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THE CHOICE OF BIOPREPARATION FOR THE CORRECTION OF INTESTINAL DYSBACTERIOSIS IN CHILDREN WITH CHRONIC HBV INFECTION AND GIARDIASIS

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ABSTRACT

Research objective: to assess efficiency of application of bio preparations in the correction of intestinal dysbacteriosis taking into account the results of "Load" test in vitro in children with chronic hepatitis B together with giardiasis.

Material and methods. We examined one hundred and fifty children with chronic hepatitis B (CHB) with concomitant giardiasis in the age from 3 to 18 years old. The diagnosis was based on data from history, results of clinical tests, biochemical and instrumental tests. Laboratory diagnostics of giardiasis was performed using the following methods: immune fluorescent method (G. Lamblia antigen in feces), polymerase chain reaction (G. Lamblia DNA in blood and feces), and triple microscopic test for precipitation components of feces. For the definition of lymphocyte sensitivity to bio preparations to choose the agent we applied loading test in vitro. For the correction of dysbacteriosis using the basic therapy we used very sensitive polycomponent probiotics in capsules such as "Bifilax-immuno", "Lacto-G", and "Narimax-plus".Lamblia eradication was performed using Macmirror (Nifuratel) 15mg/kg two times a day for 10 days. Assessment of the efficiency of the prescribed therapy was done according to the clinical, biochemical, and bacteriological data.

Results. We determined a direct proportional decrease of lymphocyte ability for E-rosette formation dependent on the expression of intestinal dysbacteriosis. The most sensitive biopreparation in in vitro test was Bifilax-immuno (62.7%), which differed by its quantitative and qualitative composition compared to Lacto-G (48.0%) and Narimax-plus (38.7%), $p<0.05$. Prescription of anti-giardiasis therapy with individually chosen very sensitive biopreparation for

the correction of intestinal dysbacteriosis in children with CHB provided achievement of stabilization (60.0%) and regression of pathological process in liver, prevention of unfavorable outcomes such as hepatic cirrhosis and hepatocellular carcinoma.

Conclusion. Differential approach to the choice of probiotic for the correction of intestinal dysbacteriosis provides the increase of the efficiency of therapy in children with CHB with concomitant giardiasis by 40.1%. Development of clinical, biochemical, and microbiological remission was achieved using Bifilax-immuno in 76.5% cases, Lacto-G in 64.4%, and Narimax-plus in 62.0% of the cases respectively ($p < 0.01$). In relation to that, children with CHB and concomitant giardiasis together with the test for intestinal dysbacteriosis have to undergo loading test in vitro for the prescription of efficient and targeted correction taking into account individual sensitivity of an organism to biopreparations.

Key words: chronic hepatitis B, giardiasis, intestinal dysbacteriosis, therapy, biopreparations, children.

INTRODUCTION

In spite of vaccination against hepatitis B and achievements in the field of chronic viral hepatitis diagnostics and therapy in children, that problem continues to be topical one for the world and local health care systems. According to the WHO data the are more than 500 million people in the world infected by Hepatitis-B virus, which is among the ten most prevalent death causes among the population, every year taking the lives of about 1.5 million people and possesses strong oncogenic potential [1]. One of the pathogenic sections in the development of pathological process in the liver of children with chronic hepatitis B is dysbiotic alterations in intestine, causing initiation of a cascade of pathological reactions promoting progression of the pathology [2,3]. Earlier in our studies we revealed high prevalence (99.7%) of intestinal dysbacteriosis (ID) among the children with CHB independently of presence or absence of giardiasis [4]. In the conditions of chronic viral persistence intestinal dysbacteriosis promotes development of disorders in secretory, motor, and barrier functions of intestine, and makes all ways of lamblia introduction real [5]. Initsturn, in the conditions of aggressive medium in cases of ID lamblia secrete more toxins promoting generalization of viral infection with outgoing complications such as polysystemic organic failure [6]. In the result it leads to the suppression of macro organism resistance, and in the conditions of combined viral-parasite infection it promotes the development of two parallel mutually-deteriorating processes. In the modern time due to the enormous variety of probiotics available on pharmaceutical market, which are blindly used, there is a pending problem of the choice of optimal bio preparation. According to the results of our studies, efficiency of existing correction methods used for intestinal micro ecology is balance is equal to 62.2% only [7]. Taking into account that fact, and recently registered characteristic development of microbiot a resistance to biopreparations [8, 9], a question of the search for new diagnostic methods allowing short-term, targeted, and maximally effective choice a bio preparation for ID correction in children with CHB appeared. The objective of the study was to assess the efficiency of bio preparation prescription for the correction of ID taking into account in vitro “load” test results in children with chronic hepatitis B with background giardiasis [10].

