ORIGINAL ARTICLE

Unveiling the Risk Factors of *Clostridioides difficile* Infection in Children; A Multicenter Retrospective Case-control Study in the Eastern Region, Saudi Arabia

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ABSTRACT

Background: *Clostridioides difficile (C. difficile) is a major cause of morbidities that ranges in severity from asymptomatic infection to antibiotic associated diarrhea (AAD), and pseudomembranous colitis (PMC). It was recognized that C. difficile infection (CDI) is a disease of advanced age with an increasing incidence worldwide. Notably, children are no longer in the safe zone due to increasing risk of associated comorbidities that predispose to a greater incidence of CDI among this age group. Few data are available to describe the incidence and the risk factors associated with CDI among children in the middle east.* ***Objective:*** *To identify the risk factors associated with (CDI) in children.* ***Methodology:*** *A multicenter retrospective case-control study was done to review the data collected electronically from the medical records during the five years of the study period. The adjusted and unadjusted regression analysis were used to identify the risk factors.* ***Results:*** *The odd ratio of developing CDI in children is higher than adults (OR:1.52, 95%CI:1.01-2.28, P = 0.0423). Children with asthma (OR:2.77, 95%CI:1.14-6.76, P=0.0251) and children with Sickle cell disease (SCD) (OR: 6.25, 95%CI: 2.03-19.27, P=0.0014) have higher risk of developing CDI.* ***Conclusion:*** *This study demonstrates a higher risk of developing CDI in hospitalized children. A significant association was found between CDI in children and certain comorbidities such as asthma and SCD.*

INTRODUCTION

The first discovery of the Gram positive, spore forming, anaerobic bacillus known as *C. difficile* was made in 1935. It was isolated from the healthy neonates' stool samples. Such bacillus proved difficult to isolate and investigate therefore, it was named *Bacillus difficilis*[1]. Historically, *C. difficile* was recognized as a commensal microorganism among infants and it has not been properly investigated in children[2].

In the late 1970s, AAD, and PMC were linked to CDI[3,4]. *C. difficile* is a main etiologic agent of both Hospital Acquired (HA)- diarrhea, and AAD. Since that time, the progressive increase in the related mortality, morbidity and recurrence raises a serious global concern about CDI[5]. Currently, *C. difficile* is considered as a significant pediatric intestinal pathogen. The incidence of CDI is increasing in children, particularly among children without traditional risk factors[6,7]. Several risk factors such as the excessive intake of antibiotics and proton pump inhibitors, repeated hospital admissions, prolonged hospital stays and the presence of numerous comorbidities increases the likelihood of developing CDI[8,9].

The two-stage algorithms enhance CDI diagnosis; using GDH detection in feces as a technique of screening for CDI and the confirmation by NAAT such as PCR to detect toxigenic strains are the two generally recommended procedures in the laboratory diagnosis of CDI[10,11]. The real-time PCR technique may be of high sensitivity and accuracy for diagnosing CDI[12]. Furthermore, the sensitivity of the different CDI diagnostic techniques including; PCR, toxin assay and culture method are known to be 99.1%, 83.3% and 51% respectively[13]. Real-time PCR sensitivity was found to be 100% according to a European study, while ELISA's toxin assay's sensitivity is 58.8%[14].

Limited data were published to describe the hazards and the factors related to CDI in children. The purpose of the present study was to identify the risk factors associated with CDI in pediatric population.

METHODOLOGY

Patients and study design:

Patient group included children and adolescent less than 18 years old, diagnosed as CDI -positive by using real-time PCR test to identify *C. difficile* toxin B genes.

The test was performed on the unformed stool samples obtained from patients with diarrhea during the study period started from January 2017 to September 2021 at King Abdulaziz Hospital, Aalhsa and Imam Abdulrahmn bin Faisal Hospital, Dammam, Eastern Region, Saudi Arabia. The control group included children, of the same age group, with other types of bacterial diarrhea admitted to the two hospitals during the same period of time. The study was approved by Institutional Review Board (IRB) of King Saud bin Abdulaziz University for Health Sciences (KSAU-HS) and King Abdullah International Medical Research Center (KAIMRC) with Reference # RA19/013/A.

