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Original Article

Microbiology Section

Fungal Culture Positivity in Patients with Perforation Peritonitis

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ABSTRACT

Background: Perforation peritonitis is the most common surgical emergency. A large number of microorganisms have been cultured from the abdominal fluid obtained from patients with gastrointestinal perforation peritonitis. The present study was undertaken to determine the frequency of positive fungal culture in perforation peritonitis as *Candida* co-infection is reported to be a bad prognostic factor in these patients.

Materials and Methods: The intraoperative specimens of abdominal fluid collected during laparotomy from 140 consecutive patients of gastro-intestinal perforation were analysed by microbial culture for bacteria and fungi. Their antimicrobial susceptibility was also studied.

Results: The mean presenting age of the patients was 35 years and 120 (85.7%) of them were males. Aerobic Gram Negative Bacilli (AGNB) were observed in 82 (79.6%) of the culture positive abdominal fluid specimens, of which 58 (70.7%) were *Escherichia coli*. Gram negative bacteria were most frequently isolated from colorectal perforation (100%) while Gram positive bacteria were from upper gastrointestinal perforation (47.2%). *Candida* was cultured in as many as 68 of 140 (48.6%) specimens. Its prevalence was highest in patients with gastroduodenal perforation (70.5%) and was altogether absent in patients having appendicular perforation.

Conclusion: High prevalence of fungal culture positivity of peritoneal fluid of patients of perforation peritonitis shows that along with the bacterial culture, fungal cultures should always be asked for in such patients. Adequate and timely antimicrobial treatment including treatment of fungal infection could help reduce mortality in this group of patients.

Keywords: *Candida*, Intra-abdominal flora, Prognosis,Significance

INTRODUCTION

Perforation peritonitis, the most common surgical emergency is associated with a high degree of morbidity and mortality. The reported mortality rate ranges between 17% and 63% [1-6]. The contaminating micro-organisms responsible for peritonitis with hollow viscous perforation are frequently polymicrobial and diverse [7]. Until recently, the leading pathogens were gram negative bacilli and anaerobic bacteria [8]. Of late, fungal micro-organisms (*Candida*) are being reported with increasing frequency [9]. Different studies show that the prevalence of different micro-organisms in intestinal perforation peritonitis varies with geographical area, patient profile and location of the perforation. As there is a paucity of similar studies from North-west Punjab, the present study was undertaken to determine the frequency of positive fungal culture and other micro-organisms involved in perforation peritonitis. This would help in the early initiation of adequate management of these patients.

MATERIALS AND METHODS

The study was conducted on 140 consecutive patients of gastro-intestinal perforation admitted during the period of two and a half years (January 2011 to June 2013) in Guru Gobind Singh Medical College & Hospital, Faridkot, India, after taking approval from the ethical committee. The inclusion criterion was any patient undergoing exploratory laparotomy for gastro-intestinal perforation except for those who presented with primary peritonitis or peritonitis due to trauma, patients on antifungal treatment before the surgery and patients less than 5 years of age. Preoperative clinical factors recorded were duration of fever, duration of abdominal pain, preoperative use of antibiotics, Preoperative medical conditions, preexisting malignancy and history of drug addiction.

Microbiological sampling

The intraoperative specimens of abdominal fluid were collected during laparotomy in sterile containers using all aseptic precautions. The specimens were immediately transferred to microbiology laboratory. In the laboratory, culture for aerobic bacteria and fungi was done. For bacterial isolation Blood agar and MacConkey agar was used and the plates were incubated at 37°C for 24 hours. Cultures positive for bacterial growth were identified by standard microbiological methods. Antimicrobial susceptibility of the identified bacteria was performed by using Kirby Bauer Disc Diffusion method following CLSI guidelines [10].

Sabouraud dextrose agar (SDA) was used as selective medium for isolation of fungi and incubation was done at 37°C for 48 hours. Lactophenol Cotton Blue mount showing budding yeast cells from the colonies obtained on SDA were identified by conventional methods such as germ tube test, sugar fermentation and assimilation reactions. Further confirmation of *Candida* species and their antifungal susceptibility was done by using automated identification and antimicrobial susceptibility testing system 'Vitek-2 Compact' (bioMerieux). Data thus obtained was compiled and results were statistically analysed using Chi-square test.

