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GIARDIASIS IN CHILDREN: MOLECULAR GENOTYPING, GROWTH AND CALPROTECTIN LEVELS

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Abstract

Introduction. Giardiasis is the most frequently reported human intestinal parasitic infection.
The objective of the study was to investigate the frequency of giardiasis, carry out the genotyping, estimate the growth and determine the level of fecal calprotectin in children.
Material and methods. 688 children aged 6-18 years were examined for Giardia duodenalis by direct microscopy. Two groups were formed: group I – children with a positive test for Giardia duodenalis (n = 90); group II – children with a negative test (n = 110). Genetic examination, anthropometry and fecal calprotectin (FC) evaluation were carried out in these children.
Results. Out of the 688 children examined, 90 had a positive result (G. duodenalis (+)). The leading clinical feature of G. duodenalis infection (+) was abdominal pain, followed by nausea and diarrhea. The FC content in the feces of the group I was significantly

Résumé

La giardiase chez les enfants: le génotype moléculaire, la croissance des enfants et la calprotectine fécale

Introduction. La giardiase est la plus sévère infection parasitaire de l'intestin humain.
L'objectif de l'étude a été d'investiguer la fréquence de la giardiase, trouver son génotype, estimer la croissance et déterminer le niveau de la calprotectine fécale chez les enfants.
Matériel et méthodes. On a utilisé la méthode directe microscopique de 688 enfants, âgés de 6 à 18 ans pour G. duodenalis. Deux groupes se sont constitués: Groupe I – avec un test positif pour G. duodenalis (n = 90); groupe II – enfants avec un test négatif (n = 110). On a effectué pour ces enfants, le dosage génétique, l'anthropométrie et la calprotectine fécale.
Résultats. Sur ces 688 enfants examinés pour G. duodenalis, 90 ont donné un résultat positif pour

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higher (p <0.05) compared to children of group II, and did not depend on sex. The analysis of the sequences characterizing the amplification of Glutamate dehydrogenase (GDH) revealed the presence of subgroups AII (54%, 13/24), BIII (8.3%, 2/24) and BIV (37.5%, 9/24). Annual body weight gain in children of group I is shifted by 1 year and 1 cm compared to the ones from group II.

Conclusions. The socio-demographic factors can be considered as predictors of the development of giardiasis in children. In the clinical course of giardiasis, the digestive tract's disease dominates. Direct and indirect methods of diagnosis are necessary to improve the diagnosis accuracy in children. Children with increased FC need further examination. Our study suggests that G. duodenalis infection is accompanied by the growth retardation and intestinal inflammation in children.

Keywords: Giardiasis, genetic testing, growth, calprotectin, children.

Abbreviations list:
Giardia duodenalis – G. duodenalis; EU/EEA – European Union/European Economic Area; ASR – Age-standardised rate; AOR – Associated odds ratio; rRNA – Ribosomal ribonucleic acid; PCR – Polymerase chain reaction; DNA – Deoxyribonucleic acid; GDH – Glutamate dehydrogenase; BG – Beta-giardin; FC – fecal calprotectin; ELISA – Enzyme-linked immuno sorbent assay; SISA calculator – Simple Interactive Statistical Analysis; CI – confidence interval; ICH GCP – International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use, Good Clinical Practice; CI – confidence interval.

(G. duodenalis (+)). Les signes cliniques de l'infection avec G. duodenalis étaient (+) maux de ventre, nausée et diarrhée. Le contenu de la FC dans les fèces du groupe I (p <0,05) était plus élevé que celui du groupe II, fait qui ne dépendait pas du sexe. Les analyses des séquences caractérisant l'amplification de GHH, ont montré la présence de sous- groupes AII (54%, 13/24), BIII (8,3%, 2/24) et BIV (37,5%, 9/24). Les gains annuels de poids corporel chez les enfants du groupe I sont décalés d'un an et 1 cm par rapport aux enfants du groupe II.