MATERIALS AND METHODS

We observed one hundred and fifty children with CHB with concomitant giardiasis in the age from 3 to 18 years old. CHB diagnosis was based on the data from case history, clinical, biochemical, and instrumental tests. Verification ofHBV-infection was done with the help of EIA and PCR (HBsAg, HBsAb, HBeAg, HBeAb, HBcorAb, HBV-DNA). Laboratory diagnostics of giardiasis was performed using the following methods: immune fluorescent method (G. Lamblia antigen in feces), polymerase chain reaction (G. Lamblia DNA in blood and feces), and triple

microscopic test for precipitation components of feces.In compliance with the guidelines proposed by I.B. Yershova (2002) [11], all patients had double test of intestinal micro flora for dysbacteriosis according to Epstain-Litvak's and F.L. Vishanskay's method (1977) using V.M. Granitov's classification(2002).

For the definition of lymphocytes sensitivity to bio preparations we used "load" test in vitro for the choice of bio preparation (Patent UZIAP 04570, 2012) [12]. In this method performance of the assessment of T-lymphocytes functional activity in E-rosette formation reaction in vitro incubation with probiotic is stipulated, providing the choice of effective bio preparation in each certain case taking into account individual sensitivity. For the control we determined amount of E-rosette forming cells (E-RFC) in blood plasma of these patients without stimulation. We calculated the number of stimulated cells (NSC) based on the difference of E-RFC number between the study and the control groups. In compliance with the method we worked out 3 types of reactions:

1-sttypereaction– *hyperergic*, increase of the number of E-rosette forming lymphocytes of the experimental sample above 5% in case of probiotic addition compared to the control sample; i.e. those not losing the ability to form E-rosettes under the influence of the agent. That reaction type indicates high sensitivity of an organism to that agent.

2-ndtypereaction–*hypoergic*, decrease of E-rosette forming lymphocytes in the experimental sample below 5% in case of the agent addition compared to the control sample; i.e. those losing ability to form E-rosettes under the influence of the preparation. Hypoergic type indicates low sensitivity of an organism to that agent.

3-rdtypereaction – *withoutalterations*, i.e. no difference between the experimental and control samples, indicating there is no sensitivity of an organism to that agents.

For the correction of dysbacteriosis we applied polycomponent probiotics in capsules such as Bifilax-immuno containing $10x10^9$ CFUL.paracaseiCRL-431 and B.animal is BB-12 (Pharmaxx International, Denmark), Lacto-G containing $5x10^9$CFUL.acidophilus, B.longum, B.bifidum, B.infantisand fructose oligosaccharides (GMP, Georgia) and Narimax-plus containing $2.2x10^8$CFUL.acidophilus, L.rhamnosus, L.bulgaricus, L.salivarius, B.bifidumandSt.thermophilus(Vitamax Ltd., Yerevan).

ID bio correction was done with background basic therapy in compliance with the results of "load" test using very sensitive bio preparation. Comparison group consisted of 60 children with CHB and giardiasis, who received dry bacterial preparations such as bifida and lactobacteria in common doses for a month together with background basic therapy. Lambliae radication was performed by means of Macmirror (nifuratel), in the dose 15mg/kg two times a day for 10 days, which is more effective and safe for children with CHB compared to other anti-giardiasis agents [13,14]. Assessment of the efficiency of the applied therapy was performed according to clinical, biochemical, and bacteriological data.

Statistical processing was done by means of variation statistics method using Student's t-criterion with special Excel-2017 software. Differences were considered to be reliable in case of $p<0.05$.

RESULTS AND DISCUSSION

Distribution of sick children according to CHB activity showed that, with background giardias is the disease progressed. So, prevailing majority (80.0%) of the patients had moderate (71.7%)and expressed (28.3%) activity of the pathology, while the rest (20.0%) of children were diagnosed with minimal activity of the pathology. The term of CHB was equal to 4.1±0.2 years. Bacteriological test of excrements for micro biocoenosis in all 75 patients displayed intestinal dysbacteriosis of the II (50), III (50), and IV (50) stages.

Analysis of the results of "load" test in vitro showed (Table 1) that, in case of IDII stage in 68.0% of the children there was notably high sensitivity f an organism to Bifilax-immuno, where the amount ofE-RFCwas reliably higher up to 58.2±1.2%, ($p<0.001$ to the control). NSC was equal to9.1±0.94% ($p<0.01$). In case of stimulation with Lacto-G hyper ergic type reaction was

determined in the blood of 60.0% of the children, number of E-RFC was increased up to 54.1±0.7% ($p<0.01$ to the control). NSC under the influence of the preparation was only 5.0±0.7%. We revealed no reliable differences in the numbers of E-RFC with Narimax-plus. It was notable that, under the influence of Bifilax-immuno NSC was reliably higher then under the influence of Lacto-G ($p<0.05$), indicating stronger immune tropic capability of Bifilax-immuno [15].

