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ORIGINAL ARTICLE

BACTERIAL TRANSLOCATION IN AN EXPERIMENTAL INTESTINAL OBSTRUCTION MODEL. C-REACTIVE PROTEIN RELIABILITY?

By
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Aim: *Bacterial translocation occurs in preseptic conditions such as intestinal obstruction. CRP is a marker of ischaemia.*
Methods: *45 albino male rats were divided into 3 groups (15 each). GI control, GII simple intestinal-obstruction and GIII strangulated obstruction. Outcome measures were: (1) Bacteriologic count & typing for intestinal contents, intestinal wall, liver, mesenteric lymph nodes and blood (cardiac & portal) (2) Histopathologic: mucosal injury score, inflammatory cell infiltrate in the wall, MLN, liver, (3) Biochemical: serum CRP, IL-10, mucosal stress pattern (glutathione peroxidase-malonyldialdhyde tissue levels).*
Results: *(1) Intestinal obstruction associates with BT precursors (Bact-overgrowth, mucosal-acidosis, immuno-incomptence), (2) Bacterial translocation (frequency & density) was found higher in strangulated I.O, that was mainly enteric (aerobic & anaerobic) and mostly E.coli, (3) The pathogen commonality supports the gut origin hypothesis but the systemic inflammatory response goes with the cytokine generating one. (4) The CRP median values for GI, II, III were 0.5, 6.9, 8.5 mg/L, for BT +ve 8 mg/L and 0.75 mg/L for BT –ve rats.*
Conclusion: *Bacterial translocation occurrs bi-directional (systemic-portal) in intestinal obstruction and the resultant inflammatory response pathogenesis is mostly 3 hit model. CRP is a reliable marker of BT, BT density and vascular compromise during I.O.*

Keywords: *presepsis, ischaemia, MODS.*

INTRODUCTION

Intestinal obstruction (IO) is a common lethal abdominal emergency resulting in high mortality, mostly due to multiorgan dysfunction syndrome (MODS),[1] significantly bacterial translocation (BT) together with septic peritonitis are the major contributors of MODS in IO.[2]

Early studies focused on BT as a unifying mechanism to explain MODS but recently other specific mechanisms are operational (immuno inflammatory).[3]

Bacterial translocation is precipitated by bacterial overgrowth disturbing the normal ecologic balance,[4,5] host immunedysfunction inciting pro & anti-inflammatory cytokines balance,[6] and mucosal barrier dysfunction, favoring oxidants release.[7]

Apart from computerized tomography, no reliable diagnostic test for intestinal strangulation is currently available, that is costy and not reproducible.[8]

Lastly the C-reactive protein (CRP) which is an inflammatory marker, is considered a marker of ischaemia and neovascularization,[9,10] hence validated in this study for detection of IO, IO subtypes and BT.

The aim of this Is to study the possible effects of I.O on the microbiologic ecologic balance, chemical and immunologic barriers that bring protection against B.T., to demonstrate BT during I.O (pathogen typing, routes and commonality) in addition to the BT local & systemic inflammatory response as well as CRP reliability in studying BT and I.O.

PATIENTS AND METHODS

This study was conducted in Mansoura Faculty of Medicine, Histology experimental laboratory unit from June 2003 to June 2006 after obtaining the required authorities permissions. It entails 45 albino male rats divided into 3 group each 15 rats. Group I as a control, Group II represents simple I.O group (simple ileal ligation 5 cm proximal to caecum) and group III comprises strangulated I.O group (symmetrical ligation of a 5 cm ileal loop with its mesentry, 5 cm proximal to caecum).

Surgical maneuver: Anaesthesia with intramuscular ketamine (HOLDEN Medical Labs, Netherlands), 5 mg/kg body weight. For GII & GIII, a midline laparotomy was done after sterilization to perform the intended I.O type and layered abdominal closure using vicryl 00 (Autosuture, US).

Subsequent to recovery all groups were held in a semi-acclimitized room at 23°C (±2), both laboratory chow and tap water were allowed for 28 hours till relaparotomy (to allow for the pathological process).