RESULTS

Preoperative profile of 140 patients enrolled in the study is shown in [Table/Fig-1]. The mean presenting age of the patients was 35 years (SD 13.0). Majority of the patients (93.6%) were less than 50 years of age. One hundred and twenty (85.7%) were males and 20 (14.3%) were females. Most common comorbid condition was renal disease (12.9%) followed by drug dependence (10.7%). The [Table/Fig-2] shows that the anatomic locations of the perforations were as follows: gastroduodenal 68 (48.6%); small gut 55 (39.3%); appendicular 9 (6.4%) and large gut 8 (5.7%).

Microbiological data

Of the 140 abdominal fluid samples cultured, 103 (73.5%) were found to be positive for various bacteria and/or fungi and 37 (26.5%) were sterile. Sixty of the 103 (58.2%) specimens showed growth of multiple microorganisms; pure growth of *Candida* was obtained in 9 (8.7%).

Parameters	Number of patients (%)
Age	
<50 years	131 (93.6)
>50 years	9 (6.4)
Sex	
Male	120 (85.7)
Female	20 (14.3)
Pre- existing comorbid conditions	
Hypertension	6 (4.3)
Diabetes mellitus	14 (10)
Renal disease	18 (12.9)
Ischaemic heart disease	3 (2.1)
HIV infection	3 (2.1)
Chronic alcoholism	5 (3.6)
Drug dependence	15 (10.7)

[Table/Fig-1]: Preoperative profile of 140 patients

Microorganism	Location of perforation			Total
	Upper (gastroduodenal, jejunal and ileal) n=123	Lower (large gut) n=8	Appendicitis n=9	
AGNB	N=66 (53.6%)	N=8(100%)	N=8(88.9%)	82
E. coli	48(39%)	5(62.5%)	5(55.5%)	58
Klebsiella	17(13.8%)	1(12.5%)	1(11.1%)	19
Enterobacter	-	-	2(22.2%)	2
Pseudomonas	-	2(25%)	-	2
GPC	58(47.2%)	3(37.5%)	3(33.3%)	64
Yeast	66(53.6%)	2(25%)	-	68
C.albicans	51(41.5%)	1(12.5%)	-	52
Non albicans *Candida*	15(12.1%)	1(12.5%)	-	16
C. krusei	8(6.5%)	1(12.5%)		
C. tropicalis	4(3.2%)			
C. glabrata	3(2.4%)			

[Table/Fig-2]: Microorganisms isolated as per location of perforation
Percentage in parenthesis

Variables	Fungal positive (n=68)		Fungal negative (n=72)		p-value
	No.	%age	No.	%age	
Superficial Surgical Site Infection*	52	76.5	29	40.3	< 0.0001
Deep Surgical Site Infection#	40	58.8	18	25	< 0.0001
Residual Abscess$	18	26.5	4	5.6	0.0009
ICU stay (>5 days)	28	41.2	10	13.9	0.0003
Hospital stay (>15 days)	49	72.1	22	30.6	< 0.0001
Mortality	10	14.7	2	2.8	0.0148

[Table/Fig-3]: Relationship of fungal culture to various parameters
*Involving only skin and subcutaneous tissue of the incision
#involving deep soft tissue (fascia, muscle) of the incision
$Abscess recurring at the site of a former abscess resulting from persistence of microbes and pus

The type of microorganisms isolated varied as per location of the perforation [Table/Fig-2]. In all, aerobic Gram Negative Bacilli (AGNB) were observed in 82 (79.6%) of the 103 culture positive abdominal fluid specimens, of which 58 (70.7%) were *Escherichia coli*. The gram negative bacteria were most frequently isolated from colorectal perforations 100% (8/8) followed by perforated appendicitis 88.9% (8/9). The results of their antimicrobial susceptibility showed that all the (100%) AGNB isolates were susceptible to aminoglycosides and 50% each to piperacillin-tazobactam and cefoperazone-sulbactam. But no strain was found to be susceptible to any of the quinolones and cephalosporins.

Gram positive bacteria were cultured most frequently from upper gastrointestinal perforations 47.2% (58/123). No strain was resistant to vancomycin. The susceptibility was highest against aminoglycosides (100%) followed by ciprofloxacin (78%) and erythromycin (72%).

Candida was cultured in as many as 68 of 140 (48.6%) specimens. The prevalence of *Candida* in abdominal fluid cultures was highest in patients with gastroduodenal perforation 70.5% (48/68) followed by small intestinal perforation 32.8% (18/55). It was not at all isolated from specimens of patients having appendicular perforation. It was obtained in combination with various bacteria in 59 (57.2%) of the culture positive specimens and as pure growth in 9 (8.7%). Their speciation showed that 52 (76.5%) of the *Candida* isolates were *C. albicans* and 16 (23.5%) were non albicans *Candida* species. Various non albicans *candida* species were *C. krusei* 8 (6.5%), C. tropicalis 4 (3.2%) and *C. glabrata* 3 (2.4%). All the *Candida* albicans, *C.tropicalis* and *C. glabrata* isolates were sensitive to fluconazole, flucytosine, amphotericin B, caspofungin and voriconazole. *C. krusei* was also found to be sensitive to these antifungal agents except fluconazole (inherent resistance).

