ISSN: 0975 -8542

Journal of Global Pharma Technology

Available Online at: www.jgpt.co.in

RESEARCH ARTICLE

Abdominal Fascia Suturing with Polypropylene Reduces intraabdominal Adhesion Incidence and Neutrophil, Macrophage, and Lymphocyte Infiltrations in Wistar Rats

Ketut Sudartana[1*], Christopher Ryalino[2]

[1.] *Departments of Surgery Faculty of Medicine, Sanglah Hospital/Udayana University Bali, Indonesia.*

[2.] *Anesthesiology Faculty of Medicine, Sanglah Hospital/Udayana University Bali, Indonesia.*

***Corresponding Author:** *Ketut Sudartana*

Abstract

Background: Intraabdominal adhesion after surgery causes pain, infertility, and chronic abdominal pain. Intraabdominal adhesion also causes longer surgery time and increases intraoperative complications. This study aimed to prove that the use of polypropylene suture causes less intraabdominal adhesion and less inflammation compared to polyglycolic acid suture. Methods: This was a randomized posttest-only control group study. Thirty-six mature male Wistar rats weighing 200-250 g were anesthetized with ketamine 50 mg/kg intramuscularly. It was incised on the inner abdominal wall layer by layer until peritoneum was seen. Wistar rats in Group A were sutured with polypropylene 4-0 with a half-centimeter apart. Rats in Group B were sutured with polyglycolic acid 4-0 suture. Peritoneum in both groups was not closed, and the abdomen was closed layer by layer, and then the lesion was enclosed with bandage. Relaparotomy was performed on day 14 and 30 to evaluate the presence of adhesion and inflammatory cells, MMP-1, TGF-ß, and TNF-α. Results: There was a significant difference between polypropylene-based sutures compared polyglycolic acid in terms of the presence of intraabdominal adhesion on 30 days after laparotomy ($p=0.016$). The infiltration of neutrophil, macrophage, and lymphocytes were significantly lower in polypropylene groups compared to polyglycolic acid ($p<0.001$). Conclusion: Polypropylene-based sutures resulted in less adhesion and inflammation compared to polyglycolic acid sutures in Wistar rats underwent laparatomy.

Keywords: *Laparotomy, Lymphocyte, Macrophage, Neutrophil, MMP-1.*

Introduction

Intraabdominal adhesion after surgery causes several things such as pain, infertility, and abdominal pain. Knowledge of the pathogenesis of intra-abdominal adhesion from cellular and molecular aspects can help effectively prevent intra-abdominal adhesion. After peritoneum is traumatized, fibrinolytic activity on the peritoneal surface decreases, causing the expression and synthesis of several cellular mediators and remodeling of connective tissue. Intraabdominal adhesion after laparotomy causes high morbidity [1].

In some recent studies reported 33% of patients who undergo laparotomy surgery come back to the hospital with various complications [2, 3]. Congenital intraabdominal adhesions or those caused by infection rarely cause intestinal obstruction. Intraabdominal adhesions also lead to longer operating times [4].Some other causes of intraabdominal adhesion include bacterial peritonitis, radiotherapy, chemical peritonitis, foreign bodies, prolonged peritoneal dialysis and infectious diseases in the pelvic cavity. Adhesion to the pelvic cavity can cause infertility and pain [5, 6].Materials commonly used as barrier adhesion are oxidized regenerated cellulose (Interceed) and polytetrafluoroethylene (PTC), both barrier materials are proven to be safe and effective for preventing intraabdominal adhesion [7].

In several studies it was reported that the barrier was good for preventing intraabdominal adhesions but could not completely eliminate adhesion in all patients. Good knowledge of the pathogenesis of intraabdominal adhesion at the cellular and

molecular level is needed to be able to find a more effective way to prevent the formation of post-laparotomy intra-abdominal adhesions. Closure of the abdominal cavity wall after surgery, especially in the peritoneum, is very varied. Experiments on rats that underwent laparotomy found that there were no significant differences in the occurrence of intraabdominal adhesion between the stitched and not sewn peritoneum [8, 9]. The goal of this study was to compare the polypropylene-based and polyglycolic acid sutures in terms of the presence of adhesion and inflammatory cells after laparotomy surgery.

