Bacteroides spp. isolation from pus or peritoneal fluid in patients with perforated peptic ulcer.

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الخلاصة

خلال الفترة ما بين تشرين الأول 2001وتشرين الثاني 2007تمت دراسة مائتين وثمانية وثمانون مريضا يشكون من اختراق أو انفجار قرحة المعدة أو الاثني عشري و الذين تمت معالجتهم في مستشفى بغداد والديوانية التعليمي بواسطة التداخل الجراحي عن طريق فتح البطن وفيها أخذت عينات من سوائل أو قيح بريتوني لغرض إيجاد نسبة الاستعمار أو الالتهاب ببكتيريا البكتيرويد اللاهوائية ولدراسة العلاقة بينها وبين بعض العوامل المساندة التي قد تتسبب في زيادة هذه النسبة لتكون كقاعدة لتحديد نوعية العلاج الوقائي من حالات التهاب الجروح وتسمم الدم الجرثومي واظهرت النتائج أن 48.6% من المرضى في الدراسة هم ذوي زروعات ايجابية للبكتيرويدأما العوامل التي قد تسبب زيادة نسبة الايجابية في الزروعات هي الوقت أو عدد الساعات منذ بدء الانفجار حتى ساعة أجراء العملية أو اخذ العينات ووجد أن الوقت هو عامل مهم إذا كان أكثر من 12 ساعة .العامل الآخر هو تعاطي بعض الأدوية قبل الجراحة مثل H2 blockers أو PPI أو الستيرويد ووجد إنها تؤثر في زيادة وبشكل معنوي في نتائج الزروعات الايجابية .تمريض من العوامل التي تمت دراستها ووجد أن الزروعات الايجابية هي في غالب المرضى التي تتجاوز أعمارهم الخمسين سنة ,أما بالنسبة إلى الأمراض المرافقة كالسكري وهبوط المناعة لم نلاحظ علاقة إحصائية معنويه في زيادة نسبة الاستعمار أو الالتهاب .

Summary

From October 2001 till November 2007,two hundred eighty eight patients with perforated peptic ulcer including duodenal and gastric ulcer attending Baghdad teaching hospital and Al-Diwanyia teaching hospital were subjected for surgery and samples from free peritoneal fluid to obtain specimens for determining the rate of peritoneal colonization by ***bacteroides spp***.

and the risk factors which may increase this rate by direct questionnaire to obtain preliminary guides for methods of prophylaxis against subsequent possible wound infection and systemic sepsis .Specimens was obtained using sterile syringe aspirating about 2-5 ml of pus or fluid which is air free Diagnostic confirmatory tests for ***Bacteroides spp***. identification

has been made by anaerobic culture utilizing selective complex medium like brain heart agar containing amikacin which inhibit growth of facultative anaerobes. Culture were incubated at 35-37c in an anaerobic atmosphere containing CO2.

Results showed that 48.6% of the studied patients were found to be ***Bacteroides*** culture positive . Some risk factors have been evaluated which may or not contribute to increasing the rate of ***Bacteroides spp.*** Colonization, among these ,time from onset of perforation till the time of surgery or sampling which was found to be a significant risk factor if the patient underwent surgery after 12hr from onset of perforation 72.5% .

Age was individually evaluated , it has appeared that increasing age is a risk factor and become significant after 50 years of age with 83.7% of patients in this age group was colonized with ***Bacteroides spp.***

The associated other medical illness , steroids consumption and immune suppression were also evaluated in which only steroids found to be a significant risk factor with a rate approaching 80.4%.

Other risk factors which has been evaluated is the preoperative use of H2 blockers or proton pump inhibitors , which found to significantly increase the rate of bacteroides isolation(80%.).

Introduction

Anaerobic bacteria are found through out the human body, and especially in high concentration in the mouth and gastrointestinal tract as part of normal flora {1},in the colon they are greatly out number the aerobic bacteria {2}.

Infection result when anaerobic and other bacteria of the normal flora contaminates normally sterile body sites {3}.

The ***Bacteroides*** species are obligate anaerobes which are very important pathogen that cause human infection {1, 5}.

The most commonly isolated bacteria are members of ***bacteroides fragilis*** group ***(B fragilis, B.ovatus, B.distasonus, B.vulgatus, B.thetaiotaemicrons***){1,2,4}, particularly from

infection associated with contamination by the bowel where they may cause suppuration eg. Peritonitis after ruptured viscus{3, 4}.

