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FEATURES OF THE ALGORITHM OF CLINICAL AND LABORATORY DIAGNOSIS OF LYME BORRELIOSIS IN CHILDREN

The aim of the study – to improve the algorithm of clinical and laboratory diagnosis of Lyme borreliosis in children.

Materials and Methods. The search and analysis of available literature sources of the PubMed database is carried out, using a combination of keywords "Lyme disease in children", " Clinical and Laboratory diagnostic". A group of children (62) aged 1 to 17 years was observed to identify pathogens of haemotransmissible infections among children. C-reactive protein (CRP) was determined, which is a protein and reagent of the acute phase. To confirm the diagnosis of PM, determine the form of the lesion and identify the antigens of the pathogen, a routine two-step method of blood analysis was used.

Results and Discussion. The algorithm for the diagnosis of Lyme borreliosis in children has been developed for use in making a diagnosis. Clinical: complaints – malaise, fatigue, headache, arthralgia, myalgia, subfebrile body temperature; anamnesis – tick bites, location of bites, time of appearance of symptoms after the bite; objective – lymphadenopathy, arthritis, pain syndrome, intoxication syndrome; laboratory and instrumental diagnostics: biochemical blood analysis (increased level of C-reactive protein, rheumatoid factor is rarely detected), ultrasound of joints, immunological examination (immunofluorescence analysis and blot test), detection of Borrelia by PCR method in synovial fluid.

Conclusions. Proposed algorithm for diagnosing the Lyme disease in children.

Key words. Lyme disease; children; immunofluorescence analysis; ELISA test; blot test.

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ОСОБЛИВОСТІ АЛГОРИТМУ КЛІНІКО-ЛАБОРАТОРНОЇ ДІАГНОСТИКИ ЛАЙМ-БОРЕЛІОЗУ В ДІТЕЙ

Мета дослідження – удосконалити алгоритм клініко-лабораторної діагностики Лайм-бореліозу в дітей.

Матеріали та методи. Проведено пошук та аналіз доступних літературних джерел бази даних PubMed з використанням комбінації ключових слів «хвороба Лайма у дітей», «клініко-лабораторна діагностика». Обстежено 62 дитини віком від 1 до 17 років для виявлення збудників гемотрансмісивних інфекцій серед дітей. Визначали С-реактивний білок, який є показником гострої фази запального процесу. Для підтвердження діагнозу Лайм-бореліозу, визначення форми ураження та ідентифікації антигенів збудника, застосовували звичайний двохетапний метод дослідження крові.

Результати дослідження та їх обговорення. Розроблено алгоритм діагностики Лайм-бореліозу у дітей для використання при встановленні діагнозу. Клінічні скарги: нездужання, втома, головний біль, артралгії, міалгії, субфебрильна температура тіла; анамнез: укуси кліщів, локалізація укусів, час появи симптомів після укусу; об'єктивні – лімфаденопатія, артрит, больовий, інтоксикаційні синдроми; лабораторна та інструментальна діагностика: біохімічний аналіз крові (підвищений рівень С-реактивного білка, рідко визначається ревматоїдний фактор), УЗД суглобів, імунологічне обстеження (імунофлуоресцентний аналіз та блот-тест), виявлення борелій методом ПЛР в синовіальній рідині.

Висновки. Запропоновано алгоритм діагностики хвороби Лайма у дітей.

Ключові слова: Лайм-бореліоз; діти; імунофлуоресцентний аналіз; Еліса тест; блот-тест.

INTRODUCTION. Lyme disease(LD) or borreliosis is an infectious disease that is a vector-borne disease, i.e. transmitted by blood-sucking ticks. You can get infected not only from a bite, but also after crushing a tick on your skin. Lyme disease is caused by three species of Borrelia burgdorferi: Borrelia *afzelii*, B. *garinii* and B. burgdorferi *sensu stricto* and has different stages of course[1]. Clinical manifestations of LB are divided into three stages: early localised stage, characterised by erythema migrans (EM) at the site of tick bite, which is a rounded red spot that gradually expands centrifugally and reaches 15–20 cm in diameter. The early generalised stage can be represented by erythema multiforme (EM), borrelial lymphocytoma, neuroborreliosis, carditis or arthritis. Late generalised stages present with Lyme arthritis and neurological symptoms [2].

THE AIM OF THE STUDY – to improve the algorithm of clinical and laboratory diagnosis of Lyme borreliosis in children.

Lyme disease can progress through a series of phases and eventually damage the joints, heart, and nervous system. Lyme disease is usually treated with antibiotics, although resistance to them has been reported. Currently, the gold standard for detecting LD is serological diagnosis, based primarily on ELISA and Western blot tests [2].

However, in the initial phase of the infection, due to the so-called serological window period, great attention is paid to the study of the genetic profile, confirmed by molecular methods - polymerase chain reaction (PCR), which is more sensitive and specific [3].

