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C-REACTIVE PROTEIN AS A BIOCHEMICAL CRITERION OF A GENERAL INFLAMMATORY SYNDROME IN LYME DISEASE IN CHILDREN

The aim of the study – to improve the methods of non-specific biochemical markers of Lyme disease.

Materials and Methods. A group of children (62) aged from 1 to 14 years was observed for the identification of pathogens of blood-borne infections among children. C-reactive protein (CRP) is a protein and acute phase reagent as well as the information base for the interpretation and analysis of clinical observations of CRP.

Results and Discussion. There were studied the concentrations of acute-phase response proteins including CRP in children with Lyme disease. We found that circulating of CRP levels as well as concentrations > 3 mg/ml was significantly higher in the group of children with Lyme arthritis cohort than in the control group ($P < 0.05$).

Conclusions. C-reactive protein concentration is a very useful but non-specific biochemical marker of inflammation, the measurement of which makes an important contribution to the screening of Lyme borreliosis in children.

Key words: Lyme disease; children; clinical symptom; C-reactive protein; inflammation.

С-РЕАКТИВНИЙ БІЛОК ЯК БІОХІМІЧНИЙ КРИТЕРІЙ ЗАГАЛЬНОГО ЗАПАЛЬНОГО СИНДРОМУ ПРИ ХВОРОБІ ЛАЙМА У ДІТЕЙ

Мета дослідження – вдосконалення методів визначення неспецифічних біохімічних маркерів хвороби Лайма.

Матеріали та методи. Для підтвердження Лайм-бореліозу проведено двохетапну діагностику. Вивчали неспецифічні маркери запалення – С-реактивний білок серед дітей із хворобою Лайма. Під спостереженням перебувала група дітей (42) віком від 1 до 14 років.

Результати дослідження та їх обговорення. Вивчали концентрації білків гострофазової відповіді, включно С-реактивний білок, у дітей із хворобою Лайма. Виявлено, що рівні С-реактивного білка, (концентрації >3 мг/мл) були значно вищими у групі дітей із когортою Лайм-артриту, ніж у контрольній групі ($P < 0,05$).

Висновки. Концентрація С-реактивного білка є корисним, але неспецифічним біохімічним маркером запалення, визначення якого полегшує скринінг Лайм-бореліозу в дітей.

Ключові слова: хвороба Лайма; діти; клінічна симптоматика; С-реактивний білок; запалення.

INTRODUCTION

Lyme borreliosis (LB) is a multisystem infectious disease caused by *Borrelia burgdorferi sensu lato* and transmitted to humans by a tick bite. The reported estimated global *Borrelia burgdorferi* seropositivity is relatively high with the top three regions: Central Europe, Western Europe and Eastern Asia. Using the western blotting (WB) to confirm *Borrelia burgdorferi* serological results could significantly improve the accuracy [1]. More studies are needed to improve the accuracy of global Lyme borreliosis burden estimates. Lyme borreliosis has become more common among children. Improving the methods of non-specific biochemical markers of Lyme disease is especially important [1]. C-reactive protein (CRP) is an acute inflammatory protein that increases up to 1,000-fold at sites of infection or inflammation [2]. C-reactive protein is produced as a homopentameric protein, termed native CRP (nCRP), which can irreversibly dissociate at sites of inflammation and infection into five separate monomers, termed monomeric CRP (mCRP) [2].

MATERIALS AND METHODS

There were 42 children with a tick bite in study group and 20 children in the control group – residents of Ternopil region, who applied to Ternopil Regional Children Hospital, 2019–2021, whose aged 8–17 years.

The group of investigated children had clinical symptoms of Lyme borreliosis and antibodies to specific proteins of *B. burgdorferi* antigen. The diagnosis of ring-shaped EM was established clinically and based on characteristic

manifestations and epidemiological history. Lyme borreliosis diagnoses were confirmed according to the classification by Y. V. Lobzyn, 2000 [3], Diagnostic criteria Serologic Diagnostic Criteria recommended by the Second National Conference on Serologic Diagnosis of Lyme Disease (1994) [4].