In the 2nd laparotomy performed after 28 hrs (same anaesthetic procedure for the three groups). Under complete sterile conditions a thoracoabdominal generous midline incision was performed and firstly direct cardiac blood sampling, second portal venous blood sampling, thirdly left hepatic lobe resection, fourthly multiple mesenteric lymph nodes excision, and fifthly ileal segment proximal to ligature i.e obstruction level in GII, strangulated ileal loop in GIII and ileal segment in GI resection together with their luminal contents.

Lastly animals were sacrificed via cervical dislocation. Disposal was performed under the supervision of Laboratory Animal House, Mansoura University.

Laboratory studies:

I. Biochemical study: (1) Ileal loop oxidant and antioxidant activity, were studied (2) Serum CRP and IL-10 levels were assayed.

(1)a. Oxidant (Malonyldialdhyde (MDA)): The tissue samples were homogenized with 0.1 ml/L phosphate buffer saline centrifuged at 2000 rpm and MDA was detected at OD (optical density) 534 nm.[11]

(1)b. Antioxidant (Glutathione peroxidase) was measured using NAD PH oxidation principle and measured at OD 340nm (12).

(2)a. CRP semiquantitative assay was performed using latex agglutination test with normal cutoff ≤ 0.5 mg/L (Human, Germany).

(2)b. IL-10 (inerleukin-10) serum level was measured using commercially available ELISA kits (Diaclone, France).

II. Histopathologic study: (1) Ileal segments were examined to score the mucosal injury.[13] (2) Ileal segment, MLN, liver samples were examined for inflammatory cell infiltrate grading (GI: one/mm3 , GII 2-4/mm3 ,GIII: ≥ 5/mm3).[14]

III. Bacteriologic study: (1) Luminal contents, (2) Intestinal wall, MLN and liver tissues, (3) Cardiac and portal blood. Samples were collected for detection of their colony forming unit (CFU) index and bacterial species by gram stain, characteristic biochemical reaction and antibiotics susceptibility.

(1) Luminal contents were homogenized in sterile isotonic saline, plated on McConkay & Columbia blood agar (aerobic and anaerobic) (Oxid-Germany), (2) For the intestinal wall, liver, MLN. The tissues were ground in phosphate buffered saline, then plated as the contents and incubated in the Gas Park system for anaerobic culture, (3) For the blood samples they are centrifuged at 3000 rpm for 30 min and the sediments were plated as the tissues.

Statistical analysis:

- Data were computed using SPSS, version 10. Sample size was determined according to statistician's request.
- The CRP values are expressed as median, other variables as mean ± SD and the CFU are logarithmically converted.
- For comparison the Chi-square test, Mann Whitney U test and one way ANOVA test are used when applicable.
- The Pearson's correlation test was used to detect CRP levels correlation with CFU density within different tissues, meanwhile the logistic regression was used to detect the significant predictors.

RESULTS

Intestinal obstruction mainly the strangulated type is significantly associated with bacterial overgrowth, oxidative stress pattern (disproportionate MDA and GPx increase) and deranged IL-10 response (significant decrement of IL-10 levels) Table 1.

The frequency and density of BT were higher in GIII than in GII but not detected in GI, their distribution was centrifugal (lumen – wall – MLN – liver – blood) Table 2.

The bacterial translocation was commonly polymicrobial (49% in GII – 60% in GIII), mostly enteric with substantial anaerobic ratio and E.coli predominance (42.2% in GII & 45.5% in GIII) Table 3.

between lumen and wall (74.0 %), lowest between lumen and blood (11.1 %) and in the strangulated group was highest between lumen and wall (66.6 %), lowest between lumen and systemic blood (4.7 %).

Mucosal injury score and inflammatory cell infiltrate were significantly higher in GIII (Fig 1), also remote inflammatory response in liver and MLN in the form of inflammatory cell infiltrate (PNL) was higher in GIII Table 4.