Conclusions. Les facteurs socio-démographiques peuvent être considérés comme des prédicteurs du développement de la giardiase. Cliniquement, la giardiase est dominée par la défaite du tube digestif. Afin d'augmenter la précision du diagnostic de la giardiase chez les enfants, il est nécessaire d'utiliser un ensemble de méthodes de diagnostic directes et indirectes. Les enfants avec FAC élevée ont besoin d'un examen plus approfondi. Notre étude suggère que l'infection à G. duodenalis est accompagnée d'une croissance lente chez les enfants, ainsi que d'une inflammation de l'intestin.

Mots-clés: la giardiase, les tests génétiques, la taille, la calprotectine, les enfants.

Introduction

Giardiasis is the most frequently reported human intestinal parasitic infection, with Giardia duodenalis (G. duodenalis, G. intestinalis, G. lamblia) as etiological factor. It has a broad variety of clinical manifestations, from asymptomatic carriers to acute or chronic illness[1]. Giardiasis is the most common parasitic infection in the European Union/European Economic Area (EU/EEA) among the five food- and water-borne parasitic diseases under mandatory EU surveillance. Surveillance of giardiasis covers the entire population in most EU/EEA countries. However, one-fourth of EU member states do not have surveillance systems for giardiasis and do not report cases[2]. Ukraine also has no surveillance systems for giardiasis. In total, 18,985 confirmed giardiasis cases have been reported by 24 countries in the EU/EEA, with an overall rate of 5.8 per 100,000 population[2]. The highest number of confirmed cases was reported by the United Kingdom (n=4 723), followed by Germany (n=3 473). These two countries accounted for 43% of all confirmed giardiasis cases in the EU/EEA. Bulgaria had the highest rate, 19.1 per 100,000 population, followed by Belgium (17.7 per 100,000 population)[2]. In both countries, there was an increase in the notification rate compared with the previous year. The number of confirmed giardiasis cases remained stable at the EU/EEA level between 2012 and 2016 (Table 1).

Half of the giardiasis cases were reported with information about importation. In the majority of countries, cases were mainly domestically acquired. In three Nordic countries (Iceland, Norway and Sweden), cases were mostly associated with travel outside the EU. In Sweden, over 80% of the cases were

Table 1. Distribution of confirmed giardiasis cases, EU / EEA, 2012-2016[2]

