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10.2478/AMB-2025-0034

CASE SERIES

CLOSTRIDIUM DIFFICILE INFECTION IN CHILDHOOD: CASE SERIES

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Abstract. *Clostridioides (formerly Clostridium) difficile is a spore-forming, anaerobic, toxin-producing gram-positive bacillus. Recently, its frequency as a cause of diarrhea in childhood has been increasing, which necessitates its further in-depth study.* ***Objective:*** *To analyze the risk factors for morbidity, clinical course and outcome in children with C. difficile infection (CDI). A series of six cases of CDI in children aged between 10 months and 11 years, hospitalized in the Clinic for Infectious Diseases at University hospital, Stara Zagora, is presented. The main clinical manifestation of the disease is a diarrheal syndrome. All of the patients underwent clinical, epidemiological, laboratory, microbiological and serological tests.* ***Conclusion:*** *The age group in our study is different from the usual one for CDI. With the exception of one, all the remaining patients lacked predisposing factors for CDI. Rapid diagnosis and initiation of adequate etiological therapy improves the condition of patients and leads to a favorable outcome.*

Key words: *childhood, clinical course, Cl. Difficile infection (CDI)*

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Received: *15 October 2024;* ***Revised/Accepted:*** *22 November 2024*

INTRODUCTION

Clostridioides (formerly Clostridium) difficile is an anaerobic gram-positive bacillus that forms spores and produces toxins. It was first described in 1935, when it was found in the feces of healthy newborn babies [1]. It was named Bacillus difficile because it was difficult to isolate. Over time, this pathogen has also proven to be a challenge in terms of treatment and control [2].

C. difficile is non-invasive. Its virulence is mainly due to enzymes such as collagenase, hyaluronidase, chondroitin sulfatase, as well as toxins A and B, which damage the cytoskeleton of epithelial cells. Glutamate dehydrogenase (GDH) is an enzyme that is produced in large quantities by C. difficile. For this reason, it can be used to diagnose it [3, 4]. There is also a third binary toxin – CDT, which is produced by certain strains of C. difficile, e.g., 027 ribotype. Its role is still being clarified. The result of toxin activity in the gut is a pathological increase in fluid and electrolyte secretion, as well as interstitial mucosal inflammation.

C. difficile may be considered a component of the normal microbiota, but under specific conditions, which disrupt its balance, it can colonize the colonic mucosa, causing varying degrees of damage ranging from mild diarrhea to toxic megacolon [5, 6].

Colonization with a toxigenic strain of C. difficile was lower than with non-toxigenic strains, peaking at 21% in infants aged 6 to 12 months. For children over 5 years of age, it is reduced to 6% [7].

Until the Covid-19 pandemic, C. difficile infection (CDI) was mainly demonstrated as a nosocomial infection. Prolonged and aggressive antibacterial therapy during the course of SARS-CoV-2 infection led to a rapid increase in the incidence of antibiotic-associated colitis. The use of proton pump inhibitors also has a synergistic effect [8, 9]. Risk factors for the occurrence of CDI are associated with weakened immunity based on advanced age, polymorbidity, tube feeding, previous hospital stay, and contact with a person infected with C. difficile, as well as long-term antibiotic therapy. Cases of CDI have been described in people with chronic diseases, transplants and cancer patients. Traditional risk factors cannot always be identified [10]. Data on the increase in cases of C. difficile infection in recent years are alarming. The incidence of CDI is approximately 20 per 100,000 children [11]. It is lowest in newborns (0.5%), gradually increases to 32.01% in the age up to 1 year and reaches 44.87% in children from 1 to 4 years of age [12]. While the germ is frequently carried by infants, it does not usually cause diarrhea in this population. It is not entirely clear why CDI is also seen in childhood [13].

Most studies indicate an increased incidence of CDI in children in the community as opposed to nosocomially acquired [14, 15]. However, infection with virulent strains of Cl. difficile type 1 (NAP1) in childhood is 19.4%, while in adults it exceeds 50% [16]. In practice, laboratory confirmation of the NAP1 strain of C. difficile is not always possible, but this does not affect the therapeutic behavior. Further studies are expected to determine the role of the NAP1 strain in severe clinical forms of CDI in the pediatric population [6, 17].

The clinical spectrum of C. difficile infection varies widely, from asymptomatic carriage to diarrhea and life-threatening colitis. Age-related incidence and severity of CDI are inversely proportional [2, 18].

