic acid. It gives its H^+ ions to the environment preventing an increase of the pH:

$$H\text{-}Hb + O2 \rightarrow [H\text{-}HbO2] \rightarrow NbO_2 + H^+$$

In the tissue capillaries a constant flow of acids (including coal) from cells leads to dissociation of oxyhemoglobin HbO2 and binding of H^+ ions in the form of H-Hb:

$$HbO_2 + H + \rightarrow [H\text{-}HbO_2] \rightarrow H\text{-}Hb + O_2$$

Tissue hemoglobin can form compounds with CO_2 - carbaminhemoglobin.

The most important functional systems of the body involved in the regulation of ABB are respiratory, urinary, digestive systems, the liver, and skin.

The lungs provide for a constant CO_2 content. The amount of CO_2 indicates the balance between its production in cellular metabolism and its excretion by the lungs with the expired air. Pulmonary ventilation ensures elimination of carbonic acid formed in the functioning of the bicarbonate buffer system. It formation of hydrogen ions is intensified, the bicarbonate system binds [H^+] with sodium bicarbonate and transforms strong acids to a weak carbonic acid with subsequent formation of water and carbon dioxide, which is excreted with the expired air. Adequate changes of ventilation are regulated by the respiratory center, which is sensitive to carbon dioxide and hydrogen ions. In hypercapnia, acidosis stimulates the respiratory center, and carbon dioxide output. Additional ventilation eliminates CO_2, and H_2CO_3 as well elevating the pH of the blood, which compensates for the acidification of interstitial fluid and blood plasma with metabolic products, organic acids first of all.

When pCO2 decreases, the intensity of stimulation drops as well, there is hypoventilation, carbon dioxide accumulates in the body. This system's ability to react quickly to changes in pH is only second to buffer systems.

The urinary system is involved in the regulation of acid-base balance. Kidneys provide for the equilibrium of the bicarbonate system. H^+ ions are eliminated and the number of bicarbonate ions is replenished.

Hydrogen ions are actively secreted into the urine by tubular epithelium, a process that restores the physiological balance in the phosphate buffer system and provides for predominance of disubstituted sodium in the blood flowing from the kidneys. The excess of hydrogen ions eliminated in this way constitutes the so-called titratable acidity of urine. Anions of strong acids are excreted together with the NH^{4+} cation, which is produced from ammonia and hydrogen in the kidney. This process is called ammonium genesis; its aim is also to remove excess hydrogen ions. Renal regulation of the ABB thus involves formation and removal of ammonium ions, secretion of hydrogen ions, as well as sparing the bicarbonate anion (bicarbonate anions from the primary urine are almost completely absorbed in the renal tubules).

In physiological conditions, excretion of [H^+] ions and reabsorption of Na^+ ions and HCO_3^- takes place in the kidneys. Carbon dioxide enters the renal tubule cells from plasma and urine, where the following interaction takes place with participation of carbonic anhydrase:

$$H_2O + CO_2 \rightarrow H_2CO_3 \rightarrow H^+ + HCO_3^-$$

The resulting [H^+] ion is secreted into the lumen of the tubules, where it is neutralized by the buffer systems of glomerular ultrafiltrate. The activity of carbonic anhydrase depends on the pH the lower the pH, the higher its activity, and vice versa.

Two mechanisms of regulation of bicarbonate in the extracellular fluid function in the kidneys: bicarbonate reabsorption and its formation in the cells of the renal epithelium.

Renal response to shifts in acid-base status develops within a few hours or even days.

Regulation of acid-base balance in the liver occurs by way of oxidation of low molecular weight organic acids (lactic acid, etc.), urea synthesis from ammonia, secretion of sodium bicarbonate in the bile, excretion of metabolic products into the intestine through the bile shunt.

Acid-base status of the blood is estimated by a range of indicators.