Country	2012		2013		2014		2015		2016			
	N	Rate	N	Rate	N	Rate	N	Rate	Con firmed cases	Rate	ASR	Reported cases
Austria	-	-	-	-	-	-	-	-	-	-	-	-
Belgium	1244	11.2	1220	11	1144	10.2	1270	11.3	198	17.7	17.6	1998
Bulgaria	1560	21.3	1873	25.7	1731	23.9	1245	17.3	1367	19.1	21.5	1367
Croatia	69	1.6	0	0.0	80	1.9	93	2.2	50	1.2	1.3	56
Cyprus	4	0.5	3	0.3	3	0.3	6	0.7	1	0.1	0.1	1
Czech R.	49	0.5	46	0.4	42	0.4	33	0.3	45	0.4	0.4	45
Denmark	-	-	-	-	-	-	-	-	-	-	-	-
Estonia	254	19.2	195	14.8	221	16.8	181	13.8	187	14.2	14.3	187
Finland	394	7.3	336	6.2	287	5.3	259	4.7	282	5.1	5.4	282
Germany	4216	5.2	4107	5.1	4014	5.0	3581	4.4	3473	4.22	4.5	3484
Greece	-	-	-	-	-	-	-	-	-	-	-	-
Hungary	81	0.8	59	0.	59	0.6	130	1.3	108	1.1	1.1	108
Iceland	22	6.9	20	6.2	22	6.8	25	7.6	19	5.7	5.1	19
Ireland	54	1.2	44	1.0	71	1.5	145	3.1	202	4.3	4.3	202
Italy	-	-	-	-	-	-	-	-	-	-	-	-
Latvia	17	08	37	1.8	73	3.6	184	9.3	76	3.9	3.9	76
Liechtenstein	-	-	-	-	-	-	-	-	-	-	-	-
Lithuania	13	0.4	13	0,4	13	0,4	9	0.3	10	0.3	0.3	10
Luxembourg	2	0.4	1	0.2	3	0.5	2	0.4	0	0.0	0.0	0
Malta	11	0.2	0	0.0	2	0.5	0	0.0	4	0.9	1.1	4
Netherlands	-	-	-	-	-	-	-	-	-	-	-	-
Norway	179	3.6	227	4.5	264	5.2	247	4,8	343	6.6	6.7	343
Poland	1622	4.3	1830	8.1	871	4.9	8687	4.4	1445	3.8	1	446
Portugal	-	-	-	-	-	-	26	0.3	30	0.3	0.3	30
Romania	260	-	328	-	796	-	959	-	892	-	-	892
Slovakia	243	4.5	180	3.3	166	3.1	228	4.2	284	5.2	5.2	284
Slovenia	35	1.7	42	2.0	38	1.8	30	1.5	54	2.6	2.6	54
Spain	859	-	885	-	1487	-	1627	-	1901	-	-	2069
Sweden	1081	1.4	1253	13.1	1260	13.1	1473	15.1	1491	15.1	15.1	1491
United Kingdom	4137	6.5	3840	6.0	3628	5.6	4536	7.0	4723	7.2	7.4	4723
EU/EEA	16396	5.8	16539	5.8	17275	5.6	17976	5.5	18985	5.8	6.3	19171

Source: Country reports; ASR: Age-standardized rate; No data reported; –: No rate calculated; N – Number; EU/EEA – European Union/European Economic Area.

infected abroad and the majority of these cases were immigrants/refugees[2].

Notification rates remain high, in particular in young children aged 0-4 years and in Eastern and Southern Europe[3]. Recent studies have identified the components of specific risk factors and ways of transmission[4]. A recent review in 19 Eastern European countries assessed the significance of Giardia spp. infections in humans and animals, as well as in the environment[5].

The review showed that Giardia spp. are common parasites of domestic animals, including pets[6]. Identified risk factors included international travel (AOR = 13.9; 95% CI 4.9-39.8), drinking water from a river, lake, stream, or spring (AOR = 6.5, 95% CI 2 , 0-20.6), swimming in natural reservoirs (AOR = 3.3; 95% CI 1.5-7.0); sexual behavior (AOR = 45.7; 95% CI 5.8-362.0), contact with children in diapers (AOR = 1.6, 95% CI 1.01-2.6), use of antibiotics (AOR = 2.5, 95% CI 1, 2-5,0) and chronic gastrointestinal disease (AOR = 1,8; 95% CI 1,1-3,0); consumption of raw foods (AOR = 0,2; 95% CI 0,1- 0.7)[7]. Research results emphasize the risk factor associated with G. duodenalis infection (23%), the presence of livestock

and, in particular, pigs near the houses[8]. Possible causes of high levels of parasites, such as G. intestinalis, E. coli, and B. hominis, are lack of education, accommodation in small houses with a large number of people, lack of sewage systems, as well as clean and safe drinking water[9]. Laboratories should be encouraged to regularly test everyone, regardless of the area of residence, for diagnosis of this forgotten pathogen, to ensure that the cases of infection in the habitat are properly identified and effectively cured[10]. However, giardiasis is the most spread in developing countries[11,12].