In Ternopil region, 172 tick bites were reported in children in 2017, while only 34 (19 %) ticks were infected with the pathogens under study. *B. burgdorferi* s.l. was detected in 19 (55.9 %) ticks, *A. phagocytophilum* – in 12 (35.3 %).

In 2018, 376 tick bites in children were reported, and 128 (34 %) ticks were infected with vector-borne diseases.

Among the 128 infected ticks removed from children's skin in 2018, *B. burgdorferi* s.l. was detected in 54 (42.3 %) ticks, and *A. phagocytophilum* in 53 (41.4 %) [4].

MATERIALS AND METHODS. A group of children (62) aged 1 to 17 years was observed to identify pathogens of haemotransmissible infections among children. Children were divided into two groups: 52 children – those who had erythema migrans (EM) after a tick bite and 10 – those who had EM + Lyme arthritis. C-reactive protein (CRP) was determined, which is a protein and reagent of the acute phase, as well as an information base for the interpretation and analysis of clinical observations of CRP. According to the recommendations of the US Centers for Disease Control and Prevention (CDC), a routine two-step method of blood sample analysis was used to confirm the diagnosis of PM, determine the forms of lesions and identify pathogen antigens [5].

RESULTS AND DISCUSSION. The survey of 62 children revealed that respondents who remembered the fact of a tick bite more often reported tick attacks in the spring and summer months, mostly in May – 30 cases, June – 22 cases, and July – 10 cases. These months are likely to be the time of the tick breeding cycle and the peak time for people to spend outdoors. According to scientific observations, the skin of the lower extremities, buttocks, abdomen and head in children are the most common areas of tick bites [6]. Most often, the bites were localised in the head (19 – 30.6 %) and lower extremities (18 – 29.03 %), trunk (9 – 14.51 %), neck (8 – 12.90 %), upper extremities (2 – 3.22 %), abdomen (4 – 6.44 %), and earlobe (2 – 3.22 %) in the study group of children, which was confirmed by other studies [6, 7].

Some of the examined children (3 (4.84 %)) reported more than 3 tick bites, 33 (53.23 %) reported a single tick bite, 21 (33.87 %) reported a double6 bite, 3 or more bites occurred in 3 (4.84 %) cases. Two children (3.23 %) did not remember any tick bites. Children aged 3 to 11 years are the most frequently affected.

According to the anamnesis, from the moment of the bite, it lasted up to 12 hours in 23 – 37.10 % of children, up to 24 hours in 20–32.26 %, 24–48 hours and more in 19–30.64 % of people.

The children were examined in the dynamics – an increase in erythema migrans (EM) by several cm was observed during the day. In a few cases, EM was 30 cm in size In most cases (45 – 72.58 %), EM was asymptomatic, sometimes (17 – 27.42 %) there was itching of the skin at the site of the tick bite.

Distribution of infected ticks of the Ixodes ricinus species.

In the 62 children examined, 105 tick bites were recorded, of which only 20 (19.05%) were infected with pathogens. *B. burgdorferi* s.l. was detected in 11 (55.00 %) ticks, *A. phagocytophilum* in 8 (40.00 %) and *B. Miyamotoi* in 1 (5.00 %). The etiopathogenic agents were detected in different life stages of ticks, such as adults, nymphs and larvae, both separately and in combination. The following combined groups of pathogens were identified: *B. burgdorferi* s.l. with *A. phagocytophilum* and *B. miyamotoi* with *A. Phagocytophilum* [8, 9].

The cause of Lyme arthritis, which was observed in 10 (16.13 %) children, is the consequences of immunopathological autoimmune reactions manifested by the development of synovitis with characteristic morphological changes, including synovial cell hyperplasia, nonspecific hypertrophy of villi, lymphoplasmacytic cell infiltration, follicle formation, and fibrin deposition. It is known that Borrelia burgdorferi antigens remain in the cartilage for a long period after etiological therapy and contribute to the stimulation of tumour necrosis factor alpha synthesis from macrophages, which leads to chronicity of the process in the joints [8]. In children with Lyme arthritis, its torpid course was observed. Often, the leading clinical sign of Lyme arthritis is monoarthritis (8 – 12.90 %), and most often (90.0 %) the knee joint is involved in the pathological process. Less commonly, the elbow, ankle, hip, and wrist joints are affected (2 – 3.23 %). All patients with Lyme arthritis have moderate synovitis; concomitant tendonitis, tendosynovitis, bursitis, enthesopathy, fibrositis; muscle involvement [9]. In 8 (12.90 %) children with arthritis and erythema migrans, an increase in CRP levels (6.8±0.6) mg/l was noted. We did not observe normal CRP levels in children with Lyme disease who did not receive antibiotic therapy [10, 11].

Laboratory tests were carried out for the purpose of differential diagnosis of arthritis. Patients underwent examination and necessary clinical and laboratory tests, including ultrasound examination of joints, magnetic resonance imaging, rheumatology tests (CRP, ASLO), daily monitoring of electrocardiogram, echocardiography.