In case of IDIII stage results of the loading test in vitro showed that under the influence of Bifilax-immuno hyper ergic type reaction was revealed in 60.0% of the children with CHB, when the number ofE-RFC was increased up to 54.3±1.2%, ($p<0.05$ to the control). NSC under the influence of the preparation was 8.0±0.95%. Lacto-G preparation did not sufficiently effect the increase of E-RFC number (49.0±1.4% $p>0.05$), while NSC was only 2.7±0.3%, indicating low immune tropic capability of the preparation in children with ID III stage. Narimax-plus also did not sufficiently stimulated the ability of lymphocytes for E-rosette formation. The number of E-RFC was 47.2±1.3% in 40.0% of the children ($p>0.05$). NSC was only 0.9±0.4%, indicating inefficiency of the preparation [16].

Analysis of the mean values of the loading test results in children with CHB and ID IV stage showed that hyperergic type reaction to Bifilax-immuno was revealed in 54.0% of the cases when the number of E-RFC was increased up to 48.0±1.1% ($p<0.05$). NSC under the influence of the preparation was equal to 8.2±0.83%. Preparations Lacto-G and Narimax-plus did not sufficiently effect the formation of E-RFC and did not reveal statistical difference between the values of the control sample, ($p>0.05$). NSC was equal to 2.3±0.12% and 1.4±0.11%, respectively.

Table 1. Results of the loading test in vitro in children with CHB with concomitant giardiasis dependently on the *ID* stage (E-RFCM±m, %).

Preparations	***ID* II stage**(n=50)		***ID* III stage** (n=50)		***ID* IV stage**(n=50)	
	Experiment	Control	Experiment	Control	Experiment	Control
Hyper ergic type reaction						
Bifilax-immuno % E-RFC,%	68.0±9.3 58.2±1.2*	49.1±1.4	64.0±9.6 54.3±1.2*	46.3±1.2	56.0±9.9 48.0±1.1*	39.8±1.1
NSC,%	9.1±0.9[a]		8.0±0.8[a]		8.2±0.8[a]	
Lacto-G % E-RFC,%	60.0±9.8 54.1±1.0*		44.0±9.9 49.0±1.4		40.0±9.8 42.1±1.1	
NSC,%	5.0±0.7		2.7±0.5		2.3±0.6	
Narimax-plus % E-RFC,%	44.0±9.9 51.1±1.1		40.0±9.8 47.2±1.3		32.0±9.3 41.2±1.1	
NSC,%	4.8±0.7[b]		0.9±0.7[b]		1.4±0.7[b]	
Hypo ergic type reaction						
Bifilax-immuno % E-RFC, %	16.0±7.3 44.2±0.7*	49.1±1.4	20.0±8.0 39.0±1.2*	46.3±1.2	24.0±8.5 33.2±1.2*	39.8±1.1
Lacto-G % E-RFC, %	16.0±7.3 43.1±1.0		24.0±8.5 37.2±1.3*		32.0±9.3 30.3±1.1*	
Narimax-plus % E-RFC, %	28.0±8.9 42.1±1.1		24.0±8.5 34.1±1.1*		32.0±9.3 28.3±1.1*	
Without alteration						
Bifilax-immuno %	16.0±7.3		16.0±7.3		20.0±8.0	

E-RFC, %	48.5±1.4		45.8±1.3		38.6±1.3	
Lacto-G % E-RFC, %	 24.0±8.5 48.8±1.1	49.1±1.4	 32,0±9,3 46.2±1.1	46.3±1.2	 28.0±8.9 38.7±1.1	39.8±1.1
Narimax-plus % E-RFC, %	 28.0±8.9 48.5±1.4		 36.0±9.6 46.9±1.1		 36.0±9.6 39.1±1.1	

Note: * - reliability of the differences to the control values ($p<0.05$)

[a] – reliability of the differences between Bifilax-immuno and Lacto-G ($p<0.05$);

[b]- reliability of differences between Bifilax-immuno and Narimax-plus ($p<0.05$);

[c]- reliability of differences between Lacto-G and Narimax-plus ($p<0.05$);

In the total number of examined children, the prevalence of hyper ergic type reaction in loading test in vitro to Bifilax-immuno was equal to 62.7±5.6%, Lacto-G to 48.0±5.8%, Narimax-plus to 38.7±5.7% cases.