G. duodenalis organisms have been sub-classified into eight genetic assemblages (designated A–H). Genotyping of G. duodenalis organisms isolated from various hosts has shown that assemblages A and B infect the largest range of host species, and appear to be the main (or possibly only) G. duodenalis assemblages that undeniably infect human subjects[13]. G. duodenalis also infects other mammals and thus has zoonotic potential. Based on molecular studies, mainly targeting the parasite small parenchyma rRNA gene locus, eight complexes (from A to H) were identified in human and other species of animals. Results showed that 18.1% of the subjects of the study were infected with G. duodenalis[14]. Among isolates, 35.9% and 21.7% were subtyped into groups A and B, respectively, while 42.4% had mixed infections A and B. Most of the isolates of group A (94%) were 100% identical to the sequences, registered in GenBank, and belonged to the AII subgroup. Similar results were obtained[15]. However, the results did not reveal mixed groups A and B[16]. High genetic variability and the frequency of double peaks make sub-genotyping problematic. The carried-out studies confirm the need for further inclusive studies, especially for heterogeneous subtypes of Group B.

The literature data point out the relationship between environmental enteropathy, intestinal disorders and development retardation, growth in particular[17]. An abnormal microflora leads to inflammation and a decrease in the intestinal barrier function[18]. The presence of inflammation of the intestine can be determined by fecal proteins, in particular, calprotectin [19-20]. It is known that a direct correlation between the content of calprotectin and linear growth is present[21]. Clarifying the relationship between intestinal pathogens[22-23], ecological enteropathy[24] and growth retardation[25] can help to develop behavioral and therapeutic interventions to reduce this disease manifestation in susceptible pediatric populations.

The objective of the study was to investigate the frequency of giardiasis, to carry out the genotyping, to estimate the growth and to determine the level of fecal calprotectin in children.

Material and methods

A coprological examination was carried out for G. duodenalis in 443 out-patient children from 12 districts of Chernivtsi region (Ukraine), who were referred by primary health care centers, as well as in 245 in-patient children who underwent treatment in the gastroenterology department of the Chernivtsi Regional Children's Hospital, during 2017-2018 (Table 2), aged 6-18 years, with clinical signs of giardiasis (periodic or persistent diarrhea, abdominal pain, nausea, vomiting, weight loss, flatulence, skin rash).

Criteria for inclusion in the study: residence in Chernivtsi region, age of patients 6-18 years old, presence of clinical signs of giardiasis, informed consent of children and their parents. Criteria for exclusion: the lack of informed written consent of the patient and his parents, age up to 6 years, the presence of chronic pathology and diseases of immune competent organs, stay abroad and/or the use of antibiotics or anti-helminthic drugs before the study.

Samples of fresh feces (at least 3 portions) were mixed and placed in containers, labeled with anonymous research codes and stored at 4°C until the further analysis. Detection of G. duodenalis was carried out by direct microscopy. Vial samples were processed using the Parasitrap ® Concentration System (Biosepar GmbH, Germany), smears were prepared which were stained with 1% solution of Lyuloh. A direct fluorescent antibody test was used (5 µl of concentrated fecal material was placed on sterile subject glass, air dried, fixed with methanol, and stained with labeled fluorescein with murine monoclonal antibodies directed against Giardia cysts (Giardia Cel, Cellabs, Sydney, Australia) for confirmation of possible microscopy results, using positive and negative controls in each series of samples. From samples of feces, which gave positive for G. duodenalis in microscopy, new fresh aliquots were sent to the laboratory for further analysis of genotyping[26,27]. For comparative analysis, 2 groups were formed: group I – children with a positive test for G. duodenalis (n = 90); Group II – children with a negative test for G. duodenalis (n = 110). 488 children were excluded from the study for various reasons (refusal to participate in the study, travel outside the region, acute viral infections, etc.).

The detection of G. duodenalis in the stool specimens was initially accomplished by a real-time PCR[28]. Amplification and detection of parasitic DNA were performed on a Corbett Rotor-Gene 6000 qPCR cycler (Qiagen Corbett, Hilden, Germany). The Rotor

Gene 6000 Series software version 1.7 was used for data analysis. Fluorescence (510 nm) was measured at the end of the annealing step of each cycle. The ramping of the machine was 10 ° C/ s in each step. A semi-nested PCR protocol targeting a 432-bp fragment of the GDH gene was performed according to[29] and anested-PCR protocol targeting a 511-bp partial sequence of the BG gene as described by[30].