Verification of CDI in children is challenging. Despite the availability of commercially available tests to detect the presence of C. difficile or its toxins, there is no gold standard for diagnosis [2]. GDH EIA for detection of C. difficile specific enzyme, Enzyme immunoassay (EIA) and Nucleic Acid Amplification Test (NAAT) for detection of toxins in stool (toxins A/B) are used in practice. Rapid tests based on GDH detection cannot distinguish toxigenic from non-toxigenic C. difficile strains, resulting in false-positive results [19]. The 2017 Infectious Diseases Society of America (IDSA) and Society for Health Epidemiology of America (SHEA) guidelines recommend the following methods as the best 2-step diagnostic algorithm:

– EIA of toxins + EIA of glutamate dehydrogenase,

– EIA + NAAT of toxins [19].

Song PH et al. confirmed that real-time PCR for the detection of toxins A and B is the most effective diagnostic method for CDI. In this way, hypervirulent strains of CD, such as ribotype 027, can be verified [20].

The guidelines for CDI therapy in childhood overlap with those in adults – in mild forms, therapy is started individually with Metronidazole or Vancomycin orally, and in severe and fulminant forms – in combination with intravenous Metronidazole. Recent recommendations concern the use of Fidaxomycine [19, 21].

Rifaximin has a potential therapeutic role in the treatment of CDI, and especially in cases of relapse [22].

The aim of this study is to analyze the predisposing factors for the incidence of CDI, the clinical course and the outcome of the disease in 6 children hospitalized in the Clinic for Infectious Diseases at University Hospital, Stara Zagora, for a period of one year (2022-2023).

Patients and Methods

6 children (4 boys and 2 girls) aged 10 months to 11 years were included in the study. Inclusion criteria were: age up to 18 years, diarrheal syndrome, proof of C. difficile toxins A and B or GDH by ELISA and PCR, negative coproculture for Shigellosis, Salmonellosis, Campylobacteriosis, Rotavirus, Norovirus, and Adenovirus.

Exclusion criteria included age over 18 years, absence of diarrhea, negative results for C. difficile.

The diagnosis was confirmed by combined GDH and toxin A/B immunoassay in stool samples – NADALR-CD toxin A/B +GDH. All cases were also confirmed by a real-time PCR assay. Data was collected using Excel office. Data analysis was performed using the SPSS 26 program.

CLINICAL CASE SERIES

Case 1

It concerns a 10-month-old boy who presented with a diarrheal syndrome of 2-3 days with up to 10 for 24 hours yellowish mushy stools with mucus and blood. There was no fever, nausea and vomiting. Clinical data for 2nd degree of dehydration was found. There are no deviations from the laboratory tests (Table 1). The immunoenzymatic and PCR examination of feces shows positive toxins A and B of C. difficile. Therapy with Metronidazole, pathogenetic and symptom-

atic agents was carried out. The diarrheal syndrome was controlled on the second day of the hospital stay. The disease showed a moderately severe course; the patient was discharged on the 5th day in an improved general condition with recommendations for a dietary regimen and continuation of probiotics at home.

Case 2

A 5-year-old girl was hospitalized due to repeated (more than 15 times a day) watery yellowish stools with impurities of mucus, started the day before. The child reported complaints of abdominal pain, without fever. On admission, she was afebrile, with evidence of dehydration of 2-3 degree – with dry oral mucosa, painful palpation in the hypogastrium, accelerated peristalsis. There were no changes in the rest of the somatic status.

No deviations were found in the laboratory tests (Table 1). Immunological and PCR tests proved the presence of toxin A of CD. Treatment with a combination of parenteral Metronidazole and oral Vancomycin in usual doses was carried out. After a 5-day stay, the child was discharged in an improved condition.

Case 3

The third patient was a 5-year-old boy who had been sick for 3 days with greenish watery stools with mucus impurities, which progressively increased up to 17 times a day. He has been maintaining a subfebrile temperature for a day. The patient was treated symptomatically at home settings without effect. He was admitted with a temperature of 37.4˚C, 3 degree of dehydration, heart rate 150 bpm. There were no deviations from the pulmonary status. Perceptible pain in the left iliac region and highly accelerated peristalsis were detected. Laboratory tests showed slightly elevated CRP – 14.8 (Table 1). The both A and B toxins of CD were proven by ELISA and PCR. Treatment with intravenous metronidazole and oral vancomycin was started. The diarrhea was controlled on the 4th day and the child was discharged on the 7th day.

