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RESULTS OF DETECTION OF COLONIC DYSBACTERIOSIS IN THYMECTOMIZED LABORATORY ANIMALS

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ABSTRACT

Experimental studies have been carried out to investigate the state of microflora of the large intestine 1, 3 and 6 months after thymectomy. The obtained data indicate the appearance of intestinal dysbacteriosis as a result of thymectomy. A decrease in indigeneous and facultative microflora in the first months after thymectomy leads to an increase in transient microflora. In 3 and 6 months after thymectomy there is a quantitative decrease of transient microflora against the background of quantitative recovery of facultative microflora. At decrease (or absence) of regulatory function of the immune system, colonisation activity and colonisation resistance of the microorganism itself come first, this provides survival of these microorganisms, as well as rapid quantitative recovery.

Key words: thymectomy, immune system, normal intestinal microflora, indigenic and facultative microorganisms, transient microflora.

INTRODUCTION

Relevance. An important place takes the immune system in the formation of normal microflora of human biotopes. The cells of this system participate in the regulation of the activity of normal microflora, quantitative and qualitative composition of microbiocenosis, as well as dosed antigenic stimulation with the help of transient bacteremia. In recent years, under the influence of various endogenous, exogenous factors develop secondary immunodeficiencies, these factors include thymectomy, which is performed during some surgical operations in children. Clinical and immunologic aspects of this condition studied by other researchers in scientific sources, but lack of data of microbiologic aspects concerning the normal microflora of the body's biotopes. In addition, there are few data on the degree of influence of thymectomy on the human intestinal

microbiocenosis. In this regard, the study and evaluation of thymectomy on the state of microflora of the large intestine in experiment remains relevant to date.

Immune mechanisms maintain the constancy of the qualitative and quantitative composition of the normal microflora of the large intestine. The role of immune mechanisms of regulation by central immune organs is important in the formation of normal intestinal microflora. Since the microbiota is a system involved in the management of homeostasis of our body, and violation of its qualitative and quantitative composition leads to the development of diseases of various organs and systems. The largest number of microorganisms' lives in the gastrointestinal tract, and their maximum number is in the lumen of the large intestine – about 10^{13} -10^{14}. This is 10 times more than the number of cells that make up the adult human body, and the sum of genes of the normal flora is approximately 150 times larger than our genome [9, 10].

Intestinal microflora performs an immunogenic role: it stimulates local immunity and development of the intestinal lymphoid apparatus. First of all, due to increased production of the key link of the local immunity system - secretory immunoglobulin - Ig class A. The intestinal microflora interacts with the innate and adaptive immune system, playing a key role in ensuring the state of immune resting of the intestine. In addition, immunogenic function of normoflora consists in stimulation of synthesis of immunoglobulins, interferon, cytokines; in activation of maturation of phagocytic mononuclear system; in increase of complement and properdin content, lysozyme activity; production of bacterial modulins. Normoflora is able to affect the differentiation of T-helper cells and thus affect the ratio of pro- and anti-inflammatory cytokines [11, 12].

Purpose of study. Study of the quantitative composition of indigenic and facultative groups of microorganisms of the microbiocenosis of the large intestine of thymectomized laboratory animals and identification of the depth of dysbacteriosis.

Material and methods. We conduct the studies in 2023-2024 in the vivarium of the Bukhara State Medical Institute.135 white mongrel rats with body weight 160-180 g of male sex, kept in standard vivarium conditions - temperature 21-22^0C, relative humidity equal to 50-60%, light regime of 12 hours of darkness and light. Methodological manual Nuraliev N.A. et al. [6] used in the maintenance of laboratory animals, feeding and care for them, selection of animals, cleaning and disinfection of vivarium premises.

Written permission was also obtained from the Ethical Committee of the Ministry of Health of the Republic of Uzbekistan to conduct experiments with

laboratory animals (Protocol No. 5/12-1679 of the Ethical Committee of the Ministry of Health of the Republic of Uzbekistan dated July 5, 2022).