Methods

This was a randomized, posttest-only, controlled-group study. Adult, healthy, male Wistar rats, weighed 200-250 g were used in this study. The rats were obtained from the Laboratory of Pharmacology of the Faculty of Veterinary Medicine, Udayana University. They were selected by simple random sampling. If, at the time of the study there were signs of intraabdominal infection, they would be excluded from the study.

Experimental animals are placed in separate cages and under controlled conditions. All subjects were given ketamine 50 mg/kg intramuscularly. Incisions were made in the midline of the abdominal wall until the abdominal fascia was identified. Then the abdominal fascia was sharply targeted to create a straight wound per the axis of the body. An intraabdominal organ evaluation was performed to determine whether there were abnormalities or not, and then the surgical wound was closed without peritoneal suturing. The control group was sewn using 4-0 atraumatic polyglycolic acid thread, four stitches at ½ cm apart. The treatment group was sewn with 4-0 atraumatic polypropylene yarn, four stitches at ½ cm apart. After 14 and 30 days we performed relaparotomy by making incisions on the right lateral side of the laparotomy wound until they penetrate the peritoneum and then observed directly whether there is adhesion between the omentum, intestine and abdominal wall (peritoneum). We took some specimens from the fascia for neutrophils, macrophages, lymphocytes, matrix metalloproteinase-1 (MMP-1), TGF-ß, and TNF-α examination.

Histological preparations were prepared and then viewed under a binocular microscope Olympus CX21 with 400 times magnification. We counted neutrophils, macrophages, and lymphocytes in the field of view. The calculation was carried out by checking the Enzym Linked Immunosorbent Assay (ELISA).We used SPSS version 22 for Windows for data analysis. The Kolmogorov-Smirnov test was used to see data normality.

Levene's test was used to assess the homogeneity of variant data between groups. Then the independent t-test is used as a comparative analysis. A p-value of <0.05 was considered statistically significant.

Results

This study observed the presence of intraabdominal adhesion between polypropylene and polyglycolic acid yarn in Wistar rats. The results are displayed in Table 1.

Table 1: The comparison between intraabdominal adhesion incidents in day-14 and day-30 between polypropylene and polyglycolic acid sutures in the Wistar rats

Observation	Suture types	Adhesion observed	Adhesion absent	CI95%	P
Day-14	Polypropylene	3 (33.3%)	6 (66.7%)	0.50 (0.178-1.410)	0.16
	Polyglycolic acid	6 (66.7%)	3 (33.3%)		
Day-30	Polypropylene	1 (11.1%)	8 (88.9%)	0.36 (0.15-0.97)	0.016
	Polyglycolic acid	6 (66.7%)	3 (33.3%)		

We also observed the difference between macrophage, neutrophil, lymphocyte, and MMP-1 levels in the Wistar rats. The infiltration of these inflammatory cells is an indicator of inflammation in the abdominal fascia. The results are shown in Table 2.

Table 2: The comparison between inflammatory cell counts between the two groups

Inflammatory cells	Polypropylene	Polyglycolic acid	CI95%	p
Neutrophils[a]	16.87±4.01	100.38±8.97	83.50 (75.72-91.28)	<0.001
Macrophages[a]	18.62±5.52	80.50±4.81	61.88 (56.31-67.44)	<0.001
Lymphocytes[a]	17.13±3.39	30.36±2.62	13.25 (9.66-16.89)	<0.001
MMP-1[b]				
Day-14	10.38±5.74	10.26±4.03	-0.12(-0.51-4.84)	0.96
Day-30	7.12±3.82	5.60±2.66	-1.51(-1.55-4.79)	0.35
TGF-ß[b]				
Day-14	60.94±24.51	66.72± 17.51	5.77(-15.69-27.25)	0.57
Day-30	65.15±13.05	62.41±16.15	-2.73(-17.41-(-11.93))	0.69
TNF-α[b]				
Day-14	250.26±133.79	268.98±92.68	18.72(-0.51-4.84)	0.73
Day-30	306.91±166.03	233.39±34.69	73.55(46.29-193.41)	0.21

[a] Data presented in mean± SD (cells per field of view); [b] Data presented in mean± SD (pg/dL);
MMP: matrix metalloproteinase; TGF: transforming growth factor; TNF: tumor necrosis factor

Discussion

This study showed that in abdominal fascia after a laparotomy, polypropylene and polyglycolic acid yarns showed a very significant difference on 30-days after the surgery (p=0.016). Normally wound healing occurs without adhesion formation. Tissue damage will be followed by fibrin formation.