Case 4

An 8-year-old boy was admitted in a moderately damaged general condition five days after discharging from our clinic, where he was treated because of diarrhea caused by C. albicans. He had temperature of 37.4˚C. Cervical micropolyadenia was established. There were no lung and heart abnormalities. The abdomen was soft, with accelerated peristalsis. Laboratory tests detected elevated leukocytes and CRP, and low values of serum sodium (Table 1). By means of PCR, the presence of toxins A and B of CD was proven with negative results for common bacterial, viral and fungal intestinal pathogens.

Treatment with Metronidazole and Vancomycin was administered. The diarrhea was controlled on the 4th day of the hospital stay. After 10 days of therapy, the child was discharged in an improved condition with normalization of laboratory indicators. Epidemiological data show that it is most likely a nosocomial infection.

Case 5

An 11-year-old patient was admitted to the clinic due to a 7-day history of diarrheal syndrome. The parents reported repeated (up to 15/24 hours) watery stools with impurities of mucus and blood. On the 5th day, the temperature raised to 38.4˚C with chills. The child was hospitalized in an impaired general condition, febrile, highly intoxicated with third-degree dehydration.

Laboratory tests showed leukocytosis – 33.87 x10^9/L, three-digit CRP – 111 ng/l. The other indicators were without deviations (Table 1).

After proving CD toxins A and B, etiological therapy was administered – intravenous Metronidazole and oral Vancomycin. Within 5 days, the laboratory parameters were normalized, the diarrheal syndrome was controlled and the patient was discharged from the hospital.

Case 6

A 7-year-old girl was acutely ill with abdominal pain, passing green, watery and mucous stools 5-7 times/24 hours. There was nausea without vomiting, without fever. There was anamnestic data on taking Pancef within the previous 10 days for the treatment of acute bronchitis.

Again, in this case, no deviations from laboratory tests are detected (Table 1). Stool examination showed the

Table 1. Laboratory indicators of clinical cases

Indices/ Patients	HB g/L	Leuc x10^9/L	Urea mmol/L	Creat mmol/L	CRP ng/L	Na mEq/L	K mEq/L	Protein g/L	Albumin g/L	Toxin
Case 1	121	12.14	4.6	37	20.9	135	4.0	68.9	40.2	A+B
Case 2	121	7.7	3.5	28	1.0	135	4.3	61.3	39.4	A
Case 3	132	8.3	1.7	47	14.8	136	4.3	67.3	45.3	A+B
Case 4	135	6.1	4.7	51	14.8	130	4.1	76	49.2	A+B
Case 5	123	33.8	5.8	78	119.4	134	3.8	53.5	32.5	A+B
Case 6	106	7.7	3.6	23	7	136	4.3	66.2	38.6	A+B+ GDH

presence of GDH antigen as well as CD toxins A and B. The presence of C. albicans was also found. Treatment included a combination of Methronidazole, Vankomycin and Nystatin, and the patient was discharged with improvement with instructions to continue probiotic therapy. After 20 days, diarrhea appeared again with 3-4 mushy stools without impurities, and the general condition was preserved. This time, only GDH antigen was demonstrated in the stool. Oral Metronidazole was prescribed for 10 days. Diarrhea disappeared on the third day. The control ELISA test was negative for markers of C. difficile infection.

DISCUSSION

In our study, all of the patients, except one, were children older than 5 years of age.

A multicenter study in 22 US pediatric hospitals over a five-year period found an increase in the annual incidence of children hospitalized for C. difficile [11].

One of the main risk factors for the development of CDI is previous antibiotic use [23]. CDI accounts for about 20% of all cases of antibiotic-associated diarrhea [24]. In a meta-analysis by Anjewierden S et al. previous exposure to antibiotics and proton pump inhibitors was found to be associated with an increased risk of CDI in children [8]. We found a similar anamnestic history of the use of a third-generation cephalosporin antibiotic for a period of 10 days in only one child. Indeed, any class of antibiotics can potentially predispose to CDI, and onset can occur within days to weeks of initiation, but of particular note are third-generation cephalosporins, clindamycin, fluoroquinolones, and amoxicillin-clavulanate [10].

One of the children had a hospitalization 5 days before the onset of CDI. The epidemiological study gave reason to think about a nosocomial infection. During the previous hospitalization, a C. albicans infection was detected. The parents deny that the child had taken antibiotics in the previous months. There is also no evidence of an existing immune deficiency. Adams DJ et al. found that 13.6% of the children with CDI did not have any identifiable risk factor [9]. We found no risk factor for CDI in the remaining four of the six children.

In the cases studied by us, there is an acute onset of the disease with a pre-hospital period of 2 to 7 days (mean 3 ± 1.78).

