Clostridium Difficile Infection in Inflammatory Bowel Disease; Does it Have an Impact?

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Background and study aim: Limited data exist on outcomes in inflammatory bowel disease (IBD) patients who develop Clostridium Difficile infection (CDI). The aim of the study to investigate prevalence of CDI in IBD with assessment of the disease behavior in affected patients.

Patients and Methods: 30 IBD patients and 15 healthy subjects of matched age & sex as control group. Patient were classified into two groups; group I (15 patients with Crohn's disease), group II (15 patients with ulcerative colitis) & control group (15 Patients not having IBD). Disease activity for group I was determined by Crohn's disease activity index (CDAI) & for group II by Truelove & Witts score. All patients & controls underwent Computed Tomography Enterocolongraphy, ileo-colonoscopy with ileal & colonic biopsies, and Clostridium Difficile detection & quantification plus detection of toxin A & B using SYBR Green Real-time PCR.

Results: 3 patients from group I had CDI and these 3 patients had active disease, one with severe activity (CDAI 550) and two with moderate activity (CDAI 380 & 420). Also 3 patients from group II had CDl and these 3 patients had moderate activity. Only one patient from the control group had CDI carriage (non-toxin producing strain).The rest of patients (38 patients) had no CDI (of them, one patient had mildly active Crohn's disease with CDAI of 200 & two patients had mildly active ulcerative colitis & the others were in remission).

Conclusion: Prevalence of toxigenic CDI was 20% among IBD patients and significantly associated with disease activity.

INTRODUCTION

Idiopathic inflammatory bowel disease (IBD) [Crohn's disease (CD) and ulcerative colitis (UC)] is a chronic or relapsing inflammatory-immune related gut disease. These two forms of IBD share epidemiologic and clinical characteristics, suggesting an underlying similar etiology **[1]**.

There is wide geographic variability in incidence and prevalence of IBD. Higher incidence rates were observed in England, Northern Europe, United States and Canada with lower incidence in Asia-Pacific region, with exception of Australia **[2]**.

The clinical manifestations of CD are more variable than UC; this may be due to transmural involvement and variability of disease extent in CD in comparison to the more superficial & less extensive distribution of UC **[3]**.

European Colitis & Crohn's Organization (ECCO) stated that disease extent is the main influencer on treatment modality and determinant to surveillance frequency **[4]**.

Diagnosis should be confirmed by clinical evaluation with combination of biochemical, endoscopic, histological &/or radiological investigations **[4,5]**.

ECCO stated: For suspected CD, ileo-colonoscopy with biopsies from terminal ileum and colonic segment for microscopic evidence of CD are first line procedures to establish diagnosis. [5] However in UC, colonoscopy with ileal intubation together with segmental biopsies including rectum is a main corner stay to establish diagnosis and disease extent **[4]**.

Clostridium Difficile infection (CDI) is defined as acute diarrheal disease caused mainly by toxigenic CDI strain **[6]**. Clostridium Difficile (C. Difficile) was first described as a cause of pseudomembranous colitis in 1978, mainly an antibiotic-associated infection **[7]**. Evidences suggested that in a process similar to that in CDI, microbial dysbiosis, consisting of an increase of detrimental bacterial populations and their toxic metabolites, together with a decrease in beneficial bacteria and their metabolic end products, alter gut luminal environment and contribute to IBD pathogenesis **[8].**

IBD patients have increased risk of acquiring CDI, have higher rates of recurrence and worse outcome. C. Difficile was associated with IBD but understanding the relationship between the two conditions is confusing. However, it is unclear how these two dysbiosis-related conditions may interact with each other, and whether, there exists a cause-consequence versus concurrent relationship **[9,10]**.

Lower abdominal pain and tenderness and watery diarrhea are typical CDI symptoms. With underlying active IBD, diarrhea can be frequently bloody **[11]**. Patients may have fever, malaise, anorexia, and leukocytosis with left shift, hypoalbuminemia and increased stool leukocytes. These findings can be seen in both CDI and IBD exacerbations, thus usually distinguish between these two conditions on the basis of laboratory testing or symptoms alone are difficult **[9]**.