The data was interpreted according to the WHO guidelines: Guidance Lyme disease: diagnosis and treatment [5].

All patients were divided into the following groups. The first group: EM+arthritis (n=16), Second group – EM (n=26).

Eight children were investigated during two phases. During the investigation it was defined the CRP, level of leucocytes taking into consideration the general blood analysis with the next definition of the correlation between them. The definition of CRP level in children with Lyme disease after tick's bite and after the treatment was investigated in the first group. The correlation comparison of CRP level with the level of leucocytes in the general blood analysis was made.

Blood samples were collected twice: before antibiotic therapy (sample 1) and after the treatment (sample 2) in about 14 days. Standard two-stage tests based on indirect fluorescent antibody (IFA) assay and immunoblotting currently serve as the primary supports for the laboratory diagnosis of LB and assessment of the disease development. The objective of this study was to identify blood proteins with altered abundances in patients with early stage Lyme disease as compared to healthy controls. Such proteins are candidate biomarkers requiring further validation that may

ultimately lead to the development of improved diagnostic tests for early Lyme disease especially in patients with a negative antibody-based serologic test.

Treatment of patients with an erythematous form of LB was performed according to the following schemes: patients received Doxycycline, tablets 100 mg, 10 pcs. 2 times/day after meals during 14 days at a dose 4 mg/kg.

The effectiveness of the treatment was assessed by the dynamics of clinical manifestations of the disease and the level of the concentrations of acute-phase response proteins including C-reactive protein (CRP) in children with Lyme disease. CRP was determined by turbidimetric method with latex reinforcement.

Less than 1 (mg/l) is a low result, from 1 to 3 (mg/l) is average result or risk, more than 3 (mg/l) is a high result.

Statistical analysis of the results was performed using the methods of parametric and nonparametric statistics "Microsoft Office Excel" and "STATISTICA". The study was conducted within the framework of the scientific research "Research on epidemiology, pathogenesis, clinics and prevention of Borreliosis", which is a part of a joint Ukrainian-Polish project under the auspices of the European Union. There were the relevant accession numbers. The tests were performed in the Laboratory of the Center for the Study of Lyme Borreliosis and other tick's infections.

Institutional Review Board Statement: "The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board (or Ethics Committee) of I. Horbachevsky Ternopil National Medical University. The Conclusion of the Commission on Bioethics of I. Horbachevsky Ternopil National Medical University, Ministry of Health of Ukraine, dated on the 1 st of November, 2022 (protocol No.66).

RESULTS AND DISCUSSION

Inclusion criteria: clinical, laboratory and instrumental signs of Lyme disease. There were no significant age and sex differences between the groups in this study.

The definition of CRP and leucocytes was made twice before the treatment and after the treatment.

The mean interval between tick suction and clinical symptoms was 12–14 days.

EM was observed in 14 patients within 24 hours, it was 31.9 % of all patients. After 24–48 hours, the appearance of EM was observed in 13 patients (29.5 %); on the 3rd day it was observed in 2 children (4.5 %); after a few months – in 1 patient (2.3 %).

Patients in the first group had arthritis syndrome, which occurred in one month after a tick bite with manifestations of EM. The children complained for persistent pain, oligoarthritis

and myalgia. The main symptoms of 12 children of the second group were: subfebrile body temperature, redness of the skin and general intoxication. Nonspecific intoxication syndrome of fatigue and myalgia was observed in 10% of patients without erythematous forms of the disease.

In the first group of the investigation it was revealed a statistic true increase of the level of CRP (Table 1).

Children with Lyme disease who had not yet received antibacterial therapy did not have normal CRP level at admission. In children of the first group, whose disease was accompanied by arthritis and EM (migration erythema), the level of CRP was higher than the CRP indicator in which Lyme disease proceeded with EM and was (6.8±0.6) mg/l and (5.2±0.5) mg/l respectively, which did not make a significant difference of 0.05. In 5 children (11.9 %) from the first group and 3 children (7.2 %), the CRP level showed high indicators.