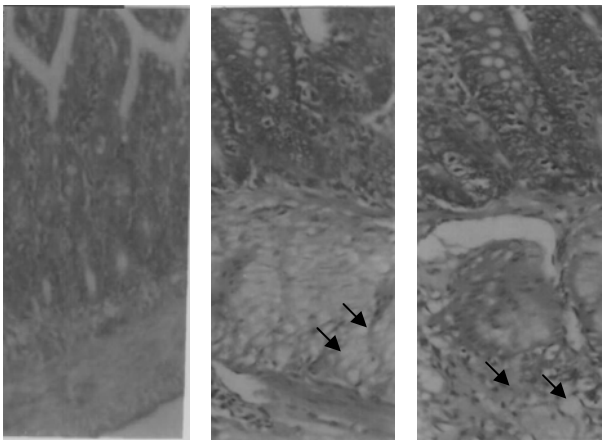

Fig 1. Photomicrographs of the local inflammatory response in the studied groups (100X).(A) Group I: no cellular infiltrate. (B) Group II: mild cellular infiltrate. (C) Group III: abundant cellular infiltrate.

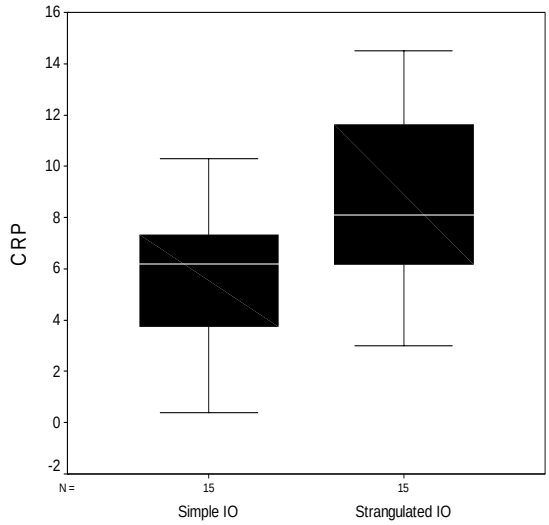

Fig 2. Box blot of CRP level in simple and strangulated intest. Obst.

The CRP median value was significantly higher in I.O and its subtypes than control Table 5. with higher 75% percentile ratio in GIII than in GII (Fig 2) but the cutoff value failed to predlict to either groups (GII & GIII in Table 5. resulting in average accurate value in I.O, subtypes detection (in simple obstruction: 56.8 %- in strangulated obstruction: 46.6 %). But the logistic regression defined the CRP value as a significant predictor of strangulated I.O [P = 0.026* OR = 1.726, 95% CI (2.78 – 0.069)].

The CRP median value was significantly higher in cases of BT during I.O compared to –ve BT Table 6. and its cutoff level significantly predicted to –ve cases resulting in good exclusion power on validation (sensitivity: 77.7 %, specificity: 33.3 %, accuracy: 66.6 %), also the logistic regression defined CRP as a significant predictor of BT during I.O [(P = 0.002*** OR = 3.074 and 95% CI (6.3 – 1.492)].

The density of BT was correlated to CRP levels in the studied tissues whether simple or strangulated obstruction (Fig 3).