The nucleic acid amplification tests (NAATs) for C-Difficile toxin genes as PCR are superior to toxins A & B enzyme immunoassay (EIA) as stated by American College of Gastroenterology (ACG) in 2013 **[12]**.

ACG guidelines in 2013 strongly recommend that all patients with IBD hospitalized with a disease flare should undergo testing for CDI. In addition ambulatory patients with IBD who develop diarrhea and known to have quiescent disease should be tested, or in the presence of risk factors such as recent hospitalization or antibiotic use **[12]**.

Limited data exist on outcomes in IBD patients who develop CDI. Most of the evidence indicates that, compared to the general population, IBD patients with CDI have worse clinical outcomes **[9]**.

Aim of the work: Investigate prevalence of CDI in IBD (CD & UC) with assessment of the disease behavior in affected patients.

PATIENTS AND METHODS

30 newly diagnosed (naïve) patients with IBD (according to clinical, laboratory, radiological, endoscopic & histopathological findings) & 15 healthy controls of matched age & sex were included in this cross-sectional study.

Only newly diagnosed patients (in our endoscopy unit) were enrolled in this study. Inclusion criteria included only newly diagnosed UC / CD adult patients of both sex who were able to give consent to participate in the study. Exclusion criteria were pervious diagnosed IBD &/or patients receiving any related drugs (5-ASA, immunosuppressants, biologics or broad spectrum antibiotics), surgical intervention or hospital admission within the past three months

These patients were further classified into three groups: **group I:** 15 patients with CD, **group II:** 15 patients with UC, & **group III:** 15 healthy control persons confirmed, not having IBD or diarrhea within the past three months.

Activity was assessed clinically for Crohn's disease by using Crohn's disease activity index (CDAI) [13] & for Ulcerative colitis patients by using Truelove and Witts Classification of Ulcerative Colitis **[4]**.

CBC, serum albumin, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), stool cultures (for exclusion of any concurrent bacterial infection of gut) & fecal calprotectin were done to all patients & controls.

All patients underwent Computed Tomography Enterocolongraphy & ileo-colonoscopy with mucosal biopsies for histopathological assessment.

Stool specimens from patients & controls were collected immediately upon defecation, kept in freezer, and delivered immediately to our laboratory frozen, where aliquots of each specimen were frozen at −80 °C until DNA extraction was done. DNA was extracted from 150 mg stool samples using ISOLATE Fecal DNA Kit (Bioline, UK). The DNA was then bound, isolated and purified using spin columns. The resulting DNA extracts were stored at -70°C until PCR assessment. Oligonucleotide primers were targeted at the 16SrRNA gene (rDNA) sequences of C. Difficile (Metabion International AG. Germany). Amplification was performed in a light cycler (Rotor Gene Q, Qiagen, Germany) using a SensiFAST ™ SYBR No-ROX PCR kit (Bioline Co. UK) **[14-16]**.

RESULTS

The study was done between January 2016 to December 2017 on 30 patients & 15 controls, no significant differences were observed between the three groups as regards the demographic data [Table (1)].

Four patients from group (I) (26.6%) and five (33.3%) from Group (II) were in active state (as assessed by using Crohn's disease activity index (CDAI) & Truelove and Witts Classification of Ulcerative Colitis, while eleven (73.3%) patients from the group (I) and ten (66.7%) patients within the group (II) were quiescent. The patients with active disease in group (I) were distributed as 1 patient with mild disease activity, 2 patients with moderate disease activity and 1 patient with severe disease activity, according to CDAI while the patients with active disease from group (II) were distributed as 2 patients with mild disease activity and 3 patients with moderate disease activity but no patients recorded with severe disease activity, according to Truelove and Witts classification [Table (2)].

From group (I), the patient with severe disease activity had extra-intestinal manifestations (EIMs) (uveitis & erythema nodosum) & the two patients with moderate disease activity had arthritis, while the patient with mild disease activity as well as the 11 quiescent patients had no EIMs. Similarly from group (II), two patients with moderate disease activity had arthritis (one of them had episcleritis as well), one more patient with moderate disease activity had episcleritis, while the two with mild disease activity as well as the 10 patients in quiescent status had no EIMs [Table (3)].

