METHODS OF MODERN LABORATORY DIAGNOSTICS OF INFECTIOUS DISEASES

Ibragimova Nadiya Sabirovna,
Isakulova Muhabbat Mardanovna
assistants at the Department of Clinical and Laboratory Diagnostics with a course of clinical and laboratory diagnostics at the Faculty of Postgraduate Education,
Daminov Feruz Asadullaevich
Associate Professor of the Department of Clinical and Laboratory Diagnostics with a course of clinical and laboratory diagnostics of FOPE,
Shahobiddinova Xosiyat Xushvaxtovna
cadet of the Department of Clinical and Laboratory Diagnostics with a course of clinical and laboratory diagnostics of FOPE,
Samarkand State Medical University,
Uzbekistan, Samarkand

МЕТОДЫ СОВРЕМЕННОЙ ЛАБОРАТОРНОЙ ДИАГНОСТИКИ ИНФЕКЦИОННЫХ БОЛЕЗНЕЙ

Ибрагимова Надия Сабировна,
Исакулова Мухаббат Мардановна
ассистенты кафедры Клинико-лабораторной диагностики с курсом клинико-лабораторной диагностики ФПДО,
Даминов Феруз Асадуллаевич
доцент кафедры Клинико-лабораторной диагностики с курсом клинико-лабораторной диагностики ФПДО,
Шахобиддинова Хосият Хушвахтовнга
курсант кафедры Клинико-лабораторной диагностики с курсом клинико-лабораторной диагностики ФПДО,
Самаркандского Государственного Медицинского Университета,
Узбекистан, Самарканд

Annotation. At the earliest stages of the disease, it is very important to identify pathology, which will significantly reduce the need to use highly invasive diagnostic methods, allow timely identification of treatment options, and prediction of possible outcomes and the likelihood of relapse of the disease. Therefore, one of the main goals of modern laboratory diagnosis is to identify biomarkers of diseases.

Key words: modern research methods, microbiomics, proteomics, metabolomics.

Аннотация. На самых ранних стадиях заболевания очень важно выявить патологию, что значительно снизит необходимость использования высокоинвазивных диагностических методов, позволит своевременно наметить пути лечения, прогнозировать возможные исходы и вероятность рецидивов болезни. Поэтому одна из основных целей современного лабораторного диагноза - выявление биомаркеров заболеваний.

Ключевые слова: современные методы исследования, микробиомика, прротеомика, метаболомика.

Introduction. Biomarkers can include various objects of study: antibodies, microbes, deoxyribo-nucleic acid (DNA), ribonucleic acid (RNA), lipids, metabolites, proteins. Changes in their concentration, structure, function or activity are associated with a state of disease progression or regression and reflect how the body responds to the disease [3, 9]. In the context of infectious diseases, it is important to distinguish between biomarkers and surrogate markers. By surrogate we mean markers that replace specific tests for identifying the causative agent of an infectious disease in hepatitis, the use of proteomic profiles in diagnosis, characterization of drug resistance of microbial pathogens or the state of the immune system of the macroorganism [1, 12], etc.

Being a specific factor, a biomarker is most often focused on identifying the cause of an infectious disease; in addition, in modern laboratory diagnostics it is widely used to determine risk, predict and monitor the disease, and also as a way to predict the success of treatment measures [2, 14]. Protocols for the development of biomarkers must contain studies of their sensitivity, specificity, and reproducibility. Для понимания значения биомаркера необходим анализ его участия в патогенезе заболевания, процессах выздоровления и реабилитации [1].

To understand the significance of a biomarker, it is necessary to analyze its participation in the pathogenesis of the disease, the processes of recovery and rehabilitation [1].

Microbiomics includes both culture-based and molecular microbial diagnostics. Despite the fact that the cultural method has not yet lost its diagnostic value in determining the etiology of infectious diseases, due to the time required for its implementation, it cannot fully comply with the tasks of early diagnosis and prognosis in infectology. In addition, the cultivation technique is associated with many limitations, including the availability of special culture media, qualified personnel, and some bacteria and large viruses cannot be cultivated [2, 11].