Thromboplastin, prothrombin, and thrombin will activate fibrinogen into fibrin. Platelet clots derived from platelet aggregation together with fibrin clots form fibrin tissue [10].Many experimental studies have proven that various forms of injury to the mesothelium significantly reduce the potential for fibrinolysis. Whawell *et al* [11].Showed that pure culture of mesothelium cells has the ability to fibrinolysis. Supported by a study of antibody inhibitors and antigenic immunoassays which explains that tissue plasminogen activator (tPA) is the main plasminogen activator in human peritoneal biopsy, which stimulates fibrin lysis and prevents adhesion [12].

The number of inflammatory cells found in this study was due to the inflammatory response in the injured tissue. The capillary capability of the blood increases and the fluid that contains a lot of protein and inflammatory cell components. Polyprofilene yarn is derived from polyamide polymer materials. This material has low toxicity to tissue, so it rarely produces an inflammatory reaction in the surrounding tissue [13].

The monofilament structure of this yarn makes it is relatively easy to penetrate tissue compared to multifilament yarn, causing smaller trauma to the tissue. Polyglicolic acid yarn is synthesized through lactic and glycolic polymers which are derived from lactic acid and glycolic acid [14, 15].

Several studies showed that during the hydrolysis of polyglicolic acid threads, acid concentrations in the extracellular environment will increase, and will trigger complementary activation and stimulate the inflammatory cascade [16]. Polyglicolic acid yarn absorption does not occur simultaneously in each section. About 30% of this yarn has not been fully absorbed for six weeks.

The involvement of inflammatory cells that dominate the injured area indicates that the inflammatory process was ongoing. The inflammatory process is useful for neutralizing and removing secondary infectious agents, destruction of tissue necrosis and tissue repair and recovery [17].The inflammatory process involves the recruitment of inflammatory cells from the blood vessels to the injured tissue. Cells that infiltrate the injured area include neutrophils, macrophages, and lymphocytes.

The inflammatory process also requires cellular responses that function to eliminate foreign bodies and dead tissue. Increased blood supply to the tissues carries nutrients needed for the healing process, so that the operating area appears red and slightly swollen [17].We found no significant difference between the two groups in MMP-1 levels. This may be related to the role of MMP-1 which appears more in the chronic wound healing process (more than four weeks).

MMP-1 is able to degrade type III collagen faster than collagen types I and II, while MMP-8 degrades type I collagen with a faster speed than type III collagen [18].The process of wound healing begins with the formation of fibrin clots, followed by the release of

various growth factors from injured cells and extracellular matrix, inflammation, granulation tissue formation, epithelialization and ultimately the production of matrix and remodeling [19, 20].During the remodeling period, the extracellular matrix is temporarily degraded and replaced by collagen. MMP-1 and MMP-8 play an important role in the regulation and wound healing process, while other MMPs, such as MMP-2, MMP-9 and MPMP-19, also play a role in wound repair [21, 22].

MMP-1 is characterized by migrating basal keratinocytes in all types of cutaneous lesions and completing the re-epithelialization process causes a decrease in the level of MMP-1 [23].TGF-ß levels in tissues are very important to describe the mechanisms involved in various physiological and pathological processes [24, 25].The results of measurements of TGF-ß levels in serum and bioassays as well as the enzyme linked imunnosorbent assay (ELISA) obtained results that were not much different.

It is recognized that an increase in TGF-ß levels can be used as a marker for the process of fibrosis [26, 27].In the process of fibrosis almost every cell transmits TGF-ß receptors, where TGF-ß is very important for the activation of fibrogenic cells. Therefore the role of TGF-ß has the ability to affect all stages of fibrosis. Three sources of fibrogenic cells have been identified, namely: activation of resident fibroblasts, fibroblast derivates from the bone marrow, and fibroblasts that result from epithelial to mesenchymal (EMT) transitions. TGF-ß is also one of the most effective chemoattaractans for macrophages and selieloid.