In Group (I): 3 patients within Crohn's disease group had CDI and these 3 patients had active disease, 1 of them had severe disease activity and 2 had moderate disease activity. The patient with mild disease activity in addition to the rest 11 patients, which were in quiescent state, had no CDI. The patient with severe disease from group (I) had Fecal calprotectin level of 830, CDAI of 550, C. Difficile PCR replication count of 1.27 X 10^{-4} and production of Both toxin A and B. First Patient with moderate disease from group (I) had fecal calprotectin level of 218, CDAI of 380, C. Difficile PCR replication count of 1.34 X 10^{-6} and production of toxin B only. Second Patient with moderate disease from group (I) had fecal calprotectin level of 280, CDAI of 420, C. Difficile PCR replication count of 2.4 X 10^{-6} and production of toxin B only. Patient with mild disease from group (I) had fecal calprotectin level of 112, CDAI of 200 and had no C. Difficile infection [Table (4)].

In Group (II): 3 patients within Ulcerative colitis group had CDI and these 3 patients had moderate active disease, the 2 patients with mild disease activity in addition to the patients in quiescent state, had no CDI. First patient with moderate disease from group (II) had fecal calprotectin level of 325, C. Difficile PCR replication count of 1.49 X 10^{-6} and production of toxin B only. Second patient with moderate disease from group (II) had fecal calprotectin level of 465, C. Difficile PCR replication count of 2.37 X 10^{-6} and production of toxin B only. Third patient with moderate disease from group (II) had Fecal calprotectin level of 902, C. Difficile PCR replication count of 1.85 X 10^{-4} and production of Both toxin A and B. First patient with mild disease from group (II) had fecal calprotectin level of 85 and had no CDI. Second patient with mild disease from group (II) had fecal calprotectin level of 97 and no CDI [Table (5)].

In control Group: only 1 subject was found to have C. Difficile carriage within the control group. The C. Difficile carrier within the control group had negative fecal calprotectin level of 42 and Cl. Difficile PCR replication counts of 1.47 x 10-7, the bacteria were non-toxin producer [Table (6)].

Table (1): Comparison between the three studied groups according to demographic data.

	Group I CD (N = 15)		Group II UC (N = 15)		Control Group (N = 15)		Test of Sig.	P
	No.	%	No.	%	No.	%		
Sex								
Male	8	53.3%	8	53.3%	7	46.7%	$\chi^2 = 0.178$	0.915
Female	7	46.7%	7	46.7%	8	53.3%		
Age								
Min. – Max.	19.0 – 37.0		19.0 – 37.0		19.0 – 36.0		F=0.481	0.622
Mean ± SD.	27.73 ± 5.48		28.13 ± 5.55		26.27 ± 5.43			
Median	28.0		28.0		25.0			

$\chi 2$, p: $\chi 2$ and p values for Chi square test for comparing between the three groups
F and P values for ANOVA test

Table (2): Distribution of the studied cases according to clinical activity.

Disease status	Group I CD (N = 15)		Group II UC (N = 15)	
	No.	%	No.	%
In remission	11	73.33%	10	66.67%
Mild disease	1	6.67%	2	13.33%
Moderate disease	2	13.33%	3	20%
Sever disease	1	6.67%	0	0%

Table (3): Extra-intestinal manifestation of IBD among patients of groups I & II.

Extra-intestinal manifestations (EIMs)	Number of patients	%	Clinical activity
Group I CD (n=15)			
No EIMs.	12	80%	Remission + mild
Arthritis	2	13.33%	Moderate
Ocular EIM	1 (Uveitis)	6.67%	Severe
Cutaneous EIM	1 (erythema nodosum)	6.67%	Severe (same patient with ocular EIM)
Group II UC (n=15)			
No EIMs.	12	80%	Remission + mild
Arthritis	2	13.33%	Moderate
Ocular	2 (episcleritis)	13.33%	Moderate (one patient in common with arthritis)
Cutaneous EIM	0	0%	

Table (4): Distribution of the studied clinically active cases of Group I according to CDAI, Fecal calprotectin, Clostridia Difficile Count and Toxins production.