Table 1. **Presence of borrelia antigens depending on disease manifestations (immunoblot method)**

	IgM				IgG						
Indicator. (n/ %)	P41 (n/ %)	OspC Ba (*B. afzelii)* (n/ %)	OspC Bb *(B. burgdorferi)*	OspC Bg (*B. garinii)* (n/ %)	VLsE (*B. afzelii)* (n/ %)	VLsE (*B. burgdorferri*) (n/ %)	VLsE (*B. garinii*) (n/ %)	Lipid Ba (*B. afzelii)*	Lipid Bb (*B. burgdorferri*)	OspC (*B. afzelii*) (n/ %)	P41 (n/ %)
Arthritis P* n=10/16.13	-	2/ 3.22	-	1/ 1.61	2/ 3.22	2/ 3.22	1/ 1,61	-	1/ 1.61	1/ 1.61	-
Arthritis H* n=10/16.13	5/ 8.06	2/ 3.22	1/ 1.61	2/ 3.22	3/ 4.84	3/ 4.84	2/ 3.22	1/ 1.61	1/ 1.61	6/ 9.68	7/ 11.29
EM P* 34/54.83	12/ 19.35	4/ 6.45	3/ 4.84	4/ 6.45	-	4/ 6.45	2/ 3.22	-	-	7/ 11.29	1/ 1.61
EM H* 20/32.26	7/ 11.29	6/ 9.68	3/ 4.84	5/ 8.06	10/ 16.13	10/ 16.13	8/ 12.90	2/ 3.22	-	8/ 12.	6/ 9.68

Notes: H – high, P – intermediate results.

Table 2. **Clinical and laboratory changes in children with Lyme disease**

Clinical and laboratory changes	Number of children with Lyme borreliosis n= 62(100%)			
	EM n=52 (83.87%)		EM+ Lyme arthritis n-10(16.13%)	
	абс	%	абс	%
Fever	17	27,42*	5	50
Headache	36	58.06	4	40
Muscle pain	8	12.90*	4	40
Joint pain	11	17.74*	10	100
Inflammatory changes (hyperaemia, swelling)	52	83.87	10	100
Itchy skin	17	27.42	4	40
Burning sensation in the soles of the feet	0	0	1	10
Limping	0	0	3	30*
Absence of symptoms	45	72.58	0	0
CRP, <5 мg/l	26	41.93	10	16,3
	5.2±0.5*		6.9±0.52	
White blood cells4.9x10^9/л	6.5±0.7		8.7± 0.43	

Note: *– significance of the difference in children with EM and EM+Lyme arthritis, p<0.05

The algorithm for diagnosing Lyme arthritis in children used in the diagnosis:

I. 1. Complaints (malaise, fatigue, headache, arthralgia, myalgia, subfebrile body temperature and lymphadenopathy).

2. Medical history (tick bite, erythema migrans).

3. Clinical manifestations:

- arthritis syndrome: mono- or oligoarticular arthritis, difficulty walking (claudication);
- pain syndrome: arthralgia, myalgia, headache;
- intoxication syndrome: fever, itching of the skin;
- asthenovegetative: anxiety, disturbance of night sleep.

II. Laboratory and instrumental diagnostics:

1. Complete blood count (elevated ESR).

2. Biochemical blood test (increased level of C - reactive protein, rheumatoid factor is rarely determined).

3. Ultrasound of the joints: thickening of the synovial membrane, increased fluid, changes in periarticular tissues, tendonitis, enteritis, thickening and swelling of the muscles are found.

4. Immunological examination. A highly sensitive ELISA test should be used as a screening test and in case of a positive or doubtful result should be confirmed by an immunoblotting test. IgM antibodies to *Borrelia* begin to rise 2-4 weeks after infection, peaking within 6-8 weeks, and then immunoglobulin production switches to IgG. High levels of IgM and IgG may persist in some patients for many years even after treatment.

5. Detection of *Borrelia* by PCR in synovial fluid.

CONCLUSIONS: 1. Tick infestations in children were most common in May

2. A study of some patterns of tick infestation in children aged 1 month to 17 years inclusive showed that the most frequently affected individuals were children aged 3 to 11 years.

3. Anamnestic exposure to ticks infected with B. *burgdorferi* and the presence of erythema migrans are sufficient to establish the diagnosis of Lyme disease and initiate treatment.

4. The types of Lyme borreliosis pathogens are determined during laboratory diagnosis of the disease by PCR.

5. The laboratory diagnosis of Lyme borreliosis is based on the identification of the pathogen itself (bacterioscopy and bacteriological diagnostic methods) or its DNA and the detection of specific blood antibodies (confirmation by immunoblot).

6. Proposed algorithm for diagnosing the Lyme diseasein children.

PROSPECTS FOR FURTHER RESEARCH. A comprehensive study and development of therapeutic and diagnostic algorithms for Lyme disease with the aim of its timely therapy and prevention of complications is promising.

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