A retrospective case-control study design was used to investigate the comorbidities and outcomes associated with CDI in children by comparing the patients' criteria which are electronically collected from their medical records through the BestCare system of the two hospitals with the support of the research center (KAIMRC) Data Management department.

Definitions:

CDI patient:

A patient suffering from diarrhea (≥ 3 unformed stool/24 hours) accompanied by a positive laboratory test results for identifying *C. difficile* toxin A and/or B (a molecular laboratory test such as PCR)[15].

Hospital- Acquired (HA-CDI):

A CDI confirmed by positive laboratory sample collected more than 3 days after hospital admission[15].

Community -Acquired (CA-CDI):

A CDI confirmed by positive laboratory sample collected in the outpatient or inpatient facility ≤ 3 days after admission to the hospital[15].

Severe CDI:

A patient with at least one of the following; Leukocytosis (WBC >15 x 10^9/L), Ileus, Megacolon, Septic shock requiring ICU admission, Serum creatinine concentration >50% above the baseline[16] .

Diagnostic Methods

(Real-Time PCR of Stool Samples):

Patients' stool samples were transported immediately to the laboratory. The Xpert *C. difficile* real time PCR (Cepheid, Sunnyvale, CA) was used to detect its toxin B gene(tcdB). The steps were performed according to the manufacturer's instructions[17]. Toxin B is a crucial component of all the *C. difficile* toxigenic strains. Using real-time PCR, the result was represented as positive toxigenic *C. difficile* or negative toxigenic *C. difficile.*

Statistical analysis:

Descriptive statistic was performed after data cleaning and continuous data were presented as median and interquartile range (IQR) while the categorical data were displayed as frequency and percentage. For the analytical part, if the P value is < 0.05 it is considered statistically significant. The unadjusted logistic regression analysis (univariate logistic regression) was performed to identify the risk factors associated with CDI, followed by the adjusted logistic regression analysis (multiple logistic regression) to elucidate the confounding between the variables. Backward elimination method was used with variables that were significant and not collinear in the unadjusted model. Death variable and laboratory data variables were excluded from the adjusted model as many laboratory results were missing. Only variables that remain significant were kept in the final adjusted model.

MS. Excel and JMP Pro 15.2.0. were used for data analysis. In case of having collinear variables, the general one is tried first, and it is excluded if it was statistically insignificant, and the next collinear variable will be used. Variables that only showed statistical significance are kept in the model.

RESULTS

The present study reviewed the records of patients with bacterial diarrhea. During the five years of the study period, 228 patients were diagnosed with CDI, and 33 (14.4%) of them were children (IQR 1-11) below 18 years, included as cases. Furthermore, 147(10.02%) out of 1467 patients with other types of bacterial diarrhea were children (IQR 1-13) included as a control group. Data analysis revealed significant increased (P = 0.0423) CDI among children in the eastern region of Saudi Arabia. The disease and patients' admission characteristics in addition to the risk factors associated with CDI are shown in Table 1. While Table 2 describes the distribution of CDI cases per year versus the control, in addition to the number and percent of HA- CDI and CA-CDI per year, misleading data were reported as unknown. Five (15.2 %) cases were identified with severe CDI. According to the definition of severity, four children were found to have WBCs >15 x10^9 /L and one child was diagnosed with PMC. No reported cases with recurrent CDI, however two children had 2 or 3 episodes of CDI during the study period.