- pH is a measure of hydrogen ions in the plasma. This is an integral indicator of the state of the buffer systems and physiological mechanisms of compensation. It changes when exposed to factors beyond the capabilities of these systems. pH is the main indicator of ABB. In healthy subjects, arterial pH is 7.40 (7.35–7.45), i.e., the blood has a slightly alkaline reaction. A decrease in pH means a shift in the acid side - acidosis (pH <7.35); an increase in pH – a shift to the alkaline side – alkalosis (pH> 7.45). shift of pH by more than 0.4 (pH under 7.0 and over 7.8) are considered to be incompatible with life. Fluctuations in pH in the range of 7.35-7.45 belong to the zone of full compensation.

- **pCO_2** is an indicator of partial tension of CO_2 in the blood. It reflects the functional state of the respiratory system. Normally, $PaCO_2$ is 40 mm Hg. Fluctuating within a range of 35 to 45 mm Hg. An increase or decrease in $PaCO_2$ is a sign of respiratory distress. Alveolar hyperventilation is accompanied by a decrease in $PaCO_2$ (arterial hypocapnia) and respiratory alkalosis, alveolar hypoventilation – higher $PaCO_2$ (arterial hypercapnia) and respiratory acidosis.

- **AB** (actual bicarbonate) means true plasma bicarbonate, that is, the content of HCO_3 ions in the blood taken from the patient in a particular setting.

- **SB** (standard bicarbonate) means standard bicarbonate in the blood plasma. This is bicarbonate content of a given patient determined in standard conditions ($pC0_2$ = 40 mm Hg, HbO_2 = 100%, $t^o = 37^0C$).

Standard and actual bicarbonates characterize the bicarbonate buffer system of the blood. The normal values of SB and AB are the same: 24.0 + 2.0 mmol / l. The number of standard and true bicarbonates decreases in metabolic acidosis and increases in metabolic alkalosis.

- **BB** (buffer base) means buffer bases in the plasma, that is, the sum of all the major components of bicarbonate, phosphate, protein, hemoglobin systems. Since the total amount of buffer bases (as opposed to the standard and actual bicarbonate) does not depend on the tension of CO_2, the BB value is an indicator of metabolic disturbances in ABB. Normally, the content of buffer bases is 48.0 + 2.0 mmol / l.

- BE (base excess): a shift of buffer bases reflects the changes in the content of buffer bases in the blood compared with the normal value for the given patient. Normally the BE value is zero; the permissible fluctuation rage is 2.3 mmol / l. When the content of the buffer bases increases, the BE value becomes positive (base excess), when it reduces, it becomes negative (base deficiency). The value of BE is the most informative indicator of metabolic disorders in ABB due to the (+) or (–) sign preceding the numeric expression. Base deficiency that exceeds normal fluctuations indicates a presence of metabolic acidosis, its excess – s presence of metabolic alkalosis.

- NBB is the sum of all major components of the patient's blood buffer systems, but estimated in standard conditions (pH = 7.38, pCO_2 = 40 mm Hg, ($t° = 37°$ C).

- **pH of the urine** is an indicator of hydrogen ions in urine; it reflects renal function, the intensity of acidogenesis and ammonium genesis.

Key indicators of blood ABB

Values	normal values
blood pH	7.40
pCO_2	40 ± 5 mm Hg
AB	19–25 mmol / L
SB	20–26 mmol / L
BB	44–52 mmol / L
BE	± 2.3 mmol / l
Urine pH	5.0–7.0

Incompetence of compensatory mechanisms in preventing a shift of hydrogen ion concentration tends to cause disturbances of acid-base balance. Depending on the mechanisms of these disorders, respiratory acidosis (or alkalosis), and metabolic acidosis (or alkalosis) are distinguished.

Acidosis is a change of ABB, in which there is absolute or relative excess of acids in the blood. Alkalosis is a change of ABB characterized by absolute or relative increase in the main valence levels.