Shirley DA, et al. reported that most children with clinical manifestation of CDI had mild to moderate watery diarrhea of 4 to 10 stools/24 hours [2]. In 4/6 patients, the number of defecation does not exceed 10/24 hours, in the remaining 2/6 defecations reach 17/24 hours. In all of them, mucus is present as pathological impurities in the feces, in two there is also blood. Morinville V, et al. described watery diarrhea in 79% of CDI cases, with blood in 12.5% [25]. Other clinical manifestations in mild and moderate-severe clinical forms include: low-grade fever, mild abdominal pain, nausea, weakness, and loss of appetite [2].

In the half of our patients, there is fever in values up to 38.4˚C, in contrast to the results of some authors, according to which this is the main clinical manifestation [26, 27]. They also indicate that the most frequent diarrheal stools are up to 10/24 hours, which is also confirmed in our study. Three of the six children have nausea, one of them have repeated vomiting. Abdominal pain of different nature was reported in four of the six patients.

The severe clinical form includes high fever, intense abdominal pain, abdominal distension, repeated vomiting, and watery diarrhea with mucus and blood leading to significant dehydration, hypoalbuminemia with peripheral edema, and subsequent circulatory shock. These cases in adult patients are defined by leukocytosis ≥ 15,000 cells/mm^3 or renal impairment with serum creatinine > 132 mmol/L, which does not apply to children [2]. In our 6 patients, we observed mostly a mild and moderate clinical form, with the exception of one child, in whom we reported up to 15 watery defecation with impurities of mucus and blood, fever up to 38.4˚C, third degree of dehydration, leukocytosis, three-digit CRP and mild hypoproteinemia. According to the literature, a severe clinical form of CDI can lead to toxic megacolon, intestinal paralysis, perforation of the colon with peritonitis, reactive arthritis, renal failure, systemic inflammatory response syndrome, sepsis and death [19]. This extreme form of CDI is less common in children than in adults – up to 8% [21]. None of our patients had such complications.

The diagnosis in our study followed the established steps to prove CDI – EIA and PCR with evidence of toxins A only in 1/6; A + B at 4/6; A+B+GDH at 1/6. This differs from the finding of Australian researchers, according to which only toxin B was most often identified in children [28]. In 2/6 parallel to C.difficile toxins, the presence of C. albicans was proven in coproculture.

Other possible etiological causes of diarrhea of viral, bacterial and fungal origin were excluded in all patients.

Vancomycin, Fidaxomicin, and Metronidazole orally, and the latter intravenously in severe cases, are the usual agents for the treatment of CDI [19]. In the Republic of Bulgaria, the preparation Fidaxomicin is not

authorized for use in practice. Among our patients, etiological therapy was started – intravenous Metronidazole alone in 1/6 and in combination with oral Vancomycin in the remaining 5/6 in the recommended doses, consistent with the recommendations in the literature [28, 29, 30]. Exactly, these guidelines were applied in our 6 cases, and in the children with added C. albicans infection, therapy was supplemented with Nystatin in 1 case and with Diflazon in 1 case. In addition, glucose-saline solutions, correction of acid-base imbalance, probiotics, symptomatic agents, and diet were also administered.

The outcome for all our patients is favorable, with a reduction of the diarrheal syndrome by day 3-4 from the start of therapy. Abnormalities in laboratory parameters normalized 5 to 10 days after hospitalization. It is considered that the recurrence of the disease in childhood has a frequency similar to that of adults (20-30%) [2]. In our group of patients, only in one of the children we noted a relapse with mild clinical manifestations and demonstration of only GDH antigen. The duration of the hospital stay was from 5 to 10 days (mean 6 ± 2.8).

CONCLUSIONS

In the children with CDI observed by us, the disease was mainly demonstrated with a mild and moderate-severe course, except for one patient who had a severe clinical course. In two of the cases, we found a risk factor for CDI – long-term antibacterial therapy in one patient, and nosocomial infection in the other patient. The outcome for all is favorable and the credit for this is due to the quick diagnosis and timely and adequate etiological therapy. Only in one case we observed a relapse, which took place in a mild form.

Therefore, in children with diarrhea and exclusion of other enteric pathogens, even in the absence of risk factors, it is appropriate to think about CDI.

Informed Consent Statement: *All data used in this manuscript are presented with the explicit consent of the patient parents, as the former are minors.*

Ethics Approval Statement: *The approval of the Ethics Committee at the University Hospital, Stara Zagora, Bulgaria, has been sought and received.*

Conflict of Interest Statement: *The authors declare no conflicts of interest related to this work.*

Funding: *The authors did not receive any financial support from any organization for this research work.*

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