Thus, we revealed interrelation between the expression of ID in children and the functional activity of T-lymphocytes in the blood plasma of patients with CHB with background giardiasis. Increase of ID was characterized by decrease of lymphocytes' ability to form E-rosettes, indicating failure of receptor tendency in T-lymphocytes to the studied preparations. The most sensitive probiotic was Bifilax-immuno containingL.paracaseiCRL-431, B. animal is BB-12. In vitro conditions these strains with antimicrobial and immune modulating properties promoted production of T-lymphocytes, by these means having a complex impact on the intensification of receptor tendency for the formation of E-RFC. Apparently, that indicated deficiency of these strains in intestine of the examined children. Nevertheless, most of the performed clinical and experimental tests testify that probiotic strains of lacto bacteria L.paracaseiCRL-431, Ent. Faecium are perceived by T-lymphocytes and stimulate inflammatory response, intensifying production of Th1 and IL-1, INF-α. They stimulate phagocyte activity of neutrophils and secretion of SIgA, while bifido bacteria stimulate Th-reg and, correspondingly, secretion of TGF-β, IL-10, by these means promoting formation of immunological tolerance [17, 18]. That can explain the necessity of decoding of the qualitative composition of intestinal micro flora, especially L.paracasei, B.animal is BB-12 subclasses in children with CHB and giardiasis.

The next stage in our study was assessment of the efficiency of biopreparations in the correction ofIDin children with CHB with background giardiasis.Prescription of individually chosen preparations had a significant effect on the dynamics of the basic clinical symptoms in children with CHB and background giardiasis. Particularly, clinically 78.4% of the children (versus 38.3% in the comparison group, $p<0.05$) had positive response. That was reflected in the improvement of children's health, leveling of complaints about fatigue, loss of appetite, and stomachache among the children of the basic group ($p<0.05$-0.001). Reliably less often we registered symptoms such as diminishing of flatulence (19.5±6.4%), rumbling in stomach (16.2±6.1%), tongue plaque and stool disorders (13.3±6.2%, $p<0.05$). The sizes of liver and spleen diminished 2.0 and 1.8 folds respectively ($p<0.05$ to the comparison group).

With the background complex therapy with individual approach to biocorrection of ID parameters of biochemical homeostasis improved by means of decrease of the expression of cytolysis syndromes (ALAT and ASAT 2.5 folds, $p<0.01$ to the control), cholestasis (total and direct bilirubin 2 folds), and mesenchymal-inflammatory (gamma-globulin and thymolturbidity test 1.8 folds, respectively, $p<0.001$).

Comparativeanalysisofquantitativeandqualitativealterationsintheintestinalmicrofloraofthe examinedchildren, independently of the activity of the disease, revealed that after the complex therapy with individually chosen probiotics the number of bifida and lactobacteria in normal limits was noted in 34.7 ± 5.5% and 30.7 ± 5.4% of the patients respectively (versus 13.3 ± 6.2% in the comparison group $p < 0.01$) (Table 2).The number of significant decrease in Logarithm values of bifida and lacto bacteria (< 105 CFU/g) in the patients of the basic group at the end of the therapy course were identified 3.7 and 3.1 folds less often ($p < 0.001$ to the

control). Normal enzyme activity of colon bacilli was registered reliably more often among the patients of the basic group ($p < 0.01$) and hemolytic E. coli were isolated 2.9 folds less often ($p > 0.05$ compared to the control). The normal number of enterococci (107—108 CFU/g) in the patients of the basic group was registered almost in half of the cases (50.7 ± 5.8% versus 16.7 ± 6.8% in the comparison group, $p<0.001$). Increase of the number of enterococci above 108 CFU/g in patients of the basic group after the therapy was not registered, while in the control group increase was observed in 13.3±6.2% of the cases ($p<0.05$). Decrease in the increased number of enterococci up to 107 CFU/g in the patients of the basic group after the therapy with probiotic was registered in 49.3 ± 5.7% of the cases ($p < 0.02$). Apparently, an individually chosen bio preparation in the conditions of impaired secretory, motor, and barrier function of intestine in children with CHB with giardiasis promotes adhesion of probiotic strains to intestinal epithelium. They attach to the epithelium by means of glycol conjugated receptors, providing colonization resistance and preventing pathogen adhesion and invasion. Finally, all these mechanisms contribute to the rise of epithelial resistance, intensifying its barrier function and protection [19].

Table 2.

Dynamic alterations in intestinal micro flora in children with CHB with background giardiasis (%).

