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Effect of mechanical bowel preparation in fibroblast, collagen density and histopathology analysis in colon anastomosis site of Wistar rat



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ABSTRACT

Backgrounds: Mechanical bowel preparation (MBP) was almost considered dogmatic in colorectal surgery. There are several methods known to perform MBP. Anastomotic leakage is considered higher in patients who had MBP, and it is thought due to alteration colonic morphologic, electrolyte and fluid imbalance.

Methods: This is an experimental study divided into two groups. This study aims to determine the difference in collagen density, amount of fibroblast and histopathologic features in the anastomotic site between Wistar rats that had MBP and without MBP to the colonic anastomosis. The first group consists of 6 Wistar rats who had colonic anastomosis without MBP, and the second group consists of 6 Wistar rats who had colonic anastomosis with BMP. On the 10th day after surgery, histopathology examination is performed with regards to collagen

density, the number of fibroblasts, infiltration of inflammatory cells and the degree of bowel wall damage at the anastomotic site. Independent t-test is used to analyze the data if it is normally distributed and Mann-Whitney test is used if the data is not normally distributed.

Results: The amount of fibroblast was significant difference between two groups ($p=0.02$), which is amount of fibroblast in the second group (3.83 ± 0.408) is higher than the first group (2.33 ± 0.816). Meanwhile, there is no significant difference regarding collagen density, infiltration of inflammatory cells and the degree of bowel wall damage ($p=0.59$, $p=0.082$ dan $p=1.00$).

Conclusion: The conclusion of this research is by performing MBP prior to colon anastomosis will exert the effect of more abundant fibroblast.

Keywords: mechanical bowel preparation, colonic anastomosis, alteration colonic morphology

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INTRODUCTION

Preoperative Mechanical Bowel Preparation (PMBP) procedure is the standard procedure to cleans the colon from the stool which is an important action to prevent post-surgical complications of colorectal infections.¹⁻⁴ Mechanical bowel preparation has changed drastically on the methods and materials used since first reported. The objective for mechanical bowel preparation is to evacuate the faeces to established good visualization of the intestinal lumen and to reduce the number of intra lumen pathogenic microorganisms which believed to decrease the occurrence of infection and leakage of the anastomosis.⁵ In addition, mechanical bowel preparation is also believed to facilitate surgeons in manipulating the intestines and avoiding spillage of the feces to the intra abdomen. However, this rationalization is not proven by recent research results on preoperative mechanical bowel preparation.²

A meta-analysis study by Pineda et al. showed anastomosis leakage 4.2 % in preoperative mechanical bowel preparation group compared to 3.5% in control group. Surgical site infection rate in PMBP was 9.9% compared to the lower rate of 8.8% in control group.² Similar results reported

in another meta-analysis study by Gravante et al. anastomosis leakage in PMBP group was 4.1% and 3.4% in control group. Surgical site infection rate was 9.6% in PMBP group and 8.7% in control group.⁶

Based on results from above studies, PMBP in elective colorectal surgery does not decrease complication rate for anastomosis leakage and surgical site infection. There is 2 major explanation related to the high number of anastomosis leakage in PMBP group. First, PMBP induced structural alteration and inflammation in intestine mucosal layer causing diminished of the mucus in superficial layer and increased in white blood cell with 52% polymorphonuclear cell in PMBP group compared to 8% in control group. Histological alteration in the mucosal layer of intestine causing disruption in structural integrity in anastomosis site.^{2,7} Second, alteration of fluid and electrolyte in mucosal layer also became the risk factor for anastomosis leakage.⁸

This study aims to determine the difference in collagen density, amount of fibroblast and histopathologic features in the anastomotic site in wistar rats model.