In children with Lyme disease, which did not have antibiotic therapy, there were no normal CRP levels. In children from the first group with Lyme disease and arthritis and ME, the level of CRP was higher than the level of CRP and in those children, which had Lyme disease and EM it was (6.8±0.6) mg/l and (5.2±0.5) mg/l correspondingly that showed no significant difference, it was 0.05. In 5 children (11.9 %) of the first group and 3 children (7.25 %) the CRP level was of a high indication.

In the 1st group of children, there were 6 examined (14.3 %), the CRP level remained at high levels after the treatment. In 10 examinees (23.8 %), the average level of CRP was (2.2±0.4) mg/l, which corresponded to the average level of the protein index. In the 2nd group of the examined children, whose disease occurred only with erythema migrans, the average CRP level was (3.0±0.3), which was not significantly different from the indicator in the first group of the sick children (EM+Arthritis) more than 0.05.

High levels of CRP after the treatment were noticed in 10 children of the second group (23.8 %) and were (3.8±0.4). In the rest children, 16 (38.1 %) were kept at the level of medium risk and increased to (2.2±0.2).

In both groups of children, after the treatment, normalization of CRP was noticed in almost the same number of children from the first group, 4 (9.5 %) and from the second group, 3 (7.1 %). In 8 children, 5 (11.9 %) from the first group and 3 (7.1 %) from the second group, CRP ranged from 0.5 to 0.9 mg/l, which corresponded to a low level of the indicator.

High indications of CRP were observed after the treatment in 10 children of the second group (23.8 %) and it was (3.8±0.4). In the rest of children 16 (38.1 %) was on the average level and it was (2.2±0.2).

Table1. Dynamics of the CRP changes like markers of inflammation in Lyme disease in children.

Normal index	Examined children			
	1 group EM+arthritis (n=16)		2 group EM (n=26)	
	before treatment	after treatment	before treatment	after treatment
CRP (mg/l)<0.5	6.8±0.6*	3.3*±0.4	5.2±0.5	3.0±0.3
Leukocytes 4.9x10 ⁹ /l	9.2±1.3	6.8±0.6	6.5±0.7	6.3±0.9

Notes*p<0.5

In both groups of children after the treatment, the normalization of CRP was admitted almost in equal quantity of children from the first group 4 children (9.5 %), from the second group 3 children (7.1 %). Among 8 children 5 of them (11.9 %) from the first group and 3 (7.1 %) from the second group, the CRP level of which was from 0.5 to 0.9, what was correspondent to a low level of its indication.

The number of leukocytes in patients of both groups before the treatment and after the treatment was normal. The comparative analysis between the children of both groups did not show a significant difference.

Therefore, in all patients with Lyme disease, an increase in CRP was observed before antibacterial treatment, the high level of which was noticed in almost 5 percent of children (19.1 %). After the treatment, the maximum results were maintained in those patients who had elevated results at the time of admission and were ((11.2±6.4) mg/l) and normalization of the results was not observed.

2. Second item; clinical manifestations of leukocytosis are increased fatigue and malaise for no apparent reason, increased body temperature more than 37.5 °C, which occurred in the examined group of patients.

Clinical manifestations of leukocytosis include increased fatigue and malaise for no apparent reason, increased body temperature in 38.0 %, which occurred in the examined group of patients.

The level of leucocytes was increased in 5 % of the examined children. In 95 % of children the level of peripheral leucocytes was within a referent norm.

It is known that Lyme disease is a polysystemic disease with a polymorphic clinical picture, we tried in the first phase of the research to establish criteria for assessing the disease [2, 3]. Inclusion criteria were as follows: -epidemiological (living in an endemic area), clinical complaints of patients (erythematous skin lesions, Lyme arthritis), infectious diagnosis confirmation: 2-stage research scheme, laboratory serological detection methods with the following standard laboratory tests for identifying Bb including conventional serological methods such as ELISA, IFA test, protein biochemical test, line blot and western blotting (WB) [4, 5]. Acute-phase proteins that are part of the innate immune system and can respond within hours of bacteria entering the skin include CRP, complement factors [6].

Nowadays, approximately CRP is a highly sensitive marker of infection and inflammation that binds to various ligands present on the surface of pathogens or exposed to stress or autologous cell death by acting through opsonin deposition and complement pathway activation, in addition to direct interaction with phagocytic cells [6].