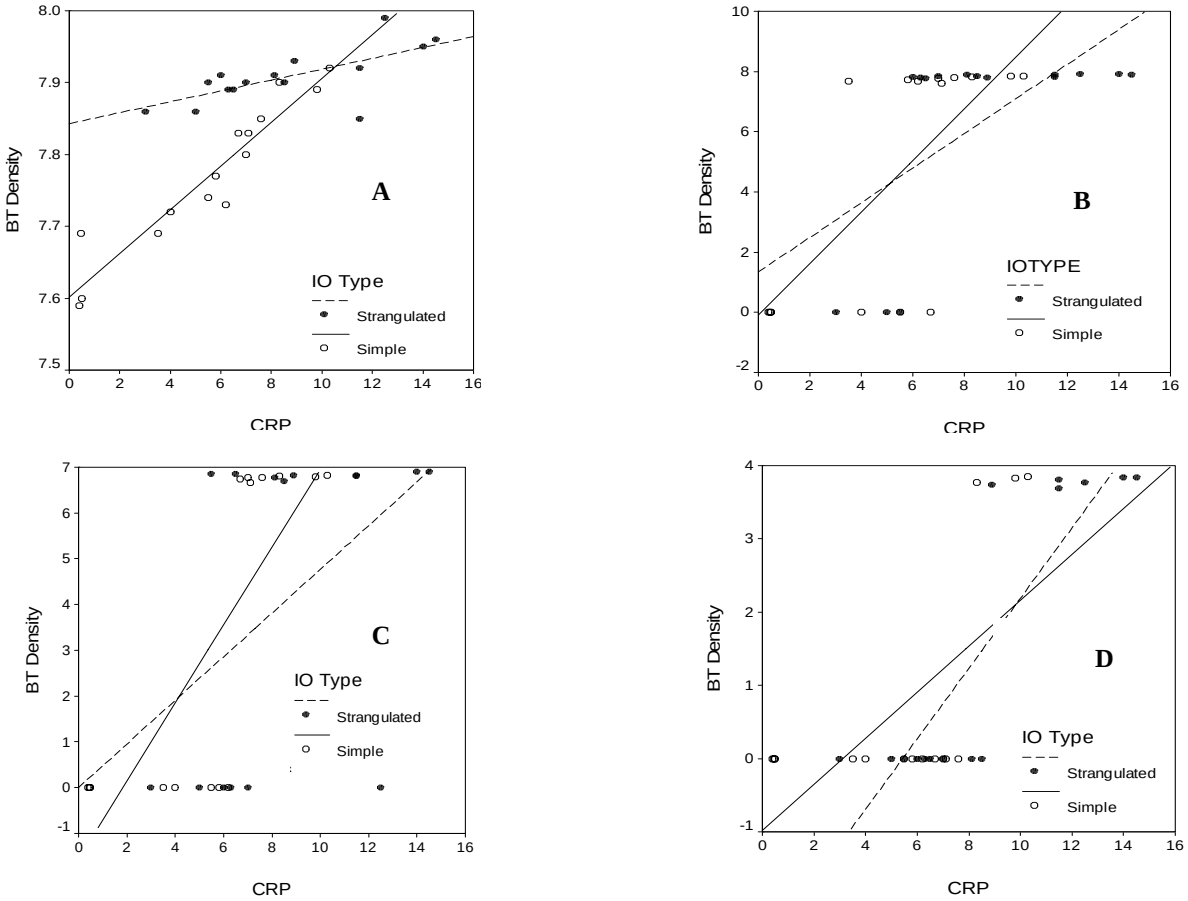

Fig 3. Scatter plots showing relation between logarithmic BT density and CRP level in both simple and strangulated I.O within the wall (a) [strangulated: r=0.675, p=0.006; simple:r=0.962, p= 0.0001], MLN (b) [strangulated: r=0.604, p=0.017 simple:r=0.82, p=0.0001], liver (c) [strangulated: r=0.472, p=0.046; simple: r=0.772, p= 0.001] and systemic blood (d) [strangulated: r=0.872, p=0.0001; simple: 0.744, p= 0.001].

Table 1. Bacterial translocation predisposing factors among the studied groups.

	Bact. count lumenal	**Intestinal wall mucosal stress**		**Serum IL-10**
	CFU/gm mean	**MDA nanomol/mg protein mean ± SD**	**GPx unit/mg protein mean ± SD**	**pg/ml mean ± SD**
GI	8.8 x 108	5.4 ± 0.4	125 ± 31	50 ± 16
GII	5.6 x 1010	11.9 ± 1.9	165 ± 41	36 ± 11.9
GIII	1.4 x 1012	21.9 ± 1.2	150 ± 37	9 ± 2.8
P value	< 0.05*	< 0.05*	< 0.05*	< 0.05*
P1	< 0.05*	< 0.05*	< 0.05*	< 0.05*
P2	< 0.05*	< 0.05*	< 0.05*	< 0.05*
P3	< 0.05*	< 0.05*	> 0.05	< 0.05*

P: One Way ANOVA test for all groups, P1: GI vs GII, P2: GI vs GIII, P3: GII vs GIII.
CFU: Colony Forming Unit. MDA: Malonyldialdhyde. GPx: Glutathione Perioxidase. IL-10: Interlekin 10.