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METHODS

An experimental study with total 12 subjects divided into 2 groups consists of the control group and PMBP group. This study was performed in Pharmacology Laboratory and Pathology Laboratory, Faculty of Medicine, Padjadjaran University, Bandung, Indonesia. The subject in this study was male Wistar rat. Inclusion criteria: healthy, age 2-4 months; weight 200-250 gram. Exclusion criteria: death prior to the sacrificial time and major anastomosis leakage marked by the accumulation of pus and faeces in intrabdominal. Preoperative mechanical bowel preparation was performed in treatment group prior to surgery and then all subjects undergo resection-anastomosis procedure at colon descendent.

All of Wistar rats had been adaptatio process for 3 days. Each wistar rat was placed in the cage/box with size 30 cm × 30 cm × 30 cm and given simillar nutrition. Preoperative mechanical bowel preparation was performed twice in treatment group in the 4th days adaptation. In 5th days all mice undergo resection-anastomosis procedure at colon descendent.

Mechanical bowel preparation

The rat in the supine position with all four limbs fixed with plaster. Insertion 5cm of a Naso-gastric

tube (NGT) into the anal canal. Then administration 20cc of normal saline through the NGT three times. The inserted liquid must wait until it returns with the feces. This procedure was performed twice one day before surgery

Bowel resection-anastomosis

Rats were anaesthetized with ketamine with 9 mg intra-muscular dose, Rats in supine position, four limbs and tail fixed with plaster on the operating table, left abdomen shaved and cleaned, disinfection of abdominal skin area with betadine, abdomen then opened by using number 15 blade to penetrate the peritoneum, identify descendent colon, Place 2 pieces of intestinal clamps on the descendent colon and perform bowel resection. Preservation of blood vessels at the end of the intestine, The colon is then stitched by using 5.0 safil with interrupted suture throughout the circle. Furthermore, the test is performed on the anastomosis site whether there is leakage or not, abdominal cavity washed with normal saline and then drained, abdominal cavity closed back with silk yarn 2.0 continuous and mass closure. The skin stitched with silk 2.0 continuous. The surgical wound is cleaned, betadine and covered Verband replaced daily.

Each rat received an intravenous amoxicillin antibiotic with a dose of 3 × 18 mg and an analgesic ketorolac 3 × 1.5 mg orally for 5 days postoperatively. On the 10th day after the bowel anastomosis resection procedure, all subjects were sacrificed and then intestinal tissue taken at the site of anastomosis and then assessed histopathologically by Hematoxylin-Eosin (HE) staining for fibroblast cell count, inflammatory cell infiltration and degree of damage to the wall colon while Van Gieson staining was used to assesses the density of collagen in the anastomosis site. Description of Histopathological analysis that was used to assess fibroblast cell count, inflammatory cell infiltration, density of collagen and intestinal wall injury showed in [table 1](#). Statistical analysis was performed using SPSS software version 13 for Windows. A Mann-Whitney test was used in this study because the data collected from each group was not normal after undergoing normality test with Kolmogorov Smirnov test and homogeneity test with Levene test.

RESULTS

All of Wistar rats live until the end of the procedure. One Wistar rats in second group had minor leakage anastomosis during reopening of the abdomen. Histopathological analysis from [table 2](#) gives us early information that group without mechanical bowel preparation have better score specially to fibroblasts count and inflammatory cell infiltration.

Table 1 Description of histopathological analysis

Histopathological Analysis	Classification
Fibroblast count	None: 0
	Low: 1
	Moderate: 2
	High: 3
	Very high: 4
Collagen density	None: 0
	Low: 1
	Moderate: 2
	High: 3
	Very high: 4
Inflamtmatory cell infiltration	None: 0
	Low: 1
	Moderate: 2
	High: 3
	Very high: 4
Intestinal wall injury	Subepithelial dilatation with capillary congestion in apical villi: 1
	Moderate separation of epithelial space: 2
	Complete separation of epithelial: 3
	Severe damage of villi with capillary dilatation: 4
	Lamina propria damage with bleeding and ulceration: 5

Table 2 The result of histopathological analysis of fibroblast count, collagen density, inflammatory cell infiltration and intestinal layer injury in each subject

