

IN VITRO TEST-RESEARCH OF THE INDIGENOUS INTESTINAL MICROFLORA OF THE PATIENTS WITH ALZHEIMER'S DISEASE

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*Annotation. At the present stage of development the priority direction of prevention and treatment of dysbiotic disorders of the intestines, especially for the patients with Alzheimer's disease (AD), in our opinion, remains the personalized therapy on the basis of one's own indigenous microflora. Studying of the adhesive properties of the representatives of the indigenous microflora (*Lactobacillus* spp. and *Bifidumbacterium* spp.) of the intestines of the patients with AD became the objective of our study. During the microbiological inspection of 21 patients with AD it had been found out that at 100% of the examined patients showed the qualitative and quantitative dysbiotic changes of the intestines of different extents and symptoms: in $38.1 \pm 0.04\%$ of cases dysbacteriosis of the I degree was registered; in $28.6 \pm 0.03\%$ – the II degree and in $33.3 \pm 0.03\%$ – III degree. After cultivation of the "intestinal" strains of the indigenous microflora on skim milk within 48 hours a reliable increase in the adhesive activity of the all studied bacteria in comparison with the control has been revealed ($p < 0.05$).*

Key words: adhesive activity, Alzheimer's disease, Bifidumbacterium, dysbiosis, gastrointestinal tract, Lactobacillus.

Introduction. The gut microbiota is a "blackbox", drawing attention of the researchers for "interpretation" and understanding of the pathogenesis of many human diseases today. More than 70% of species of microorganisms resist microbiological cultivation, i.e. they cannot be isolated in a pure culture in artificial nutrient media and are identified by means of classical methods of microbiology. The technological breakthrough allowed taking a new step from a simple detection of microorganisms to understanding of their functions and a role in organism homeostasis. New methodological approaches allow analyzing all the set of the composite processes, happening at the cellular level or in the whole live organism – genomics, transcriptomics, metagenomic sequencing, proteomics and metabolomics, which completely transformed our ideas of the structure and function of the "invisible body" [1].

The gut microbiota actively affects the transformation processes of the molecules of proteins, fats and carbohydrates, synthesis of vitamins in the GIT, intestinal peristalsis regulation, takes part in the processes of detoxication and regulates permeability of

some substances through the mucous membrane of the gut. Studying of the interrelation between the gut and the brain, that is the so-called gastrobrain axis (gut-brain-axis), by means of which the brain has an impact of the function of the gastrointestinal tract and the last one – vice-versa, is modern and relevant [2, 3]. At the same time, the main basic components of the microbiota-gut-brain axis is the central nervous system, neuroendocrinal and the neuroimmune systems, the autonomic nervous system, and the system of the nerve ganglia of the intestines and the gut microbiota. These components form a complex multiple-factor network, by means of which the signals from the brain can affect not only the motor, sensory and secretory activity of the intestines, but also its microbiota. And on the contrary, visceral signals from the microbiota-mediated area of intestines, significantly influence brain functions [4].

It is important to emphasize that the probiotics bacteria, applied at certain human diseases and morbid conditions colonize the intestines, can affect the central nervous system through the products of a number of neurotransmitters and biological substances: serotonin, melatonin, gamma-aminobutyric acid (GABA), catecholamines, histamine and acetylcholine. A part of these substances is capable to take effect not only on the mesenterial, but, first of all, on central nervous system [5].

Today more than 60% of the functional foodstuffs are used for the purpose of the influence on the digestive system. Prebiotics and probiotics are the drugs, widely used in the world [6].

Despite various formulations of the concept of “probiotics”, most of researchers call them the medicines containing certain strains of microflora of a healthy human body in a quality of the active ingredient. It is known that normally bacteria, living in the mucous membrane have their antagonistic effect, concerning the pathogenic and opportunistic microflora and provide a vitamin-providing and enzymatic function [7].