According to the degree of compensation, all condiitons can be divided into:

- Compensated - pH = 7.40 ±.04
- Subcompensated acidosis - pH 7.35–7.31
  alkalosis - pH 7.45–7.49
- Decompensated
  acidosis - pH <7.30
  alkalosis - pH> 7.50

Metabolic acidosis is the most common form of ABB disturbance due to excessive formation and accumulation of organic acids or loss of bases in the tissues and the blood. The alkaline reserve of the blood decreases primarily due to depletion of bicarbonate. This takes place in diabetes mellitus (an increase in ketone bodies: Beta-hydroxybutyric and acetoacetic acid), malnutrition, starvation, fever, toxic conditions, renal glomerular disease, cardiovascular disease, contagious and inflammatory diseases, gastrointestinal diseases, shock. In metabolic acidosis urine acidity and its content of ammonia is increased.

Metabolic acidosis disturbs microcirculation, vascular walls aredamaged, its permeability increases. Hypotension develops. These changes in microvascular blood flow promote thrombosis and bleeding. When the blood pH is under 7.2, cardiac output decreases.

Laboratory indicators of metabolic acidosis:

Compensated acidosis: the absolute amount of HCO_3^- ions and pCO_2 decreases, but their ratio (20/1) remains unchanged. Depletion of the buffer systems results in reduced blood pH and decompensation.

Decompensated acidosis: blood pH, pCO_2, AB, SB, BB are reduced. The negative BE value is increased indicating depletion of alkaline reserve of the blood.

Respiratory acidosis is characterized by an increased concentration of hydrogen ions in the blood due to accumulation of carbon dioxide. Respiratory acidosis is caused by hyperventilation of the lungs. This can occur in asthma, pneumonia, disorders of blood circulation with congestion in the pulmonary circulation, pulmonary edema, emphysema. Disturbances of central regulation of respiration in cerebral injuries and tumors, stroke, poisoning with morphine, barbiturates or alcohol, incorrect selection of artificial lung ventilation can cause respiratory acidosis. The result is hypercapnia, i.e. PSO_2 increase in arterial blood, with increased content of H_2CO_3 in the blood plasma, which in turn leads to compensatory rise of bicarbonate ions (HCO^{3-}) in the plasma (the so-called alkaline reserve of the blood increases). Simultaneously with the decrease in the pH of the blood in respiratory acidosis, urinary excretion of free and bound acids (in the form of ammonium salts) increases.

In hypercapnia paralytic vasodilatation of the brain develops, the production of cerebrospinal fluid increases, intracranial pressure elevates. Severe disturbances can lead to generalized CNS depression. Hypercapnia and hypoxia cause an elevation of catecholamines stimulating the vasomotor center. The cardiac function (heart rate, minute blood volume, cardiac output) intensifies, the tone of the arterioles increases, hypertension develops. With progressing respiratory acidosis tissue hypoxia increases, arrhythmia develops, sensitivity to adrenergic catecholamines decreases. Heart failure, hypotension, disorders of the gastrointestinal tract, pulmonary hypertension progresses.

Laboratory indicators of respiratory acidosis:

pH of the blood is reduced;

pCO_2, AB, SB and BB are increased;

BE: a moderate shift in the positive direction;

Chloropenia as a result of enhanced urinary excretion;

Hyperkalemia at initial stages of acidosis later replaced by hypokalemia (within 5-6 days).

Metabolic alkalosis is characterized by deficiency of $[H^+]$ ions in the blood in combination with an excess of bicarbonate ions. Metabolic alkalosis may result from the loss of a large number of acid equivalents (with uncontrollable vomiting, gastro-intestinal disorders), and an enhanced flow of substances that were not neutralized by acid gastric juice and retained basic properties, as well as accumulation of these agents in the tissues (in tetanus in particular), and in cases of excessive and uncontrolled introduction of alkaline solutions for correction of metabolic acidosis. Metabolic alkalosis increases the content of bicarbonate (HCO^{3-}) in the plasma and, therefore, increases the blood alkaline reserve. Hypercapnia developing in this case should be regarded as compensation of metabolic alkalosis; it develops as a result of reduced excitability of the respiratory center at a high pH and decreased frequency of respiration. This type of AB disturbance is accompanied by reduction of urine acidity and ammonia content in urine.