Variable	Group I (n=6)						Group 2 (n=6)					
	1	2	3	4	5	6	1	2	3	4	5	6
Fibroblast count	3	2	1	2	3	3	4	3	4	4	4	4
Collagen density	3	2	2	3	3	3	3	2	3	2	2	2
Inflammatory cell infiltration	4	4	4	2	3	1	2	2	2	2	2	2
Intestinal wall injury	5	4	5	4	4	4	5	5	4	4	4	2

Table 3 Comparison of fibroblast count, collagen density, inflammatory cell and intestinal layer injury

Variable	Control		p
	I	II	
	Mean ± SD	Mean ± SD	
Fibroblast	2.33 ± 0.816	3.83 ± 0.408	0.02*
Collagen	2.5 ± 0.547	2.33 ± 0.516	0.59*
Inflammatory cell	3 ± 1.264	2 ± 0	0.082*
Intestinal layer injury	4.33 ± 0.492	4.33 ± 0.492	1.00**

Note: * Independent t-test; ** Mann Whitney test

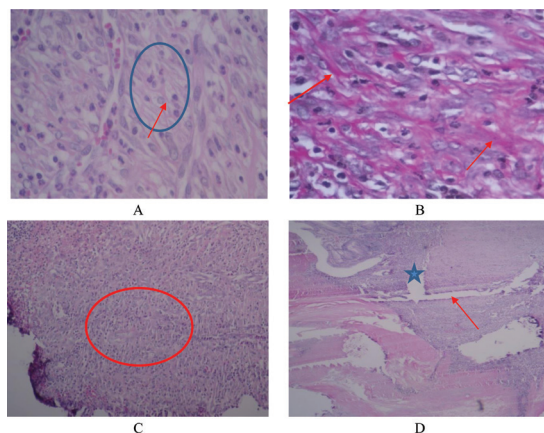


Figure 1 Histopathology image: A) Very high fibroblast count (red arrow) with 100x magnification; B) Moderate collagen density (red arrow) with 400x magnification; C) Moderate inflammatory cell infiltration (blue nucleus) with 40x magnification; D) Lamina propria (red arrow) damage with ulceration (blue asterisk) at intestinal wall with 4x magnification.

Statistical analysis from all variable (table 3) concluded the number of fibroblasts higher in the treatment group 3.83 compared to the control group that was 2.33. Analysis with Independent T-test at 95% confidence level showed that mean of fibroblast of the treatment group was significantly higher ($p = 0.02$) than mean of fibroblast in control group.

DISCUSSION

Based on Table 3, the number of fibroblasts higher in the treatment group 3.83 compared to the control group that was 2.33. Statistical analysis with Independent T-test at 95% confidence level showed that mean of fibroblast of the treatment group was significantly higher ($p = 0.02$) than mean of fibroblast in control group. According to Bucher et al. PMBP in the intestine by will cause significant damage to the epithelial layer and colonic superficial mucus. This damage can be seen in 4-8 hours after PMBP.⁹ Also, normal saline administered in PMBP also contributes to worsening bowel wall damage. In another study that compared the use of Ringer's lactate, polyethylene glycol and normal saline solution in rat undergo PMBP, the results reported that intestinal damage and inflammation were higher at normal saline group compare to others.¹⁰

In the process of good wound healing, 24 hours after tissue damage the fibroblasts will migrate and proliferate actively. The migration of fibroblasts is also reinforced by Transforming Growth Factor- β (TGF- β) produced by platelets, Interleukin-1 produced by keratinocytes and Fibroblast Growth Factor (FGF) produced by macrophages. On the fourth day of the 21st day, fibroblasts actively synthesise collagen, elastin, and glycosaminoglycans as extracellular matrix material.¹¹

In this study, the density of collagen was not significantly different between the two groups. It can be explained that the process of collagen synthesis and collagen degradation is balanced. A study by