Relationship of fungal culture to surgical site infection, residual abscess, ICU stay, hospital stay and mortality is shown in [Table/Fig-3].

DISCUSSION

Perforation peritonitis is a frequently encountered surgical emergency. In tropical countries like India, it commonly affects young men in the prime of life in comparison to the studies from the west [11] where the mean age is between 45–60 years. The mean presenting age in the present study was 35 years (SD 13) which collaborates with another study from India [12]. Worldwide there is predominance of males presenting with this life-threatening problem [12-14]; our study also shows a similar trend, with a male to female ratio of 6:1.

The gastrointestinal tract is a major reservoir of microorganisms and an important portal for intra abdominal infections and sepsis. Results of the present study revealed that the composition of the microbial flora in the abdominal fluid varied depending on the location of the perforation. From the upper gastrointestinal perforation high prevalence of AGNB (53.6%) & GPC (47.2%) was observed along with 53.6% prevalence of *Candida*. From the lower digestive tract more of AGNB (100%) and GPC (37.5%) were isolated than *Candida* (25%). *Candida* was not isolated from appendicular perforation. These results are in concordance with the study of Ruiter et al., who had also reported maximum isolation of *Candida* from gastric perforation (41%) followed by small gut perforation (34.1%) [15]. *Candida* was not isolated from appendicular perforation in their study too.

In all, *Candida* was recovered from as many as 48.6% (68/140) of intraoperative intra-abdominal specimens. The isolation rate of *Candida* in different studies varies considerably. *Candida* has apparently not been detected in some of the studies [16,17] while in others their isolation rate ranged from 1% to 38% of patients with secondary peritonitis [1,2,18-20]. The differences in the rate of fungal isolation in different studies could be because of different patient populations studied, differences in the microbiological methods used for the isolation of fungus or a combination of both. There are two important reasons for the high recovery rate of *Candida* in our study. Firstly, all the specimens were cultivated on a selective yeast medium (Sabouraud's Agar). Specimens of peritoneal fluid after an abdominal perforation nearly always consist of a mixture of different aerobic and anaerobic bacteria and in such situations, the isolation of *Candida* may easily be missed if only culture media for bacterial isolation are used. Secondly, majority of our patients reported history of long duration of intake of antibiotics. Prolonged treatment with broad spectrum antibiotics gives the yeasts (*Candida*) a further growth advantage.

As a commensal of the digestive tract, *Candida* may leak into the peritoneal cavity after perforation of a hollow viscus or surgical

section of the intestinal wall. However, under most circumstances, *Candida* will be cleared quickly from the peritoneum. Nevertheless, in some patients, peritoneal seeding could result in the development of an intra-abdominal *Candida* infection, with a risk of dissemination to the bloodstream and to extra-abdominal tissues and organs [21].

The question remains of whether routine antifungal therapy would benefit the patients with perforation peritonitis. In our study, patients with positive fungal culture had higher incidence of surgical site infection, residual abscess formation, longer ICU stay, longer hospital stay and higher mortality rates in comparison to fungal culture negative patients and results were statistically significant (p- value = <0.0001, <0.0001, 0.0009, 0.0003, <0.0001, 0.0148 respectively). These patients may be considered for early antifungal therapy which could minimise the overall morbidity and mortality. However, as *Candida* was not at all isolated in appendicular perforation, this suggests that *Candida* is not an important pathogen in appendicitis and antifungal treatment is unnecessary in perforated appendicitis. This is in agreement with other studies too [15,22]. Here it is important to mention that before initiating antifungal treatment, the isolated *candida* strain should be speciated and subjected to antifungal susceptibility testing as some of the species (e.g. *C krusei*) are inherently resistant to azoles.

CONCLUSION

It can thus be concluded that bacterial as well as fungal cultures and antimicrobial sensitivities of peritoneal fluid specimens are imperative for the treatment of patients of perforation peritonitis. As there is high prevalence of positive peritoneal fluid fungal cultures and fungus being a significant risk factor for adverse outcome in these patients, surgeons should be made aware of the usefulness of the prophylactic antifungal therapy, especially in patients with upper gastrointestinal perforation.

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