Significant progress in the diagnosis of infectious diseases has been achieved with the introduction of non-culture research methods based on amplification of nucleic acids - polymerase chain reaction (PCR diagnostics), determination of the

content of microbial antigens and antibodies to them in samples of biological material. These biomarkers are so widely introduced into modern clinical practice that they can rightfully be classified as routine. However, these methods are constantly being improved. Thus, the PCR method has undoubted advantages: high accuracy and speed of reproduction, low cost, maximum level of automation with good sensitivity and specificity. However, the method is not without drawbacks - the efficiency of PCR decreases when determining RNA or DNA containing viruses with a large number of genotypes. One of the directions in eliminating these shortcomings is the creation of multiplex systems for PCR, which make it possible to identify several microbial agents or a set of biotypes of representatives of the same species at once. The development of such multiplex systems became possible due to the accumulation of a wide range of DNA/RNA samples of microorganisms in the world and the emergence of a megabase of these molecules [2, 12].

In addition to the cultural, immunological and molecular genetic characteristics of microbial pathogens, the genetics of the person himself is of great importance in infectious diseases [1, 16]. For example, at one time it was found that the development of the infectious process caused by the H5N1 influenza virus depends not only on the genotype of the virus, but is also largely determined by a person's genetic predisposition [3, 17]. Similar conclusions were obtained in the study of other infectious diseases.

In addition to direct research of the human genome, epigenomic directions related to the expression of individual genes have developed. Various approaches are being implemented for such studies: determining the relationship between gene polymorphisms and their regulation with the participation of characteristic histones, detection of genome methylation sites, the presence of chromatin conformations, which in general makes it possible to decipher the phenotypic characteristics of a healthy organism and an organism in a particular pathological state. In particular, methylomics studies the processes of DNA methylation, which makes it possible to obtain unique characteristics for the control and modulation of the expression of various genes. This direction is widely used in the analysis of the pathology of reproduction, age-related diseases, tumor and infectious pathologies, as well as a wide range of immune-mediated diseases (Crohn's disease, other autoimmune and allergic processes) [1, 7].

Another direction is transcriptomics, based on the use of messenger RNA molecules as biomarkers, of which about 3000 and about 300 microRNAs have currently been identified [1, 6]. Transcriptomics is widely used in the diagnosis of tumor and autoimmune diseases; it has also shown its importance in infectious pathology, in particular in the study of macrophage reactions in tuberculosis, as well as in the form of one of the biomarkers of infection caused by Clostridium difficile - transcript (RNA) of the receptor for interleukin-8 , or the chemokine CXCL8, in feces.

Proteomics is widely used to analyze clinically relevant biological fluids to identify protein biomarkers of pathological conditions, as well as to monitor therapeutic interventions for infectious diseases. At the present stage, “omics” technologies make it possible not only to determine biomarkers suitable for specific diagnostic tasks, but also to deeply study the pathogenesis of infectious diseases, obtain new generations of vaccine preparations, etc. [64]. Thus, with the help of proteomic studies, the role of a number of HIV proteins in viral replication has now been clarified, and the pathogenesis of pulmonary lesions in HIV infection has been deciphered [2, 8].

A widely used method of proteomic analysis is also two-dimensional gel electrophoresis 2D-DIGE (Two-dimensional Difference Gel Electrophoresis). Using this technology, it was possible, for example, to differentiate virus-induced protein expression within cells, as was shown in the rotavirus model in relation to calmodulin inhibitors and chelators. The 2D-DIGE method has been used to refine new protein targets for the treatment of severe infections caused by enteroviruses [1, 5].