In these children, in addition to the above-mentioned methods of research, anthropometry was carried out [31,32], as well as the determination of fecal calprotectin (FC)[33].

Anthropometric measurements in children were performed twice. If two measurements of length differed > 0.5 cm, a third measurement was performed and the average value recorded. The length was measured according to the standard method using a stadiometer (accuracy of 0.1 cm). Feces for the FC detecting was collected in a plastic container, then frozen in an Eppendorf vial at –80C. The FC was measured using the ELISA kit for EK-CAL (Bühlmann Laboratories AG, Switzerland) according to the manufacturer's methodology.

Statistical analysis of the results (quantitative and qualitative analysis with the calculation of the average and relative values, identification of statistical significance by the $\chi 2$ criterion for absolute values as well as with the Fisher's angle transformation method $p\phi$ for relative values) was conducted with statistical modules such as Statistica v.6.0 and MedStat and on-line SISA calculator (Simple Interactive Statistical Analysis), using correlation and parametric analysis. Average values are given as (M±m), where M is the average value of the index, m is the standard error of the mean; n – the number of the experimental group.

Both parametric and nonparametric statistical methods were used depending on the normality of the distribution of the indices. The index values were presented as absolute and relative values and median. A confidence interval (CI) is set at 95%. A comparison of two independent samples was performed using Student's t-criterion for independent variables with their proper distribution. In the presence of incorrect distribution of the variables in the groups of comparison, the quantitative characteristics of the indices were calculated using the U-criterion Mann-Whitney and the N-criterion Kruskal-Wallis (for three or more groups). The differences between the values were considered reliable with the correlation coefficient $p < 0,05$.

All studies were conducted after the informed consent was signed by the children (aged over 6 years) and their parents. The work follows the ethical principles of the people who act as subjects of the study taking into account the main provisions of the ICH GCR and the Helsinki Declaration of the World Medical Association for Biomedical Research, where a person acts as their object (World Medical Association Declaration Helsinki 1964, 2000, 2008), The Council of Europe Convention on Human Rights and Biomedicine (2007).

Results

The analysis of socio-demographic indices of the examined children on the basis of sex (boys/girls), age (6-18 years), attendance of the organized educational establishments (kinder-garden, school, college), residence (city/village) (Table 2) was conducted.

Out of 688 children examined for G. duodenalis by the above listed methods, in 90 (13.1%) cases a positive result (G. duodenalis (+)) was detected. The age distribution of children from G. duodenalis (+) is presented in Figure 1. The highest rate of G. duodenalis (+) was recorded in children aged 6-10 years (52, 57.8%), in the age group of 11-14 years, the positive test was detected in 24 children (26.6%) and the lowest index was in children aged 15-18 years – 14,15.6%. The ratio between girls and boys is 0.78 and 1.28, respectively. Most often, G. duodenalis was diagnosed in children from rural areas (66 out of 90, 73.3%).

The leading clinical feature of G. duodenalis (+) was abdominal pain (100%), in second place, nausea and diarrhea (94.4%) and headache (88.8%) (Table 3).

The FC was described by Fagerhol et al in 1979[33], its content is stable in feces for 7 days at room temperature and it is used as a non-specific marker of intestinal inflammation. The mean FC index in the examined children was 32.5±6.4 mg/kg. The results of the study on the FC content in children with G.

Table 2. Socio-demographic indices of the examined children (years 2017-2018).