Clinical signs of the underlying disease usually prevail over the clinical signs of metabolic alkalosis. Cramps and tetanus attacks are most pronounced (due to hypocalcemia) as well as disturbance of myocardial function, increased neuromuscular excitability on account of increased permeability of cell membranes.

Laboratory indicators of metabolic alkalosis:

Increased pH, AB, SB, BB;

strongly positive BE;

moderately elevated pCO_2;

hypernatremia, chloropenia, hypokalemia, hypocalcemia.

Respiratory alkalosis develops in hyperventilation (upon inhalation of pure oxygen, compensatory dyspnea accompanying a number of diseases including neurotoxic syndrome, infectious viral conditions). Moreover, respiratory alkalosis may be caused by stimulation of the respiratory center in pathological processes in the central nervous system (trauma, tumor.) In this case, due to the rapid excretion of CO_2, hypocapnia develops, i.e. a lowering of PCO_2 in arterial blood (under 35 mm Hg.); reduction of carbon dioxide content in the arterial blood is accompanied by a decrease in plasma bicarbonates (reduced alkaline reserve of the blood), as a part of them turns into carbonic acid by way of compensation. However, this mechanism is often insufficient to offset the decrease in H_2CO_3. In respiratory alkalosis the acidity of urine and its ammonia content decreases.

Clinical manifestations of respiratory alkalosis are associated with decreased tissue blood flow, impaired microcirculation, decreased tissue metabolism in vital organs. CNS disorders, cardiac and neuromuscular disorders are noted.

Laboratory indicators of respiratory alkalosis:

increased pH of the blood and urine pH;

drastic decline in pCO_2;

lowered AB, SB, BB;

moderately negative BE;

hypocalcemia.

In practice, isolated forms of respiratory or metabolic ABB disorders are rare, most often there is a combination of these. Thus, for example, mixed acidosis is a result of a change in both "metabolic" and "respiratory" indicators; such ABB disturbances are often observed in bronchopulmonary disease.

If the blood pH remains within normal limits in ABB disturbances of varying nature, such ABB changes can be considered compensated, but if the pH is beyond normal values, then ABB disturbances may be either partially compensated or uncompensated (depending on the extent of pH deviation) .

Compensatory mechanisms in disorders of acid-base balance

Types of ABB disturbance	Compensatory mechanisms
Carbon dioxide acidosis	pH reduction is offset by increased reabsorption of bicarbonates by the kidneys and its return to the blood. Arterial hypoxemia is compensated for with an increase in the number of red blood cells
Respiratory alkalosis	Compensation due to buffer systems: Kidneys: increased excretion of bicarbonates in the urine due to decreased reabsorption in the kidneys
Metabolic acidosis	Due to respiratory mechanisms: Decrease in the partial pressure of carbon dioxide
Metabolic alkalosis	Due to respiratory mechanisms: Elimination of CO_2 from the lungs

Changes in the $BHCO_3/H2CO_3$ ratio may take place due to both the numerator and denominator by. In the first case, changes are metabolic in nature, they indicate an active reaction of the buffer systems. In the second case there is a reaction of the respiratory system leading to a slowdown or acceleration of CO_2 elimination by the lungs.

If $BHCO_3$ is initially increased, compensation is aimed at increasing H_2CO_3 by hypoventilation to recover the $BHCO_3/H_2CO_3$ ratio = 20:1 and a return to normal pH (metabolic alkalosis compensated for by respiratory acidosis). In this case the pH tends to increase.