For experimental studies, all animals (n=135) were categorized into the following groups: Main group - white mongrel rats that underwent thymectomy and after 1, 3, 6 months in which the normal microflora of the large intestine was studied (n=60);

Comparison group - white mongrel rats that did not undergo thymectomy but had normal colonic microflora studied - "falsely operated" (n=60);

Control group - intact white mongrel rats (n=15).

The main and comparison groups, in turn, were divided into 3 more subgroups - the results of the study of normal colon microflora 1 month (O1), 3 months (O2) and 6 months (O3) after thymectomy, 1 month (C1), 3 months (C2) and 6 months (C3) after "false surgery" are presented.

For the experiment, we used the method of exclusion of one of the central organs of the immune system of experimental animals - thymus (thymus gland). Thymectomy technique Victoria R. Rendell et al. (2014) used to perform thymectomy in adult white mongrel rats, which is a simple method of rat thymectomy that uses mini sternotomy and endotracheal intubation.

Biological material for studying the quantitative composition of microflora in all laboratory animals we took from the large intestine (intestinal contents) after killing. We took liver, spleen, lungs, peripheral blood of experimental animals to study translocation of microorganisms to internal organs.

Results and discussion. We studied the effect of thymectomy on the microflora of the large intestine in the dynamics of studies 1, 3 and 6 months after thymectomy of experimental animals. The results of the studies are summarized in the form of Table 1.

Table 1.

Comparative parameters of quantitative composition of colonic microbiocenosis in thymectomized animals in the dynamics of studies, 1g CFU/ml.

Microorganisms	Intact, n=15	Timeline after thymectomy		
		After 1 month, n=20	After 3 months, n=20	After 6 months, n=20
Lactobacillus spp	10,79±0,41	7,12±0,53*↓	6,56±0,49*↓	5,05±0,18*↓^
Bifidobacterium spp	9,18±0,63	6,57±0,62*↓	5,71±0,26*↓	5,20±0,49*↓^
Bacterioides spp	7,82±0,41	5,89±0,12*↓	4,37±0,24*↓^	4,62±0,25*↓^
Enterococcus spp	5,24±0,38	4,27±0,29*↓	4,78±0,32*↓^	5,05±0,42↔^
Escherichia coli (lactose «+»)	7,34±0,29	4,36±0,44*↓	4,17±0,63*↓	4,36±0,17*↓
Streptococcus spp	5,46±0,19	3,71±0,58*↓	3,98±0,37*↓	4,83±0,41*↓^
Streptococcus spp (hemolytic)	0	6,81±0,46*↑	3,48±0,19*↑^	2,05±0,19*↑^
Staphylococcus spp	6,16±0,47	5,32±0,39*↓	5,73±0,40*↔	6,00±0,10↔^

Enterobacter spp	4,74±0,39	3,05±0,25*↓	3,41±0,37*↓	4,20±0,30↔^
Escherichia coli (lactose «-»)	0	3,00±0,00*↑	2,00±0,00*↑^	2,22±0,13*↑^
Proteus spp	4,93±0,51	2,05±0,19*↓	3,00±0,00*↓^	4,00±0,00*↓^
Candida spp	2,00±0,23	5,11±0,31*↑	5,49±0,38*↓	6,67±0,54*↑^

Note: * - sign of reliable difference of data in relation to the data of intact rats; ↓, ↑ - direction of changes; ^ - sign of reliable difference in relation to the data of animals 1 month after thymectomy.

Table 1 shows that we identified 12 microorganisms, 6 of which are representatives of the indigenic microflora of the large intestine (*Lactobacillus spp, Bifidobacterium spp, Bacteroides spp, Enterococcus spp, E. coli l*actose "+", *Streptococcus spp*, and 6 of which are representatives of the facultative microflora (*Streptococcus spp (hemolytic, Staphylococcus spp*). *E. coli* lactose "+", *Streptococcus spp*) and six of them are representatives of facultative microflora *(Streptococcus spp* (hemolytic), *Staphylococcus* spp, *Enterobacter* spp, *E. coli* lactose"-", *Proteus spp* and *Candida spp*).