Index	N (n=688)	%
Sex		
Boys	386	56.1
Girls	302	43.9
Age (years)		
6-10	380	55.2
11-14	208	30.2
15-18	100	14.5
Territory of residence:		
City	210	30.5
Village	478	69.5
Organized establishment:		
Kindergarten	130	18.9
School	478	69.5
College	80	11.6

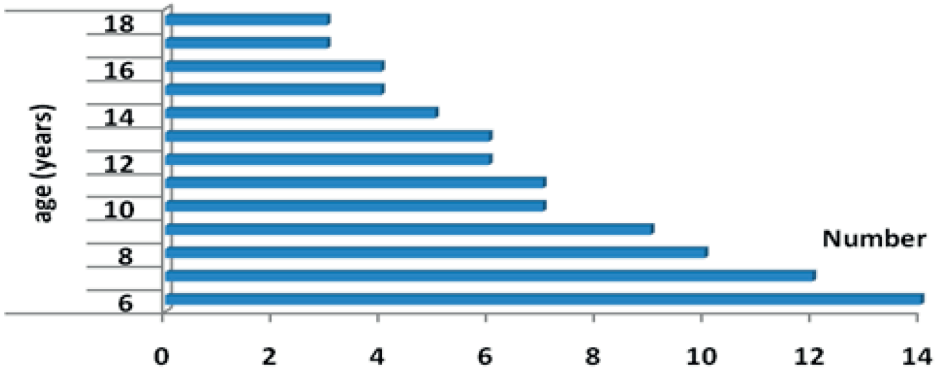

Figure 1. The age distribution of children with G. duodenalis (+).

Table 3. Frequency of clinical symptoms in the examined children.

Sign	Main group (children infected with G. duodenalis, n = 90)		Comparison group (children not infected with G. duodenalis, n = 110)	
	Number	%	Number	%
Abdominal pain	90	100*	60	54.5
Nausea	85	94.4*	10	9.1
Vomiting	21	23.3*	3	2.7
Intestinal dysbiosis	30	33.3*	10	9.1
Diarrhea	85	94.4*	12	10.1
Weight loss	20	22.2*	10	9.1
Low grade fever	19	21.1*	3	2.7
Skin rash	30	33.3*	7	6.3
Flatulence	25	27.7*	2	1.8
Alopecia	5	5.5*	0	0
Sleep disturbance	14	15.5*	2	1.8
Itching	15	16.6*	4	3.6
Jaundice	7	7.7*	0	0
Eosinophilia	24	26.6*	10	9.1
Lymphocytosis	17	18.8*	3	2.7
Neutropenia	9	10*	0	0
Headache	80	88.8*	30	27.3

Note. * – probability values at p <0.05.

Table 4. FC indices in the examined children, depending on gender.

Sex	Group I (n=90)			Group II (n=110)		
	Number, %	Average FC concentration, mg / kg [95DI]	p	Number, %	Average concentration of FC, mg / kg (95DI)	p_1
Boys	50 (55.5)	39.2 [30-41]	<0.05	55 (50)	27.4 [23-39]	>0.05
Girls	40 (44.5)	37.5 [31-38]	<0.05	55 (50)	27.9 [21-37]	>0.05

Note FC – fecal calprotectin; p – the reliability of the difference between group I and II; p1 – the reliability of the difference between boys and girls.

duodenalis (+) and in children G. duodenalis (-) are presented in Table 4. There were 50 boys (50 out of 90, 55.5 %) and 40 girls (40 out of 90, 44.5%) in group I, group II (G. duodenalis (-) included 55 boys, as well as 55 girls (55 out of 110, 50%).