Young et al. reported there was an increase in the collagen degradation in the anastomosis site up to the fourth day of intestinal anastomosis in experimental animals.¹² This result also consistent with the study by Kosmidis et al. where anastomosis leakage occurs mostly on the second to fourth day of the post-intestinal anastomosis.¹³ In addition, Bucher et al. also reported there is significant inflammatory cells infiltration in the colon after PMBP.⁹ The imbalance between the collagen synthesis and collagen degradation is strongly influenced by the presence or absence of infection around the anastomosis area. This can be seen from histopathology and matrix metalloproteinase (MMP) 1 and 13.¹⁴

After the fourth day, the collagen synthesis would appear to be dominant which would increase the strength of bowel anastomosis.¹¹ In another study, on the fourth-day hydroxyproline concentrations (an amino acid found almost exclusively in collagen) in anastomosis site showed no significant difference between PMBP and control groups.¹⁵ Because in this study histopathologic examination was examined on the tenth day, it can be concluded that statistically there was no significant difference in collagen density in place of the anastomosis.

Furthermore, in [table 3](#) showed the infiltration of inflammatory cells and degree of intestinal wall damage were not significantly different in statistical analysis. A study Fa-Si-Oen et al. reported the same thing in their study, where there was no significant difference in the infiltration of inflammatory cells and degree of intestinal wall damage in PMBP group compared to control groups in humans.¹⁶ This results in both our and Fa-Si-Oen et al. studies which PMBP was performed 1 day before surgery and then the sample for histopathologic examination is taken 24 hours later.¹⁶ This correlates with the turn-over of intestinal epithelial cells which mostly happen in 2-3 days after the breakdown or death epithelial cells and will form a new cell 2-3 days later.⁴¹ In another study by Bucher et al. there is a significant difference in the infiltration of inflammatory cells and damage to the lining of the intestine in mice colon undergo PMBP. The histopathologic sample was obtained from the colon, 4 hours after PMBP was performed. According to Bucher et al. concluded that even though there are significant changes in both variables at the beginning, it is not the main cause of anastomosis leakage.⁹

Research by Gravante et al. confirmed that there is no clear advantage in PMBP in reducing complication rates such as leakage of bowel anastomosis, surgical wound infections, and intra-abdominal abscesses.⁶ There are two explanations of the high number of intestinal leakage in PMBP group, first the PMBP cause alteration of structure and inflammation of the intestinal

mucosal lining which includes loss of mucus in the superficial layer and an increase in polymorphonuclear lymphocyte by 52% in PMBP group compared to 8% in control group. The impact of histologic structure changes on the intestinal mucosa is not yet known, but this may cause a disruption of structures integrity in the site of bowel anastomosis.^{2,7} Second, there is a fluid and electrolyte imbalance in intestinal wall which is a high-risk factor for leakage of anastomosis.⁸

Jung et al. concluded in his study that PMBP was no more favourable than without PMBP in managing patients with elective colorectal surgery. In addition, PUM also causes abdominal pain, nausea, and vomiting before and after surgery. The most common complication is the resumption of normal bowel work longer if compared with PMBP. This is due to PMBP disturb the fluid and electrolyte balance resulting in slower rate of bowel to return to the normal bowel work.¹⁸

Limitation of this study is we only do the mechanical bowel preparation in one day before surgery and one type of bowel preparation. Usually, the preparation is combined and start 2 or 3 days before surgery. Furthermore, for the good results for the next research is do the histopatlogy analysis in each phase of anastomosis healing.

CONCLUSION

In general, it can be concluded that morphological changes in the intestinal wall are not the main factors causing intestinal leakage. This study showed a significant difference in the number of fibroblast cells in which the number of fibroblast cells in the PMBP group is higher than the control group. In addition, the density of collagen, infiltration of inflammatory cells and the degree of intestinal wall damage were not different between the 2 groups, this was possible because of sampling at the tenth day after PMBP.

ETHICAL CONSIDERATION

All protocol in this study has been reviewed by Ethical Committee Hasan Sadikin General Hospital, Bandung-Indonesia with ethical clearance reference number LB.04.01/A05/EC/013/II/2012.

CONFLICT OF INTEREST

There is no conflict of interest.

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