Table 1: The risk factors associated with CDI in children

Variable	All (n=180)	Control (n=147)	CDI (n=33)	Unadjusted model			Adjusted Model		
				OR	95% CI	P	OR	95% CI	P
Age: Median (IQR)	3 (1-12)	3 (1-13)	3 (1-11)	0.98	0.92-1.05	0.5724	-		
Gender (Female)	87(48.33%)	69 (46.94%)	18 (54.55%)	1.36	0.64-2.89	0.4304	-		
Hospital (Hospital 1)	146 (81.11%)	119 (80.95%)	27 (81.82%)	1.06	0.40-2.81	0.9086	-		
Admitted to ICU	16 (8.89%)	14 (9.52%)	2 (6.06%)	0.61	0.13-2.84	0.5312	-		
HA-CDI	108 (60.00%)	85 (57.82%)	23 (69.70%)	1.68	0.75-3.78	0.2114	-		
Previous hospital stays	43 (23.89%)	32 (21.77%)	11 (33.33%)	1.80	0.79-4.09	0.1628	-		
Comorbidities									
Diabetes	4 (2.22%)	4 (2.72%)	0 (0.00%)	-	-	0.9929	-		
Hypertension	5 (2.78%)	4 (2.72%)	1 (3.03%)	1.12	0.12-10.33	0.9222	-		
Asthma	33 (18.33%)	22 (14.97%)	11 (33.33%)	2.84	1.21-6.67	**0.0166***	2.77	(1.14-6.76)	**0.0251***
Inflammatory bowel disease	7 (3.89%)	5 (3.40%)	2 (6.06%)	1.83	0.34-9.88	0.4813	-		
Gastroenteritis	35 (19.44%)	30 (20.41%)	5 (15.15%)	0.70	0.25-1.96	0.4923	-		
Urinary tract infection	6 (3.33%)	4 (2.72%)	2 (6.06%)	2.31	0.40-13.16	0.3469	-		
Cancer	1 (0.56%)	1 (0.68%)	0 (0.00%)	-	-	0.9916	-		
Kidney Disease	14 (7.78%)	12 (8.16%)	2 (6.06%)	0.73	0.15-3.41	0.6847	-		
Stroke	1 (0.56%)	0 (0.00%)	1 (3.03%)	-	-	0.9897	-		
SCD	15 (8.33%)	7 (4.76%)	8 (24.24%)	6.40	2.13-19.23	**0.0009***	6.25	(2.03-19.27)	**0.0014***
Medication history									
Antibiotics	52 (28.89%)	42 (28.57%)	10 (30.30%)	1.09	0.48-2.48	0.8428	-		
Proton pump inhibitors (PPI)	18 (10.00%)	13 (8.84%)	5 (15.15%)	1.84	0.61-5.58	0.2809	-		
Immunosuppressive	4 (2.22%)	3 (2.04%)	1 (3.03%)	1.50	0.15-14.89	0.7292	-		
Corticosteroids	5 (2.78%)	3 (2.04%)	2 (6.06%)	3.10	0.50-19.32	0.2262	-		
Lab results									
WBC >15x10^{9} /L	9 (26.47%)	5 (17.86%)	4 (66.67%)	9.2	1.30-64.89	**0.0260***	-		
Creatinine >133 µmol/L	26 (89.66%)	21(87.50%)	5 (100.00%)	-	-	0.9977	-		
Albumin <30 gm/L	8 (40.00%)	6 (37.50%)	2 (50.00%)	1.67	0.18-15.13	0.6499	-		
Outcome(death)	2 (1.11%)	2 (1.36%)	0 (0.00%)	-	-	0.3666	-		

Table 2: The yearly distribution of CDI cases (HA, CA and unknown) versus the control

Year	HA-CDI		CA-CDI		Unknown		Total Cases		Total control	
	N	%	N	%	N	%	N	%	N	%
2017	4	80.00%	1	20.00%	0	0.00%	5	23.81%	16	76.19%
2018	7	63.64%	4	36.36%	0	0.00%	11	23.40%	36	76.60%
2019	3	75.00%	1	25.00%	0	0.00%	4	8.51%	43	91.49%
2020	5	71.43%	0	0.00%	2	28.57%	7	20.00%	28	80.00%
2021	4	66.67%	2	23.33%	0	0.00%	6	20.00%	24	80.00%
Total	23	69.70%	8	4.24%	2	6.06%	33	18.33%	147	81.67%

DISCUSSION

Children have a long history with *C difficile* dating back to the discovery of the pathogen as a component of the gut flora in newborns. Different studies on the pediatric records have confirmed an increased prevalence of CDI among the USA children. A retrospective study analyzed the data extracted from the record of the Pediatric Health Information System, has reported an increased annual incidence density (53%) during the period from 2001 to 2006 (p=0.04)[18] which is agreed with the results of the present study that showed

a significant increase in CDI among children (P = 0.0423). During each year of the study period (2017-2021) it was noticed that the majority of the reported cases were HA-CDI.