The results of the conducted studies showed that 1 month after thymectomy there were quantitative changes in all identified microorganisms. It should be emphasized that decrease of gram-positive cocci and gram-negative bacteria was noted, the exception was the quantitative increase of *Candida spp* from 2.00±0.23 CFU/ml to 5.11±0.31 CFU/ml by 2.56 times (P<0.001). It is noteworthy that 1 month after thymectomy, hemolytic strains of streptococci (6.81±0.46 CFU/mL) and lactose-negative Escherichia coli hemolytic strains (2.00±0.00 CFU/mL) were isolated in large numbers in the large intestine.

Identical studies we performed 3 months after thymectomy to determine further changes in the microbiocionosis of the large intestine in the experiment. The obtained results showed that the quantitative decrease of indigeneous microorganisms 3 months after thymectomy continued. The quantitative parameter of facultative microorganisms, as the results mostly remained below the data of intact rats (P<0.05). But in comparison with O1 subgroup in O2 subgroup all parameters tended to increase, it concerned quantitative parameters of *Streptococcus spp* (3, 98±0, 37 CFU/ml), *Staphylococcus spp* (5, 73±0, 40 CFU/ml), *Enterobacter spp* (3, 41±0, 37 CFU/ml), *Proteus spp* (3, 00±0,00 CFU/ml). Quantitative difference between these groups of microorganisms practically did not remain. If in intact subjects the difference was 2.57 times in favor of indigenes, then 1 month after thymectomy it decreased to 1.53 times, and 3 months later to 1.04 times.

Quantitative parameters of facultative microorganisms remained below the data of intact rats (P<0.05), but in comparison with O1 subgroup in O2 subgroup all parameters tended to increase, it concerned quantitative parameters *Streptococcus spp* (3, 98±0, 37 CFU/ml), *Staphylococcus spp* (5,73±0,40 CFU/ml),

Enterobacter spp (3,41±0,37 CFU/ml), *Proteus spp* (3,00±0,00 CFU/ml). The quantitative difference between these groups of microorganisms remained practically unchanged. If in intact subjects the difference was 2.57 times in favor of indigenes, then 1 month after thymectomy it decreased to 1.53 times, and 3 months later to 1.04 times. This fact indicates that the regulatory function of the immune system is more concerned with indigenic microorganisms than with facultative representatives of the normal microflora of the large intestine in white mongrel rats in the experiment. The further decrease of indigenic microorganisms and increase of facultative microorganisms explains by the fact that the latter have higher colonization activity and therefore recover faster. In addition, these microorganisms have higher colonization resistance, which provides protection against internal factors, such as mechanical factors and resistance factors of local immunity.

The mechanism of transient microorganisms is somewhat different, as their colonization activity depends on the colonization activity of representatives of the normal microflora of the large intestine. Transient microorganisms do not identified during normal activity of the immune system and intestinal microbiocenosis (hemolytic strains of *Streptococcus spp* and lactose-negative *E.* coli. *coli*). However, 1 month after thymectomy they were isolated in large quantities (respectively 6.81±0.46 CFU/ml and 2.00±0.00 CFU/ml), but 3 months after thymectomy their quantity decreased or remained unchanged (respectively 3.48±0.19 CFU/ml and 2.00±0.00 CFU/ml). this is closely related to the increased colonization activity of representatives of normal microflora of the large intestine (facultative microorganisms).

As for fungi of *Candida* genus, the quantitative parameter after thymectomy steadily increased and reached 5.49±0.38 CFU/mL in 3 months after thymectomy, which is 2.75 times more than intact rats ($P<0.001$) and 1.07 times more than the parameters of O1 subgroup - 1 month after thymectomy ($P>0.05$). The increased colonization activity and colonization resistance of Candida spp, as well as the impossibility of its passage through the intestinal mucous membranes to other organs and tissues (absence of bacterial translocation phenomenon) explain this. This makes it difficult for the immune system to regulate the quantitative and qualitative composition of *Candida spp*.

In 6 months after thymectomy the decrease of lactobacilli and bifidobacteria continues, other representatives of indigenic microorganisms, including anaerobes, insignificant increase in the number of facultative microorganisms in relation to the data of O1 and O2 subgroups and their recovery to normal values, further decrease in the number of hemolytic strains of streptococci, but increase of *Candida spp*.