The FC content in the feces of the group I was significantly higher (p <0.05) compared to parameters in children of group II and did not depend on sex. The highest levels of FC were registered in children aged 6-10 years (Figure 2). The FC level lower than

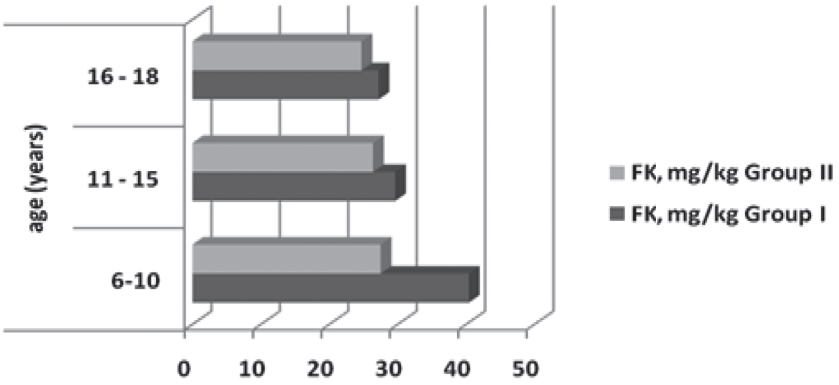

Figure 2. Mean indices of FC in the examined children, depending on age.

Table 5. Results of genotyping of clinical isolates G. duodenalis.

Assemblage	Sub-assem Blage	No. isolates	Locus	Reference sequence	Stretch	Single nucleotide polymorphism	Genbank accession no.
A	AII	3	CDH	L49510	88-470	T139C	KY499033
		2	CDH	L49510	88-470	T139C, T432C	KY499034
		6	CDH	L49510	88-470	A221C	KY499035
	BIII	2		AF069059	88-470	G306A, G309T, G315A, G336T	KY499036
	BIV	3		L40508	88-470	None	KY499037
		5		L40508	88-470	T183C, T290Y, C396T, C423T, N387C	KY499038
				L40508	88-470	T183C, T387C, C396T, C423T	KY499039
				L40508	88-470	T183C, T335C, T387C, C396T, C423C	KY499040
	AII	1	BG	AY972723	99-594	None	KY499041
		1	BG	AY972724	99-594	A186G	KY499042

Table 6. Indicators of growth in observed children (cm)

Age	N	Group I		N	Group II	
		Girls	Boys		Girls	Boys
		M ± m	M ± m		M ± m	M ± m
6	14	118 ± 3.21	119± 2.91	18	121 ± 2.83	122 ± 3.11
7	12	122.15 ± 4.25	123.15 ± 4.50	18	126.38 ± 3.16	125.42 ± 3.02
8	10	126.85 ± 4.29	127.29 ± 3.18	15	128.04 ± 2.63	128.67 ± 2.72
9	9	132.00 ± 4.62	133.12 ± 3.63	11	132.25 ± 3.39	134.59 ± 3.61
10	7	137.86 ± 3.81	136.91 ± 4.85	6	136.18 ± 3.54	140.36 ± 4.80
11	7	141.75 ± 4.21	141.05 ± 3.56	6	142.10 ± 4.23	144.39 ± 4.23
12	6	143.79 ± 5.16	146.47 ± 5.85	6	152.56 ± 4.15	148.71 ± 3.50
13	6	152.13 ± 7.29	151.86 ± 6.45	6	154.89 ± 5.48	154.60 ± 4.39
14	5	157.35 ± 5.51	154.13 ± 5.46	6	156.08 ± 5.85	167.94 ± 6.52
15	4	159.03 ± 4.28	166.44 ± 6.62	6	159.64 ± 4.79	169.18 ± 5.49
16	4	162.98 ± 4.33	169.82 ± 6.67	6	162.58 ± 8.95	172.63 ± 6.03
17	3	165. 08 ± 4.54	160.12 ± 5.37	6	166.78 ± 7.75	176.63 ± 5.93
18	3	167.98 ± 5.22	170.18 ± 5.33	6	169.58 ± 4.94	178.13 ± 7.13

50 mg/kg was determined in 70 out of 90 children (77.7%) and only in 20 of 90 children (22.3%) FC levels exceeded 50 mg/kg, but no index higher than 100 mg/kg was detected. In all children in group II, FC did not exceed the threshold of 50 mg/kg.