	Case 1	Case 2	Case 3	Case 4	Min	Max	Med.	Mean
CDAI	200	380	420	550	200	550	387.5	394.3±65.3
Fecal Calprotectin	112	218	280	830	112	830	390	412±38.32
Clostridia Difficile count	Negative	1.34×10^{-6}	2.4×10^{-6}	1.27×10^{-4}	1.34×10^{-6}	1.27×10^{-4}	1.67×10^{-5}	$1.67 \pm 0.32 \times 10^{-5}$
Clostridia difficile toxin	A-ve/B-ve	A-ve/B+ve	A-ve/B+ve	A+ve/B+ve	-	-	-	-
P_c value				0.0063				

P_c value positive if ≤ 0.05 i.e.: p value for individualized comparison.

Table (5): Distribution of the studied clinically active cases of Group II according to True and Love activity index, fecal calprotectin, Clostridia Difficile PCR replication count and production of toxins.

	Case 1	Case2	Case 3	Case 4	Case 5	Min	Max	Med.	Mean
Disease activity	MILD	MILD	MOD	MOD	MOD	-	-	-	-
Fecal Calprotectin	85	97	325	465	902	85	902	375	399±22.32
Clostridia Difficile count	Negative	Negative	$1.49x10^{-6}$	$2.37x10^{-6}$	$1.85x10^{-4}$	$1.49x10^{-6}$	$1.85x10^{-4}$	$1.93x10^{-5}$	$1.99±0.64 x10^{-5}$
Clostridia difficile Toxin	-	-	A-ve/ B+ve	A-ve/ B+ve	A+ve/ B+ve	-	-	-	-
P_c value	0.0047								

P_c value positive if ≤ 0.05 i.e.: p value for individualized comparison.

Table (6): Comparison between all cases of the three studied groups according to Presence of Clostridia Difficile.

	Group I CD (N = 15)		Group II UC (N = 15)		CONTROL GROUP (N = 15)	
	No.	%	No.	%	No.	%
Clostridia Difficile						
Negative	12	80.0	12	80.0%	14	93.33%
Positive	3	20.0%	3	20.0%	1	6.67%
Relation to disease clinical activity (N = 3)	**3**	**100%**	**3**	**100%**	-	-
Mild	0	0%	0	0%	-	-
Moderate	2	66.7%	3	100%	-	-
Sever	1	33.3%	0	0%		

DISCUSSION

IBD patients have a risk for developing special enteric infection more than general population especially CDI. IBD itself is an independent risk factor for CDI: a threefold increased risk over general population has been reported. [1] Analysis of a registry database suggests that 10% of IBD patients will develop a CDI at some point, and in approximately 10% of patients, CDI occurs at time of IBD diagnosis **[18]**.

According to CDAI, most Group I patients were in clinical remission (73.33%), those with active disease, 6.67% have severe disease activity, 13.33% have moderate disease activity and 6.67% have mild disease activity. Crohn's disease activity index (CDAI) used as index for the CD assessment **[13,19-21]**.

Regarding Truelove and Witts classification, most Group II patients (66.67%) were in clinical remission, 20% of patients had moderate disease activity and 13.33% had mild disease activity but no patients had severe disease activity.

Prevalence of Clostridium Difficile infection among patients' groups was 20% for both CD and UC groups and 6.67% for control group. All patients in both groups with CDI were in activity. Only patients in both groups showing mild disease activity were negative for Clostridium Difficile infection.

Those with severe disease activity within CD group have highest level of Clostridium Difficile relative count $1.27x10^{-4}$ with production of both cytotoxic toxin A and B and highest level of fecal calprotectin 830.

While UC patients, highest Clostridium Difficile count $1.85x10^{-4}$ was found in patients with moderate disease activity with highest fecal calprotectin level of 902. Infection was associated with production of both toxin A and B.

Other two Clostridium Difficile count were $2.37x10^{-6}$ & $1.49x10^{-6}$ related to moderate disease activity but less active than patient with highest Clostridium Difficile count, with fecal calprotectin of 465 and 325 respectively of these patients and production of only toxin B.