When making pro-biotic drugs the strains of microorganisms, which fulfill certain requirements, have to be chosen. They are as follows: 1) safety of the strains, intended for their introduction to the structure of probiotics; 2) the existence of antagonistic properties to the pathogenic and opportunistic microflora; 3) stability to lytic enzymes of saliva (lysozyme), digestive enzymes (pepsin, lipase) and to bile 4) resistance to the action of gastric juice 5) the adhesive activity and colonial resistance; 6) resistance to antibiotics; 7) the higher specific growth rate of pro-biotic cultures in comparison with a comensalna microflora that allows them to master nutritious substrate faster and consequently, to increase the productivity of cells of pro-biotic strains; 8) the strain has to be technological by production (stable during cultivation and other stages of the technology process); 9) immune and modulatory and immunogenous probiotic action [7].

That is, probiotic strains of microorganisms have to be resistant to antibiotics, due to the synthesis of antibiotic-like substances as well, so a probiotic and an antibiotic have to work in common, that is the additive effect has to be implemented.

However, the widespread introduction of gene-modified strains of microorganisms in the medical practice is limited to a potential unpredictable impact of such microorganisms on the host's organism (a person or an animal) and also on ecosystems.

Some researchers consider that it can be connected with the emergence of the new properties in introduced species, enhancing their competitiveness and also disturbance of the balance of ecosystems. Besides, the possibility of uncontrollable transferring of a recombinant DNA to new hosts is actively discussed. At the same time many researchers experimentally confirmed the environmental safety of the recombinant microorganisms used as the effective bacteriotherapeutic drugs [8]. There are still a lot of tasks to be solved.

Proceeding from the stated above, at the present stage of development the priority direction of prevention and treatment of dysbiotic disorders of the intestines, especially for the patients with Alzheimer's disease, in our opinion, remains the personalized therapy on the basis of one's own indigenous microflora.

Studying of the adhesive properties of the representatives of the indigenous microflora (*Lactobacillus* spp. and *Bifidumbacterium* spp.) of the intestines of the patients with AD became the objective of our study.

Materials and methods. During the research the condition of the gut microflora was specified according to the methodical instructions [9]. For the purpose of obtaining of *Lactobacillus* spp. and *Bifidumbacterium* spp. isolates we have conducted a microbiological research of the gut microflora of the patients with Alzheimer's disease (AD) (n = 21). Studying of the adhesive activity of *Lactobacillus* spp. and *Bifidumbacterium* spp. isolates was carried out according to the technique of V.I. Brilis and coauthors. [10].

The statistical processing of the obtained results was carried out by means of the Statistica 6.1 software package with the use of the parametrical Student's t-test.

Results and their discussion. Analyzing the results of the microbiological research of the excrements of the patients with AD we had found out that 100% of the examined patients showed the qualitative and quantitative dysbiotic changes of the intestines of different extents of manifestations: I dysbacteriosis degree was registered in 38.1±0.04% % of cases; II degree – in 28.6 ± 0.03%; III degree – in 33.3 ± 0.03%.

Analyzing the qualitative and quantitative structure of the indigenous microbiota of the patients with AD, it had been established that the most evident qualitative and quantitative disturbances of the content of *Lactobacillus* spp. and *Bifidumbacterium* spp. were revealed in the patients with the II and III degree of gut dysbiosis. The quantity of the indigenous microflora authentically decreased ($p < 0,05$) in comparison with the indices of the patients with dysbiosis degree I; critically low quantitative indices of *Lactobacillus* spp. were registered in the patients with AD and the dysbiosis degree III ($\lg 4.48 \pm 0.15$ CFU), and *Bifidumbacterium* spp. – the patients with dysbiosis degree II ($\lg 3.7 \pm 0.2$ CFU).

Such qualitative and quantitative changes in the gut microflora of the patients with AD reduce colonial resistance and can be connected also with a decrease in the adhesive potential of the representatives of the indigenous microflora.