CRP as a marker of inflammation shows high serum levels in invasive bacterial infections [7]. There are scientific studies of the potential for measuring CRP in different phases of acute bacterial meningitis in children to predict the results [8]. Thus, Peltola and Heikki [9] in their study determined the initial values of CRP in 669 children. There were obtained useful prognostic information, CRP measurements on the 3rd or 4th day carried out in 275 children with pathology of

the nervous system. Hearing impairment although not total deafness was found to be 3–7 times more likely if CRP was above the median level shortly after hospitalization.

So Elsammak, Mohamed [12] studied serum procalcitonin (PCT) and C-reactive protein (CRP) in children with streptococcal tonsillopharyngitis or non-streptococcal tonsillopharyngitis which was similar to healthy children. PCT had greater specificity than CRP for the detection of bacterial tonsillopharyngitis. Differentiating between bacterial and non-bacterial conditions is very difficult. On the example of differential diagnosis of viral pneumonia and bacterial community-acquired pneumonia in children [10] in the works of Robert G. Badik, 2008, evaluated serum concentrations of C-reactive protein (CRP) as a predictor of bacterial pneumonia [13].

It should be noted that Yong Zhou, Shizhen Qin (2019) [14] in a longitudinal cohort of 40 Lyme disease patients and 20 healthy controls, identified 10 proteins with significantly altered serum levels in patients at the time of diagnosis. In an independent cohort of patients with erythema migrans, six of these proteins, APOA4, C9, CRP, CST6, PGLYRP2 and S100A9, were confirmed to show significantly altered serum levels in patients at time of presentation. According to (Elrod, Julia, 2022) [15], the most important predictors of a long-term increased frequency of infectious complications are an increased preoperative level of C-reactive protein, a high intraoperative assessment of severity, and insufficient primary antibiotic treatment [16].

We examined the relationship between baseline peripheral white blood cell count and C-reactive protein (CRP) values with outcomes in 42 children. A highly sensitive method of determining CRP established that the initial level of peripheral leukocytes corresponded to normal limits, while the level of CRP ranged from the normal reference values to (7.2 mg/l).

The same data were obtained by researchers Derek and Williams, 2015, [10] who studied the relationship between baseline peripheral leukocyte and C-reactive protein (CRP) values with outcomes among 153 children hospitalized with pneumonia.

To our opinion the CRP concentration is thus a very useful nonspecific biochemical marker of inflammation, measurement of which contributes importantly to:

- a) screening for organic disease;
- b) monitoring of the response to treatment of inflammation and infection;
- c) detection of intercurrent infection in immunocompromised individuals, and in the few specific diseases characterized by modest or absent acute-phase responses.

CRP is a biochemical criterion of a general inflammatory syndrome in Lyme disease in children.

CONCLUSIONS.

1. Nowadays, the markers of inflammation in Lyme disease in children have not been sufficiently investigated.

2. Taking into consideration that the level of C-reactive protein fluctuates within (up to 3.0), it shows that this is a marker of high bacterial load in Lyme disease in children.