If metabolic processes result in increased levels of acid metabolic products in the blood, metabolic acidosis develops, buffer base (SB, BB) decrease, their deficiency (BE) progresses, compensatory hyperventilation develops, the $BHCO_3/H_2CO_3$ ratio is balanced by reducing the denominator; pH returns to normal, pO_2 increases (metabolic acidosis compensated for by respiratory alkalosis).

If primary hyperventilation leads to removal of carbon dioxide from the blood and reduced $PCO_2>$, compensation by metabolic changes develops: SB, BB decrease, BE increases, and the pH returns to normal. Usually, pO_2 is increased, pCO_2 is decreased (respiratory alkalosis compensated for by metabolic acidosis).

It is not always possible to distinguish between primary and secondary shifts of ABB. Usually primary ABB indicators are more pronounced than compensatory. To avoid errors in the interpretation of acid-base status as well as an assessment of all components of the ABB analysis, one should take into account pO_2 and the overall clinical picture of the patient's condition.

To neutralize acidotic ABB shifts, alkaline solutions (sodium bicarbonate, trisamin, etc.) are used; for the correction of alkalosis, on the contrary, solutions containing acidic valence (hydrochloric acid or hydrochloric acid, etc.) are used. It is important that the correction of ABB should be accompanied by monitoring of the changes of acid-base balance.

Emergency laboratory tests are a set of qualitative and / or quantitative analyses of various biological materials, which provide the findings within a short time. In emergency laboratory tests the time from withdrawing biological material to the outcome of study should not exceed 40 minutes at specialized hospitals, and 1 hour at express laboratories of multi-department medical institutions. For successful resuscitation the run-time of emergency tests should not exceed 3-5 minutes. Such tests include: investigation of acid-base status, hemoglobin, hematocrit, blood glucose, investigation of electrolytes (potassium, sodium, calcium, chloride), lactate.

Acid-base state of the blood is an important indicator in assessment of the condition of the body in extreme situations in critical care. Nowadays an investigation of the acid-base balance of the blood is made with gas analyzers, which, given the temperature and blood pressure, directly determine the concentration of H^+ ions (pH) and the index of pCO_2 (the amount of CO_2).

Structure of tests at an express laboratory

coagulation invesigation	6–8%
hematological invesigation	23–26%
immunogematologic invesigation	1–1.5%
general clinical tests	5–7%
biochemical tests	58–65%
ABB and electrolytes	24–32%

Addendum to the order № 10 by the Ministry for Public Health in Russia of April 13, 2011 № 315n "On establishing provision of anesthetic and intensive care to adult population," which includes "Regulation of the organization of medical anesthesiology and intensive care." Article 11 of the addendum states that while administering intensive care, an anesthesiologist-resuscitator implements the whole range of therapeutic, prophylactic and **diagnostic procedures** to restore, stabilize and normalize the disturbed functions of vital organs and systems, including symptomatic and pathogenetic therapy, temporary prosthesis of disturbed functions, their timely diagnosis and monitoring. This article is of significant practical importance for performance of laboratory tests in the recovery room and the operating unit (if there are no intensive care units), i.e. in those cases when there are no the laboratory professionals in the staff.

Orders	Recommendations
Order of July 6, 2009 N 389n "On establishing provision of medical care to patients with acute ischemic stroke"	Neurological department for patients with acute stroke should conduct a test for peripheral blood glucose, INR, aPTT within 20 minutes after blood withdrawal.
Order of August 19, 2009 N 599n "On establishing provision of routine and emergency medical care to the population of the Russian Federation with cardiologic diseases of the circulatory system"	In the health care setting, where patients with cardiovascular disease receive emergency care, the following tests are provided on emergency basis and at any time of the day: hematocrit, glucose, sodium, potassium, magnesium, creatinine, troponin, CK-MB, D-dimer, fibrinogen in the blood serum, activated partial thromboplastin time (aPTT), activated clotting time (ACT), acid-base balance and blood gases. The standard equipment of the intensive care unit at an emergency cardiology department, the standard equipment of the emergency department of cardiology should include: Laboratory equipment for automatic testing for hemoglobin, hematocrit, and coagulation parameters (activated clotting time, aPTT, fibrinogen, INR, D-dimer), electrolytes (K, Na), troponin, glucose, creatinine, bilirubin, blood gases.
Order of December 8, 2009 N 966n "On establishing the procedure of providing medical care to patients with urological diseases"	In the health care setting with a urology department, the following emergency and routine tests should be provided: hematocrit, glucose, sodium, potassium, creatinine and urea in the serum, acid-base balance.