Table 5 shows the results of genotyping of G. duodenalis isolates, which are completely sub-genotyped in this study. The analysis of the sequences characterizing the amplicon of GDH revealed the presence of subgroups of AII (54%, 13/24), BIII (8.3%, 2/24) and BIV (37.5%, 9/24).

The analysis of the dynamics of the main anthropometric indices of children from 6 to 18 years showed a gradual uneven increase in height in boys (125.42 – 175.63 cm, n = 105) and in girls (122.15 – 167.98 cm, n = 95). The reliable difference in growth rates between children with G. duodenalis (+) and G. duodenalis (-) was not detected (Table 6).

However, the largest annual linear growth in group I is shifted for 1 year and 1 cm compared to children in group II (group I: for girls aged 12 to 13 years old +9 cm, for boys aged 14 to 15 years +12 cm and group II: in girls 11-12 years +10 cm, in boys 13-14 years +13 cm).

Discussion

Unlike most EU countries, in Ukraine giardiasis is mandatory to be reported. The prevalence of giardiasis in children in Ukraine is 0.051 per 100,000 population[34]. For comparison, Bulgaria registered 19.1 per 100,000 population and Belgium 17.7 per 100,000 population[35]. These data convincingly indicate that the actual number of giardiasis in Ukraine should be much higher than the available official data. With this assumption, we conducted a clinical trial (688 children) and a laboratory-genetic (90 children) study. Our studies have confirmed the data showing that the highest number of giardiasis is registered at junior school age (6-10 years)[36]. There is an evidence that giardiasis is often found in travelers returning from the endemic regions[37], but in our study, staying in these regions was one of the criteria for excluding from the study. As in other European countries. Microscopic examination remains the method of choice for the detection of G. duodenalis[38-39]. Most often, G. duodenalis was diagnosed in rural areas, which is confirmed by other researchers[40-41]. Among clinical symptoms, abdominal pain and diarrhea were the most frequent in our patients, as in other studies[42-44], but it is noteworthy that intestinal dysbiosis has been diagnosed in one-third of patients with giardiasis. Results of a molecular genetic study of isolates of G. duodenalis need to be analyzed, since such studies have been conducted in Ukraine for the first time on a small number of patients, though genotyping has been widely used in recent years[45-47].

Most recently, fecal calprotectin levels have also been found to be associated with persistent giardia and microscopic duodenal inflammation[48]. Our studies showed a link between the presence of G. duodenalis in feces, increased concentrations of FC and growth disturbances. This is consistent with literature data, which show the connection between intestinal pathogens and increased fecal markers[49-50].

Physical development is one of the integral indicators of the biological maturity of the body systems, since it determines, the course and consequences of many diseases, on one hand, and depends on health indicators on the other hand. Informativeness of the indicators of physical development is confirmed by high correlation with many functional and structural systems of the body and serves as one of the criteria for assessing the capacity to work[51, 52].

Limitations of the study

This study has several limitations. The study was conducted over a short period of time. The presence of G. duodenalis in the soil was not examined as well as the level of antibodies and the immunological status of children wasn't detected. It must be taken into account that human growth depends on many factors, such as diet, socio-economic status, and associated infections. Thus, an accurate estimation of the G. duodenalis infection effect on growth is a complex task due to the number of potential factors that were not taken into account in the assessment of the growth of children. A large-scale further research accruing to large numbers of patients is required.

Conclusions

The established socio-demographic factors can be considered as predictors of the development of giardiasis in children. In the clinical course of giardiasis, the digestive tract's disease dominates. A complex of direct and indirect methods of diagnosis is necessary to improve the accuracy of the diagnosis of giardiasis in children, Children with increased FC need further examination. This prospective study suggests that G. duodenalis infection is accompanied by the growth retardation in children, as well as by intestinal inflammation.

Compliance with Ethics Requirements:

„The authors declare no conflict of interest regarding this article"

„The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law. Informed consent was obtained from all the patients included in the study"

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