Ig (CFU/g) of intestinal micro flora	Before the therapy n=105	After the therapy		P
		Basic group n=150	Comparison group n=60	
Bifida bacteria:				
- normal (10^9-10^{10})	4.7±2.1	34.7±5.5	13.3±6.2	<0.001
- moderate decrease (10^6-10^5)	20.9±4.0	49.3±5.7	23.3±7.7	<0.05
- significant decrease (<10^5)	74.4±4.3	16.0±4.3	63.4±8.8	<0.001
Lactobacteria:				
- normal(10^7-10^8)	4.7±2.1	30.7±5.4	13.3±6.2	<0.01
- moderate decrease(10^6-10^5)	29.5±4.5	50.7±5.8	33.3±8.6	>0.05
- significant decrease (<10^5)	65.8±4.6	18.6±4.5	53.4±9.1	<0.001
E.colitипичные:				
- normal(10^7-10^8)	5.7±2.3	34.7±5.5	16.7±6.8	<0.01
- decrease (<10^7)	74.3±4.3	49.3±5.7	70.0±8.4	<0.02
- increase (>10^8)	20.0±3.9	16.0±4.3	13.3±6.2	>0.05
E.colilactose negative	30.5±4.5	18.6±4.5	26.7±8.0	>0.05
E.colihemolytic	20.0±3.9	5.3±2.6	16.7±6.8	>0.05
Enterococci:				
- normal(10^7-10^8)	6.7±2.4	50.7±5.8	16.7±6.8	<0.001
- decrease (<10^7)	74.3±4.3	49.3±5.7	70.0±8.4	<0.02
- increase (>10^8)	19.0±3.8	-	13.3±6.2	<0.05
Staphylacoccus aureus	29.5±4.5	9.3±3.4	20.0±7.3	<0.05
Staphylococcus epidermidis	29.5±4.5	5.3±2.6	20.0±7.3	<0.05
Proteus	14.3±3.4	-	10.0±5.5	<0.02
Klebsiella	14.3±3.4	9.3±3.4	10.0±5.5	>0.05
Candida	55.2±4.9	18.6±4.5	36.7±8.8	<0.02
2-component RPM associations	20.0±3.9	9.3±3.4	16.7±6.8	>0.05
3-component RPM associations	10.5±3.0	-	10.0±5.5	<0.02
4-component RPM associations	5.7±2.3	-	3.3±3.3	>0.05

Note: P – statistically reliable differences of the values with background therapy.

Among the representatives of relatively pathogenic micro flora (RPM) — St. aureus et St. epidermidis were revealed 2.3 and 3.5 folds less often (9.3 ± 3.4% and 5.3 ± 2.6% versus 20.0 ±

7.3% of the cases in comparison group, $p < 0.05$) among the children of the basic group after the therapy. After the therapy we could not detect non-fermenting Proteus among the children of the basic group, while in the control group the values almost did not change ($p<0.05$). In the basic group of patients, the number of yeast-like Candida fungi and revealed RPM associations diminished notably, while 3 and 4-component associations disappeared at all ($p<0.05$—0.001). Thus, performed correction of ID in children with CHB with background giardiasis, taking into account their individual sensitivity to a bio preparation, different from the control group, promoted significant improvement of micro ecological status in intestine due to more expressed normalizing impact on qualitative and quantitative composition of the micro flora. Application of anti-giardiasis therapy with individually chosen very sensitive bio preparation for the correction of intestinal dysbacteriosis in children with CHB provided achievement of stabilization (60.0%) and regression of the pathological process in liver, prevention of unfavorable outcomes such as liver cirrhosis and hepatocellular carcinoma [20].

CONCLUSION

1. We revealed an interrelation between the functional activity of T-lymphocytes and expression of ID in children with CHB and concomitant giardiasis. Decrease of the number of E-RFC directly proportional to the expression of ID was determined, which indicated the failure of receptor tendency in T-lymphocytes and sensitivity to bio preparations.
2. In children with CHB and giardiasis the most sensitive bio preparation in vitro test was Bifilax-immuno (62.7%) in comparison with Lacto-G (48.0%), and Narimax-plus (38.7%, $p < 0.05$).
3. Differentiated approach to the choice of probiotic for ID correction provided the rise of therapy efficiency in children with CHB with concomitant giardiasis by 40.1%. development of clinical, biochemical, and microbiological remission was achieved using Bifilax-immuno in 76.5%, Lacto-G in 64.4%, and Narimax-plus in 62.0% of the cases respectively ($p < 0.01$).
4. Children with CHB with concomitant giardiasis should have loading test in vitro together with ID test for the prescription of effective and targeted correction taking into account individual sensitivity of an organism to biopreparations.

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