DISCUSSION

1. Acid-base balance.
2. The mechanism of the buffer system of hemoglobin.
3. The role of physiological systems in maintaining the acid-base balance:
    a. Pulmonary system.
    b. Renal system.
    c. Hepatic system.

4. Forms of disturbance of acid-base balance. Alkalosis and acidosis:
    a. Respiratorya.
    b. Metabolica.
    c. Compensateda.
    d. Decompensateda.
5. Clinical and diagnostic value of changes in ABB indicators.
6. Diagnosis of urgent conditions in anesthesiology and critical care medicine:
    a. General clinical tests.
    b. Express diagnostics.

INDEPENDENT WORK OF STUDENTS

1. Write down the practice session protocol indicating its objectives and outcomes, schemes and methods to determine the acid-base balance of the body.

2. Interpret a test of acid-base balance in various pathological conditions of the human body. Give your opinion, enter it in the protocol.

3. Write down tests for acid-base balance used in clinical practice. Give your opinion, enter it in the protocol.

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Tests for control

Select one correct answer.

01. EXCRETORY ENZYME SYNTHESIZED IN THE LIVER

1) Prothrombinaza
2) cholinesterase
3) alkaline phosphatase
4) LDH
5) AST
6) ALT

02. ORGAN-SPECIFIC ENZYMES FOR LIVER ARE

1) AST
2) ALT
3) LDH
4) glutamate dehydrogenase

03. ALPHA2-GLOBULINS INCLUDE SUCH PROTEINS

1) haptoglobin
2) transferrin
3) β-lipoprotein
4) hemopexin

04. BETA-GLOBULINS INCLUDE PROTEINS

1) haptoglobin
2) transferrin
3) ceruloplasmin
4) immunoglobulins

05. PLASMA PROTEINS ARE

1) keratins
2) elastin
3) globulins
4) skleroproteins
5) collagens

06. GROUNDMASS (60%) OF CELLS, LOCATED IN THE ISLETS OF LANGERHANS, ARE

1) a-cells
2) β-cells
3) D-cell
4) PP-cells
5) E-cells

07. INHIBITOR OF MOST SERINE PROTEASE IS

1) alpha-amylase
2) alpha-1-atnitripsin

3) strychnine
4) fibrinolizin
5) no right answer

08. HORMONE, THAT INCREASES THE LEVEL OF BLOOD SUGAR LEVEL, IS
1) Insulin
2) glucagon
3) PTH
4) aldosterone

09. THE RENAL THRESHOLD FOR GLUCOSE, RANGES
1) 8.0-9.0 mmol / l
2) 8,9-10 mg / l
3) 10-15 mmol / l
4) no right answer

10. NORMAL LEVEL OF GLYCATED HEMOGLOBIN IN THE BLOOD IS
1) 1%
2) 3%
3) 5.7%
4) 10%

11. INCREASE OF TROPONIN I IN THE BLOOD DURING ACUTE HEART ATTACK IS
1) 2 hours after an acute attack
2) 3 hours after an acute attack
3) 4-6 hours after an acute attack
4) no right answer

12. THE MAIN FUNCTIONAL UNITS OF THE KIDNEY IS
1) neuron
2) nephron
3) acinus
4) islet

13. CONDITION IN WHICH THE DAILY VOLUME OF URINE IS MORE THAN 2 LITERS IS CALLED
1) izostenuria
2) oliguria
3) polyuria
4) nocturia

Keys

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