Table 2. Frequency and density of bacterial translocation in the tissues among the studied groups.

	Wall		MLN		Liver		Systemic		Portal	
	No (%)	Mean 10^7 log	No (%)	Mean 10^7 log	No (%)	Mean 10^7 log	No (%)	Mean 10^3 log	No (%)	Mean 10^3 log
GI	-	-	-	-	-	-	-	-	-	-
GII	12 (80)	7.77	9 (60)	7.15	7 (46.6)	6.78	3 (20)	3.81	-	-
GIII	15 (100)	7.9	12 (80)	7.85	10 (66.6)	6.83	6 (40)	3.78	3 (20)	3.93

The mean density of BT is logarithmically expressed.

Table 3. Frequency and types of isolated organisms.

	GII I.O									GIII I.O								
Isolates	**No**									**No**								
Enteric(E) & Non-enteric NE)	**64**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**84**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Luminal	20	9	1	2	3	2	3	2	3	21	10	3	3	3	3	4	4	3
Wall	16	7	1	1	2	2	3	2	3	19	9	-	2	2	2	2	2	1
MLN	13	5	-	1	1	-	1	1	1	17	8	-	2	1	2	1	3	1
Liver	11	4	-	-	1	-	-	-	1	15	6	1	1	1	-	-	1	1
BL	4	2	-	1	1	-	-	1	-	8	3	1	1	1	-	-	2	-
Portal	0	-	-	-	-	-	-	-	-	4	2	-	1	-	-	-	1	-

Enteric (E) 1. E.coli 2. Klebsila oxytoca 3. Enterococcusfeacalis 4. Enterobacter 5. Clostrodium 6. Bacteroids
Non-enteric (NE) 7. Coagul –ve staph 8. Pseudomonas
* E.coli in GII 42.2% & GIII 45.5%
* Polymicro in GII 49% & GIII 60%

Table 4. The local and remote inflammatory response.

	Wall				Liver			MLN	
	Score of mucosal injury	cellular infiltrate			cellular infiltrate			Hyperplasia	
		1	2	3	1	2	3	+	–
GI	0.9	1	11	3	12	2	1	1	14
GII	2.4	1	5	9	1	4	10	9	6
GIII	4.3	0	1	14	0	0	15	15	0
P value	< 0.05*		< 0.05*			< 0.05*		> 0.05	

One Way ANOVA test.

Table 5. CRP median value and frequency of positive cases (cutoff level 0.5 mg%).

		Median value	
G I	n 15	0.5	
G II	n 15	6.9	
G III	n 15	8.5	
G II + III	n 30	7.0	
P1	GI vs GII	# 0.0001 ***	
P2	GI vs GIII	0.0001 ****	
P3	GI vs GIII	0.02 *	
P4	GI vs GII+GIII	0.0001 ***	
		No	%
GII		12	80
GIII		15	100
P value		## 0.23	

Mann-Whitney test.
Chi-square test.
P < 0.05*: significant, P < 0.01**: highly significant, P< 0.001***: extremely signifjcnat.

Table 6. CRP relation with bacterial translocation in intestinal obstruction and frequency of positive cases.

				Median CRP		P value
B.T	BT	+	n 22	8.0 #		0.0001***
		–	n 8	0.75		
GII.I.O	BT	+	n 10	7.25		0.001**
		–	n 5	0.5		
GIII.I.O	BT	+	n 12	12.5		0.004**
		–	n 3	0.50		
				No	%	P
	BT	+	n 22	17 ##	77	< 0.05*
		–	n 8	2	25	

Mann Whitney U test.
Chi-Square test.
P < 0.05*: significant,P < 0.01**: highly significant, P< 0.001***: extremely signifjcnat.