A special group of biomarkers consists of immunological parameters. At first, these parameters were perceived only as surrogate markers of infectious diseases, but as immunological research technologies improved, it became possible in some cases to consider test results as specific biomarkers, especially in immunocompromised patients. A special group of biomarkers consists of immunological parameters. At first, these parameters were perceived only as surrogate markers of infectious diseases, but as immunological research technologies improved, it became possible in some cases to consider test results as specific biomarkers, especially in immunocompromised patients.

Cytokines and other soluble factors are widely used as biomarkers of the effectiveness of the immune response to microbial antigens. This test is nonspecific, so in this case we are most often talking about either infections in general or a group of infectious processes; in some cases, an entire molecular complex is used as a marker.

Metabolomics, from the point of view of the technologies used, is the most advanced section of modern laboratory diagnostics. The metabolome is understood as the cumulative characteristics of a large number of metabolic products (amino acids, nitrogenous bases, organic acids, carbohydrates, heterocyclic and many other compounds), which is obtained using various methods of molecular biology. Among these methods, the leading place is occupied by spectroscopic technologies such as nuclear magnetic resonance (NMR) and mass spectrometry. The obtained data are assessed using special systems of mathematical models (processed databases), for example, the Metabolome Wide Association Screening or Human Serum Metabolome Database system [1]. In addition to spectroscopic technologies, other methods of molecular biology, in particular gas and liquid chromatography, and capillary

electrophoresis, are used to characterize the metabolome in various biological materials. Existing database-based systems make it possible to recognize many diseases of the cardiovascular system, metabolic syndrome, tumors, and neurodegenerative processes. Metabolomics is being introduced into clinical practice and in infectious diseases.

The NMR method made it possible to establish specific changes in the metabolism of phospholipids, phosphocholine, phosphoethanolamide, amino acids, nucleotides, glycolysis products and oxidative stress during infection of the body with virulent strains of Mycobacterium tuberculosis [3].

Metabolomics characterizes metabolism not only from the standpoint of determining biomarkers of individual infectious processes at the level of the human body, but also makes it possible to diagnose infectious diseases using the state of normal human microflora. In particular, it was shown that stool samples contain a variety of amino- and phenol-containing metabolites from both the person himself and the metabolic products of his microflora. Thanks to this, by characterizing microbial metabolites such as bile acids, glucose, free fatty acids, dipeptides and other intestinal microbiota, it is possible to predict the development of infection caused by Clostridium difficile. Metabolomics of microorganisms plays a huge role in the development of our understanding of biological processes affecting the structure, mechanism of formation and antibiotic resistance of mucosal biofilms [2].

Metabolomics techniques can directly identify pathogenic microorganisms. Using a model for recognizing mixed infection pathogens (Escherichia coli, Corynebacterium glutamicum, Acinetobacter calcoaceticus, Staphylococcus aureus), it was shown that capillary electrophoresis for identifying single-strand conformational polymorphism (CE-SSCP) is not inferior in diagnostic value to the PCR method [2]. Pulsed gel electrophoresis in the form of Phenotype MicroArray technology makes it possible to differentiate between about 29 species of pathogenic Salmonella. Secondary metabolites from the group of siderophores play a role in the identification of uropathogenic Escherichia coli variants in urine [1, 4].

Conclusions. Concluding the presented review, it should be noted that the practice of laboratory diagnosis of infectious diseases is experiencing a new stage of development. New technologies have appeared, the relevance of the problem of identifying microbial pathogens is gradually decreasing, and the primary tasks of laboratory diagnostics themselves have changed. The tasks of early diagnosis of infectious diseases, prediction of the course of the disease and the effectiveness of therapeutic measures are currently coming to the fore, which, in accordance with modern terminology, is expressed in the development of biomarkers of infectious diseases as specific signs of the pathological process.