As the main source of TGF-ß from injured tissue is tissue macrophages and recruited macrophages (ilfiltrating macrophages). There are several other cells that also excrete TGF-ß, including: parenkhim cells undergoing apoptosis and myofibroblasts. The multifunctional cytokine TGF-ß during the process of fibrogenesis is the formation of fibrous tissue, healing and repair of damaged parenkhim tissue [28]. The initial trigger for the inflammatory response is TNF-α and this can stimulate the production of other cytokines and adhesion molecules such as ICAM and VCAM. The main sources of TNF-α after abdominal fascia laparotomy surgery are macrophages and fibrinolytic cells [24].

Cytolytic cytokines or TNF-α have the ability to induce apoptosis and inflammation that is highly dependent on the cellular environment [29].In studies involving humans as clinical objects, the role of immunization and immunoregulation that TNF-α can be illustrated is the administration of anti-TNF-α to patients to reduce the symptoms of the disease.

From the results of this study, there was no difference in the two treatments due to the disruption of the fibrinolysis process, the macrophages would survive and the fibroblasts proliferate. Within five days the fibrin tissue that is formed will be replaced by fibroblast cells and the formation of new blood vessels, will bring antiplasm to counteract the effects of fibrinolysis and thicken the fibrous tissue to form permanent fibrous adhesion [10].

Conclusion

In rats that underwent laparotomy, polypropylene-based sutures showed less intraabdominal adhesion compared to polyglycolic acid on 30 days after the laparotomy. The number of neutrophils, macrophages, and lymphocytes in the abdominal fascia was less in polypropylene-based sutures compared to polyglycolic acid.

References

1. Van Goor H (2007) Consequences and complications of peritoneal adhesions. Color Dis., doi:10.1111/j.1463-1318.2007.01358.x
2. Singh K, Hariharan S (2019) Detecting major complications and death after emergency abdominal surgery using the surgical apgar score: A retrospective analysis in a caribbean setting. Turkish J. Anaesthesiol. Reanim, doi:10.5152/TJAR.2019.65872
3. Cauley CE, Panizales MT, Reznor G, et al (2015) Outcomes after emergency abdominal surgery in patients with advanced cancer: Opportunities to reduce complications and improve palliative care. J. Trauma Acute Care Surg., doi:10.1097/TA.0000000000000764
4. Brüggmann D, Münstedt K, Tinneberg HR, Hackethal A, Tchartchian G,

Wallwiener M (2010) Intra-abdominal adhesions - Definition, origin, significance in surgical practice, and treatment options. Dtsch Arztebl., doi:10.3238/arztebl.2010.0769