DISCUSSION

Likely to the disturbed dynamic milieu of the intestinal tract during intestinal obstruction, this study defined bacterial overgrowth association with I.O in line with other authors,[15,16] also the oxidative stress pattern during I.O produce ATP depletion,[17] cytoskeleton disruption,[18,19] neutrophil priming[20] resulting in mechanical gut barrier dysfunction, and significantly the I.O association with impaired IL-10 response as reported by a previous report[21] and Souza et al.,[22] declared the immune incompetence status. Others[23] deny those associations.

In accordance with many researchers,[24-26,22] BT occurred during I.O as reported here, and the centripetal decrement in the frequency and density of the pathogens supports tissue colonization is gut derived not blood derived. This study also defined BT is bidirectional during I.O specially in the ischaemic variant as reported by Wells et al.[27] and Mainous et al.[28] and that is related to direct intestinal wall insult.

Further support for the gut origin hypothesis during I.O from this study is the enteric bacteria predominance as detected by some authors.[29,30] Moreover, E.coli outnumber other pathogens and this is mostly related to its facultative nature and its fimbriated surface i.e. colonizing factor thus supporting lymphatic route for BT. Furthermore, the

obligate anaerobic organisms isolates detection as reported by Boedeker,[31] O'Boyle et al.[32] and Cevikel et al.[1] define the colonization resistance failure in the ischaemic intestinal obstruction. So bacterial overgrowth, bacterial virulence and wall integrity (structure and function) work together.

In this study the commonality of pathogens define the gut-origin hypothesis, provides additional support for local transmural route (Phagocytes or enterocytes) in line with many researchers[4,29,30] who defined lymphatic route predominance in the simple I.O and venous portal predominance in the ischaemic variant as found by previous works.[33-35,26]

The observed local and systemic immunoinflamamtory histopathologic changes as Akcay et al.[24] reported, might be related to cytokines release producing inflammatory cell influx resulting in tissue injury supporting the cytokine generating hypothesis.[36] Consequently we believe the 3 hit model[2] during I.O 1st (increased intestinal pressure or ischaemia), 2nd (reperfusion injury) (increased secretion & decreased absorption – prostaglandin release – collaterals opening – distension) resulting gut barrier failure and 3rd bacterial and cytokine translocation.

Despite CRP surge during I.O, its cutoff value didn't predlict to any subtypes with consequent average accuracy in detection of I.O subtypes, so CRP is a non selective marker in suspected cases and confirming information from medical history and physical examination must be scrutinized to see if it support or contradicts CRP + ve cases. But significantly once I.O is diagnosed the CRP is a significant predictor of the ischemic variant as reported by Willet et al.[37]

The high CRP level was associated with B.T during I.O and its subtypes. The ability of its cutoff level to define BT with high sensitivity, specificity and accuracy was associated with a statistically significant predictor value in accordance with Cevikel et al.[1] making CRP a reliable test to detect BT during I.O.

Complementary to previously found the parallel relation between CRP level and BT (frequency & density) specially the ischemic variant is mostly related to the cascades of systemic inflammatory response mediators as described by Moore.[38] So, CRP can be considered a predictor of vascular compromise and BT severity.

Conclusively, intestinal obstruction by its types is associated with BT precursors. BT is functioning during I.O, has bidirectional routes most pathogens are enteric, specially E.coli with obligate anaerobe occasionally in the ischaemic variant. Bacterial overgrowth and virulence and intestinal wall structure and function work together. Both the BT and cytokine generating hypothesis are operational during I.O, and the 3 hit model is the appropriate model. The CRP is a non selective diagnostic marker in suspected cases of I.O but once diagnosed is a significant predictor of its subtypes. The CRP is a reliable test of BT during I.O. The CRP is a predictor of vascular compromise and BT severity.

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