Researchers proclaimed an increasing symptomatic CDI among hospitalized children with risk factors such as; administration of antibiotics, immunosuppressive medication and those with intestinal dysfunction. They also reported a poor CDI outcome, and greater incidence of morbidity and mortality in hospitalized children[19]. Prolonged hospital stay is another associated risk factor in children[20]. This was parallel to the results of the present study where the prolonged hospital stays and the hospital acquired infection were greater in CDI patients compared to the control group, though the relationship was insignificant. Similar insignificant higher results were recognized in CDI patients with medication history including antibiotics, PPI, immunosuppressive and corticosteroids.

In the present study, the unadjusted and the adjusted models unveiled that the most frequent comorbidities associated with CDI during the childhood period include bronchial asthma (P= 0.0251) and sickle cell disease (P= 0.0014). Sickle cell gene prevalence was estimated by the Saudi Premarital Screening program and the highest prevalence was eminent in the Eastern Province[21]. This may show why SCD represents a significant comorbid condition in children. In general, SCD patients receive repeated doses of empirical antibiotics which poses a potential for increasing the rate of CDI, that is ultimately associated with excessive antibiotic treatment[22]. Other authors[23], had observed the increased CDI prevalence among SCD over the last decade, which is also consistent with our results. Nevertheless, the rate of CDI in both pediatric and adult SCD patients was found to be low because the SCD microbiome has protected the patients from CDI by changing their intestinal metabolome[24–26]. Although the rate of CDI in the mentioned studies among SCD patients was low, yet the authors indicated the presence of an association between CDI and SCD in the same age group as identified in our research. Furthermore, it was reported that hematologic disorders are other chronic comorbidities that increases the risk of exposure to CDI in the pediatric population[27].

Infants with *C. difficile* colonization and/or infection (CDCI) are more prone to develop allergic disorders during their early childhood. These findings imply that CDCI during infancy may refer to a reduction in the diversification of the gut microbiota, which is linked to a higher risk of developing allergic sensitization[28]. This was in concordance with the present study that showed a higher risk of acquiring CDI among asthmatic patients less than 18 years. Dysregulation of the microbiota has been linked to the occurrence of asthma and other allergic manifestations. For example, *C. difficile* colonization of infants was linked to higher risk of developing atopy such as; allergic sensitization, wheezy chest, and eczema[29]. Likewise, neonatal colonization with *C. difficile* was related to the development of asthma at the age of six to seven years[30].

The majority of the laboratory data was missing and the analysis of available laboratory showed a significant (P=0.0260) association between CDI and a high WBCs count (WBCs $>15x10^9$ /L). The neutrophil: lymphocyte ratio is a predictor for ICU admission and death within two days of CDI diagnosing[31]. Another retrospective study found a link between neutrophilia and the greater possibility of CDI resolution[32]. Considering the diversity of the WBCs subpopulations in CDI patients, the total WBCs count may not be an accurate indicator of disease severity[33]. Though, the CDI severity was studied widely in another study and four different definitions of severity were discussed, the WBCs count (WBCs $>15x10^9$ /L) was a common predictor of the disease severity in three of them[16]. In the present study four children with significant high WBCs count, according to the unadjusted model, were recognized with severe CDI; a significant result that was lost in the adjusted model.

CONCLUSION

The rate of CDI is high among children in Saudi Arabia. Notably, children with sickle cell disease and asthma are at greater risk than others. Early and accurate disease diagnosis is crucial for proper case management to reduce the unfavorable outcomes and recurrence.

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other reviewed media. I have contributed sufficiently to the project to be included as author. To the best of my knowledge, no conflict of interest, financial or others exist. All authors have participated in the concept and design, analysis, and interpretation of data, drafting and revising of the manuscript, and that they have approved the manuscript as submitted.

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