The value of the adhesive characteristics of the bacteria for a macroorganism can be considered from two aspects. On the one hand, the adhesive potential of the indigenous

microflora is one of the factors of realization of colonial resistance of the mucous membrane of the intestines and an obstacle of joining to the receptors of the mucous membrane of the pathogenic microorganisms. On the other hand, in case of development of dysbiotic disorders the adhesive properties of the opportunistic microflora are considered as a pathogenicity factor as they allow microbes to be fixed on the surface of the skin, mucous membrane and to colonize this biotope, reaching a certain population level [11-12].

In the course of studying of the indicators of *Lactobacillus* spp. (n = 41) and *Bifidumbacterium* spp. (n = 33) adhesion to erythrocytes of blood 0 (1) of the blood type we have determined that among the isolated strains from the patients with AD there was no one with a high adhesion degree (fig. 1).

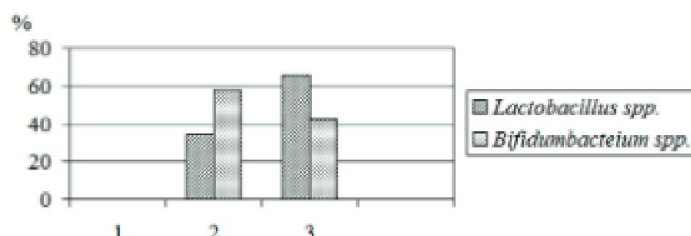


Fig. 1. The quantity of the lactobacteria and bifidobacteria, isolated from the intestines of the patients with AD on the adhesion index (1 – the high AI, 2 – the medium AI, 3 – the low AI).

In the analysis of the indicators of the adhesion index of microorganisms (AIM) we have established the distinctions: among the “intestinal” *Lactobacillus* spp. isolates, obtained from the patients with Alzheimer’s disease, the average degree of adhesion (the total value of AIM 3.61 ± 0.05 bact. cell / er.) showed the strains, isolated from the patients with I and II degree the dysbiotic changes of the intestines. All the patients with the III intestinal dysbiosis degree *Lactobacillus* spp. isolates had their low adhesive activity (the total value of AIM 1.93 ± 0.03 bact. cell / er.).

According to the obtained results on studying of the adhesive properties of *Bifidumbacterium* spp. isolates it has been determined that according to AIM these strains were characterized as low (42.4%) and medium (65.9%) on the level of adhesion (fig. 1), and all clinical *Bifidumbacterium* spp. isolates, which had their low adhesive activity (AIM values = 2.12 ± 0.03 bact. / red cell), were isolated from the patients with the I and II degree of dysbiotic intestine changes.

In the second series of the experiment with the isolates of indigenous intestinal microflora, we carried out cultivation of the clinical strains of *Lactobacillus* spp. and *Bifidumbacterium* spp., obtained from the patients with AD on skim milk within 48 hours: 1 ml of *Lactobacillus* spp. suspension or *Bifidumbacterium* spp. in the number of

0.5 units, according to the McFarland standard were added to 10 ml of sterile skim milk. After cultivation of these strains their adhesive activity were studied.

As a result of the analysis of the obtained results an increase in the adhesive activity of the all studied bacteria in comparison with control is considered to be reliable ($p < 0.05$) (the first series of the experiment – primary isolation from the patients): *Lactobacillus* spp. and *Bifidobacterium* spp. were characterized by their medium adhesion activity. So, the AIM of *Lactobacillus* spp. ($n = 41$) in the sampling made $3,86 \pm 0.07$ bact./red cell (from 2.91 to 6.9 bact./red cell.); 12.2% of lactobacteria had the high adhesive activity, 24.4% – the low adhesive activity and 63.4% – the medium adhesive activity.

Among bifidobacteria isolates ($n = 33$) after cultivation in the sterile skim milk the AIM of *Bifidobacterium* spp. in the sampling made $3,86 \pm 0.07$ bact./red cell. (The AIM indices fluctuated from 2.91 to 6.9 bact./red cell.): 21.1% were considered as high-adhesive strains, whereas most of them showed the medium (66.7%) adhesion ability and only 12.2% of bifidobacteria strains had their low adhesive potential.