(P<0.05). The trend of quantitative increase of all studied facultative microorganisms continued 6 months after thymectomy, which started 3 months after thymectomy (P<0,05). The number of transient microflora - hemolytic streptococci also significantly decreased, amounting to 2.05±0.19 CFU/mL, at the same time the number of lactose-negative Escherichia coli (2.22±0.13 CFU/mL) remained unchanged.

For a more complete picture of the effect of thymectomy on the state of the large intestine of experimental animals, we calculated the dysbacteriosis index (DI) in thymectomized animals 1, 3, 6 months after thymectomy. We used the method of diagnostics of dysbacteriosis of the large intestine proposed by Uzbek scientists Garib F.Y., Adylov Sh.K., Narbaeva I.E.. The simplicity characterizes this method, where only two indicators assess microflora disorders. If there is a decrease in the number of representatives of normal microflora is considered dysbacteriosis I degree, if the number of indigenic microflora decreases, where on the contrary quantitative indicators of conditionally pathogenic microflora increased from the norm is considered dysbacteriosis II degree.

In intact animals ID, I was 0.2 units; ID II was 0.5 units (Table 2).

Table 2

Detection rates of dysbacteriosis index in white mongrel rats after thymectomy, units.
(according to Garib F.Y. et al. 1993)

Study groups	Dysbacteriosis Index	
	ID I < 0.1, units	ID II < 0.5, units
Intact	0,2	0,5
1 month after thymectomy	0,2	0,5
3 months after thymectomy	0,4	0,6
6 months after thymectomy	0,2	0,8

The obtained results show that the depth of dysbacteriosis increases with increasing time after thymectomy, indicating a quantitative imbalance of indigenic and facultative representatives of normal microflora of the large intestine in experimental animals. We found the connection of depth of dysbacteriosis and terms after thymectomy, even 6 months after thymectomy the state of intestinal microbiocenosis, indicating the main regulatory role of the immune system on the microbiocenosis of human biotopes.

Conclusions.

In 1 month after thymectomy in white mongrel rats, there is a significant quantitative decrease in the number of indigenous in 1.23-1.68 times. The facultative microorganisms of the large intestine in 1.16-2.40 times in relation to intact, with the appearance of transient microflora and increase of *Candida spp*. with the decrease of immune system activity (thymectomy), as immune system

factors are a regulating factor for normal microflora of the large intestine and deterrent factors for the growth of transient microflora and *Candida spp*.

The number of indigenic microorganisms continued to decrease in 3, 6 months after thymectomy, and facultative in these terms begin to recover, at the same time the number of transient tends to decrease, *Candida spp* to increase. With a decrease in the regulatory function of the immune system, (thymectomy) colonization activity and resistance took the leading place, which provides their quantitative recovery after thymectomy.

Detection of hemolytic strains of streptococci and Escherichia coli, increase of *Candida spp* in the large intestine of animals 1 month after thymectomy are precursors of intestinal dysbiosis formation (pre-pathological state). The colonization activity and resistance of transient microorganisms directly depends on colonization activity of representatives of normal microflora of the large intestine, as they were not isolated at normal activity of the immune system and intestinal microbiocenosis. The depth of dysbiosis increases with increasing time after thymectomy.

Quantitative decrease of indigenic microorganisms of the large intestine in all periods of the experiment (1, 3, 6 months), restoration of the number of facultative ones in 3-6 months after thymectomy. The determination of the dysbacteriosis index, isolation of transient, hemolytic strains of *Streptococcus spp* and *E. coli only after thymectomy we recommend as immuno-microbiological prognostic criteria of the degree of influence of the immune system on the microbiocenosis of the large intestine and E. coli* only after thymectomy. Against the background of a decrease in representatives of normal microflora, we recommend as immuno-microbiological prognostic criteria of the degree of influence of the immune system on the microbiocenosis of the large intestine and as immuno-microbiological prognostic criteria of the degree of development of dysbiosis of the large intestine in experiment.

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