LITERATURE

1. Global seroprevalence and sociodemographic characteristics of *Borrelia burgdorferi* sensu lato in human populations: a systematic review and metaanalysis / Y. Dong, G. Zhou, W. Cao [et al.] // *BMJ Global Health*. – 2022. – Vol. 7. e007744. DOI:10.1136/bmjgh-2021-007744.
2. Nykytyuk S. O. Lyme borreliosis in children / S. O. Nykytyuk, S. I. Klymniuk // *Achievements of Clin. and Experim. Med.* – 2020. – Vol. 1. – P. 14–25.
3. Лобзин Ю. В. Лайм-боррелиоз (иксодовие клещевие боррелии) / Ю. В. Лобзин, А. П. Усков, С. С. Козлов. – СПб : Фолиант, 2000. – 156. с.
4. Centers for Disease Control and Prevention. Recommendations for test performance and interpretation from the Second National Conference on Serologic Diagnosis of Lyme Disease // *MMWR Morb. Mortal Wkly Rep.* – 1995. – Vol. 44. – P. 590–591.
5. Information on laboratory diagnosis and treatment for Lyme disease. – From: Public Health England.
6. Sproston N. R. Role of C-reactive protein at sites of inflammation and infection / N. R. Sproston, J. J. Ashworth // *Front Immunol.* – 2018. – Vol. 9. – P. 754. DOI: 10.3389/fimmu.2018.00754. PMID: 29706967; PMCID: PMC5908901.
7. Kozlovska A. Lyme disease: clinical picture, diagnosis and treatment / A. Kozlovska // *Ukrainian medical journal*. [Electronic resource]. Available from: <https://www.umj.com.ua/article/158253/hvoroba-lajma-klinichna-kartina-diagnostika-ta-likuvannya>.
8. Selective apheresis of C-reactive protein for treatment of indications with elevated CRP concentrations / S. Kayser, P. Brunner, K. Althaus [et al.] // *Journal of Clinical Medicine*. – 2020. – Vol. 9 (12). – P. 2947. DOI:10.3390/jcm9092947, PMID 32932587, PMC 7564224 (free full text) – (Online [accessed 29 March, 2021]).
9. Heikki P. Predicting outcome of childhood bacterial meningitis with a single measurement of C-reactive protein / P. Heikki, P. Tuula, R. Irmeli // *The Pediatric Infectious Disease Journal*. – 2016. – Vol. 35 (6). – P. 617–621.
10. Association of white blood cell count and C-reactive protein with outcomes in children hospitalized for community-acquired pneumonia / D. J. Williams, M. Hall, K. A. Auger [et al.] // *The Pediatric Infectious Disease Journal*. – 2015. – Vol. 34 (7). – P. 792–793.
11. Prat C. Procalcitonin, C-reactive protein and leukocyte count in children with lower respiratory tract infection / C. Prat, J. Domínguez, C. Rodrigo // *The Pediatric Infectious Disease Journal*. – 2003. – Vol. 22 (11). – P. 963–967.
12. Elsammak M. Diagnostic value of serum procalcitonin and c-reactive protein in Egyptian children with streptococcal tonsillopharyngitis / M. Elsammak, H. Hanna, A. Ghazal // *The Pediatric Infectious Disease Journal*. – 2006. – Vol. 25(2). P. 174–176.
13. Robert G. The utility of serum C-reactive protein in differentiating bacterial from nonbacterial pneumonia in children: A meta-analysis of 1230 children flood / G. Robert, J. Badik, S. C. Aronoff // *The Pediatric Infectious Disease Journal*. – 2008. – Vol. 27 (2). – P. 95–99.
14. Measurement of organ-specific and acute-phase blood protein levels in early Lyme disease / Yong Zhou, Shizhen Qin, Mingjuan S Yong Zhou [et al.] // *Journal of Proteome Research*. Inst for systems biology. – 2019. – Vol 11 (16). – P. 22–27 (UTC).
15. Elrod J. Are we hitting the target? The effectiveness of distinct antibiotic regimens in pediatric appendectomies / J. Elrod, F. Yarmal, Ch. Mohr // *The Pediatric Infectious Disease Journal*. – 2022. – Vol. 41 (6). – P. 460–465.
16. Pepys M. B. Acute phase proteins with special reference to C-reactive protein and related proteins (pentaxins) and serum amyloid A protein / M. B. Pepys, M. L. Baltz // *Adv. Immunol.* – 1983. – Vol. 34. – P. 141–212. [PubMed] [Google Scholar]