The pathogen city of bacteroides depend on its virulent factors including capsular polysaccharide which induces abscess formation and its lipopolysaccharide (endotoxin)which less toxic than those of other gram negative bacteria {5,6,10}.

The morbidity and mortality of intra abdominal infection vary dramatically with the level of gastrointestinal perforation {2,15}.

Under normal circumstances the stomach contains fewer than 10^3 bacteria per ml^3 , largely because the action of hydrochloric acid , and when patient use H2 blockers or proton pump inhibitors that raise the gastric pH the no. of bacteria rapidly approaches the level seen in the small bowel which is approximately 10^8 including many organisms usually prevalent in faces {2,7,8}.
Infection after peptic ulcer perforation present acutely , and the patient is commonly able to give the exact time at which the perforation occurred {2,3,15} . This form of peritonitis is initially chemical , but with time it becomes infected by various endogenous bacteria , the no. and type of which has a direct relation to the time and the preoperative use of H2 blockers or PPI.{2,4,6,7,8}.

Aim of the study

To find out the rate of peritoneal colonization or infection by ***Bacteroides spp***. following gastro-duodenal ulcer perforation and the risk factors that might be associated with increase of this rate .

Patient and method

During the period from October 2001 till November 2007 two hundred eighty eight patient with perforated peptic ulcer (gastric and duodenal) attending Baghdad teaching hospital and Al diwaniya teaching hospital were subjected to surgery inform of laparotomy and surgical management . During surgery samples from free peritoneal fluid or pus was obtained to asses the rate of ***Bacteroides spp,*** colonization or infection of the peritoneal cavity.

All patient were assessed post operatively by direct history taking regarding some risk factors which might or not contribute to increasing rate of ***Bacteroides spp***. isolation , including the preoperative use of H2 blockers or PPI , chronic steroid use , the preoperative existence of co-morbid variables like diabetes mellitus or other immune suppression(leukemia , lymphoma , or immune suppresive drugs).

Method

Sample collection : Specimens of peritoneal fluid or pus was obtained immediately following laparotomy using a sterile syringe aspirating about 2-5 ml of pus or fluid which is air free . The specimens was immediately transported to the laboratory in average time 30-120 min .In cases when samples can not be transferred to the media expeditiously, 2ml of pus or fluid is injected into a sealed anaerobic tube (port –a-cult) and kept over night at room temperature {1,2,5,9}.

Cultivation: The material was plated on brain heart infusion agar or brucella blood agar which is highly enriched with hemin , vitamin K and blood . Anaerobic blood agar plate containing Amicacin,kanamycin or vancomycin to inhibit the growth of facultative anaerobes and permit growth of obligate anaerobes .{1,5,4}.The anaerobic plates were incubated in a Gas pak jars in 35-37c which provide an anaerobic atmosphere containing co2 , and examined at 48 and 96 hrs {11,14,15}.anaerobes especially bacteroides fragilis species were identified by colony characteristics of which that appear 1-3 mm diameter ,convex, smooth, white to gray non haemolytic , non pigmented colonies

Direct microscopy of members of bacteroides fragilis are pale staining gram negative bacilli with rounded cell.{1,5,11,12,13}.

Statistical analysis: The data were analyzed by EPI ver6.2softwre system the statistical significance of the calculated estimate of the risk (OR) was assessed by chi square test. P value less than the 0.05 level of significance considered statistically significant, (reference*) referred to the group with which other groups are compared.

Results

Table 1: demonstrate the overall rate of ***bacteroides spp.*** isolation in which the rate reach 48.6%of the studied population .

Table 1: Rate of ***Bacteroides spp.*** Isolation from peritoneal fluid or pus in patients with perforated peptic ulcer .

	Culture +ve	Culture - ve	total
No.	140	148	280
%	48.6	51.4	100

Table 2: demonstrate the rate of *Bacteroides spp.* isolation in relation to the preoperative use of H2 blockers or PPI in which the rate was found significantly higher 80%in patients who use these drugs.