REFERENCES

1. Ray S., Patel S.K., Kumar V., Damahe J. et al. Differential expression of serum/plasma proteins in various infectious diseases: specific or nonspecific signatures. Proteomics Clin Appl. 2014; Vol. 8 (1-2): 53-72.
2. Yeh Y.T., Nisic M., Yu X., Xia Y. et al. Point-of-care microdevices for blood plasma analysis in viral infectious diseases. Ann Biomed Eng. 2014; Vol. 42 (11): 2333-43.
3. Reuter S., Ellington M.J., Cartwright E.J., K ser C.U. et al. Rapid bacterial whole-genome sequencing to enhance diagnostic and public healthmicrobiology. JAMA Intern Med. 2013; Vol. 173 (15): 1397-404.
4. Бердиярова Ш.Ш., Юсупова Н.А. Особенности иммунометаболических нарушений иммунологической реактивности при гематогенных остеомиелитах, Вестник науки и образования, 29-32
5. Tursunov Feruz O'Ktam O'G'Li, Raximova Gulchiroy Olim Qizi, Isroilova Umidaxon, Turayeva Shaxnoza ASSESSMENT OF CARBOHYDRATE METABOLISM IN PATIENTS WITH DIABETES AND COVID-19 // ReFocus. 2022. №4.
6. S.T. Eshqobilova, N.A. Yusupova ,F.S. Oripov. Influence of energy drinks on individual systems of the human body //International Journal of Early Childhood Special Education, - 2022 - № - 14-5 P.7176-7184
7. Ибрагимова Н. и др. ДИАГНОСТИКА, ЛЕЧЕНИЕ И ПРОФИЛАКТИКА САХАРНОГО ДИАБЕТА 2-го ТИПА //Центральноазиатский журнал академических исследований. – 2024. – Т. 2. – №. 1. – С. 9-13.
8. Ibragimova N. S., Keldiyorova S. H. K. GSh Nazarova The value of folic acid, homocysteine and endothelin-1 in the development of polycystic ovary syndrome in women of reproductive age //Central Asian Research Journal for Interdisciplinary Studies (CARJIS). – 2022. – Т. 2. – №. 10.
9. Dushanova G. A., Nabiyeva F. S., Rahimova G. O. FEATURES OF THE DISTRIBUTION OF HLA-ANTIGENS AMONG PEOPLE OF THE UZBEK NATIONALITY IN THE SAMARKAND REGION //Open Access Repository. – 2023. – Т. 10. – №. 10. – С. 14-25.
10. Berdiyarova Sh.Sh., Ahadova M.M., Ochilov S.A. COMPLICATIONS OF TREATMENT OF ACUTE HEMATOGENOUS OSTEOMYELITIS, LITERATURE REVIEW, Galaxy International Interdisciplinary Research Journal 293-298
11. Бердиярова Ш.Ш., Юсупова Н.А., Ширинов Х.И. Клинико-лабораторная диагностика внебольничных пневмоний у детей, Вестник науки и образования, 80-83

12. Kudratova Zebo Erkinovna, Karimova Linara Alixanovna Age-related features of the respiratory system // ReFocus. 2023. №1. URL: https://cyberleninka.ru/article/n/age-related-features-of-the-respiratory-system.
13. Sabirovna I. N., Kizi U. S. I. FEATURES OF THE COURSE OF POSTPONED PREGNANCY //Research Focus. – 2023. – Т. 2. – №. 1. – С. 236-240.
14. Nabiyeva F. S. et al. CREATION OF OPTIMUM CONDITIONS FOR PROPAGATION OF SACCHAROMYCES CEREVISIAE YEAST //Journal of new century innovations. – 2023. – Т. 23. – №. 1. – С. 85-91.
15. Isomadinova L.K, Qudratova Z.E., Babaxanova F.Sh.clinico-laboratory features of the course of covid-19 with hepatitis b journal of new century innovations №-3. 2023 P. 60-65.
16. Ибрагимова Н. и др. РАССТРОЙСТВА ИММУННОЙ СИСТЕМЫ. ПАТОГЕНЕТИЧЕСКИЕ ОСНОВЫ //Центральноазиатский журнал академических исследований. – 2024. – Т. 2. – №. 1. – С. 4-8.