When studying the sensitivity of the isolated microorganisms (*Lactobacillus* spp. or *Bifidobacterium* spp.), which was carried out by disco-diffusion method with the use of paper disks by LLC Pharmaktiv production (Kiev, Ukraine.) and Himedia (India), it has been established that 29.27% of *Lactobacillus* spp. strains showed their resistance to gentamycin; 24.39% – to chloramphenicol and 9.75% p – to erythromycin. When studying the results of the antibiotic record of *Bifidobacterium* spp. it has been revealed that the greatest number of strains (24.24%) was resistant to erythromycin.

It has been considered that antibiotic-resistance is a useful property of pro-biotic strains of *Bifidobacterium* and *Lactobacillus* bacteria that provides the high performance of probiotics for the complex therapy of infectious diseases (joint reception of probiotics and antibiotics). However, in recent years the situation cardinaly changed, it is connected, first of all, with the global distribution among antibiotic-resistance phenomenon microorganisms that can lead to a number of consequences, adverse for the person. It is connected with the fact that such strains of pro-biotic bacteria can bear the “silent” genes of antibiotic resistance or have their own mechanisms of resistance to antibacterial drugs.

For this reason, creation of the personalized bank of indigenous intestinal microflora (bacteria of *Bifidobacterium* and *Lactobacillus* type) which can form the further basis for creation of autoprobiotics for prevention and treatment of intestinal dysbiotic disorders is relevant and perspective direction for the future of the population.

Conclusions. During the microbiological inspection of 21 patients with AD it had been found out that at 100% of the examined patients showed the qualitative and quantitative dysbiotic changes of the intestines of different extents and symptoms: in $38.1 \pm 0.04\%$ of cases dysbacteriosis of the I degree was registered; in $28.6 \pm 0.03\%$ – the II degree and in $33.3 \pm 0.03\%$ – III degree. The critically low ($p < 0,05$) quantitative indices of *Lactobacillus* spp. were registered in the patients with AD and the III degree of dysbiosis ($\lg 4.48 \pm 0.15$ CFU), and *Bifidobacterium* spp. – the patients with the II degree have dysbiosis ($\lg 3.7 \pm 0.2$ CFU).

34.1% of strains of *Lactobacillus* spp. obtained from the patients with AD showed the medium adhesion degree (AIM values = 3.61 ± 0.05 bact. /red cell), obtained from the patients with I and II degree of dysbiotic changes of intestines; in 33.3% of the patients with the III degree of the intestine dysbiosis *Lactobacteria* spp. isolates had their low adhesive activity (AIM values = 1.93 ± 0.03 bact. / red cell).

Bifidumbacteium spp. isolates were characterized by the low (42.4%) and medium (65.9%) adhesion level, and all the clinical isolates, which had their low adhesive activity (AIM values = 2.12 ± 0.03 bact. /red cell.), were isolated from the patients with I and II extent of dysbiotic changes of intestines.

After cultivation of the "intestinal" strains of the indigenous microflora on skim milk within 48 hours a reliable increase in the adhesive activity of the all studied bacteria in comparison with the control has been revealed ($p < 0.05$) (the first series of the experiment – primary isolation from the patients): *Lactobacillus* spp. and *Bifidumbacteium* spp., were characterized by the medium adhesion activity.

The prospects of further researches in this direction. It is expedient to carry out restoration of the colonial resistance of the intestines in the patients with Alzheimer's disease on the basis of using of high-adhesive strains of bifidobacteria and lactobacilli. One of the ways of the long-time storage of microorganisms is drying that allows keeping their main properties. However, it is reached not in case of all the ways of drying therefore studying of the various ways of freeze drying of *Lactobacillus* spp. and *Bifidumbacteium* spp. will be the following step of our researches that will allow keeping their main biological properties (adhesive properties and the antagonistic activity, concerning the opportunistic intestinal microflora).

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