REFERENCES

1. Dong, Y., Zhou, G., & Cao, W. (2022). Global seroprevalence and sociodemographic characteristics of *Borrelia burgdorferi* sensu lato in human populations: a systematic review and metaanalysis. *BMJ Global Health*, 7, e007744. DOI:10.1136/bmjgh-2021-007744.
2. Nykytiuk, S.O., & Klymniuk, S.I. (2020). Lyme borreliosis in children. *Achievements of Clin. and Experim. Med.*, 1, 14-25.
3. Lobzyn, Yu.V., Uskov, A.P., & Kozlov, S.S. (2000). *Laim-borrelioz (iksodovye kleshchevye borrelilzy) [Lyme borreliosis (ixodid tick-borne Borrelia)]*. SPb: Foliant [in Russian].
4. (1995).Centers for Disease Control and Prevention. Recommendations for test performance and interpretation from the Second National Conference on Serologic Diagnosis of Lyme Disease. *Morb. Mortal. Wkly Rep.*, 44, 590-591.
5. Information on laboratory diagnosis and treatment for Lyme disease. From: Public Health England.
6. Sproston, N.R., & Ashworth, J.J. (2018). Role of C-reactive protein at sites of inflammation and infection. *Front. Immunol*, 9, 754. DOI: 10.3389/fimmu.2018.00754. PMID: 29706967; PMCID: PMC5908901.
7. Kozlovska A. Lyme disease: clinical picture, diagnosis and treatment. *Ukrainian Medical Journal*. [Electronic resource]. Available from: <https://www.umj.com.ua/article/158253/hvoroba-lajma-klinichna-kartina-diagnostika-ta-likuvannya>. Ukrainian.
8. Stefan Kayser, Patrizia Brunner, Katharina Althaus, Johannes Dorst, & Ahmed Sheriff (2020). Selective apheresis of C-reactive protein for treatment of indications with elevated CRP concentrations. *Journal of Clinical Medicine*, 9 (12). 2947 DOI:10.3390/jcm9092947, PMID 32932587, PMC 7564224 (free full text) – (Online [accessed 29 March, 2021]).
9. Peltola, Heikki; Pelkonen, Tuula; Roine (2016). Irmeli. Predicting outcome of childhood bacterial meningitis with a single measurement of C-reactive protein. *The Pediatric Infectious Disease Journal*. 35 (6), 617-621.
10. Williams, D.J., Hall, M., & Auger, K.A. (2015). Association of white blood cell count and C-reactive protein with outcomes in children hospitalized for community-acquired pneumonia. *The Pediatric Infectious Disease Journal*, 34 (7), 792-793. DOI: 10.1097/INF.0000000000000724
11. Prat, C., Domínguez, J., & Rodrigo, C. (2003). Procalcitonin, C-reactive protein and leukocyte count in children with lower respiratory tract infection. *The Pediatric Infectious Disease Journal*, 22 (11), 963-967. DOI: 10.1097/01.inf.0000095197.72976.4f

12. Elsammak, M., Hanna, H., & Ghazal, A. (2006). Diagnostic value of serum procalcitonin and c-reactive protein in Egyptian children with streptococcal tonsillopharyngitis. *The Pediatric Infectious Disease Journal*, 25 (2), 174-176.

DOI: 10.1097/01.inf.0000199273.37314.b2

13. Robert, G., Badik, J., Aronoff, S.C. (2008). The utility of serum C-reactive protein in differentiating bacterial from nonbacterial pneumonia in children: A meta-analysis of 1230 children flood. *The Pediatric Infectious Disease Journal*, 27 (2), 95-99. DOI: 10.1097/INF.0b013e318157aced

14. Yong Zhou, Shizhen Qin, Mingjuan S Yong Zhou, Shizhen Qin, Mingjuan Sun, & Li Tania (2019). Measurement of Organ-

Specific and Acute-Phase Blood Protein Levels in Early Lyme Disease. *Journal of Proteome Research*. 4, (16), 22-27 (UTC).

15. Elrod, Ju., Yarmal, F., & Mohr, Ch. (2022). Are we hitting the target? The effectiveness of distinct antibiotic regimens in pediatric appendectomies. *The Pediatric Infectious Disease Journal*, 41 (6), 460-465.

16. Pepys, M.B., & Baltz, M.L. (1983). Acute phase proteins with special reference to C-reactive protein and related proteins (pentaxins) and serum amyloid A protein. *Adv. Immunol.*, 34, 141-212. [PubMed] [Google Scholar]

DOI: 10.1016/s0065-2776(08)60379-x

Received 19.01.2022

Accepted 20.01.2022

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