Table 2: ***Bacteroides spp.*** Isolation according to the use of antisecratory agents

Bacteroide Isolation	Positive		Negative		Total
Positive	No.	%	No.	%	140
	120	80	20	14.5	
Negative	No.	%	No.	%	148
	30	20	118	85.5	
total	150		138		288
OR=Pvalue	0.04 significant				

Table 3: Shows the relation between the rate of *Bacteroides spp.* isolation to the time elapse between onset of perforation and time of surgery or sampling in which the rate found significantly higher 72.5% in patient who present late more than 12hrs from onset of perforation and only 27.5%of patients who present more than 12hrs are culture negative .

Table 3: rate of ***Bacteroides spp***. isolation according to the time elapse from onset of perforation to the time of surgery

Bacteroid isolation	Time in hours .						Total
	0 - 6		6 - 12		> 12		
	No	%	No	%	No	%	
+ ve	2	5.1	17	20.7	121	72.5	140
- ve	37	94.9	65	79.3	46	27.5	148
Total	39	100	82	100	167	100	288
OR= P value	reference *		0.2 not significant				o.o2 significant

Table 4: Demonstrate the rate of *bacteroides spp.* isolation in relation to age in which the rate appear significantly higher in patients who are older than 50 years 83.7%

Table 4. rate of ***bacteroides spp***. isolation according to the age of the patients

Bacteroides Isolation	age in years						Total
	18 - 35		35 – 50		50>		
	NO	%	NO	%	NO	%	
+ ve	17	14.9	41	35.9	82	83.7	140
- ve	149	85	35	46.1	16	16.3	148
Total	114	100	76	100	98	100	288
OR= P value	Reference *		0.1 Not significant		0.03 Significant		

Table 5: demonstrate the rate of *bacteroides spp.* isolation in relation to the preoperative existence of medical illness of which only chronic steroid use increase the rate of bacteroides isolation .

Table 5: Rate of ***bacteroides spp*** isolation in relation to certain medical illness

Bacteroide Isolation	medical problem								Total
	None		D M		Steroid		Immune suppression		
+ ve	No	%	No	%	No	%	No	%	140
	19	15.2	27	61.4	90	80.4	4	57.1	
-ve	106	84.8	17	38.6	22	19.6	3	42.9	148
Total	125	100	44	100	112	100	7	100	288
OR= P value	Reference *		0.1 Not significant		0.04 Significant		0.1 Not significant		

Discussion

Perforation of peptic ulcer occur in 3-10% of patients with peptic ulcer , which will produces chemical peritonitis initially but after few hours ultimately becomes infected and bacterial peritonitis ensue {2,6,4} . the infection is usually mixed due to synergetic aerobic gram –ve bacilli and anaerobic bacteria (***bacteroides fragilis*** group {4}.In our study ,the overall rate of bacteroides isolation was 48.6% , Which is higher than other studies 22.1%{ 17} , this may be attributed to the late presentation of most patients . The relation between bacteroid isolation and the time elapse from onset of perforation to the time of surgery was also evaluated , and it has been found that , late presentation More than 12hr significantly increase the rate 72.5% as compared to 27.5% who are ***Bacteroides*** culture –ve , this might be attributed to the time allowed for bacteria to proliferates while no action done or antibiotic administered {2,3,6}. Age is one of the parameters evaluated in our study and it is found that increasing age increase the rate of ***bacteroides spp***. colonization and become significant in older patients (more than 50) , in whom the rate approach 83.7 %, again this may be attributed to late surgery due to a combination of obesity and most surgeons

reluctant to do surgery earlier because of minimal and masked features of peritonitis {2,3,15}. . Associated medical illness like diabetes , preoperative chronic use of steroids , and other immune suppression were evaluated individually among which only steroids use found to significantly increase the rate of bacteroides isolation 80.4 % and un doubtfully the rate of peptic ulcer perforation {2,15}, this may be attributed to lower immunity and possible change in the no. and type of microflora {2,15,16}.

Among the most important and well studied factors that increase the intra gastric bacterial growth and may change the type of bacterial microflora is the use of H2 blockers or PPI which in our study found to be a significant factor that increase the rate of ***bacteroides spp***. isolation this certainly due to the increase of intra gastric pH {2,7,8,15}.

Conclusion

Bacteroides spp are important pathogen following peptic ulcer perforation and especially significant in late presentation, in older age group, those patients who use steroids and in most patients who use PPI or H2 blockers preoperatively.

Recommendation

We recommend to add an antibiotic effective against ***bacteroides*** of which the most effective, safe and cheep is metronidazol or clindamycin (as a second choice) as a prophylactic or therapeutic measures {2, 14, 15}.

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