The only one healthy control with Clostridium Difficile carriage has Clostridium Difficile relative count of $1.47x10^{-7}$ and his bacteria were non-toxin producers. Comparing this control Clostridium Difficile count with mean of Clostridium Difficile count of other IBD groups [$1.67\pm0.32x10^{-5}$ for CD group and 1.99 ± 0.64 $x10^{-5}$ for UC group], A strong significant statistical difference regarding Clostridium Difficile count of both groups versus control Clostridium Difficile relative count was noted. i.e. $P^3 = 0.00012$.

Issa et al. **[22]** showed 16% prevalence of CDI among IBD patients and 50 % of infected patients were hospitalized and concluded that CDI is significantly increased in IBD patients and negatively impacted clinical outcome.

Balamurugan et al **[23]** found that the prevalence of CDI was so high to the level that 34 patient from the 37 patients with UC had CDI and also 21 form healthy controls were positive to CDI, but only 8 patients from UC group & none from controls were toxin producer, no statistical significant difference between active and quiescent disease was found. In contrast to the present study, this could be attributed to most patients in Indian study have non toxin-producing infection and for complete pathognomonic action of CDI to occur, toxin production is mandatory.

Gwen et al **[24]** study found no statistical difference regarding CDI and disease activity. They stated that 10% of IBD flares reported to be result of microbial pathogen invasion, and testing CDI is mandatory.

Thus ECCO in 2013, strongly recommended CDI testing in any IBD patient presented with disease flare at any time during disease course **[12,18]**.

Ananthakrishnan et al studied the impact of CDI on IBD and documented an increase in incidence among those with underlying IBD and substantial morbidity association, the surgical need and even mortality. Also, they mentioned similarity of clinical presentation between CDI and a flare of underlying IBD that highlighted the importance of diagnosis as essential mean to prevent deterioration and further need for immunosuppression escalation in absence of appropriate antibiotic therapy **[25]**.

In 2017, Gillespie et al investigated epidemiology and risk factors of CDI in patients with IBD. Their results showed that incidence of CDI among IBD patients was 6.7% with equal prevalence of CDI among CD and UC, concluding that IBD patients are more vulnerable to CDI at a younger age and that IBD patients with CDI would require biologic therapy with increased rates of extra-intestinal manifestations. [26] These results are in agreement with our results, where some patients with both IBD & CDI had extra-intestinal manifestations (arthritis, uveitis, episcleritis or erythema nodosum).

Shoaei et al in 2015 stated that the prevalence of C. Difficile isolates was 31.8% (27/85) in UC patients and rather prevalent in UC patients & all patients with CDI experienced moderate to severe disease and so need for close monitoring and appropriate treatment including early detection and treatment of CDI would lead to better UC outcomes **[27]**. These results are matching with results of this study.

AGA (2017) conducted an expert review from the clinical practice updates on management of CDI in IBD and recommended that clinicians should test patients who present with a flare of underlying IBD for CDI, consider hospitalization for close monitoring and aggressive management for IBD with CDI who have profuse watery &/or bloody diarrhea, severe abdominal pain, markedly increased peripheral leukocyte count, or evidence of sepsis and referral for fecal microbiota transplantation to IBD patients with recurrent CDI **[28]**.

Kim et al concluded that CDI was not that rare cause of flare-up in patients with UC in Korea. However, CDI did not appear to influence the course of UC flare-up among Korean patients **[29]**.

D'Aoust J et al identified 70 articles including a total of 932141 IBD patients or IBD-related hospitalizations, reporting that in IBD, CDI could be associated by increased morbidity, with subsequent escalation in IBD medical therapy, urgent colectomy and increased hospitalization, as well as excess mortality **[30]**.

CONCLUSION

IBD has been found to be associated with CDI. The prevalence of toxigenic CDI was 20% among studied IBD patients and significantly associated with initial disease activity.

RECOMMENDATIONS

Further investigations about the trigger of IBD associated with CDI are needed. Also more research is needed including larger number of patients to show the impact of immunosuppressant as part of treatment of IBD on vulnerability to CDI.

Ethical approval and informed consent: The Ethics Review Board of the Faculty of Medicine, Alexandria University, approved the study. Informed consent was obtained from all patients.

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