5. Rosenthal RH, Ling FW, Rosenthal TL, Gene McNeeley S (1984) Chronic pelvic pain: Psychological features and laparoscopic findings. Psychosomatics, doi:10.1016/S0033-3182(84)72944-6
6. Stout AL, Steege JF, Dodson WC, Hughes CL (1991) Relationship of laparoscopic findings to self-report of pelvic pain. Am J. Obstet. Gynecol., doi:10.1016/0002-9378(91)90630-A
7. Ahmad G, Duffy JMN, Farquhar C, et al (2008) Barrier agents for adhesion prevention after gynaecological surgery. Cochrane Database Syst. Rev., doi:10.1002/14651858.CD000475.pub2
8. Gungor B, Malazgirt Z, Topgül K, Gök A, Bilgin M, Yürüker S (2010) Comparative Evaluation of Adhesions to Intraperitoneally Placed Fixation Materials: A Laparoscopic Study in Rats. Indian J. Surg., doi:10.1007/s12262-010-0168-3
9. Ribeiro WG, Rodrigues DVS, Atta FFM, et al (2019) Comparative study of peritoneal adhesions after intraperitoneal implantation in rats of meshes of polypropylene versus polypropylene/polyglecaprone versus polyester/porcine collagen. Acta Cir. Bras., doi:10.1590/s0102-865020190060000003
10. Moretti AIS, Souza Pinto FJP, Cury V, et al (2012) Nitric oxide modulates metalloproteinase-2, collagen deposition and adhesion rate after polypropylene mesh implantation in the intra-abdominal wall. Acta Biomater, doi:10.1016/j.actbio.2011.08.004
11. Whawell SA, Scott- Coombes DM, Vipond MN, Tebbutt SJ, Thompson JN (1994) Tumour necrosis factor- mediated release of plasminogen activator inhibitor 1 by human peritoneal mesothelial cells. Br J. Surg., doi:10.1002/bjs.1800810218
12. Chegini N (1997) The role of growth factors in peritoneal healing: Transforming growth factor ß (TGF-ß). In: European Journal of Surgery, Acta Chirurgica, Supplement.
13. Wong VWY, Rao SK, Lam DDC (2007) Polyglactin sutures versus nylon sutures for suturing of conjunctival autograft in pterygium surgery: A randomized, controlled trial. Acta Ophthalmol Scand doi:10.1111/j.1600-0420.2007.00904.x
14. Kalu E, Sumar N, Giannopoulos T, et al (2007) Cytokine profiles in serum and peritoneal fluid from infertile women with and without endometriosis. J. Obstet. Gynaecol. Res., doi:10.1111/j.1447-0756.2007.00569.x
15. Munton CGF, Phillips CI, Martin B, Bartholomew RS, Capperauld I (1974) VICRYL® (Polyglactin 910): A new synthetic absorbable suture in ophthalmic surgery: A preliminary study. Br J. Ophthalmol., doi:10.1136/bjo.58.11.941
16. Ceonzo K, Gaynor A, Shaffer L, Kojima K, Vacanti CA, Stahl GL (2006) Polyglycolic acid-induced inflammation: Role of hydrolysis and resulting complement activation. Tissue Eng., doi:10.1089/ten.2006.12.301
17. Bachus KE, Doty E, Haney AF, Weinberg JB (1995) Differential Effects of Interleukin-Iα, Tumor Necrosis Factor-α, Indomethacin, Hydrocortisone, and Macrophage Co-Culture on the Proliferation of Human Fibroblasts and Peritoneal Mesothelial Cells. J. Soc. Gynecol. Investig., doi:10.1177/107155769500200410
18. Knuutinen A, Kokkonen N, Risteli J, et al (2002) Smoking affects collagen synthesis and extracellular matrix turnover in human skin. Br J. Dermatol., doi:10.1046/j.1365-2133.2002.04694.x
19. Eming SA, Martin P, Tomic-Canic M (2014) Wound repair and regeneration: Mechanisms, signaling, and translation. Sci. Transl. Med., doi:10.1126/scitranslmed.3009337
20. Reinke JM, Sorg H (2012) Wound repair and regeneration. Eur. Surg. Res., doi:10.1159/000339613
21. Mueller MD, Tschudi J, Herrmann U, Klaiber C (1995) An evaluation of laparoscopic adhesiolysis in patients with chronic abdominal pain. Surg. Endosc., doi:10.1007/BF00190085
22. Bremers AJA, Ringers J, Vijn A, Janss RAJ, Bemelman WA (2000) Laparoscopic adhesiolysis for chronic abdominal pain: An objective assessment. J. Laparoendosc.

Adv. Surg. Tech - Part A. doi:10.1089/109264200421577

23. Caley MP, Martins VLC, O'Toole EA (2015) Metalloproteinases and Wound Healing. Adv Wound Care, doi:10.1089/wound.2014.0581
24. Kaidi AA, Nazzal M, Gurchumelidze T, et al (1995) Preoperative administration of antibodies against tumor necrosis factor-alpha (TNF-α) and interleukin-1 (IL-1) and their impact on peritoneal adhesion formation. In: American Surgeon.
25. Kanzaki G, Nagasaka S, Higo S, et al (2016) Impact of anti-glomerular basement membrane antibodies and glomerular neutrophil activation on glomerulonephritis in experimental myeloperoxidase-antineutrophil cytoplasmic antibody vasculitis. Nephrol. Dial. Transplant., doi:10.1093/ndt/gfv384
26. Epstein FH, Border WA, Noble NA (1994) Transforming growth factor ß in tissue fibrosis. N. Engl. J. Med., doi:10.1056/NEJM199411103311907
27. Verrecchia F, Mauviel A (2007) Transforming growth factor-ß and fibrosis. World J. Gastroenterol., doi:10.3748/wjg.v13.i22.3056
28. Kisseleva T, Brenner DA (2008) Fibrogenesis of parenchymal organs. In: Proceedings of the American Thoracic Society, doi:10.1513/pats.200711-168DR
29. Saba AA, Godziachvili V, Mavani AK, Silva YJ (1998) Serum levels of interleukin 1 and tumor necrosis factor α correlate with peritoneal adhesion grades in humans after major abdominal surgery. Am Surg.

